# HERBICIDE CONCENTRATIONS IN SELECT WATERFOWL PRODUCTION AREA WETLANDS IN WEST CENTRAL MINNESOTA, 1993

by

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## ABSTRACT

Twenty-eight emergent, seasonally to semipermanently flooded wetlands in an intensively farmed area of west central Minnesota were sampled before and during the 1993 crop growing season to determine surface water concentrations of widely used herbicides. Specifically, water obtained from eight isolated Waterfowl Production Area (WPA) wetlands ("reference") and twenty co-owned WPA wetlands ("treatments") (bordering agricultural croplands which had high potential for exposure to chemical influences) was analyzed to determine whether, and to what extent, wetland basins both adjacent to, and isolated from, agricultural lands contained measurable concentrations of herbicides. Surface water samples were screened for alachlor and triazine class herbicide types using enzyme-linked immunosorbent (ELISA) assays. Identification of individual herbicides were determined by chemical analyses.

Although alachlor and triazine concentrations are no longer used on Service lands, both were found by ELISA detection at concentrations in surface waters from WPA reference wetlands. Chemical analyses failed to detect (when measured) any individual common or acid herbicides in any of the eight reference wetlands. Herbicide occurrence in reference wetlands at the maximum observed concentrations is not known to significantly impact aquatic vegetation or invertebrates. However, while concentrations of these herbicides in reference WPA wetlands were found to be low, timing of increases in their respective concentrations tended to follow trends observed in the treatment WPA wetlands. Such results strongly suggest the existence of aerial drift and/or precipitation deposition of herbicides in "isolated" WPA wetlands.

Based on a limited number of samples, it is apparent that some co-owned WPA wetlands can acquire herbicide concentrations which exceed concentrations typically detected in isolated WPA wetlands. Several herbicide concentrations (triazine and alachlor classes, dicamba, bentazon, imazethapyr, atrazine, cyanazine, alachlor) in some wetlands were up to several orders of magnitude higher in co-owned treatment WPA wetlands than concentrations found in isolated WPA reference wetlands. The FY 93 survey maximum alachlor and triazine concentrations derived by ELISA were similar to the FY 92 maximum concentrations found in privately owned wetlands (individual herbicides were not measured in FY 92 survey). Herbicide concentrations in some of the treatment WPA wetlands suggest that concentrations of those herbicides being introduced into WPA wetlands by way of direct runoff from adjacent croplands may be biologically significant. Also, apparent differences in herbicide concentrations occurred within the four WMDs and may reflect geographic climatic, cropping pattern differences, or local biases toward specific herbicides for given crop types or application rates across the district.

Lastly, comparisons of ELISA and analytical chemistry determinations of triazine and alachlor-related compounds showed measurement differences at low and high concentrations in some wetlands. Dr. Jim Zajicek (Chemist, Immunochemistry Research Section) of the National Biological Survey reviewed the data and provided an important discussion to explain the discrepancies. His review (provided in Appendix I) will be extremely beneficial in future ELISA studies for the Environmental Contaminants Program of the Service.

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## INTRODUCTION

Vegetative habitat structure, specifically the interspersion of macrophyte cover and open water, has been identified as an important biotic factor controlling the selection of wetlands by breeding waterfowl (Keith 1961; Kaminski and Prince 1981; Murkin et al. 1982; Nelson and Kadlec 1984; review by Kantrud 1986). Nelson and Kadlec (1984) described the interactions occurring among macrophytes, macroinvertebrates, and waterfowl in freshwater wetlands as a complex interdependency in which "dynamic changes in the abundance and distributional pattern of macrophytes influence functional processes in litter decomposition and macroinvertebrate communities that, in turn, affect reproductive effort and success of waterfowl." Lillie and Evrard (1994) supported these findings and suggested that the morphometry or vegetative structure of a wetland may modify the strength of the interrelationships between waterfowl and macroinvertebrates. Thus, the vegetative structure of a wetland may influence associations between waterfowl breeding pair densities and selected limnological habitat variables.

Herbicides that enter wetland systems may have direct toxic effects on aquatic plants and invertebrates and, therefore, may indirectly affect the reproduction and survival of waterfowl by altering food and cover (Grue et al. 1988). At low concentrations, herbicides can directly impact algae, protozoans, plankton, seeds, foliage, roots and tubers that invertebrates and hen waterfowl (e.g., gadwalls, mallards, pintails) depend upon for food and survival.

Wildlife inhabiting seasonal and semipermanent wetland types may have the greatest potential for exposure to agricultural chemicals. These wetlands are typically relatively small in size and interspersed within private agricultural croplands, which increases the probability of pesticide inputs from direct overspray and aerial drift (Grue et al. 1986). A more detailed discussion on the importance of prairie potholes to waterfowl and the factors relating to increased potential for herbicide runoff into wetlands is provided elsewhere (Ensor and Smith 1994).

While many herbicides degrade relatively rapidly in the environment, many persist for longer periods of time than organophosphate, carbamate and synthetic pyrethroid insecticides. In addition, most herbicides are applied to cropland in spring and early summer--seasons that coincide with periods of maximum precipitation runoff and migratory bird reproductive activity. U.S. Fish and Wildlife Service Wetland Management Districts (WMDs) currently

manage thousands of acres of Federal lands in Minnesota for waterfowl production, including Waterfowl Production Areas (WPAs). In 1990, 15,825 pounds of active ingredients representing 20 herbicides were applied to 15,533 acres of Service-managed lands in Minnesota (USFWS 1990). The most heavily and most frequently used chemical was 2,4-D. In 1987, approximately $100,000 was spent on noxious weed control on approximately 16,000 acres of WMD lands (USFWS 1992). This amount does not include the cost of chemicals for nesting cover establishment. In light of an increased emphasis on implementation of Integrated Pest Management (IPM) and reduction of chemical use on Service lands, several concerns come to light: (a) implications of the authorized use of agricultural chemicals on Service lands in close proximity to wetlands and other surface waters have not been determined; (b) implications of private agricultural chemical use directly adjacent to Service WPA wetlands have not been determined; and (c) how such Service uses compare with private agricultural chemical uses in terms of resulting wetland water quality.

To address these concerns, the Twin Cities ES Field Office (TCFO) conducted a two-year (FY 92 and 93) water quality investigation to determine the frequency with which west-central Minnesota wetland basins, both adjacent to and isolated from private croplands, contained measurable concentrations of the most widely used herbicides. This report addresses the second year of that two-year investigation on herbicide use in Minnesota's prairie pothole region.

*Results of the FY 92 TCFO Investigation*

A Twin Cities Field Office (TCFO) investigation was undertaken in FY 92 to determine whether, and to what extent, prairie pothole wetlands in or adjacent to agricultural lands in Minnesota are non-target receptors for agricultural herbicides. Results from the survey indicate that many temporary, seasonal and semipermanent wetlands within agricultural settings in west central Minnesota may be receiving herbicides via runoff at concentrations which can adversely impact their flora and fauna (Ensor and Smith 1994). Private wetlands adjacent to croplands contained higher concentrations of both triazine and alachlor herbicides than wetlands on WPAs isolated by distance and topography from herbicide use. The higher concentrations of each herbicide group measured in the 1992 survey have been shown by others to impact primary producers, such as planktonic and filamentous algae, floating plants (e.g., *Lemna*), and some shallow-rooted broadleaved submergents (e.g., *Vallisneria*). Grass-like perennials such as cattails, rushes, sedges, and reeds were likely unaffected by herbicide concentrations measured in the study. Concentrations of 2,4-D were consistently low and similar among both private and WPA wetlands. The 2,4-D concentrations measured in the study have not been shown to have adverse impacts on aquatic life.

In addition, some WPA wetlands adjacent to private croplands and some drainage ditches flowing through WPAs contained herbicide concentrations which exceeded those found in isolated WPA wetlands. WPA wetlands on such ditch systems could be receiving biologically significant concentrations of several herbicides. Concentrations of some herbicides measured in wetlands under joint private/Federal ownership at the boundaries of WPAs exceeded those of the same herbicides in isolated wetlands on the same WPA.

Apparent differences in herbicide concentrations occurring in wetlands within the Fergus Falls, Morris, and Litchfield WMDs may reflect geographic, climatic, or cropping pattern differences, or local biases toward specific herbicides for given crop types or application rates across the Districts. Subtle increases in triazine and alachlor herbicide concentrations in isolated WPA wetlands tended to parallel the timing and direction of herbicide concentrations in private wetlands within the same Districts. In addition to frequent, localized herbicide inputs to wetlands from agricultural runoff, results of the survey suggest that widespread, low-level herbicide deposition is occurring across the Prairie Pothole Region of western Minnesota even prior to the onset of spring planting. Probable herbicide transport mechanisms include long-range aerial drift and subsequent precipitation of herbicides applied in more southern agricultural regions.

The FY 92 survey was essentially a reconnaissance, examining both anticipated worst- and best-case scenarios from an exposure standpoint. Several limitations inherent in the survey, and recognized beforehand, included: (a) a relatively small sample size; (b) the inability to determine in advance which crop types and associated herbicide usage would develop adjacent to previously selected "treatment" wetlands; and (c) the inability of the enzyme-linked immunosorbent assay (ELISA) to identify major corn and small grain herbicides other than those in the triazine, alachlor, and phenoxy acid herbicide classes (ELISA methodology described in Materials and Methods section).

*Relevance of the FY 93 TCFO Investigation*

Because herbicides are, by definition, phytotoxic, their potential impacts on wetland macrophyte and algal communities are of interest to the Service. The amounts and types of herbicides entering wetlands partially or wholly within WPAs has generated interest among Minnesota WMD personnel. Because "co-owned" wetlands (wetlands only partially within the boundaries of WPAs) are numerous within the WMDs, it was suggested that a logical next step following the FY 92 survey would be to determine whether, and to what extent, co-owned WPA wetlands were receiving herbicides via runoff from adjacent private agricultural lands. A comparison of herbicide concentrations in co-owned wetland basins with those in isolated WPA wetlands would provide WMD personnel information concerning the relative water quality of wetlands under their management. It could also provide Service management with information necessary to decide whether herbicide runoff into Federal WPAs is sufficiently well defined and severe to warrant a request to pesticide regulatory agencies for corrective action.

Further attempts in the FY 93 study to characterize the herbicide "soups" existing in prairie pothole wetlands could be greatly advanced through examination of an expanded list of herbicides commonly used on soybean, corn and small grain crops. The addition of analytical chemistry capability would allow the identification of approximately 25 individual herbicides and their metabolites, and expand the list of target crops to include soybeans, the second most widely grown crop in Minnesota. This approach would help improve our understanding of herbicide influxes to wetlands by: (a) affirming the present study's ELISA method results; (b) identifying specific chemicals

applied (rather than herbicide groups); (c) identifying many herbicides not detected by the ELISA method; and (d) allowing analysis of a more comprehensive list of target crop types, thus providing a better understanding of the chemical soup to which wetland biota in west central Minnesota are being exposed.

The overall goal of the FY 93 portion of the multi-year project was to provide Service wetland managers, private landowners, and the public with an assessment of the degree to which wetland ecosystems in Minnesota's Prairie Pothole Region are receiving agricultural pesticide inputs under a range of geographic, cultural, physical and institutional settings. This information may be used to further public education and demonstration efforts, such as the Partners for Wildlife Program, to guide research into areas of operationally relevant pesticide effects, and to lessen impacts, if any, of private sector agricultural chemical use to wetlands and the trust resources they support. Finally, a comparison of herbicide concentrations in co-owned wetland basins within WPAs versus WPA wetlands isolated from agricultural use would provide Wetland Management Complex personnel information concerning the relative water quality of each.

## STUDY AREA DESCRIPTION

Emergent, seasonally to semipermanently flooded wetland basins were selected within the west-central agricultural area of Minnesota for surface water sampling. The study sites consisted of twenty Service co-owned WPA wetlands (referred to as "treatments") and eight isolated WPA wetlands (referred to as "references") within the Detroit Lakes, Fergus Falls, Morris, and Litchfield WMDs (Figure 1). Treatment WPA wetlands were partially-owned Service wetlands bordering agricultural croplands which had high potential for exposure to chemical influences. Reference WPA wetlands situated in interior portions of WPAs were isolated physically and topographically from agricultural influence. Reference wetlands in each WMD were surveyed in order to provide a "best case" herbicide scenario for purposes of comparison.

Target wetland basin selection criteria included:

(1) emergent, seasonally to semipermanently flooded basins;
(2) co-owned WPA wetlands bordered by target crops on at least 50% of their perimeters;
(3) reference WPA wetlands isolated from cropland to the greatest extent possible;
(4) size, depth, and vegetative community, where present, were to be as similar as possible among reference and treatment wetlands.

Selection criteria of wetlands and counties were similar to those used in the FY 92 survey (Ensor and Smith 1994).

## MATERIALS AND METHODS

### *Selection of Wetlands for Sampling*

Attempts were made to sample as many reference wetlands from the FY 92 survey as possible (included Grandokken, Reger, and Larson WPAs). Each WMD initially identified specific WPAs for consideration based on the initial wetland criteria. The eight selected WPA reference wetlands included two wetlands from each of the following WMDs: Detroit Lakes, Fergus Falls, Morris and Litchfield.

Treatment wetlands were also initially identified by WMD personnel for consideration based on the initial wetland criteria. TCFO staff then visited each recommended WPA site in order to select the final twenty treatment wetlands. The final selections included five wetlands from each of the following WMDs: Detroit Lakes, Fergus Falls, Morris and Litchfield.

### *Collection and Analyses of Surface Water*

Surface water samples were collected four times at roughly monthly intervals from April through July, 1993 at eight reference and twenty treatment WPA wetlands in Detroit Lakes (Figure 2), Fergus Falls (Figure 3), Morris (Figure 4) and Litchfield WMDs (Figure 5). After the initial sampling (pre-herbicide application) in April, dates of sampling followed heavy rainfall events to the extent possible in order to determine maximum runoff concentrations. Collection dates were: April 12-16, May 10-13, June 9-12, and July 13-16.

For each sampling episode at each treatment wetland site, surface water samples were obtained at the most likely area of runoff influence at the perimeter of the wetland. Samples at both reference and treatment wetlands were obtained at the same location in each wetland each sampling period. Individual wetland surface water samples were collected in both a chemically clean 8 oz. amber jar for ELISA and one-liter clear jars for each type of chemical analyses desired. Jars were placed on ice in coolers in the field. Clear liter jars were shipped within 24 hours (Overnight Express) to the University of Iowa Hygienic Laboratory (IHL) for surface water herbicide analyses. Amber jars were kept on ice and refrigerated immediately upon return to the Field Office.

Within 7 days following the first day of sampling, surface water samples collected for immunoassay analyses were brought to room temperature and screened for total triazine and alachlor concentrations using ELISA kits (EnviroGuard® plate kits). A Bio-Tek Model EL307 photometer using a 450 nanometer wavelength filter was used to measure the optical density (absorbance) of each test well (wells included blanks, negative controls, calibrators and samples). Blanks, controls, calibrators and samples were run in triplicate and results were averaged for each sample. The range of $R^2$ values (correlation coefficients for fit of the calibration curves to the actual data) for alachlor and triazine assays were 0.99 and 0.98-0.99, respectively. Bio-Tek software was used to automatically derive the herbicide concentration (ppb) from optical density data.

Surface water samples were analyzed by IHL for the following herbicides: atrazine, cyanazine (Bladex), metolachlor (Dual), alachlor (Lasso), desisopropyl atrazine, desethyl atrazine, metribuzin (Sencor), butylate (Sutan), trifluralin (Treflan), 2,4-D (Weedar, Weedone), dicamba (Banvel), bentazon (Basagran), MCPA, EPTC (Eptam or Eradicane), bromoxynil (Buctril), imazethapyr (Pursuit), and fenoprop (Silvex). Metribuzin (Sencor), butylate (Sutan), trifluralin (Treflan), EPTC (Eptam), MPCA, 2,4-D (Weedar or Weedone) and fenoprop (Silvex) were analyzed but were not detected in any samples throughout the sampling period. Therefore, no values for these seven herbicides are presented in the tables or figures. The U.S. Environmental Protection Agency (EPA) Methods 507 and 515.1 were utilized by IHL to generate the analytical chemistry results. Quality control (QC) data, which includes the pesticide QC data (spike recovery summary data), surrogate compound recovery (recovery of surrogate compounds added to each sample to monitor system performance), and spike recovery data (recovery of spikes prepared and analyzed with the samples as an additional check on system performance) are available upon request.

Comparisons of ELISA and analytical chemistry determinations of triazine and alachlor-related compounds showed measurement differences at low and high concentrations in some wetlands. Dr. Jim Zajicek (Chemist, Immunochemistry Research Section), of the National Biological Survey at the Midwest Science Center, reviewed the differences between ELISA and confirmation methods and provided a discussion to explain the discrepancies. His review is provided in the Appendix.

## RESULTS

Depending upon the observed or anticipated crop types in their respective watersheds, concentrations of up to 25 different corn, soybean, and small grain herbicides could be obtained for each co-owned wetland (Table 1). Alachlor and triazine surface water concentrations derived from ELISA are presented in Tables 2 and 3, respectively. Phenoxy acid and common herbicide surface water concentrations derived from chemical analyses are presented in Tables 4 and 5, respectively.

### *Reference WPA Wetlands*

Although alachlor and triazine herbicides are no longer used on Service lands, both were found at low concentrations in the WPA reference wetlands. ELISA-derived herbicide concentrations indicated consistently low concentrations of alachlors and triazines in all reference WPA wetlands (see Tables 2 and 3). Total alachlor and triazine concentrations within those wetlands ranged from 0.01-0.15 ppb and 0.03-0.22 ppb, respectively. The maximum total alachlor concentration (0.15 ppb) occurred in June at Grandokken WPA (Fergus Falls WMD). The maximum total triazine concentration (0.22 ppb) occurred in June at Froland WPA (Morris WMD). Mean monthly and mean overall concentrations of ELISA-derived alachlors and triazines were similar and not significantly different among or within WMD reference WPA wetlands (Table 6 and Figures 6 and 7, respectively). Detroit Lakes WPA reference wetlands, however, tended to have slightly lower concentrations of those herbicide groups than the other

WMDs. Chemical analyses failed to detect any common or acid herbicides in any of the eight reference WPA wetlands (Tables 4 and 5). A comparison of ELISA and chemically analyzed herbicide concentrations is presented in more detail in Appendix I. Herbicide occurrence in reference wetlands at the maximum observed concentrations is not known to significantly impact aquatic vegetation or invertebrates.

While concentrations of these herbicides in reference WPA wetland samples were found to be low, the timing of increases in their respective concentrations tended to follow the trends observed in the treatment WPA wetlands. This finding also complements findings from the FY 92 survey where reference WPA wetlands followed trends observed in privately-owned agricultural wetlands (Ensor and Smith 1994). Such results strongly suggests the existence of aerial drift and/or precipitation deposition of herbicides into "isolated" wetlands.

*Treatment WPA Wetlands*

Concentrations of alachlor and triazine herbicide groups in surface water samples from co-owned WPA wetlands varied among WMDs and within individual wetlands over time. The range of alachlor and triazine concentrations measured by ELISA within the 20 treatment wetlands was 0.02-6.09 ppb and 0.04-5.17 ppb, respectively (Tables 2 and 3). The maximum alachlor concentration (6.09 ppb) occurred in May on Bjur WPA in the Litchfield WMD. The maximum triazine concentration (5.17 ppb) occurred at Rosby Lake WPA in July in the Morris WMD. These maximum concentrations are similar to the FY 92 maximum concentrations found in privately owned wetlands (6.56 ppb alachlors in Morris WMD and 5.27 ppb triazines in Litchfield WMD). Alachlor and triazine herbicide concentrations in some of the treatment WPA wetlands suggests that concentrations of those herbicides being introduced into WPA wetlands by way of direct runoff from adjacent croplands may be biologically significant.

Mean treatment wetland alachlor and triazine concentrations derived from ELISA for the total sampling period within the Litchfield WMD were significantly greater than for wetlands in the other three WMDs (Table 6) for both reference and treatment wetlands. Fergus Falls WMD mean surface water alachlor concentrations for the entire sampling period was significantly higher than the Detroit Lakes WMD treatment wetlands and all reference wetlands. Again, these comparisons suggest that significantly higher herbicide concentrations may be entering some Service "co-owned" WPA wetlands than isolated WPA wetlands.

Mean ELISA-derived monthly concentrations of alachlors for treatment WPA wetlands within the Litchfield WMD were significantly greater than for the Fergus Falls, Morris, or Detroit Lakes WMD wetlands for the May, June, and July sampling period (Figure 8). Litchfield WMD treatment WPA wetlands had ELISA-derived mean monthly concentrations of triazines that were also significantly higher from Morris, Fergus Falls, and Detroit Lakes for the April, May, and June sampling period. Morris and Litchfield WMD treatment wetland triazine concentrations were significantly higher from Fergus Falls and Detroit Lakes WMD for the sampling month of July (Figure 9).

Individual ELISA-derived treatment and reference wetland monthly alachlor concentrations are provided for Detroit Lakes, Fergus Falls, Morris, and Litchfield WMDs in Figures 10, 11, 12, and 13, respectively. Similar triazine values for the four WMDs are provided in Figures 14-17, respectively.

Detroit Lakes WMD

The five co-owned wetlands sampled in the Detroit Lakes WMD were adjacent to soybeans (n=2), small grains (n=2), and small grains/corn (n=1). Minimal or no target herbicides were detected throughout the growing season.

Minimal concentrations of alachlor and triazine herbicides were detected by ELISA throughout the growing season (Tables 2 and 3, Figures 10 and 14, respectively). No detections of the specific common herbicides (atrazine, cyanazine, metolachlor, alachlor, atrazine metabolites) determined by chemical analyses were found in any Detroit Lakes WMD co-owned wetlands (Table 5). Also, no detections by chemical analyses were found in any Detroit Lakes WMD co-owned wetlands for dicamba (Banvel), bentazon (Basagran), MCPA or bromoxynil (Buctril) (Table 4). Arneson WPA, however, had a treatment wetland concentration of 5.4 ppb imazethapyr (Pursuit) in April. No other herbicides were detected in that wetland during the sampling season.

Fergus Falls WMD

Five co-owned wetlands sampled in the Fergus Falls WMD were adjacent to small grains (n=2), soybeans (n=1), small grains/alfalfa (n=1), and small grains/soybeans (n=1). Minimal or no target herbicides were detected from April through June. Elevated concentrations of various herbicides were detected in several of the July samples.

Lillemoen WPA had elevated ELISA-derived alachlor concentrations throughout the growing season. However, minimal concentrations of alachlor and triazine herbicides were detected by ELISA throughout the growing season at the other WPAs (Tables 2 and 3, Figures 11 and 15, respectively). None of the specific common herbicides (atrazine, cyanazine, metolachlor, alachlor, atrazine metabolites) were found by chemical analyses in any Fergus Falls WMD co-owned wetlands throughout the sampling period (Table 5). Also, no dicamba (Banvel) or bromoxynil (Buctril) was identified by chemical analysis during the growing season (Table 4). An elevated concentration of bentazon (Basagran) (1.8 ppb) was measured in July at Lillemoen WPA. Elevated concentrations of imazethapyr (Pursuit) were measured at Knollwood, Lillemoen, and Spink WPAs during different time periods. April detections of the herbicide (1.7 ppb) occurred at Lillemoen WPA. July detections of the chemical (3.0 ppb and 6.3 ppb) occurred at Knollwood and Spink WPAs, respectively.

Morris WMD

Co-owned wetlands sampled were adjacent to soybeans (2), soybean/small grains (2), and corn (1). Scattered low herbicide concentrations were found in the April through June samples. Some elevated and potentially biologically significant concentrations of multiple herbicides were found in several individual wetlands during July.

Minimal concentrations of alachlors or triazines were detected by ELISA for Gorder, Froland, and Kolstad WPA throughout the growing season (Tables 2 and 3, Figures 12 and 16, respectively). However, elevated concentrations of alachlor were detected from April - July (0.65-1.17 ppb and 0.57-1.70 ppb) at Chokio and Rosby Lake WPAs, respectively. Similarly, elevated ELISA-derived July concentrations of triazines (2.00 and 5.17 ppb) were detected at Chokio and Rosby Lake WPAs, respectively. The elevated Chokio WPA triazine concentration was strongly influenced by upstream croplands. This was deduced from the fact that the wetland in question was surrounded by soybeans and small grains to which triazines are not legally applied.

Chemical analyses also detected elevated concentrations of specific triazine herbicides at Chokio and Rosby Lake WPAs (Table 5). The parent compound, atrazine, was present at 1.1 ppb and 8.5 ppb in July at Chokio and Rosby Lake WPAs, respectively. Coincidentally, the atrazine metabolites were also detected in July at 0.1 ppb and 1.0 ppb, respectively at those WPAs. Concentrations at or near the detection limit for cyanazine (Bladex) (0.1 ppb and 0.2 ppb) occurred in June at Gorder and Froland WPAs, respectively. No detections of atrazine, cyanazine, alachlor, metolachlor or atrazine metabolites were detected by chemical analyses at any other Morris WMD co-owned WPA wetlands.

No analytical chemistry detections of dicamba (Banvel) or bromoxynil (Buctril) occurred for Morris WMD co-owned WPA wetlands (Table 4). Two elevated detections of bentazon (Basagran) (4.4 ppb and 54.0 ppb) occurred at Gorder and Froland WPAs, respectively, in July. Imazethapyr (Pursuit) was detected in July (1.9 ppb and 6.3 ppb) at Froland and Kolstad WPAs, respectively, whereas April and May water samples from the Rosby Lake WPA treatment wetland were found to contain 3.1 ppb and 1.6 ppb, respectively. The Rosby Lake imazethapyr concentrations may have been influenced by upstream croplands. This was deduced from the fact that the wetland in question was surrounded by corn to which the herbicide is not legally applied. However, another possibility is that the chemical was applied to a previous year's non-corn crop, had persisted, and was slowly degrading: the values continued to decline during the sampling season.

Litchfield WMD

Co-owned wetlands sampled were adjacent to soybeans (3), corn (1), and corn/alfalfa (1). Herbicide concentrations ranging from below detection to significantly elevated were found in the April water samples. Analysis of May, June, and July samples indicated that several wetlands contained potentially biologically significant concentrations of various herbicides throughout the period, with numerous wetlands containing multiple herbicides.

Minimal to significantly elevated ELISA-derived concentrations of both alachlors and triazines occurred in treatment WPA wetlands in the Litchfield WMD (Tables 2 and 3, Figures 13 and 17, respectively). Elevated concentrations of alachlors (1.16-6.09 ppb) occurred in May, June and July with the highest concentrations occurring at Bjur (6.09 ppb) and Lindgren Lake (6.0 ppb) WPAs in May. Norway Lake WPA, however, had low concentrations of alachlors (0.20-0.48 ppb) throughout the sampling period. Elevated triazine concentrations

occurred throughout the sampling period (1.15-4.80), with highest concentrations occurring at Bjur WPA (4.80 ppb) and Lindgren Lake (4.0 ppb) in June and July, respectively.

Specific triazine herbicides were detected by chemical analyses at each WPA within the Litchfield WMD (Table 5). Norway Lake and Greenleaf WPAs had concentrations at or near the limits of detection (0.1 ppb and 0.4 ppb, respectively) for atrazine and/or cyanazine (Bladex). Significantly elevated atrazine concentrations of 28.0 ppb were found at Bjur WPA in June. Other elevated concentrations of atrazine occurred at Sunberg and Lindgren Lake (1.2 ppb and 3.1 ppb, respectively) in April and July, respectively. Cyanazine (Bladex) concentrations were elevated at Sunberg WPA for the three months (April, June, July) the wetland was sampled. The Sunberg and Lindgren Lake WPA atrazine and cyanazine concentrations were strongly influenced by upstream croplands. This was deduced from the fact that the particular wetlands were surrounded by soybeans to which triazines are not legally applied.

Specific alachlor herbicides (Table 5) were analytically detected in water from three of the five WPAs (Greenleaf, Bjur, and Lindgren Lake WPAs). Metolachlor (Dual) and alachlor (Lasso) concentrations were each detected in water from two of the five WPAs (Greenleaf and Bjur WPA for metolachlor; Bjur and Lindgren Lake WPAs for alachlor) during the sampling period. Significantly elevated concentrations of metolachlor occurred at Greenleaf (30 ppb in May) and Bjur (13 and 19 ppb in May and June) WPAs, respectively. Elevated concentrations of alachlor (Lasso) occurred at Bjur WPA (2.4 ppb in April) and Lindgren Lake WPA (4.2 ppb in May).

No analytical detections of bentazon (Basagran) were found from water obtained from any Litchfield WMD treatment WPA wetlands (Table 4). Elevated concentrations of dicamba (Banvel) occurred at Bjur (9.3 and 1.0 ppb in June and July) and Lindgren Lake WPAs (6.8 ppb in May). The only detected concentration (3.4 ppb) of bromoxynil (Buctril) occurred in water obtained from the Bjur WPA in June. Greenleaf and Lindgren Lake WPAs had imazethapyr (Pursuit) concentrations of 1.3 ppb and 5.1 ppb, respectively, in July. Presence of imazethapyr at the Greenleaf WPA was strongly influenced from sources other than runoff from the adjacent corn field since this herbicide is not applied to corn.

*Findings For Two WPAs Containing Both Reference and Treatment Wetlands*

Two sampling locations (Froland and Grandokken WPAs) included measurements of both reference and treatment wetlands on the individual WPA. The Froland site, located in Morris WMD, had a treatment wetland surrounded by soybeans (Figure 18). The Grandokken site, located in Fergus Falls WMD, had a treatment wetland surrounded by small grains (Figure 19). The reference and treatment wetland on Grandokken WPA was also measured in the FY 92 study. Although the Froland WPA treatment wetland had no individual alachlors detected by instrumental analysis, an ELISA alachlor concentration (0.36 ppb) peak occurred in July. A cyanazine detection (0.2 ppb) by instrumental analyses and increase (0.22 ppb) by ELISA in June corresponded very closely, although this herbicide is not applied to soybeans. Apparently, other influences outside of the immediate crop type caused both the alachlor and

triazine peaks. In general, reference wetland herbicide concentrations (both from ELISA and from instrumental analyses) were less than the treatment wetland, although the concentrations were not statistically significant. No individual herbicides were detected by instrumental analyses at Grandokken WPA treatment or reference wetlands. Although not statistically significant, Grandokken treatment WPA wetland had higher ELISA alachlor concentrations than the reference wetland. Triazine concentrations by ELISA detection did not exhibit any specific trend. Neither alachlors or triazines were likely applied to the crop adjacent to the treatment wetland (small grains). Therefore, herbicide presence presumably occurred from other sources.

## DISCUSSION

### *Herbicide Concentrations in WPA Wetlands*

Based on a limited number of samples, it is apparent that some co-owned WPA wetlands can acquire herbicide concentrations which exceed concentrations typically detected in isolated WPA wetlands. Concentrations of triazine and alachlor classes and individual herbicides (dicamba, bentazon, imazethapyr, atrazine, cyanazine, alachlor) in some wetlands were up to several orders magnitude higher in co-owned treatment WPA wetlands than concentrations found in isolated WPA reference wetlands (Tables 6 and 7). More importantly, some co-owned WPA wetlands can acquire herbicide concentrations equal to those measured in privately-owned wetlands (based on ELISA determinations from FY 92 study).

A result of our survey also indicates that prairie pothole wetlands may involve interactions of multiple herbicides (and potentially insecticides) comprising chemical "soups" unique to individual wetlands. The majority of the WPA treatment wetlands sampled in the two year survey had more than one chemical present in the surface waters. The various ingredients comprising a particular "soup" may be individually, additively, or even synergistically toxic in certain combinations. Concurrent contamination of wetlands by more than one agricultural pesticide may alter the persistence of compounds, and thus, their potential toxicity to wetland wildlife. The individual and interactive effects of herbicides in concentrations likely to be encountered in natural wetlands have received minimal study to date, although some individual herbicide impacts on experimental wetland meso- or microcosms have been investigated (Stay et al. 1989; Johnson 1986; Huckins et al. 1986; Jenkins and Buikema 1990). The above findings suggest that certain agricultural herbicides applied by farmers in west central Minnesota are entering, and may be impacting, WPA wetlands situated adjacent to or surrounded by croplands.

### *Herbicide Deposition in Wetlands*

Results from a limited sampling of both isolated reference and co-owned treatment WPA wetlands indicate that widespread, low-level aerial herbicide deposition in wetlands is occurring in the Prairie Pothole Region of Minnesota. While the relative importance of various potential routes of entry of herbicides to wetlands (e.g., field runoff, aerial drift or precipitation) is not clear, evidence from this survey and the results of our FY 92 survey

strongly suggests that this deposition form (volatilization followed by deposition in rain) may be a contributing factor to the overall deposition measured in the wetlands. While concentrations of herbicides in reference WPA wetland samples were found to be low, the timing of increases in their respective concentrations tended to follow the temporal trends observed in the treatment WPA wetlands within the same districts. Observed concentrations in the isolated WPA wetlands in the FY 92 survey also followed the general temporal trends observed in private wetlands adjacent to fields which received herbicide treatments (Ensor and Smith 1994).

Preliminary results of a Minnesota Department of Agriculture (MDA) three-year study indicates that a variety of pesticides are observed in the rain falling on Minnesota (Capel et al. 1994). Some of the most commonly detected pesticides in rain were the normally broadcast, preemergent herbicides -- alachlor, atrazine, cyanazine and metolachlor. These are the same herbicides that were most commonly detected in our FY 92 and 93 surveys as well as studies on Minnesota's rivers and streams (Thurman et al. 1991). Although the MDA study did not sample any rain fallen in the area of the state where our study occurred, maximum concentrations of the four most commonly identified herbicides in rainfall in other agricultural areas of the state were 12 ug/L (alachlor), 2.9 ug/L (atrazine), 3.5 ug/L (cyanazine), and 0.92 ug/L (metolachlor). Recent studies in Iowa have found small concentrations of herbicides (usually under 1 ppb, but up to 40 ppb) in rainfall samples. The compounds most often detected (also atrazine, alachlor, metolachlor and cyanazine) were found in almost every rainfall event in Iowa from mid-April through July (Nations 1992). Capel et al. (1994) also suggested that there is a strong local influence on the herbicide content on rain superimposed on a significant regional content of herbicides in rain. A comparison of the magnitude of these two processes (volatilization followed by deposition in rain versus field runoff induced by rainfall) needs to be measured in future studies. In addition, the environmental effects of the herbicides deposited in rain to the surface water of the wetlands measured is largely unknown and needs to be addressed.

*Geographic and Cropping Pattern Influences*

Apparent differences in herbicide concentrations occurring within the four WMDs may reflect geographic, climatic, or cropping pattern differences, and local biases toward specific herbicides for given crop types or application rates across the districts. For instance, Detroit Lakes and Fergus Falls WMD counties have a higher percentage of small grain bushels produced than Morris or Litchfield WMDs (Minnesota Agriculture Statistics 1992). Conversely, Morris and Litchfield WMD counties have a higher percent of corn and soybean bushels produced than Detroit Lakes and Fergus Falls WMDs (ibid). Uses of different herbicide types depending on crop types prevalent in the districts may heavily influence the potential damage to water quality in these respective districts. For example, only Morris and Litchfield WMDs had detectable analytically derived individual triazine and alachlor herbicides (Figure 20) in the surface waters of wetlands we measured. These particular herbicides are the primary herbicides for these crop types and is reflected in the percentage of occurrence in these districts. Litchfield, which had the highest number of soybeans and corn crops adjacent to the measured wetlands,

also had the highest percentage of these particular herbicides. The picture is somewhat confounded, however, when imazethapyr (soybean only) and bentazon (corn and soybean) use by the individual districts is compared (Table 21). Highest percent of occurrence of imazethapyr occurred most at Fergus Falls, then Morris, Litchfield, and Detroit Lakes WMDs, respectively. Highest occurrence of bentazon occurred at Morris then Fergus Falls WMDs. These differences within districts may not only reflect crop type differences, but also local biases toward specific herbicides or application rates across the districts. Bromoxynil, which is used on corn and small grains, were detected only in Morris and Litchfield districts. Dicamba, which is applied to corn and small grains, was detected only in Litchfield WMD.

*Biological Significance of Herbicide Concentrations*

The wetland complex as well as the land use associated with wetland basins and the surrounding uplands has been recognized as an important factor influencing waterfowl recruitment in the agriculturally-influenced prairie pothole region of Minnesota. Pesticides (herbicides and insecticides) that enter wetland systems may have direct or indirect toxic effects on aquatic plants and invertebrates, and may directly or indirectly affect the reproduction and survival of aquatic birds (including federally-listed endangered species) by altering food and cover.

Findings from this survey suggest that "typical agricultural use" of herbicides in the west central agricultural area of Minnesota may potentially impact the water quality of prairie potholes and thus the flora and fauna that inhabit and utilize the wetlands. Specifically, some temporary, seasonal and semipermanent wetlands are acquiring surface water concentrations of some herbicides from agricultural runoff which may adversely influence the wetlands' biota. Concentrations detected have been determined to impact primary producers (such as planktonic and filamentous algae) (Hartman and Martin 1985; DeNoyelles et al. 1982; Johnson 1986) as well as floating plants and some shallow-rooted broadleaved submergents (Hamilton et al. 1987). Grasslike perennials such as cattails, rushes, sedges, and reeds were likely unaffected by herbicide concentrations measured in this study. Herbicide concentrations in some wetland surface waters reached 30 ug/L (metolachlor), 54 ug/L (bentazon), 6.6 ug/L (alachlor), and 28 ug/L (atrazine).

When survey maximum and mean concentrations are compared to guidelines established to protect freshwater aquatic life, several discerning facts are realized (Table 9). FY 93 survey maximum concentrations for atrazine, cyanazine, and metolachlor exceeded the Canadian guideline concentrations for protection of freshwater aquatic life. In addition, the overall mean atrazine and metolachlor concentrations for the FY 93 survey exceeded the Canadian guidelines. It is highly probable that exceedence of herbicide concentrations in Minnesota's wetlands is occurring more often than this survey indicates. The peak concentrations of herbicides occurring in most of the study wetlands were probably higher than the respective measured concentrations because sampling was delayed for one to three days after a rainfall event. Thus, more severe adverse impacts to the wetlands due to higher concentrations of herbicides is likely. The extent of herbicide runoff from treated fields is determined to a large extent by rainfall intensity and the time elapsed

between herbicide applications and rainfall events (Weber et al. 1980). Biological significance of several herbicide concentrations (54 ug/L bentazon, 6.3 ug/L imazethapyr) is not known at this time.

*Implications to Guidelines for Protection of Freshwater Aquatic Life*

Currently, the Minnesota Pollution Control Agency (MPCA) exempts "normal farming practices" from the State's "Specific Standards of Quality and Purity for Class 2 Waters of the State; Aquatic Life and Recreation" (Minnesota Chapter 7050, 1994) to protect wetlands. Under Part 7050.0210, Subpart 13a., the MPCA added the following language to its 7050 rule:

> "Wetland pollution prohibited. Wetland conditions shall be protected from **chemical**, physical, **biological**, or radiological changes to prevent significant adverse impacts to the following designated uses: **maintaining biological diversity, preserving habitat....**" (emphasis added).

Additionally, under Part 7050.0222, Subpart 6, the MPCA added the following language:

> "Subp. 6. Class 2D waters. The quality of Class 2D wetlands shall be such as to **permit the propagation and maintenance of a healthy community of aquatic and terrestrial species indigenous to wetlands, and their habitats.**" (emphasis added).

However, the standards later state that:

> **"Activities in wetlands which involve the normal farm practices of planting....including the recommended applications of fertilizers and pesticides, are excluded from these standards..." (emphasis added).**

This portion of the standard dilutes the focus of wetland protection by excluding an activity affecting a high percentage of Minnesota's wetlands. An exemption appears to give the agricultural community great latitude to neglect appropriate wetland protection measures, thus compromising the diversity and well being of indigenous wetland plants and animals. Utilizing the Service's NWI mapping, we have determined the percentage of wetlands within a select group of counties that would not be protected against agricultural chemical runoff, drift or direct application by the proposed language. The numbers provided are underestimates of the actual number of potentially affected wetlands in agricultural areas because we limited our search to palustrine emergent and forested temporary, seasonally, and semipermanently flooded wetlands (shrub or permanently flooded wetlands were not included). Clay, Faribault, Fillmore, Freeborn, Grant, Norman, Otter Tail, Polk, and Stearns counties would continue to have 79%, 85%, 75%, 86%, 74%, 70%, 47%, 59% and 75% respectively of their wetlands exposed to unrestrained agricultural chemical inputs under the proposed standards.

The exclusion of such practices may severely undermine the protection of the state's wetlands. The fact that wetlands may serve as sinks for agricultural

chemicals, whether they persist or not, necessitates that the protection of the water quality standards be extended to encompass pesticide application. Agricultural chemicals pose unacceptable risks to life in wetlands whether they are found in invertebrates, fish, wildlife, vegetation, sediments, or the water column. Inclusion of this exemption allows agricultural chemicals, which are likely to be found throughout the agricultural-related wetlands, to potentially cause a significant risk to aquatic life and wildlife.

## RECOMMENDATIONS

1. The individual and interactive effects of herbicides in concentrations likely to be encountered in natural wetlands is largely unknown. Thus, a better understanding of the chemical "soups" and their interactions to which wetland biota in west central Minnesota are being exposed needs to be addressed.

2. Insecticide and herbicide mutual presence and their interactions in wetlands are largely unknown and should be measured in the future. Concurrent contamination of wetlands by more than one agricultural pesticide may alter the persistence of compounds, and thus, their potential toxicity to wetland wildlife.

3. Great care should be given to future purchases of "co-owned" wetlands where potential negative impacts to water quality of wetlands adjacent to croplands is likely. Stronger attempts should be made in future acquisitions, where possible, to purchase complete wetlands with bordering uplands in order to preserve the water quality of the wetland ecosystem.

4. Comparisons of ELISA and analytical chemistry determinations of triazine and alachlor-related compounds showed measurement differences at low and high concentrations in some wetlands. Dr. Jim Zajicek (Chemist, Immunochemistry Research Section) of the National Biological Survey at the Midwest Science Center, reviewed the differences and provided a detailed discussion to explain the discrepancies. His review, addressed in Appendix I, will be extremely beneficial in future ELISA studies for the Environmental Contaminants (EC) Program of the Service. Dr. Zajicek's findings should be dispersed throughout the EC program in order to inform other Environmental Contaminants Specialists on appropriate interpretations of such data.

5. A study to examine the effects of habitat and agricultural practices on birds breeding on farmland in Ontario determined that the most important variable affecting total bird species abundance was herbicide use. The comparative field study on organic farms and chemical farms indicated that sites that were sprayed with herbicides had fewer birds (Freemark and Csizy 1993). This finding has importance to Midwest agroecosystems, where the intensification of agriculture and extensive use of herbicides have significant implications for not only aquatic-dependent but also farmland wildlife and their habitats. A similar study in Minnesota may be warranted.

TABLES

Table 1. Herbicides detectable by ELISA and instrumental analyses.

I. Instrumental Analyses:

| Common Name | Brand Name | Quantitation Limit (ug/L) |
|---|---|---|
| Atrazine | Aatrex | 0.1 |
| Desethyl atrazine | -- | 0.1 |
| Desisopropyl Atrazine | -- | 0.1 |
| Cyanazine | Bladex | 0.1 |
| Metolachlor | Dual | 0.1 |
| Alachlor | Lasso | 0.1 |
| Metribuzin | Sencor, Lexone | 0.1 |
| Trifluralin | Treflan, Trific, Trilin | 0.1 |
| Butylate | Sutan | 0.1 |
| EPTC | Eptam, Eradicane | 0.1 |
| 2,4-D | Weedar, Weedone | 0.2 |
| Dicamba | Banvel | 0.2 |
| Imazethapyr | Pursuit | 1.0 |
| Bentazon | Basagran | 0.2 |
| MCPA | -- | 200.0 |
| Bromoxynil | Buctril | 0.2 |

II. ELISA analyses:

| Compound | 50% Bo* (ug/L) | LDD (90% Bo)* (ug/L) |
|---|---|---|
| Alachlor Plate Kit: | | |
| Alachlor | 0.60 | 0.05 |
| Metalaxyl | >1000.00 | 10.00 |
| Metolachlor | 40.00 | 0.60 |
| Triazine Plate Kit: | | |
| 6-Hydroxy Atrazine | 5.20 | 0.01 |
| Ametryn | 0.11 | 0.001 |
| Atrazine | 0.32 | 0.037 |
| Cyanazine | 12.50 | 0.23 |
| Deethylated atrazine | 11.60 | 0.30 |
| Didealkylated atrazine | no reaction | no reaction |
| Prometon | 1.00 | 0.01 |
| Prometryn | 0.34 | 0.015 |
| Propazine | 0.29 | 0.014 |
| Simazine | 0.67 | 0.004 |
| Simetryn | 0.34 | 0.003 |
| Terbuthylazine | 2.40 | 0.060 |
| Trietazine | 5.30 | 0.008 |

*100% Bo equals the maximum amount of alalchlor- or triazine-enzyme conjugate that is bound by the antibody in the absence of any alachlor or triazine in sample (i.e. negative control)

Table 2. ELISA-derived alachlor concentrations in surface waters of reference and treatment Wetland Management District Waterfowl Production Area wetlands in west central Minnesota, April - July, 1993 (in ppb).

| WPA Name | April | May | June | July | Mean |
|---|---|---|---|---|---|
| Detroit Lakes WMD | | | | | |
| References | | | | | |
| Noreen | ----[a] | 0.04 | 0.06 | 0.05 | 0.05 |
| Larson | 0.01 | 0.03 | 0.05 | 0.04 | 0.03 |
| Treatments | | | | | |
| Nord | 0.02 | 0.04 | 0.05 | 0.05 | 0.04 |
| Hillestad | 0.04 | 0.04 | 0.07 | 0.11 | 0.07 |
| Arneson | 0.03 | 0.04 | 0.06 | 0.08 | 0.05 |
| Tatlie Lake | ---[a] | 0.05 | 0.06 | 0.06 | 0.06 |
| Kasin | ---[a] | 0.03 | 0.07 | 0.28 | 0.13 |
| Fergus Falls WMD | | | | | |
| References | | | | | |
| Grandokken | 0.05 | 0.07 | 0.15 | 0.07 | 0.09 |
| Reger | 0.03 | 0.04 | 0.12 | 0.07 | 0.07 |
| Treatments | | | | | |
| Julsrud | 0.04 | 0.04 | 0.05 | 0.08 | 0.05 |
| Knollwood | 0.11 | 0.14 | 0.12 | 0.11 | 0.12 |
| Lillemoen | 3.58 | 1.30 | 2.61 | 1.80 | 2.32 |
| Grandokken | 0.10 | 0.17 | 0.26 | 0.20 | 0.18 |
| Spink | ---[a] | 0.08 | 0.10 | 0.07 | 0.08 |
| Morris WMD | | | | | |
| References | | | | | |
| Froland | 0.02 | 0.07 | 0.12 | 0.08 | 0.07 |
| Larson | 0.03 | 0.07 | 0.09 | 0.11 | 0.08 |
| Treatments | | | | | |
| Chokio | 0.96 | 1.17 | 1.06 | 0.65 | 0.96 |
| Rosby Lake | 0.92 | 0.57 | 1.04 | 1.70 | 1.06 |
| Gorder | 0.05 | 0.07 | 0.21 | 0.16 | 0.12 |
| Froland | 0.08 | 0.12 | 0.20 | 0.36 | 0.19 |
| Kolstad | 0.03 | 0.16 | 0.10 | 0.09 | 0.10 |
| Litchfield WMD | | | | | |
| References | | | | | |
| Weber | 0.03 | 0.07 | 0.10 | 0.05 | 0.06 |
| Burr Oak | 0.03 | 0.06 | 0.13 | 0.08 | 0.08 |
| Treatments | | | | | |
| Sunberg | 0.45 | 0.79 | 0.87 | 1.16 | 0.82 |
| Norway Lake | 0.20 | 0.45 | 0.48 | 0.31 | 0.36 |
| Greenleaf | 0.20 | 2.42 | 0.58 | 0.22 | 0.86 |
| Bjur | ---[a] | 6.09 | 5.31 | 4.59 | 5.33 |
| Lindgren Lake | ---[a] | 6.00 | 1.75 | 3.08 | 3.61 |

[a] Did not sample

Table 3. ELISA-derived triazine concentrations in surface waters of reference and treatment Wetland Management District Waterfowl Production Area wetlands in west central Minnesota, April - July, 1993 (in ppb).

| WPA Name | April | May | June | July | Mean |
|---|---|---|---|---|---|
| Detroit Lakes WMD | | | | | |
| References | | | | | |
| Noreen | ----[a] | 0.06 | 0.09 | 0.15 | 0.10 |
| Larson | 0.04 | 0.04 | 0.08 | 0.11 | 0.07 |
| Treatments | | | | | |
| Nord | 0.10 | 0.13 | 0.17 | 0.16 | 0.14 |
| Hillestad | 0.09 | 0.07 | 0.13 | 0.10 | 0.10 |
| Arneson | 0.07 | 0.11 | 0.14 | 0.15 | 0.12 |
| Tatlie Lake | ---[a] | 0.05 | 0.07 | 0.10 | 0.07 |
| Kasin | ---[a] | 0.05 | 0.08 | 0.08 | 0.07 |
| Fergus Falls WMD | | | | | |
| References | | | | | |
| Grandokken | 0.04 | 0.06 | 0.15 | 0.16 | 0.10 |
| Reger | 0.03 | 0.04 | 0.12 | 0.16 | 0.09 |
| Treatments | | | | | |
| Julsrud | 0.09 | 0.10 | 0.19 | 0.19 | 0.14 |
| Knollwood | 0.05 | 0.06 | 0.09 | 0.08 | 0.07 |
| Lillemoen | 0.04 | 0.07 | 0.14 | 0.11 | 0.09 |
| Grandokken | 0.04 | 0.07 | 0.11 | 0.15 | 0.10 |
| Spink | ---[a] | 0.08 | 0.11 | 0.10 | 0.10 |
| Morris WMD | | | | | |
| References | | | | | |
| Froland | 0.04 | 0.08 | 0.22 | 0.15 | 0.12 |
| Larson | 0.04 | 0.06 | 0.10 | 0.12 | 0.08 |
| Treatments | | | | | |
| Chokio | 0.04 | 0.08 | 0.13 | 2.00 | 0.56 |
| Rosby Lake | 0.08 | 0.14 | 0.20 | 5.17 | 1.40 |
| Gorder | 0.05 | 0.08 | 0.16 | 0.10 | 0.10 |
| Froland | 0.05 | 0.09 | 0.21 | 0.22 | 0.14 |
| Kolstad | 0.05 | 0.09 | 0.13 | 0.13 | 0.10 |
| Litchfield WMD | | | | | |
| References | | | | | |
| Weber | 0.03 | 0.06 | 0.13 | 0.11 | 0.08 |
| Burr Oak | 0.03 | 0.06 | 0.12 | 0.09 | 0.08 |
| Treatments | | | | | |
| Sunberg | 2.22 | 2.29 | 1.80 | 2.13 | 2.11 |
| Norway Lake | 0.25 | 0.93 | 0.42 | 0.54 | 0.54 |
| Greenleaf | 0.05 | 0.11 | 0.19 | 0.96 | 0.33 |
| Bjur | ---[a] | 0.29 | 4.80 | 0.17 | 1.75 |
| Lindgren Lake | ---[a] | 1.15 | 0.26 | 4.00 | 1.80 |

[a] Did not sample

Table 4. Analytically-derived phenoxy acid concentrations in surface waters of reference and treatment Wetland Management District Waterfowl Production Area wetlands in west central Minnesota, April - July, 1993 (in ppb).

| Reference WPA | dicamba (Banvel) | bentazon (Basagran) | bromoxynil (Buctril) | imazethapyr (Pursuit) |
|---|---|---|---|---|
| Detroit Lakes WMD | | | | |
| Noreen | | | | |
| April | NA | NA | NA | NA |
| May | <0.2 | <0.2 | NA | <1 |
| June | NA | NA | NA | NA |
| July | NA | NA | NA | NA |
| Larson | | | | |
| April | <0.2 | <0.2 | NA | <1 |
| May | NA | NA | NA | NA |
| June | NA | NA | NA | NA |
| July | NA | NA | NA | NA |
| Fergus Falls WMD | | | | |
| Grandokken | | | | |
| April | <0.2 | <0.2 | NA | <1 |
| May | NA | NA | NA | NA |
| June | NA | NA | NA | NA |
| July | NA | NA | NA | NA |
| Reger | | | | |
| April | <0.2 | <0.2 | NA | <1 |
| May | NA | NA | NA | NA |
| June | NA | NA | NA | NA |
| July | NA | NA | NA | NA |

* NA = Not analyzed

Table 4 (con't). Analytically-derived phenoxy acid concentrations in surface waters of reference and treatment Wetland Management District Waterfowl Production Area wetlands in west central Minnesota, April - July, 1993 (in ppb).

| Reference WPA | dicamba (Banvel) | bentazon (Basagran) | bromoxynil (Buctril) | imazethapyr (Pursuit) |
|---|---|---|---|---|
| Morris WMD | | | | |
| Froland | | | | |
| April | <0.2 | <0.2 | NA | <1 |
| May | NA | NA | NA | NA |
| June | NA | NA | NA | NA |
| July | NA | NA | NA | NA |
| Larson | | | | |
| April | <0.2 | <0.2 | NA | <1 |
| May | NA | NA | NA | NA |
| June | NA | NA | NA | NA |
| July | NA | NA | NA | NA |
| Litchfield WMD | | | | |
| Weber | | | | |
| April | <0.2 | <0.2 | NA | <1 |
| May | NA | NA | NA | NA |
| June | NA | NA | NA | NA |
| July | NA | NA | NA | NA |
| Burr Oak | | | | |
| April | <0.2 | <0.2 | NA | <1 |
| May | NA | NA | NA | NA |
| June | NA | NA | NA | NA |
| July | NA | NA | NA | NA |

* NA = Not analyzed

Table 4 (con't). Analytically-derived phenoxy acid concentrations in surface waters of reference and treatment Wetland Management District Waterfowl Production Area wetlands in west central Minnesota, April - July, 1993 (in ppb).

| Treatment WPA | dicamba (Banvel) | bentazon (Basagran) | bromoxynil (Buctril) | imazethapyr (Pursuit) | Crop Type |
|---|---|---|---|---|---|
| Detroit Lakes WMD | | | | | |
| Nord | | | | | small grains |
| April | <0.2 | <0.2 | <0.2 | <1.0 | |
| May | <0.2 | <0.2 | NA | NA | |
| June | <0.2 | <0.2 | NA | NA | |
| July | 0.3 | <0.2 | <0.2 | <1.0 | |
| Hillestad | | | | | soybeans |
| April | <0.2 | <0.2 | <0.2 | <1.0 | |
| May | <0.2 | <0.2 | NA | NA | |
| June | <0.2 | <0.2 | <0.2 | <1.0 | |
| July | <0.2 | <0.2 | <0.2 | <1.0 | |
| Arneson | | | | | soybeans |
| April | <0.2 | <0.2 | <0.2 | 5.4 | |
| May | <0.2 | <0.2 | NA | NA | |
| June | <0.2 | <0.2 | <0.2 | <1.0 | |
| July | <0.2 | <0.2 | <0.2 | <1.0 | |
| Tatlie Lake | | | | | small grains |
| April | NA | NA | NA | NA | |
| May | <0.2 | <0.2 | <0.2 | <1.0 | |
| June | <0.2 | <0.2 | <0.2 | NA | |
| July | <0.2 | <0.2 | <0.2 | <1.0 | |
| Kasin | | | | | small grains and corn |
| April | NA | NA | NA | NA | |
| May | <0.2 | <0.2 | <0.2 | <1.0 | |
| June | <0.2 | <0.2 | <0.2 | NA | |
| July | <0.2 | <0.2 | <0.2 | <1.0 | |

* NA = Not analyzed

Table 4 (con't). Analytically-derived phenoxy acid concentrations in surface waters of reference and treatment Wetland Management District Waterfowl Production Area wetlands in west central Minnesota, April - July, 1993 (in ppb).

| Treatment WPA | dicamba (Banvel) | bentazon (Basagran) | bromoxynil (Buctril) | imazethapyr (Pursuit) | Crop Type |
|---|---|---|---|---|---|
| Fergus Falls WMD | | | | | |
| Julsrud | | | | | small grains |
| April | <0.2 | <0.2 | <0.2 | <1.0 | alfalfa |
| May | <0.2 | <0.2 | NA | NA | |
| June | <0.2 | <0.2 | <0.2 | NA | |
| July | 0.3 | <0.2 | <0.2 | <1.0 | |
| Knollwood | | | | | small grains |
| April | <0.2 | <0.2 | <0.2 | <1.0 | |
| May | <0.2 | <0.2 | NA | NA | |
| June | <0.2 | <0.2 | <0.2 | NA | |
| July | <0.2 | <0.2 | <0.2 | 3.0 | |
| Lillemoen | | | | | small grains |
| April | <0.2 | <0.2 | <0.2 | 1.7 | soybeans |
| May | <0.2 | <0.2 | NA | NA | |
| June | <0.2 | <0.2 | <0.2 | <1.0 | |
| July | <0.2 | 1.8 | <0.2 | <1.0 | |
| Grandokken | | | | | small grains |
| April | <0.2 | <0.2 | <0.2 | <1.0 | |
| May | <0.2 | <0.2 | NA | NA | |
| June | <0.2 | <0.2 | <0.2 | NA | |
| July | <0.2 | <0.2 | <0.2 | <1.0 | |
| Spink | | | | | soybeans |
| April | NA | NA | NA | NA | |
| May | <0.2 | <0.2 | <0.2 | <1.0 | |
| June | <0.2 | <0.2 | <0.2 | <1.0 | |
| July | <0.2 | <0.2 | <0.2 | 6.3 | |

* NA = Not analyzed

Table 4 (con't). Analytically-derived phenoxy acid concentrations in surface waters of reference and treatment Wetland Management District Waterfowl Production Area wetlands in west central Minnesota, April - July, 1993 (in ppb).

| Treatment WPA | dicamba (Banvel) | bentazon (Basagran) | bromoxynil (Buctril) | imazethapyr (Pursuit) | Crop Type |
|---|---|---|---|---|---|
| Morris WMD | | | | | |
| Chokio | | | | | soybeans |
| April | <0.2 | <0.2 | <0.2 | <1.0 | small grains |
| May | <0.2 | <0.2 | <0.2 | <1.0 | |
| June | <0.2 | <0.2 | <0.2 | <1.0 | |
| July | <0.2 | <0.2 | <0.2 | <1.0 | |
| Rosby Lake | | | | | corn |
| April | <0.2 | <0.2 | <0.2 | 3.1 | |
| May | <0.2 | <0.2 | NA | 1.6 | |
| June | <0.2 | <0.2 | <0.2 | NA | |
| July | 0.7 | <0.2 | 0.3 | <1.0 | |
| Gorder | | | | | small grains |
| April | <0.2 | <0.2 | <0.2 | <1.0 | soybeans |
| May | <0.2 | <0.2 | <0.2 | <1.0 | |
| June | <0.2 | <0.2 | <0.2 | <1.0 | |
| July | <0.2 | 4.4 | <0.2 | <1.0 | |
| Froland | | | | | soybeans |
| April | <0.2 | <0.2 | <0.2 | <1.0 | |
| May | <0.2 | <0.2 | <0.2 | <1.0 | |
| June | <0.2 | <0.2 | <0.2 | <1.0 | |
| July | <0.2 | 54.0 | <0.2 | 1.9 | |
| Kolstad | | | | | soybeans |
| April | <0.2 | <0.2 | <0.2 | <1.0 | |
| May | NA | NA | NA | NA | |
| June | NA | NA | NA | NA | |
| July | <0.2 | <0.2 | <0.2 | 6.3 | |

* NA = Not analyzed

Table 4 (con't). Analytically-derived phenoxy acid concentrations in surface waters of reference and treatment Wetland Management District Waterfowl Production Area wetlands in west central Minnesota, April - July, 1993 (in ppb).

| Treatment WPA | dicamba (Banvel) | bentazon (Basagran) | bromoxynil (Buctril) | imazethapyr (Pursuit) | Crop Type |
|---|---|---|---|---|---|
| Litchfield WMD | | | | | |
| Sunberg | | | | | soybeans |
| April | <0.2 | <0.2 | <0.2 | <1.0 | |
| May | NA | NA | NA | NA | |
| June | <0.2 | <0.2 | <0.2 | <1.0 | |
| July | <0.2 | <0.2 | <0.2 | <1.0 | |
| Norway Lake | | | | | soybeans |
| April | <0.2 | <0.2 | <0.2 | <1.0 | |
| May | NA | NA | NA | NA | |
| June | <0.2 | <0.2 | <0.2 | <1.0 | |
| July | <0.2 | <0.2 | <0.2 | <1.0 | |
| Greenleaf | | | | | corn alfalfa |
| April | <0.2 | <0.2 | <0.2 | <1.0 | |
| May | <0.2 | <0.2 | <0.2 | <1.0 | |
| June | <0.2 | <0.2 | <0.2 | NA | |
| July | <0.2 | <0.2 | <0.2 | 1.3 | |
| Bjur | | | | | corn |
| April | NA | NA | NA | NA | |
| May | <0.2 | <0.2 | <0.2 | <1.0 | |
| June | 9.3 | <0.2 | 3.4 | NA | |
| July | 1.0 | <0.2 | <0.2 | <1.0 | |
| Lindgren Lake | | | | | soybeans |
| April | NA | NA | NA | NA | |
| May | 6.8 | <0.2 | <0.2 | <1.0 | |
| June | 0.4 | <0.2 | <0.2 | NA | |
| July | <0.2 | <0.2 | <0.2 | 5.1 | |

* NA = Not analyzed

Table 5. Analytically-derived common herbicide concentrations in surface waters of reference and treatment Wetland Management District Waterfowl Production Area wetlands in west central Minnesota, April - July, 1993 (in ppb).

| Reference WPA | atrazine | cyanazine (Bladex) | metolachlor (Dual) | alachlor (Lasso) | desethyl atrazine | desisopropyl atrazine |
|---|---|---|---|---|---|---|
| Detroit Lakes WMD | | | | | | |
| Noreen | | | | | | |
| April | NA* | NA | NA | NA | NA | NA |
| May | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| June | NA | NA | NA | NA | NA | NA |
| July | NA | NA | NA | NA | NA | NA |
| Larson | | | | | | |
| April | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| May | NA | NA | NA | NA | NA | NA |
| June | NA | NA | NA | NA | NA | NA |
| July | NA | NA | NA | NA | NA | NA |
| Fergus Falls WMD | | | | | | |
| Grandokken | | | | | | |
| April | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| May | NA | NA | NA | NA | NA | NA |
| June | NA | NA | NA | NA | NA | NA |
| July | NA | NA | NA | NA | NA | NA |
| Reger | | | | | | |
| April | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| May | NA | NA | NA | NA | NA | NA |
| June | NA | NA | NA | NA | NA | NA |
| July | NA | NA | NA | NA | NA | NA |

* NA = Not analyzed

Table 5 (con't). Analytically-derived common herbicide concentrations in surface waters of reference and treatment Wetland Management District Waterfowl Production Area wetlands in west central Minnesota, April - July, 1993 (in ppb).

| Reference WPA | atrazine | cyanazine (Bladex) | metolachlor (Dual) | alachlor (Lasso) | desethyl atrazine | desisopropyl atrazine |
|---|---|---|---|---|---|---|
| Morris WMD | | | | | | |
| Froland | | | | | | |
| April | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| May | NA | NA | NA | NA | NA | NA |
| June | NA | NA | NA | NA | NA | NA |
| July | NA | NA | NA | NA | NA | NA |
| Larson | | | | | | |
| April | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| May | NA | NA | NA | NA | NA | NA |
| June | NA | NA | NA | NA | NA | NA |
| July | NA | NA | NA | NA | NA | NA |
| Litchfield WMD | | | | | | |
| Weber | | | | | | |
| April | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| May | NA | NA | NA | NA | NA | NA |
| June | NA | NA | NA | NA | NA | NA |
| July | NA | NA | NA | NA | NA | NA |
| Burr Oak | | | | | | |
| April | <0.2 | <0.2 | <0.2 | <0.2 | <0.2 | <0.1 |
| May | NA | NA | NA | NA | NA | NA |
| June | NA | NA | NA | NA | NA | NA |
| July | NA | NA | NA | NA | NA | NA |

* NA = Not analyzed

Table 5 (con't). Analytically-derived common herbicide concentrations in surface waters of reference and treatment Wetland Management District Waterfowl Production Area wetlands in west central Minnesota, April - July, 1993 (in ppb).

| Treatment WPA | atrazine | cyanazine (Bladex) | metolachlor (Dual) | alachlor (Lasso) | desethyl atrazine | desisopropyl-atrazine | Crop Type |
|---|---|---|---|---|---|---|---|
| Detroit Lakes WMD | | | | | | | |
| Nord | | | | | | | small grains |
| April | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |
| May | NA | NA | NA | NA | NA | NA | |
| June | NA | NA | NA | NA | NA | NA | |
| July | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |
| Hillestad | | | | | | | soybeans |
| April | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |
| May | NA | NA | NA | NA | NA | NA | |
| June | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |
| July | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |
| Arneson | | | | | | | soybeans |
| April | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |
| May | NA | NA | NA | NA | NA | NA | |
| June | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |
| July | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |
| Tatlie Lake | | | | | | | small grains |
| April | NA | NA | NA | NA | NA | NA | |
| May | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |
| June | NA | NA | NA | NA | NA | NA | |
| July | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |
| Kasin | | | | | | | small grains |
| April | NA | NA | NA | NA | NA | NA | |
| May | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |
| June | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |
| July | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |

* NA = Not analyzed

Table 5 (con't). Analytically-derived common herbicide concentrations in surface waters of reference and treatment Wetland Management District Waterfowl Production Area wetlands in west central Minnesota, April - July, 1993 (in ppb).

| Treatment WPA | atrazine | cyanazine (Bladex) | metolachlor (Dual) | alachlor (Lasso) | desethyl atrazine | desisopropyl-atrazine | Crop Type |
|---|---|---|---|---|---|---|---|
| Fergus Falls WMD | | | | | | | |
| Julsrud | | | | | | | small grains alfalfa |
| April | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |
| May | NA | NA | NA | NA | NA | NA | |
| June | NA | NA | NA | NA | NA | NA | |
| July | 0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |
| Knollwood | | | | | | | small grains |
| April | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |
| May | NA | NA | NA | NA | NA | NA | |
| June | NA | NA | NA | NA | NA | NA | |
| July | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |
| Lillemoen | | | | | | | small grains soybeans |
| April | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |
| May | NA | NA | NA | NA | NA | NA | |
| June | NA | NA | NA | NA | NA | NA | |
| July | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |
| Grandokken | | | | | | | small grains |
| April | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |
| May | NA | NA | NA | NA | NA | NA | |
| June | NA | NA | NA | NA | NA | NA | |
| July | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |
| Spink | | | | | | | soybeans |
| April | NA | NA | NA | NA | NA | NA | |
| May | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |
| June | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |
| July | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |

* NA = Not analyzed

Table 5 (con't). Analytically-derived common herbicide concentrations in surface waters of reference and treatment Wetland Management District Waterfowl Production Area wetlands in west central Minnesota, April - July, 1993 (in ppb).

| Treatment WPA | atrazine | cyanazine (Bladex) | metolachlor (Dual) | alachlor (Lasso) | desethyl atrazine | desisopropyl-atrazine | Crop Type |
|---|---|---|---|---|---|---|---|
| Morris WMD | | | | | | | |
| Chokio | | | | | | | soybeans small grains |
| April | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |
| May | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |
| June | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |
| July | 1.1 | <0.1 | <0.1 | <0.1 | 0.1 | <0.1 | |
| Rosby Lake | | | | | | | corn |
| April | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |
| May | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |
| June | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |
| July | 8.5 | <0.1 | <0.1 | <0.1 | 1.0 | 0.3 | |
| Gorder | | | | | | | soybeans small grains |
| April | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |
| May | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |
| June | <0.1 | 0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |
| July | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |
| Froland | | | | | | | soybeans |
| April | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |
| May | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |
| June | <0.1 | 0.2 | <0.1 | <0.1 | <0.1 | <0.1 | |
| July | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |
| Kolstad | | | | | | | soybeans |
| April | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |
| May | NA | NA | NA | NA | NA | NA | |
| June | NA | NA | NA | NA | NA | NA | |
| July | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |

* NA = Not analyzed

Table 5 (con't). Analytically-derived common herbicide concentrations in surface waters of reference and treatment Wetland Management District Waterfowl Production Area wetlands in west central Minnesota, April - July, 1993 (in ppb).

| Treatment WPA | atrazine | cyanazine (Bladex) | metolachlor (Dual) | alachlor (Lasso) | desethyl atrazine | desisopropyl-atrazine | Crop Type |
|---|---|---|---|---|---|---|---|
| Litchfield WMD | | | | | | | |
| Sunberg | | | | | | | soybeans |
| April | 1.2 | 3.5 | <0.1 | <0.1 | 0.4 | 0.3 | |
| May | NA | NA | NA | NA | NA | NA | |
| June | 0.7 | 2.0 | <0.1 | <0.1 | 0.3 | 0.3 | |
| July | 0.6 | 3.1 | <0.1 | <0.1 | 0.4 | 0.4 | |
| Norway Lake | | | | | | | soybeans |
| April | 0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |
| May | NA | NA | NA | NA | NA | NA | |
| June | 0.1 | 0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |
| July | 0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |
| Greenleaf | | | | | | | corn alfalfa |
| April | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |
| May | <0.1 | <0.1 | 30.0 | <0.1 | <0.1 | <0.1 | |
| June | <0.1 | <0.1 | 2.0 | <0.1 | <0.1 | <0.1 | |
| July | 0.4 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |
| Bjur | | | | | | | corn |
| April | NA | NA | NA | NA | NA | NA | |
| May | <0.1 | <0.1 | 13.0 | 2.4 | <0.1 | <0.1 | |
| June | 28.0 | <0.1 | 19.0 | 0.9 | 0.8 | 0.2 | |
| July | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |
| Lindgren Lake | | | | | | | soybeans |
| April | NA | NA | NA | NA | NA | NA | |
| May | 0.4 | <0.1 | <0.1 | 4.2 | 0.2 | <0.1 | |
| June | 0.1 | 0.1 | <0.1 | <0.1 | <0.1 | <0.1 | |
| July | 3.1 | 0.1 | <0.1 | <0.1 | 0.8 | 0.3 | |

* NA = Not analyzed

Table 6. ELISA-derived mean alachlor and triazine concentrations in surface waters of reference and treatment Wetland Management District Waterfowl Production Area wetlands in west central Minnesota, April - July, 1993 (in ppb).

| | Reference Wetlands | | Treatment Wetlands | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Detroit Lakes | | Fergus Falls | | Morris | | Litchfield | |
| Herbicide Class | n | Mean | n | Mean | n | Mean | n | Mean | n | Mean |
| Alachlors | 8 | 0.07 A* | 5 | 0.07 A | 5 | 0.67 B | 5 | 0.49 AB | 5 | 1.94 C |
| Triazines | 8 | 0.09 A | 5 | 0.10 A | 5 | 0.11 A | 5 | 0.46 AB | 5 | 1.25 C |

* Values sharing similar letters are not significantly different.

Table 7. Analytically-derived mean herbicide concentrations in surface waters of reference and treatment Wetland Management District Waterfowl Production Area wetlands in west central Minnesota, April - July, 1993.

| | Reference Wetlands | | Treatment Wetlands | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Detroit Lakes | | Fergus Falls | | Morris | | Litchfield | |
| Herbicide | n | Mean | n | Mean | n | Mean | n | Mean | n | Mean |
| 2,4-D | 8 | LD* | 18 | LD | 19 | LD | 18 | LD | 16 | LD |
| fenoprop | 8 | LD | 18 | LD | 19 | LD | 18 | LD | 16 | LD |
| dicamba | 8 | LD | 18 | LD | 19 | 0.20 | 18 | 0.04 | 16 | 1.10 |
| bentazon | 8 | LD | 18 | LD | 19 | LD | 18 | 3.25 | 16 | LD |
| MPCA | 8 | LD | 18 | LD | 19 | LD | 18 | LD | 16 | LD |
| bromoxynil | NA** | -- | 14 | LD | 15 | LD | 17 | 0.02 | 16 | 0.22 |
| imazethapyr | 8 | LD | 12 | 0.45 | 12 | 0.96 | 17 | 0.79 | 13 | 0.53 |
| atrazine | 8 | LD | 13 | LD | 11 | LD | 18 | 0.54 | 16 | 2.18 |
| cyanazine | 8 | LD | 13 | LD | 11 | LD | 18 | LD | 16 | 0.56 |
| metolachlor | 8 | LD | 13 | LD | 11 | LD | 18 | LD | 16 | 4.00 |
| alachlor | 8 | LD | 13 | LD | 11 | LD | 18 | LD | 16 | 0.47 |
| desethyl-atrazine | 8 | LD | 13 | LD | 11 | LD | 18 | LD | 16 | 0.18 |
| desisopropyl-atrazine | 8 | LD | 13 | LD | 11 | LD | 18 | LD | 16 | 0.10 |

* LD=less than detected value
**NA=not analyzed

Table 8. Timings of herbicide applications for individual crop types.

| Herbicide | Preplant | Pre-emergence | Post-emergence | Sequential |
|---|---|---|---|---|
| Atrazine | C* | C | C | |
| Cyanazine (Bladex) | C | C | C | C |
| Alachlor (Lasso) | C,S | C,S | C | |
| Metolachlor (Dual) | C,S | C,S | C | |
| De-ethyl atrazine | C | C | C | |
| Desisopropyl atrazine | C | C | C | |
| Bentazon (Basagran) | | | C,S | S |
| Dicamba (Banvel) | C,O,W,B | C,O,W,B | C,O,W,B | |
| 2,4-D | S | C,S | C,O,W,B | |
| MPCA | | | B,O,W | |
| Bromoxynil (Buctril) | | C | C,O,W,B | |
| Imazethapyr (Pursuit) | S | S | S | |

*C=corn, S=soybean, B=barley, O=oats, W=spring wheat

FIGURES

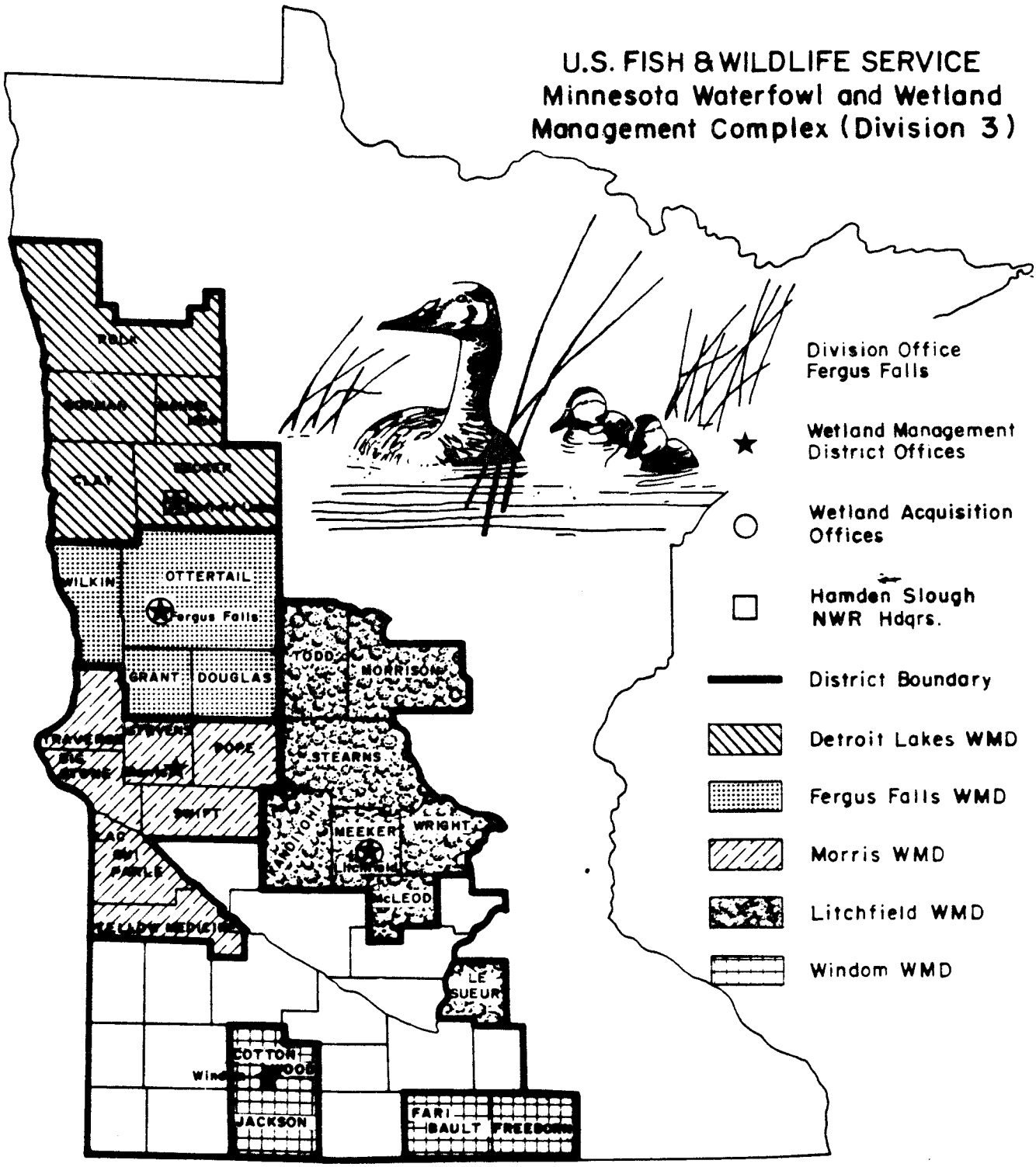


Figure 1. West-central counties of Minnesota within Wetland Management Districts selected for wetland sampling, April - July, 1993.

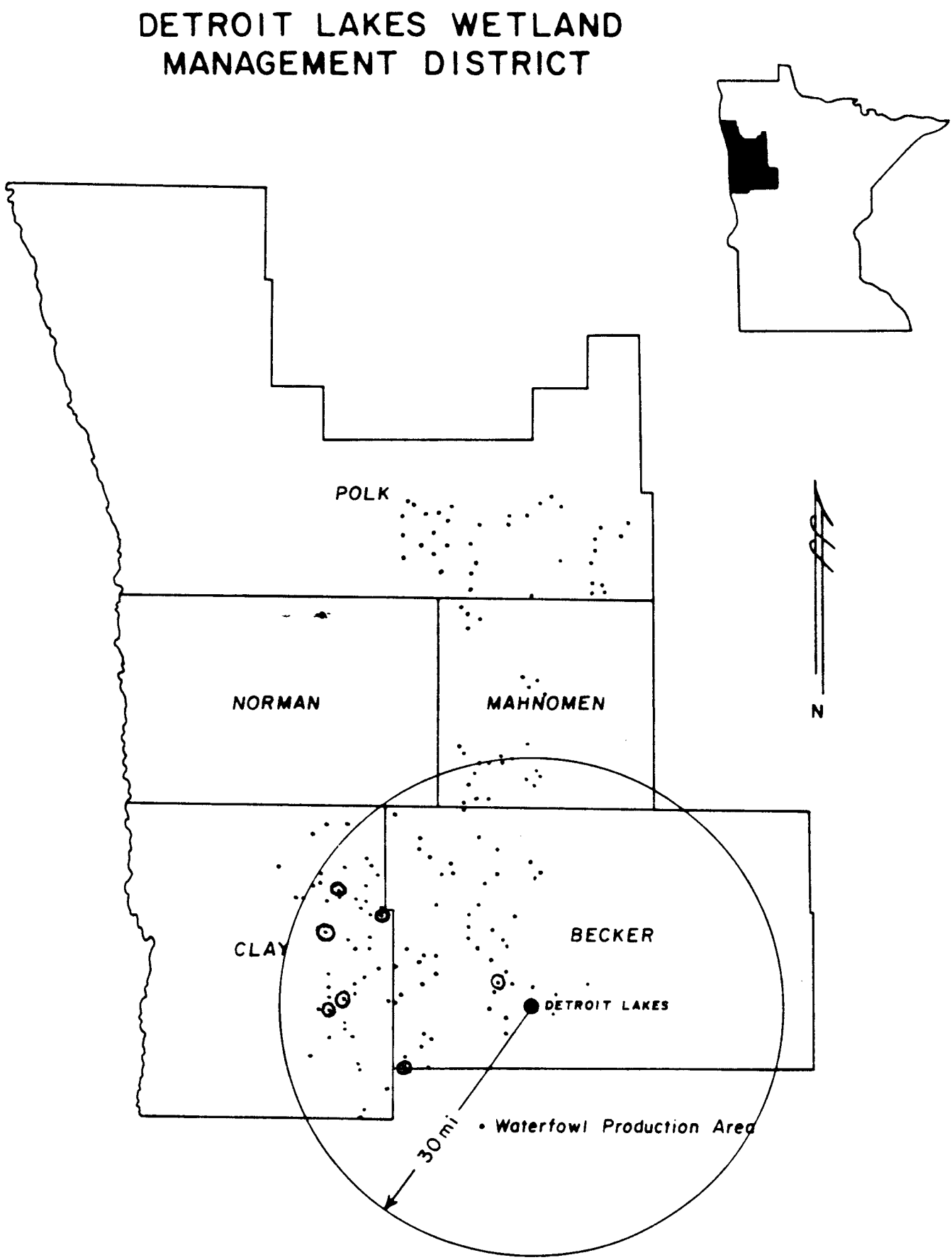


Figure 2. Location of Waterfowl Production Area wetlands selected for sampling in Detroit Lakes Wetland Management District, April - July, 1993.

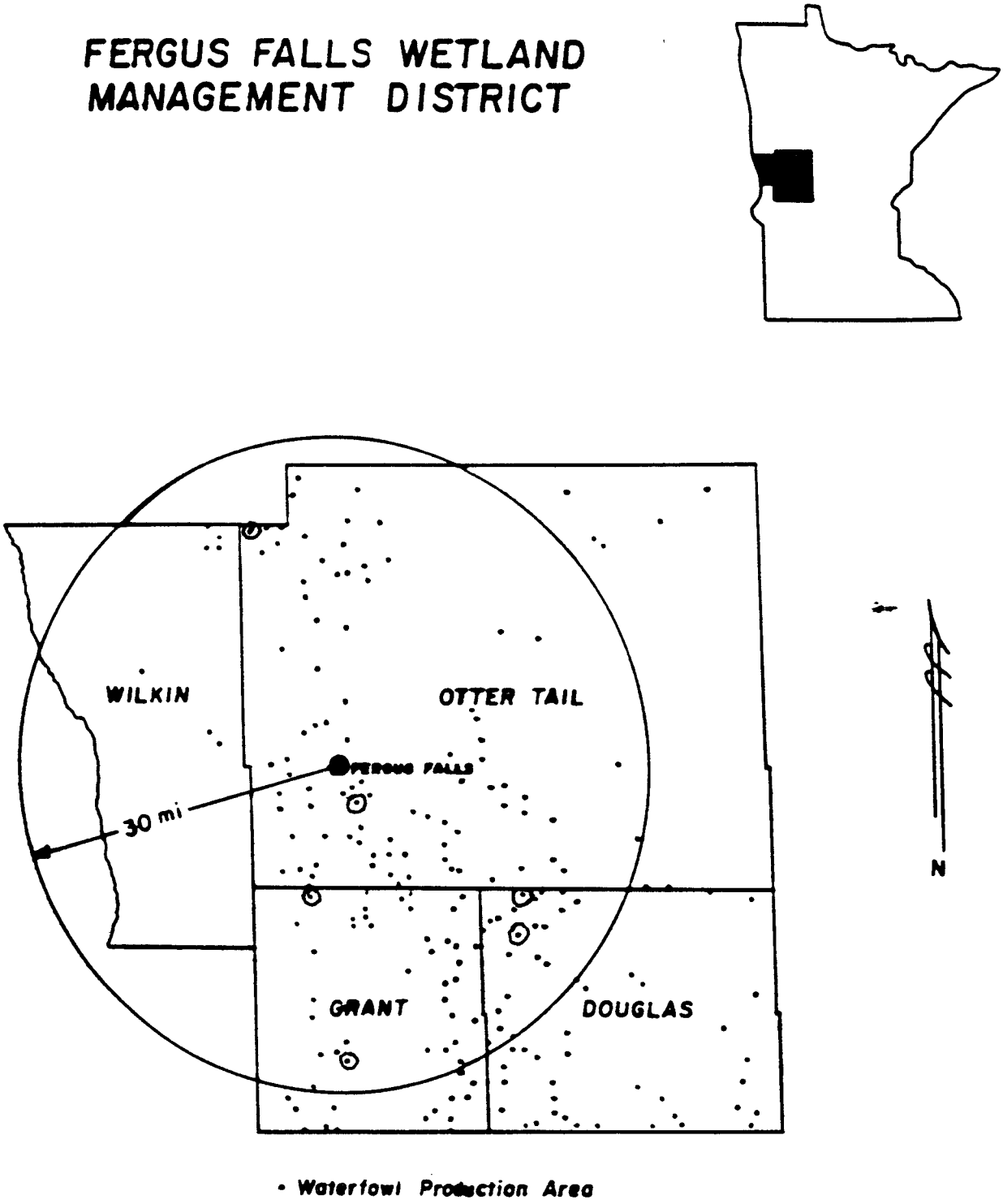


Figure 3. Location of Waterfowl Production Area wetlands selected for sampling in Fergus Falls Wetland Management District, April - July, 1993.

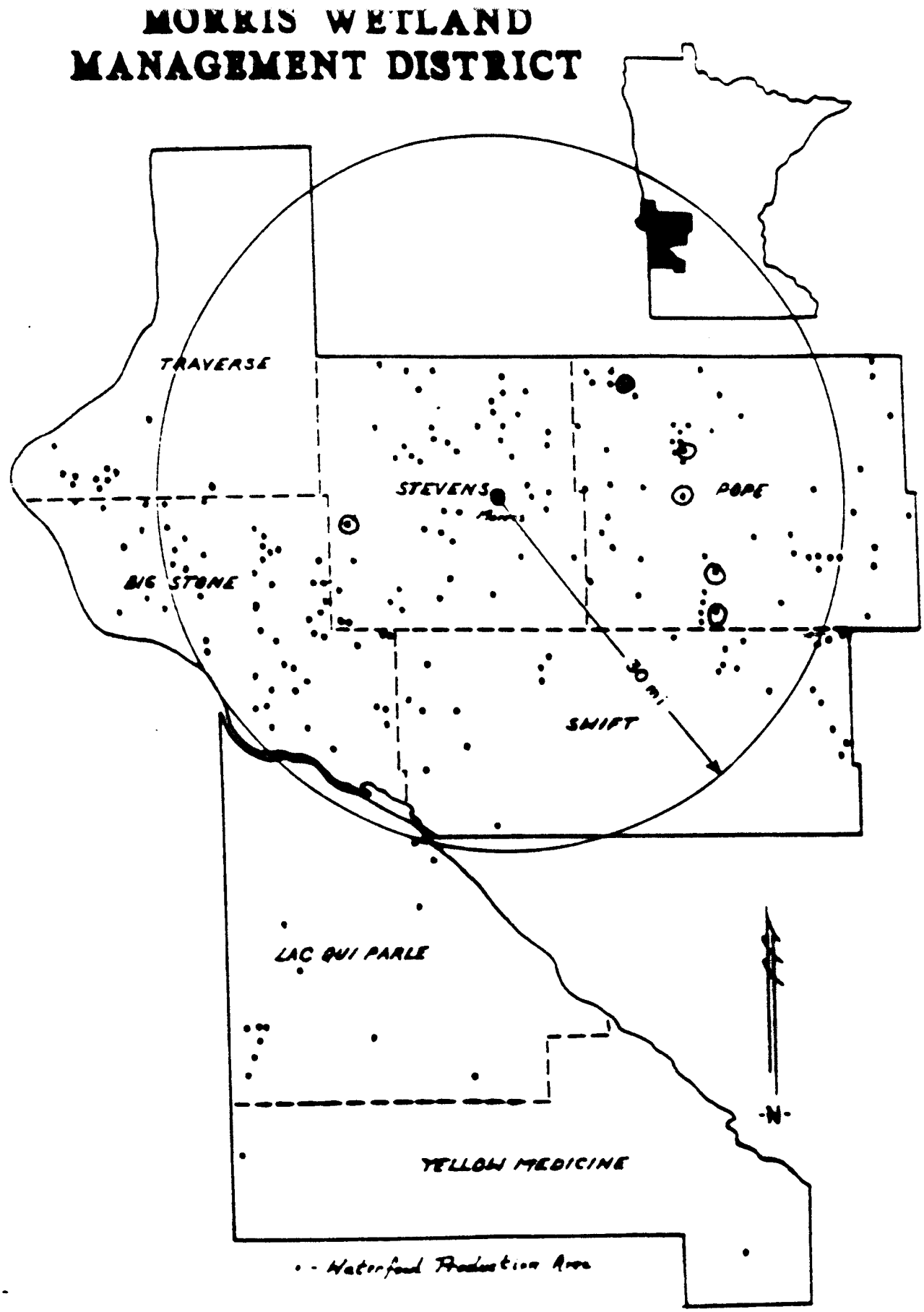


Figure 4. Location of Waterfowl Production Area wetlands selected for sampling in Morris Wetland Managment District, April - July, 1993.

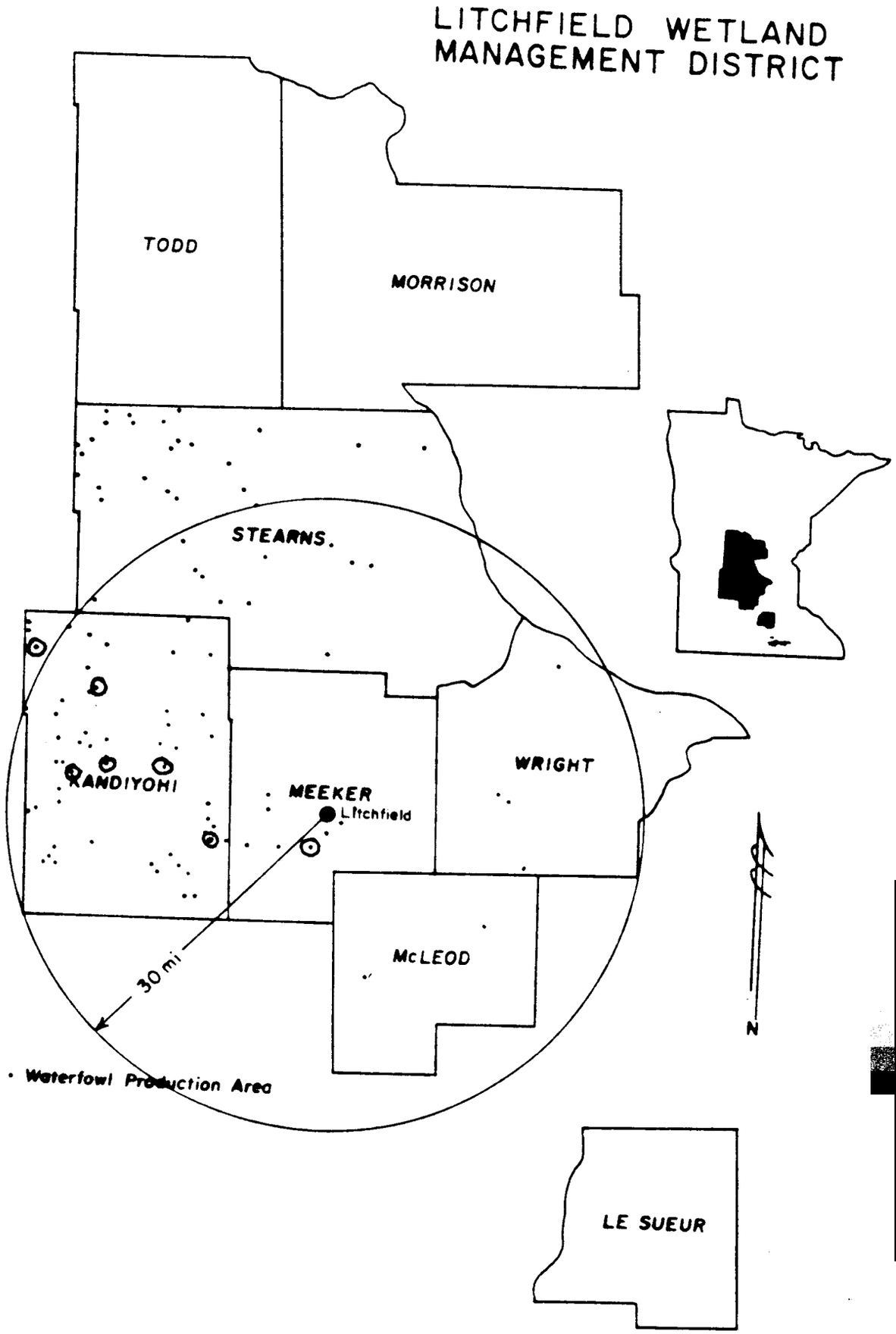


Figure 5. Location of Waterfowl Production Area wetlands selected for sampling in Litchfield Wetland Management District, April - July, 1993.

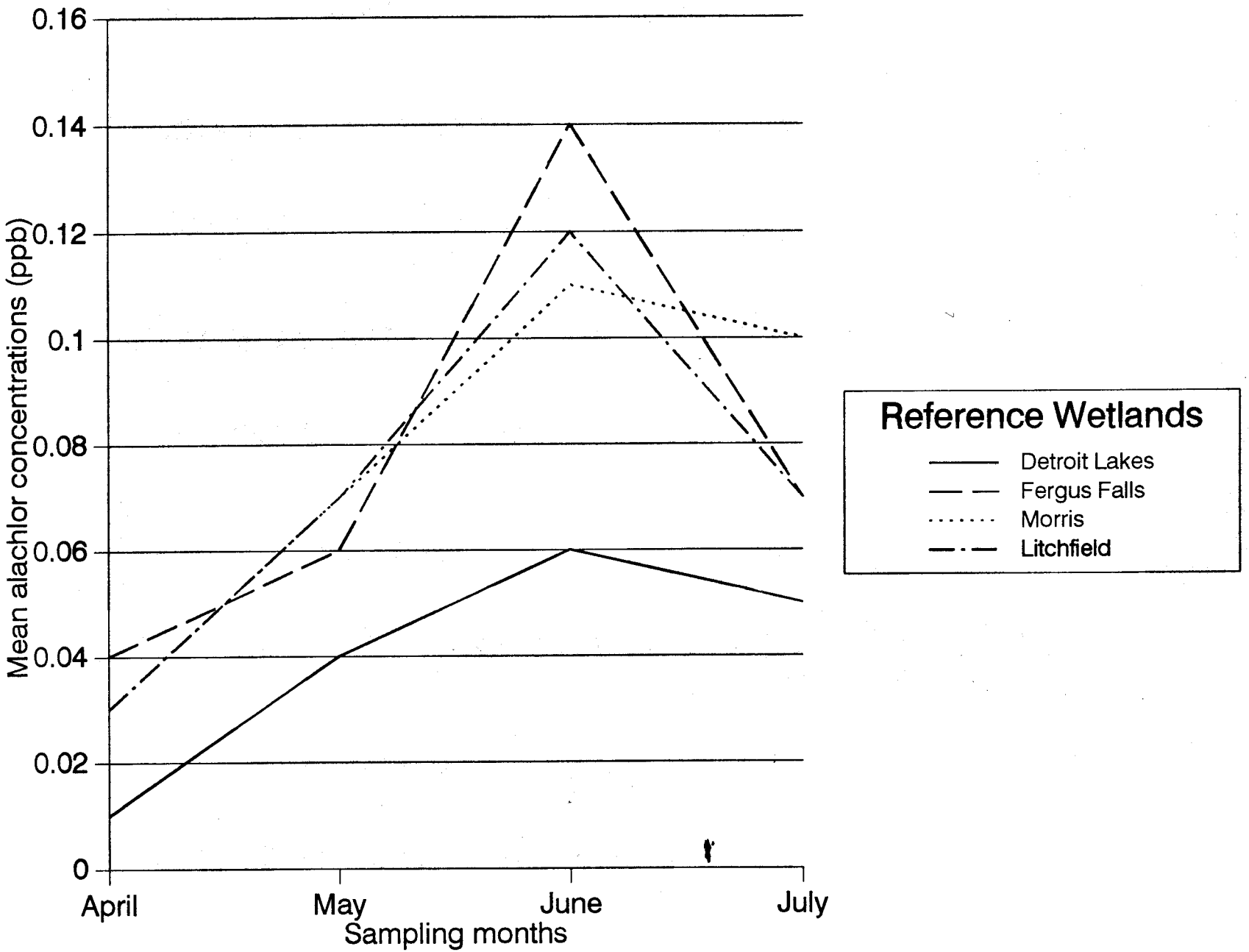


Figure 6. ELISA-derived mean monthly alachlor concentrations (ppb) in surface waters of reference Waterfowl Production Area wetlands in Detroit Lakes, Fergus Falls, Morris, and Litchfield Wetland Management Districts, April - July, 1993.

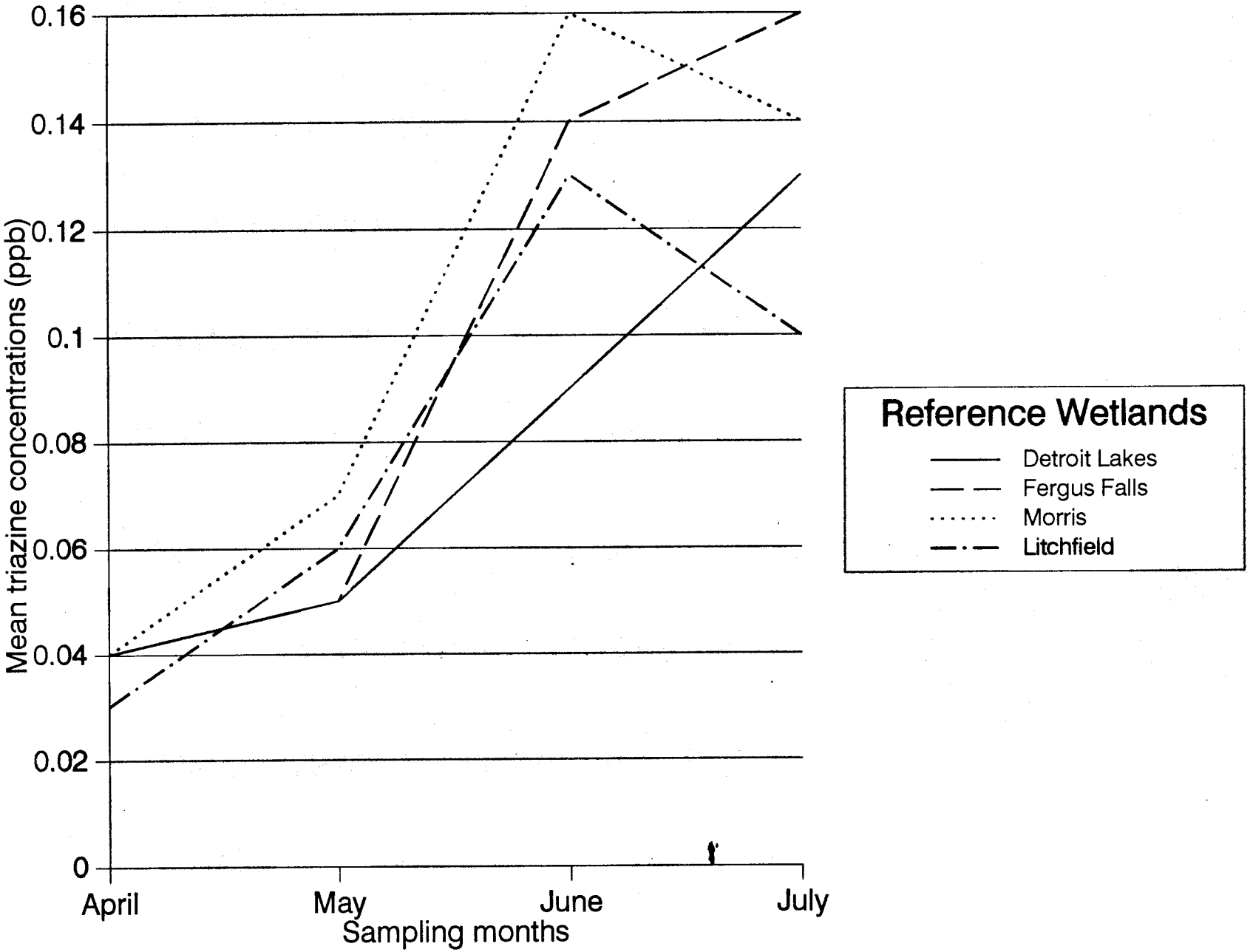


Figure 7. ELISA-derived mean monthly triazine concentrations (ppb) in surface waters of reference Waterfowl Production Area wetlands in Detroit Lakes, Fergus Falls, Morris, and Litchfield Wetland Management Districts, April - July, 1993.

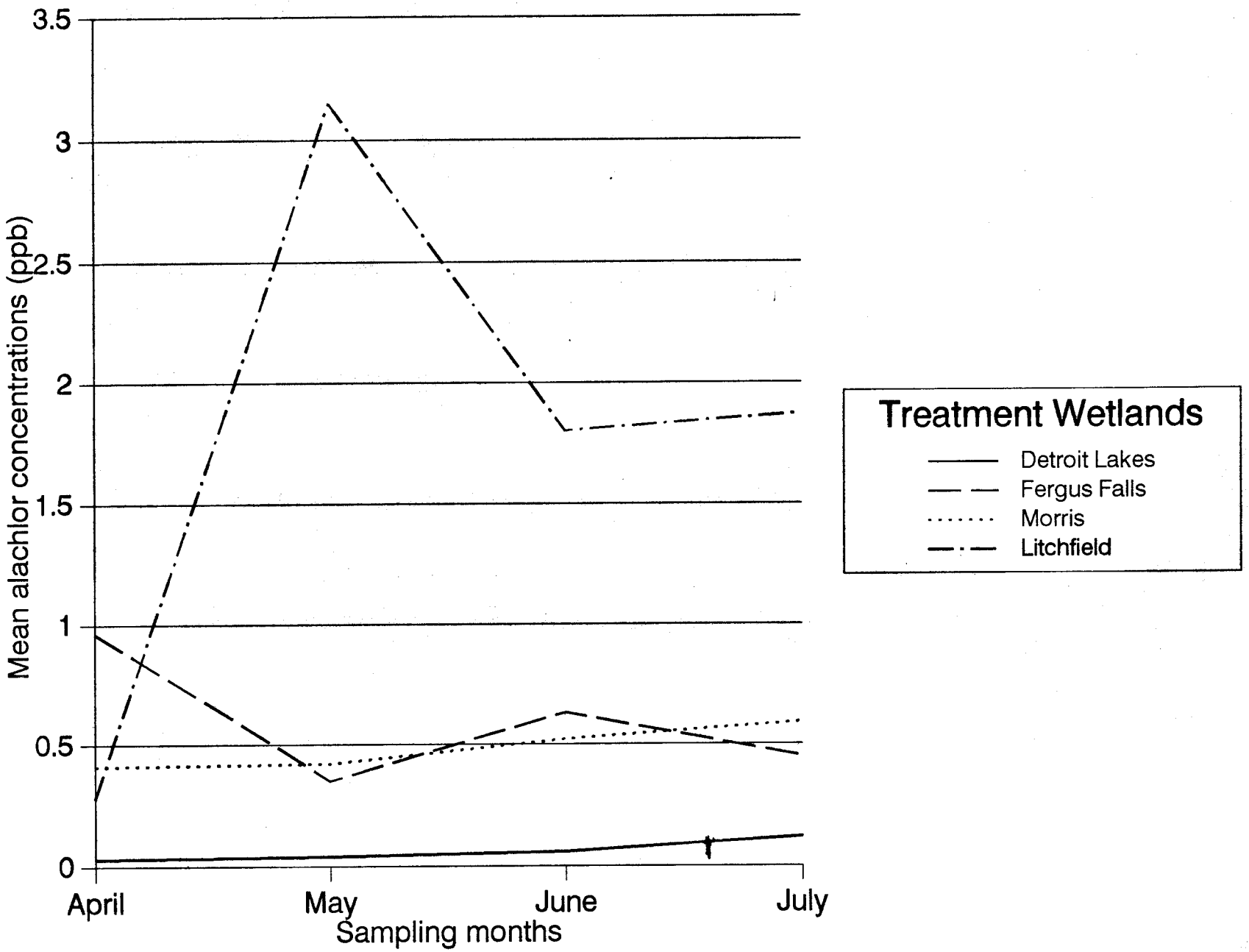


Figure 8. ELISA-derived mean monthly alachlor concentrations (ppb) in surface waters of treatment Waterfowl Production Area wetlands in Detroit Lakes, Fergus Falls, Morris, and Litchfield Wetland Management Districts, April - July, 1993.

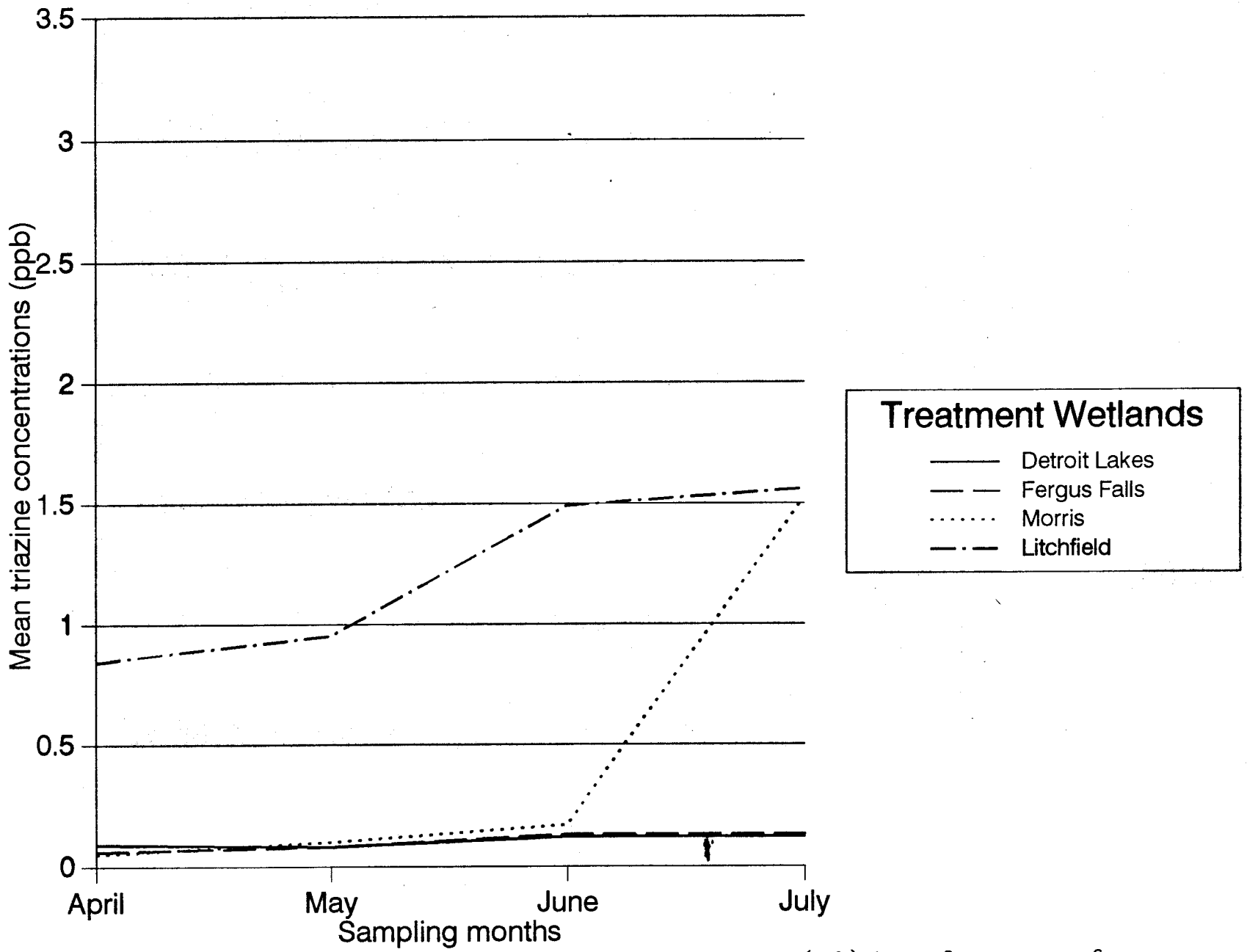


Figure 9. ELISA-derived mean monthly triazine concentrations (ppb) in surface waters of treatment Waterfowl Production Area wetlands in Detroit Lakes, Fergus Falls, Morris, and Litchfield Wetland Management Districts, April - July, 1993.

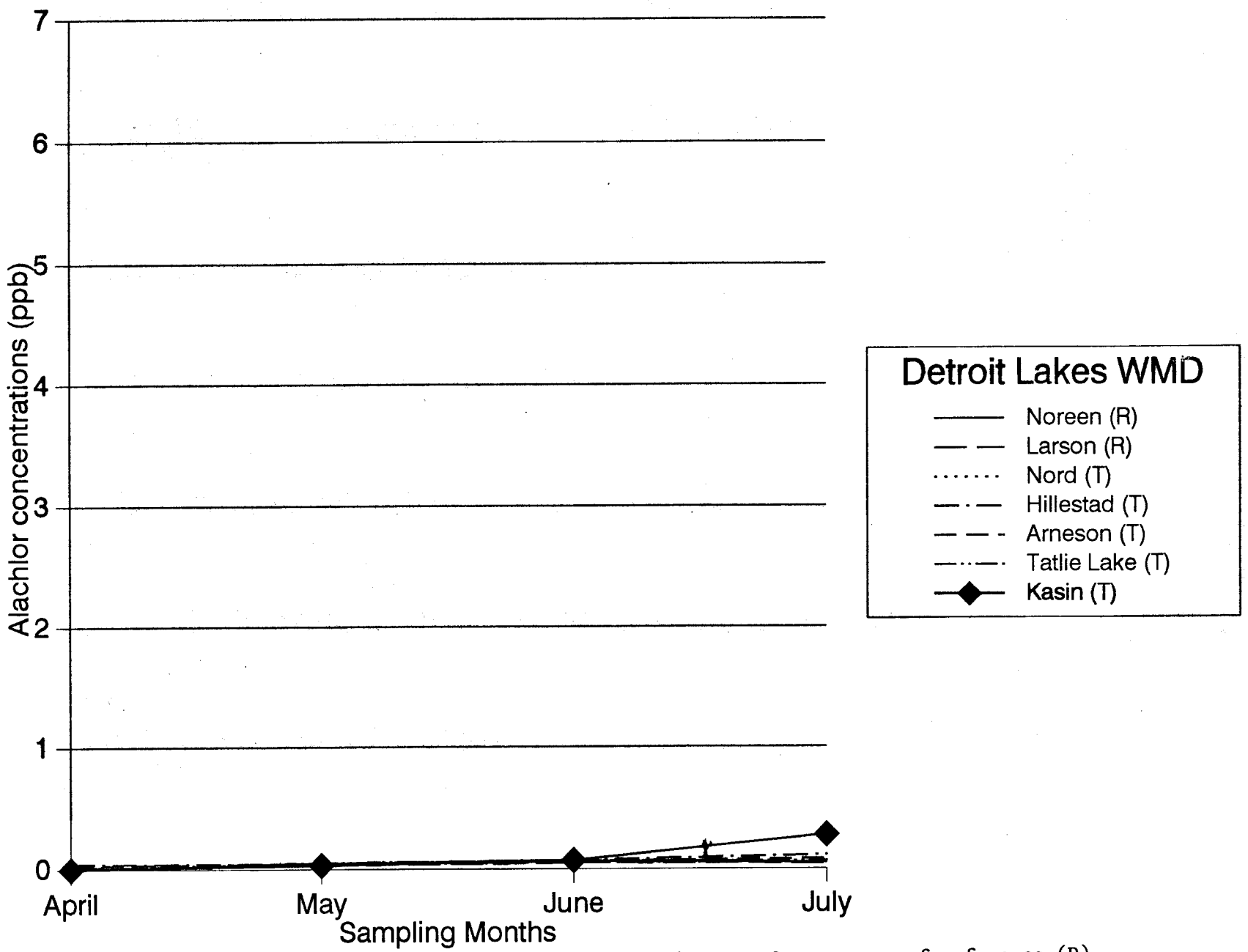


Figure 10. ELISA-derived alachlor concentrations (ppb) in surface waters of reference (R) and treatment (T) Waterfowl Production Area wetlands in Detroit Lakes Wetland Management District, April - July, 1993.

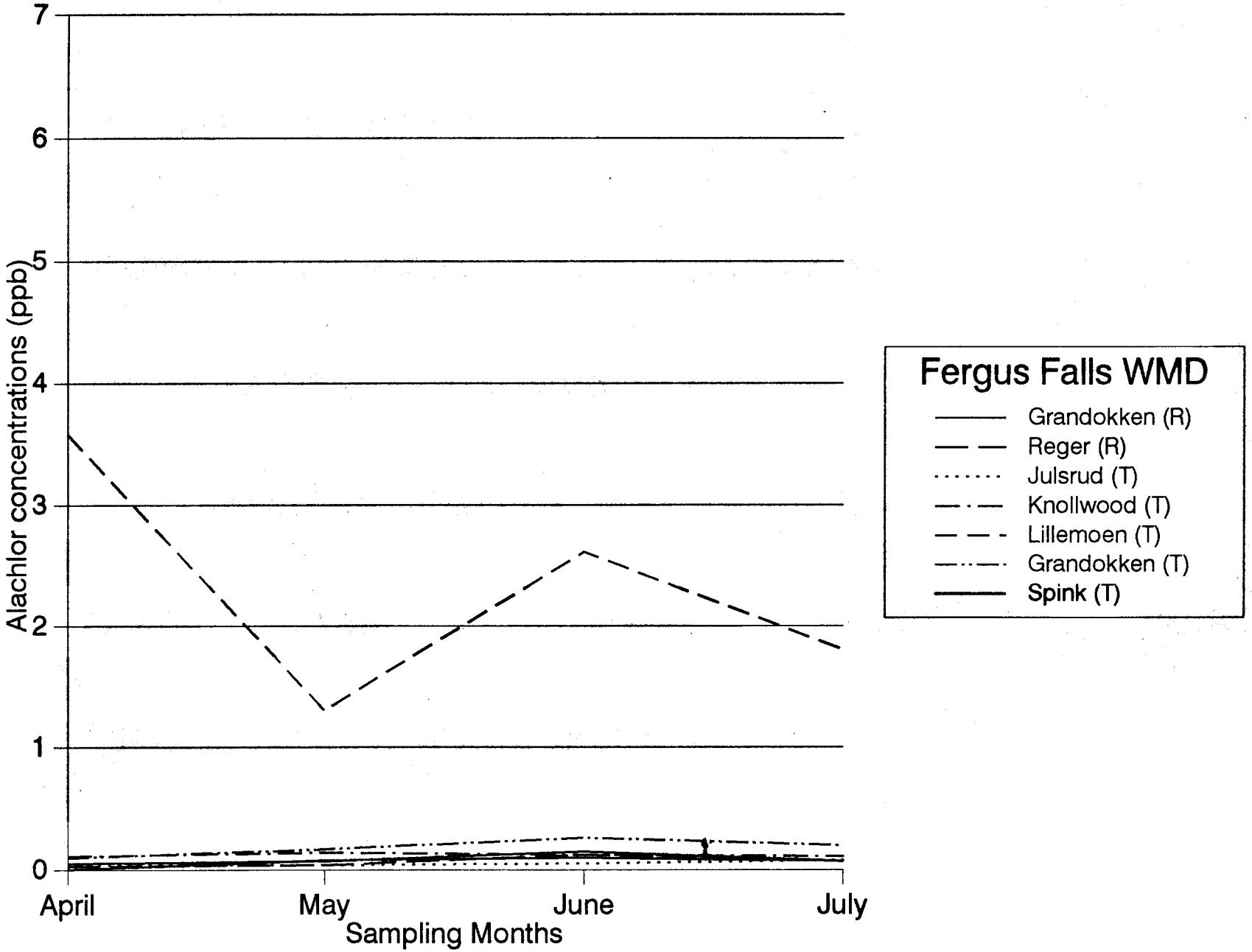


Figure 11. ELISA-derived alachlor concentrations (ppb) in surface waters of reference (R) and treatment (T) Waterfowl Production Area wetlands in Fergus Falls Wetland Management District, April - July, 1993.

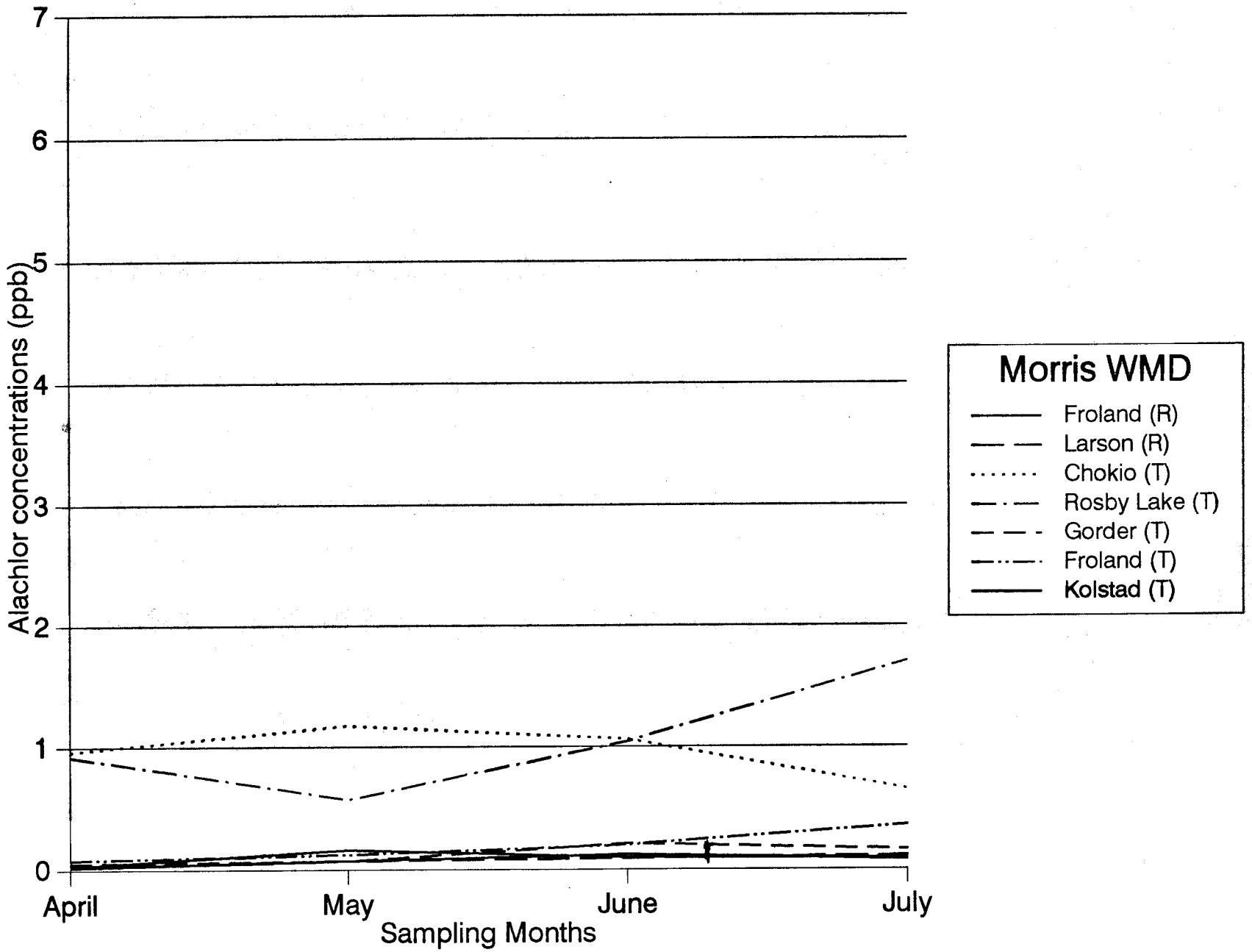


Figure 12. ELISA-derived alachlor concentrations (ppb) in surface waters of reference (R) and treatment (T) Waterfowl Production Area wetlands in Morris Wetland Managment District, April - July, 1993.

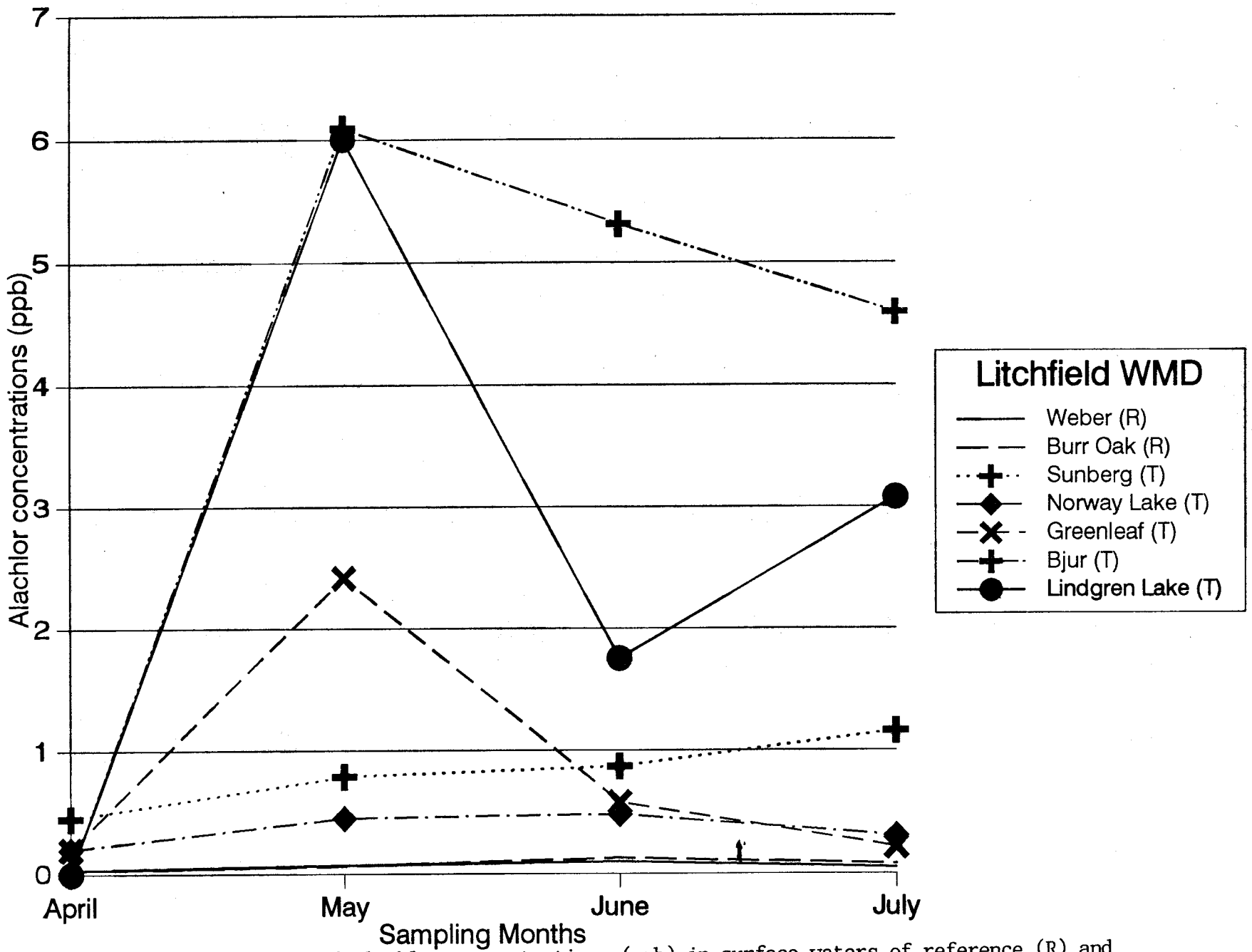


Figure 13. ELISA-derived alachlor concentrations (ppb) in surface waters of reference (R) and treatment (T) Waterfowl Production Area wetlands in Litchfield Wetland Management District, April - July, 1993.

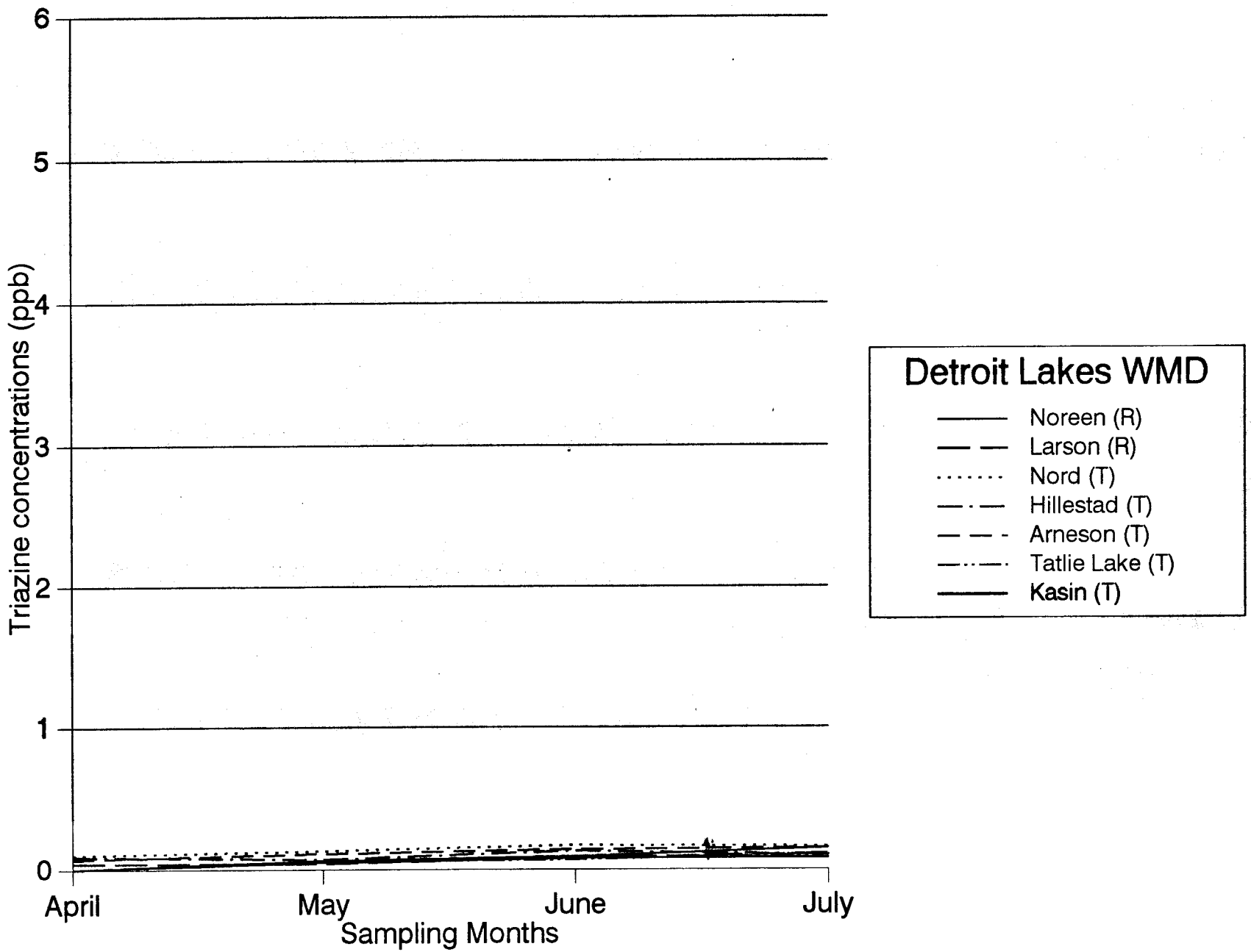


Figure 14. ELISA-derived triazine concentrations (ppb) in surface waters of reference (R) and treatment (T) Waterfowl Production Area wetlands in Detroit Lakes Wetland Management District, April - July, 1993.

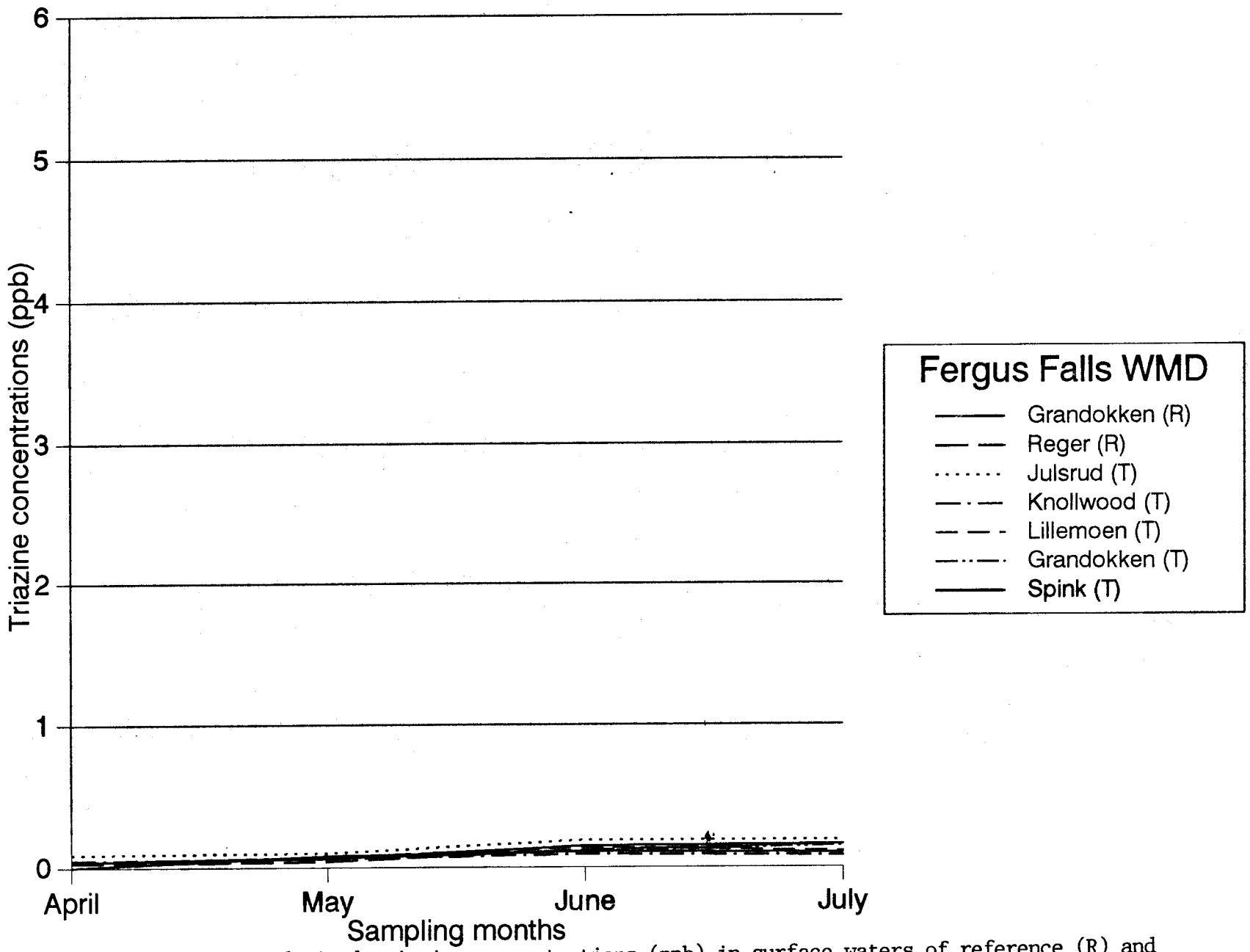


Figure 15. ELISA-derived triazine concentrations (ppb) in surface waters of reference (R) and and treatment (T) Waterfowl Production Area wetlands in Fergus Falls Wetland Managment District, April - July, 1993.

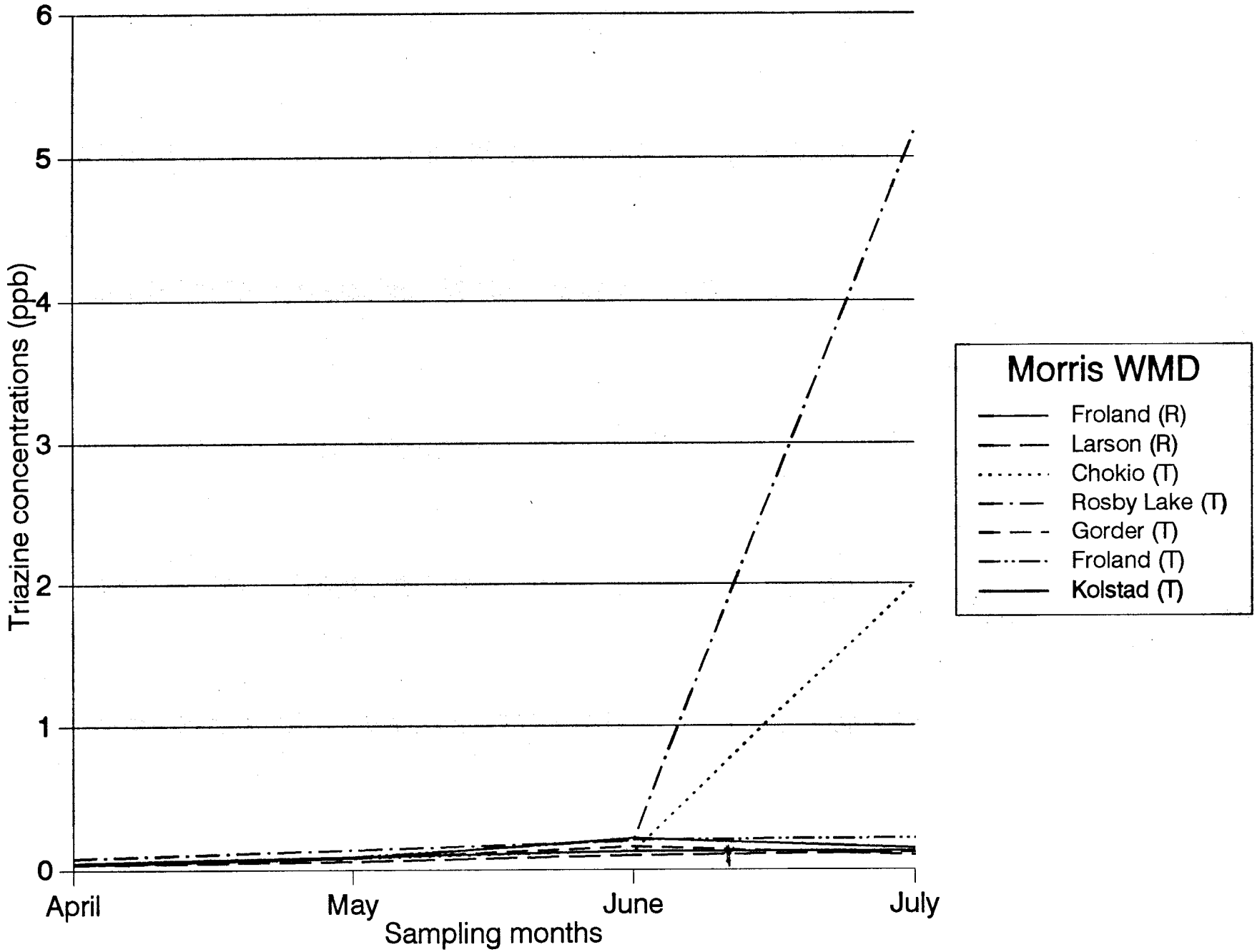


Figure 16. ELISA-derived triazine concentrations (ppb) in surface waters of reference (R) and treatment (T) Waterfowl Production Area wetlands in Morris Wetland Management District, April - July, 1993.

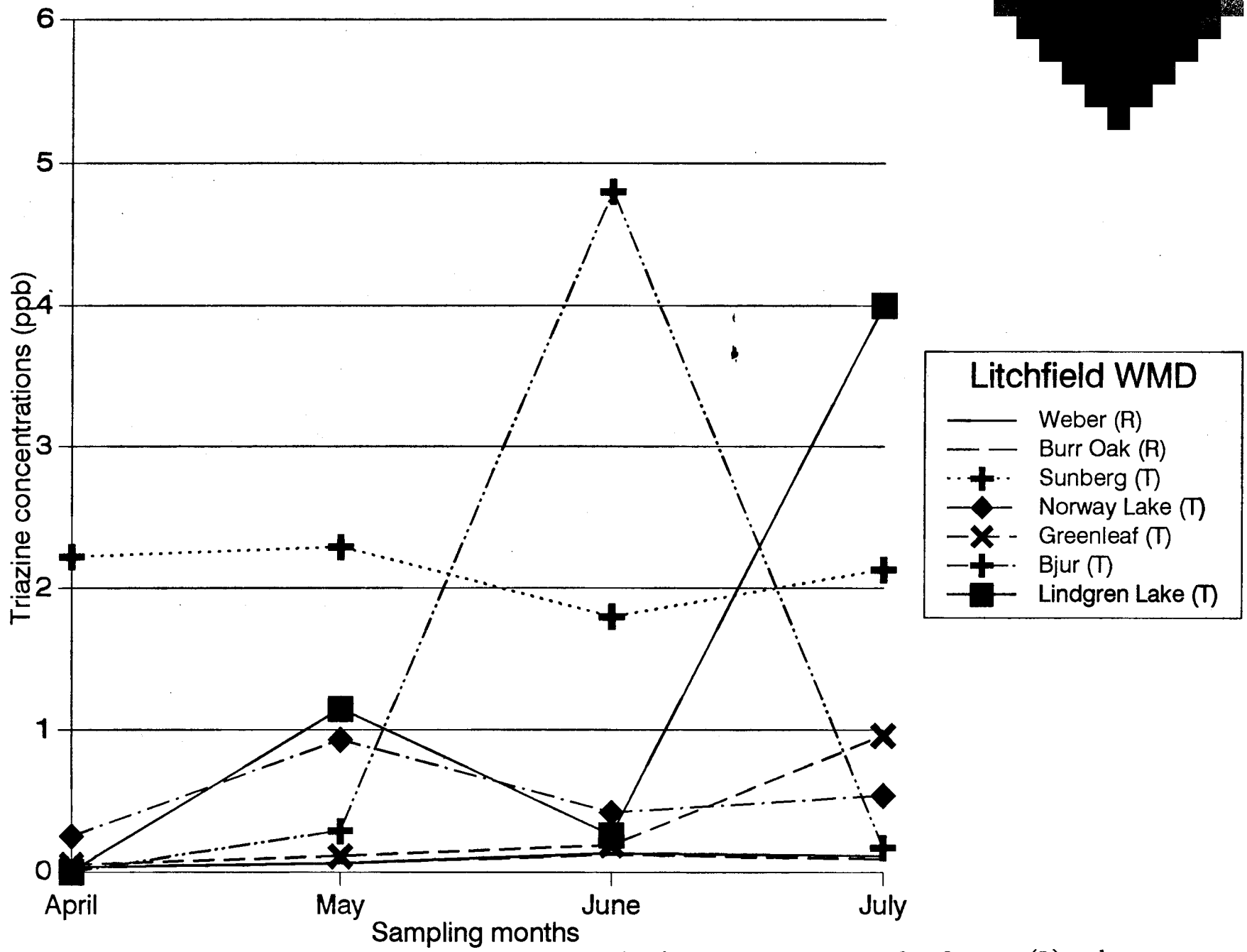


Figure 17. ELISA-derived triazine concentrations (ppb) in surface waters of reference (R) and treatment (T) Waterfowl Production Area wetlands in Litchfield Wetland Managment District, April - July, 1993.

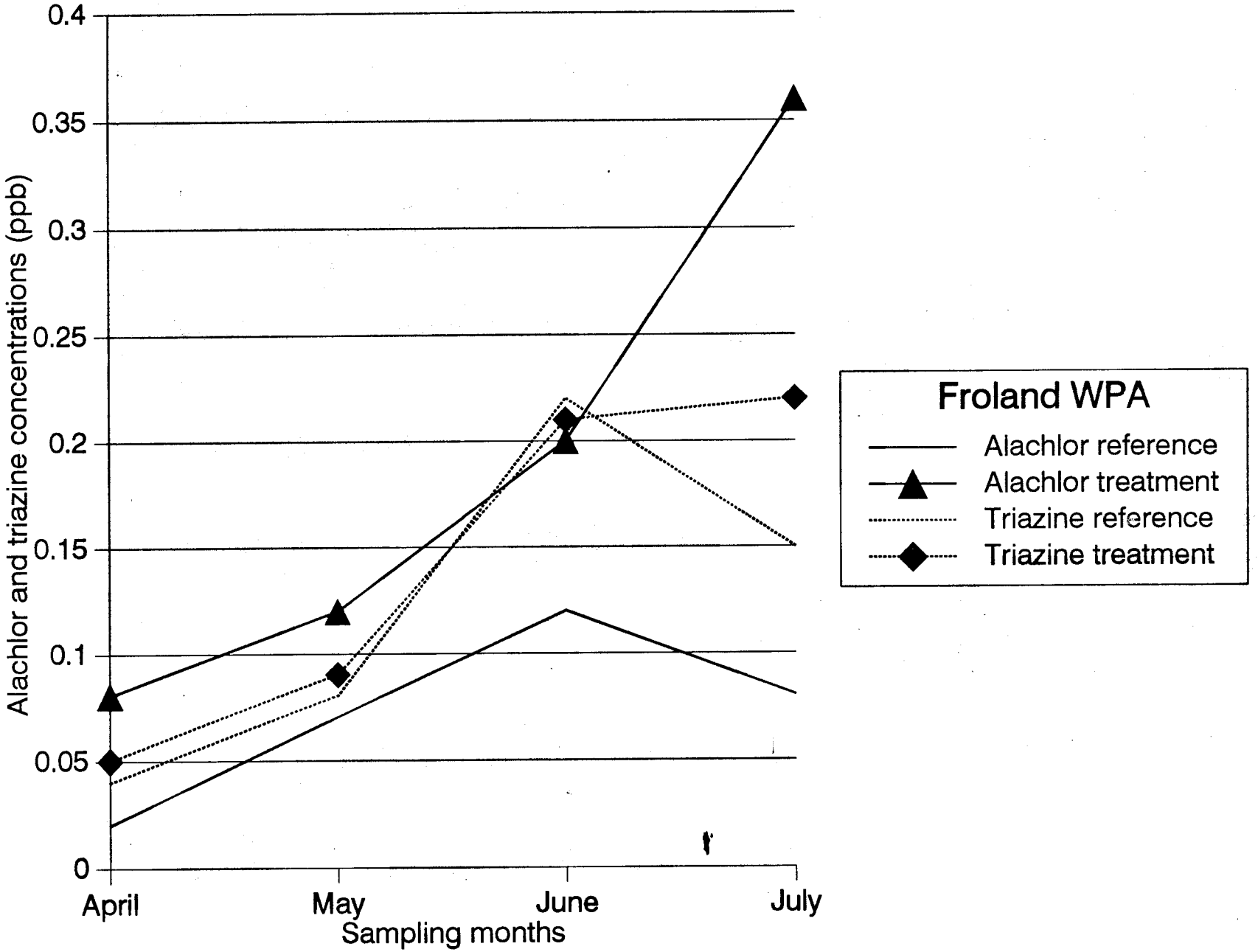


Figure 18. ELISA-derived alachlor and triazine concentrations (ppb) in surface waters of a reference and treatment wetland on Froland Waterfowl Production Area, Morris Wetland Management District, April - July, 1993.

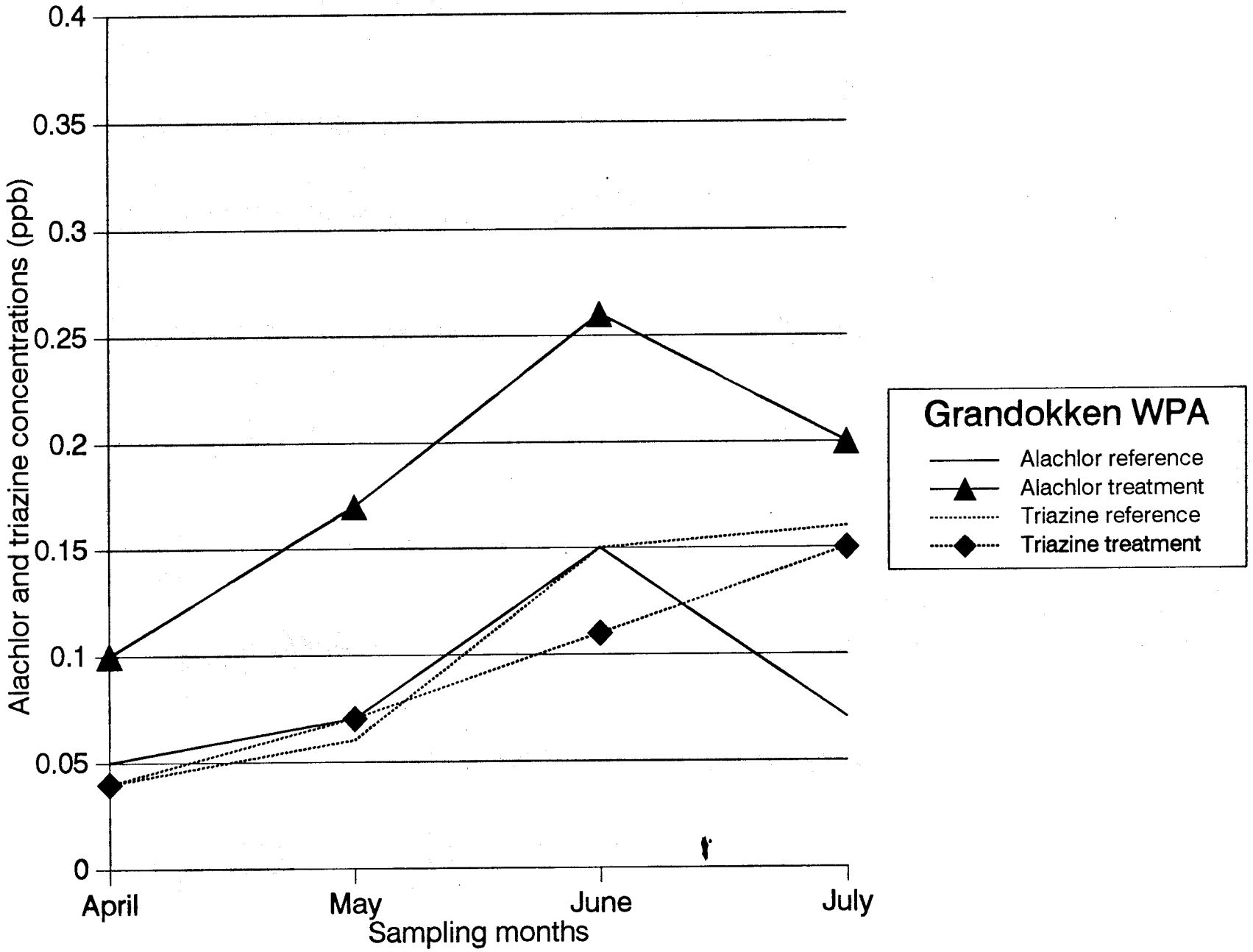


Figure 19. ELISA-derived alachlor and triazine concentrations (ppb) in surface waters of a reference and treatment wetland on Grandokken Waterfowl Production Area, Fergus Falls Wetland Management District, April - July, 1993.

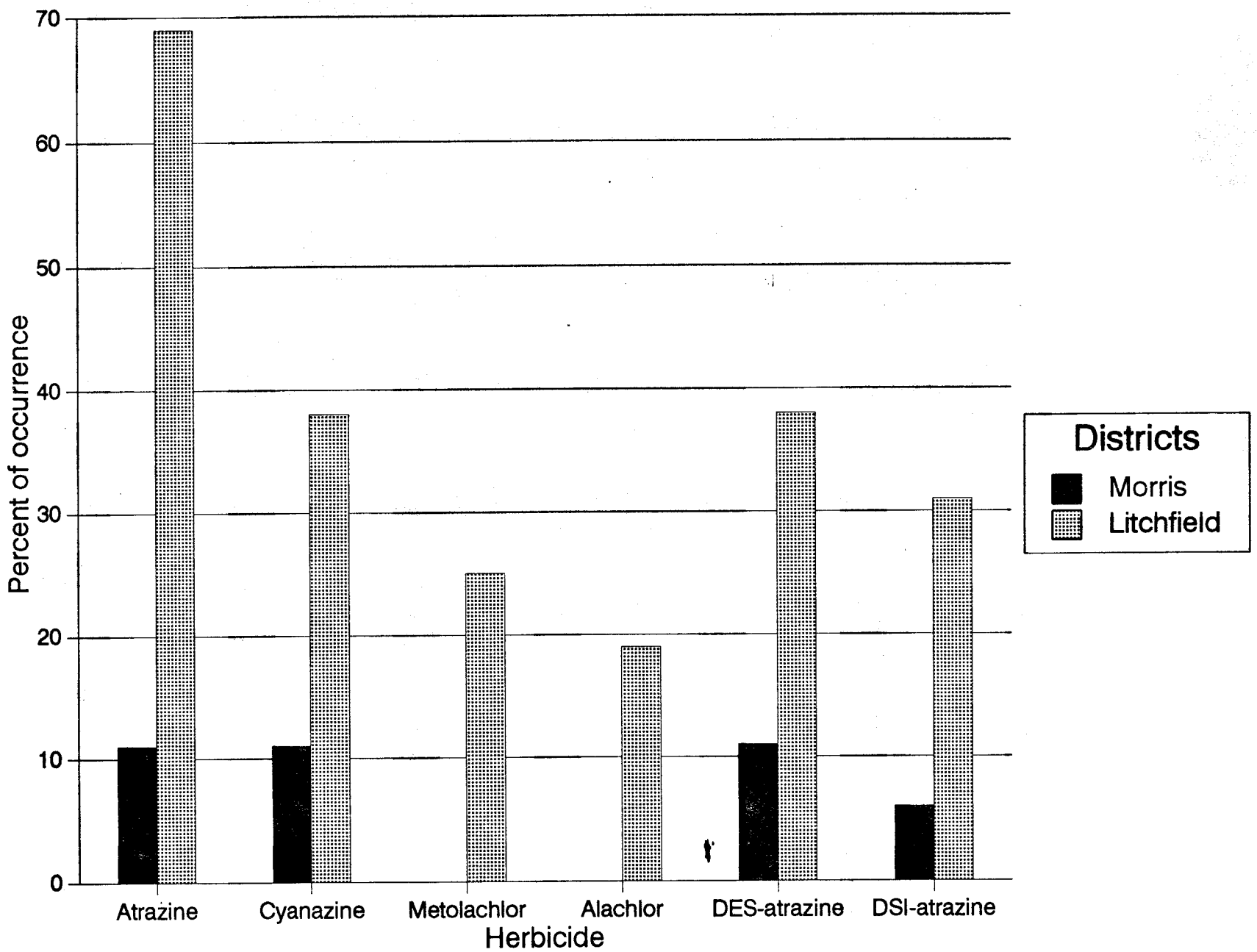


Figure 20. Percent occurrence of surface water common herbicide detections derived from analytical chemistry in Morris and Litchfield Wetland Management Districts, April - July, 1993.

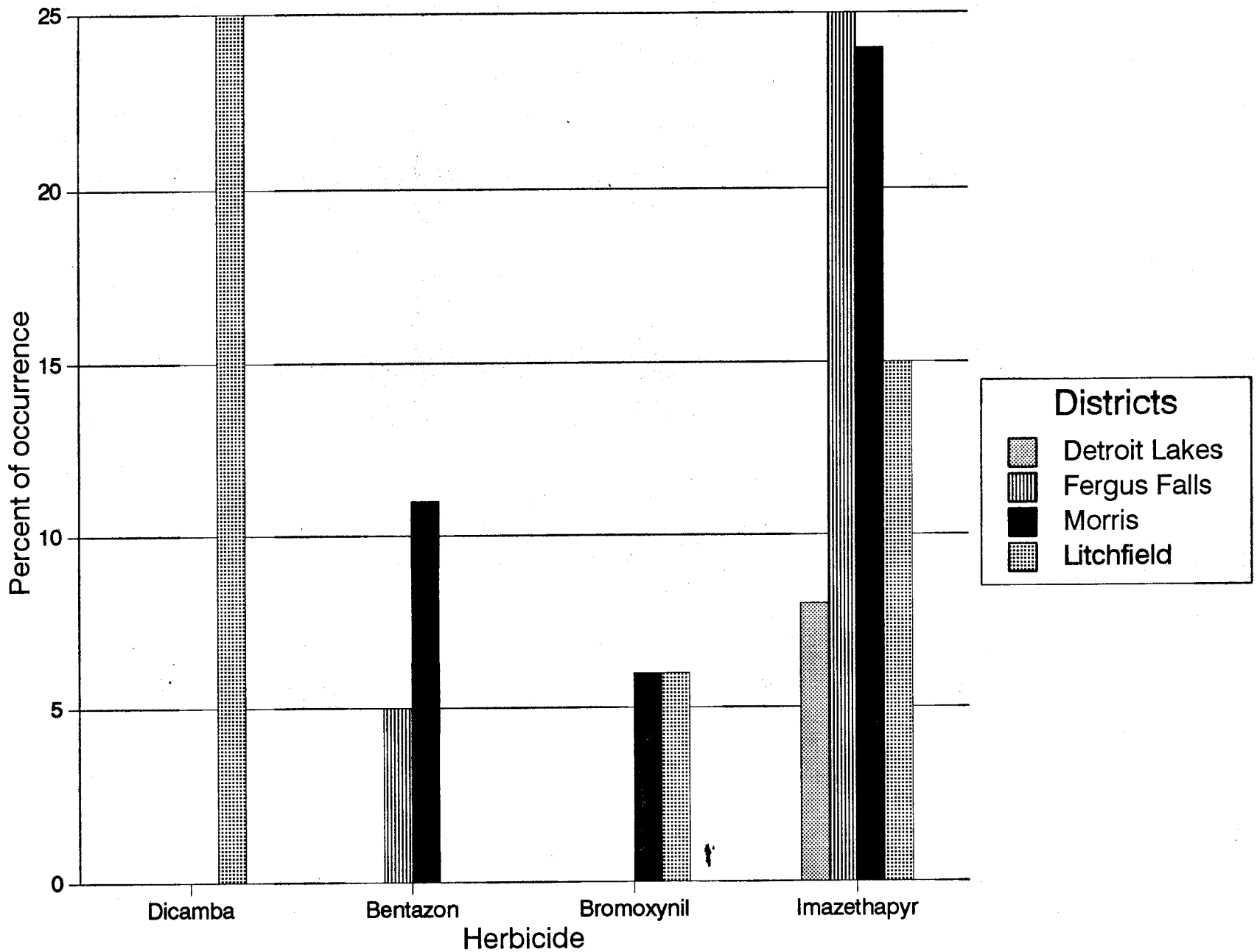


Figure 21. Percent occurrence of surface water phenoxy acid herbicide detections derived from analytical chemistry in Detroit Lakes, Fergus Falls, Morris, and Litchfield Wetland Managment Districts, April - July, 1993.

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# APPENDIX

# United States Department of the Interior

NATIONAL BIOLOGICAL SURVEY

Midwest Science Center
4200 New Haven Road
Columbia, Missouri 65201


November 4, 1994

Keren Ensor
Fish and Wildlife Service
Twin Cities Field Office
4101 East 80th Street
Bloomington, Minnesota 55425-1665

Dear Keren:

This is my written ELISA-GC data review that I promised you back in July. As you know I spent several hours, in early July, reviewing your raw ELISA data obtained for surface water samples collected in selected Waterfowl Production Areas (WPAs) of federal Wetland Management Districts in West Central Minnesota during 1992 and 1993. The two ELISAs that you used were designed to be class specific for 1) triazines (as atrazine) or 2) chloroacetanilides (as alachlor); you used dilutions of the chemical listed in parentheses to calibrate each class-specific ELISA's response. Also, you provided me with the results of independent capillary gas chromatography with nitrogen phosphorous detection (GC/NPD) confirmation analyses performed by the Iowa Hygienic Laboratory (IHL) on a selected number of duplicate water samples collected in 1993. The IHL results included measurements of a number of triazines, selected atrazine metabolites, and the chloroacetanilides: alachlor and metolachlor.

You also provided me a table comparing the ELISA herbicide class results with the IHL GC/NPD results. In general, you had observed that the total herbicide concentrations for each class, based on the IHL GC/NPD results, were much greater than those obtained from the ELISA measurements. You also indicated that there were other data inconsistencies that you hoped that I could resolve.

As we discussed in our July phone conversation, I had several general observations in my review of the 1993 ELISA raw data which are as follows:

> **Definition**: In the following discussions the ELISA cross-reactivity (CR) of a compound is defined as the ratio:
>
> $$\frac{\text{50\% } B/B_0 \text{ concentration of the reference compound (e.g atrazine)}}{\text{50\% } B/B_0 \text{ concentration of the cross-reacting compound (e.g. cyanazine)}}$$
>
> Therefore, the CR of cyanazine is: $0.25\ \mu g/L \div 27\ \mu g/L = 0.0093$

**Triazines (Refer to summary Table 1):**

1 None of the water samples were reanalyzed after being diluted. Samples that had measured ELISA absorbances at 450 nm (B's) lower than the highest calibration standard or greater than that of the zero calibration standard ($B_0$) should have been reanalyzed after dilution. Refer to the discussion below concerned with matrix effects, high sample concentrations and Millipore results printed in parentheses.

2 Several water samples (esp. LT1 Apr., May, and July and LT4 June in Table 1) had measured B's that were less than that of the highest calibration standard (pertinent samples in Table 1 are boxed). It is highly probable that these sample results have underestimated the true ELISA concentrations. IHL GC/NPD results for each of these samples gave total triazines that exceeded those measured by ELISA (for these undiluted water samples).

For the ELISA measurements that you used the ratio $B/B_0$ is inversely proportional to the analyte concentration. This relationship is log-linear and the calibrated concentration-response range is typically limited by the responses of the highest and lowest non-zero standards. Unlike many conventional determinations, **for these ELISA determinations the higher the concentration the lower the response ($B/B_0$)**, and above a certain concentration the response ($B/B_0$) becomes nearly constant and cannot be accurately calibrated. Therefore, any sample whose measured $B/B_0$ is less than that of the highest calibration standard should be diluted and reanalyzed (typically by 1/2 to 1/4 th). Furthermore, when using an ELISA that is class specific, it may be important to make dilutions of any positive samples, since this may be useful in diagnosing high concentrations of less cross-reactive compounds. Compounds with low cross-reactivity may also have lower efficacy, such that, the minimum $B/B_0$ achievable (at high concentrations) is only 0.4 to 0.7. In this case it may require several dilutions of the sample to observe a significant increase in the measured $B/B_0$. In other words the apparent ELISA concentration for each serial dilution will remain nearly constant, while the calculated sample concentration corrected for the dilutions, will appear to increase by the product of the dilution. Sample concentration will appear to double for serial 1/2 dilutions.

3 Table 1 also shows that predicted ELISA concentrations for total-triazines, based on the sum of the IHL GC/NPD results for all measured cross-reacting compounds multiplied by their respective cross-reactivities, were much lower than the IHL GC/NPD totals, and usually lower than the ELISA results.

Possible explanations include:

a) The cross-reactivities are in error (i.e. too low for the non-atrazine triazines)

b) Metabolites not measured by GC/NPD are being measured by the ELISA

c) The GC/NPD or ELISA results are inaccurate. This would mean that the ELISA results are too high which is not likely; actually, as discussed above, ELISA results are probably too low due to insufficient sample dilution. The alternative is that the IHL GC/NPD results are too low. The recoveries of spiked target herbicides appear to be acceptable, but it is still possible that some cross-reacting triazines or metabolites/breakdown products were not measured by GC/NPD due to the selectivity of the prior extraction and clean-up steps employed by IHL.

**Chloroacetanilides (refer to summary Table 2):**

1 Similar results were observed for the total-chloroacetanilides (as alachlor) analyses. Briefly, a few samples had $B/B_0$s less than the highest calibration standard, suggesting that their true ELISA concentrations were higher. The corresponding IHL GC/NPD results for chloroacetanilides gave higher total concentrations than the ELISA results. Table 2, also shows that predicted ELISA concentrations for total-chloroacetanilides based on the sum of the IHL GC/NPD results for all cross-reacting compounds multiplied by their respective cross-reactivities, were lower than the IHL GC/NPD totals, and usually lower than the actual ELISA results.

2 Selected water samples collected in June and July of 1994 consistently had relatively high concentrations of total-chloroacetanilides by ELISA, while IHL GC/NPD measured total-chloroacetanilides were not-detected (0.05 ng/mL = 0.5 X MDL of 0.1 ng/mL, Table 2).

Possible explanations for these results include:

a) The ELISA is measuring metabolites and other undocumented cross-reacting parent compounds (e.g. see discussion of ESA below), and some of these compounds are measured by GC/NPD, while others are not. Certain alachlor metabolites that cross-react with the chloroacetanilide ELISA may not be extracted or recovered through the sample clean up steps prior to GC/NPD.

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b) The ELISA calibrations are positively biased and/or the IHL GC/NPD calibrations are negatively biased.

c) The ELISA is measuring the same parent chloroacetanilides as GC/NPD, but portions of the parent chloroacetanilides are lost during sample storage and preparation prior to GC/NPD.

d) Cross-reactivities used to calculate predicted ELISA total-chloroacetanilide concentrations are too low.

The most likely explanation for the observed differences between the ELISA and IHL GC/NPD results is possibility a) above. Recently, several research groups have documented similar false positive results for the determination of alachlor in surface and ground waters by ELISA and a reference confirmatory method (1-3). Based on follow up analyses (1,2), they have determined that 2-[(2,6-diethylphenyl)(methoxymethyl)amino]-ethane sulfonic acid (ESA) is a major metabolite of alachlor, that accumulates in surface and ground waters, and has nearly equivalent cross-reactivity (0.44) to that of its parent, alachlor (1). ESA is readily detected by the ELISA, and is typically not extracted or recovered by sample preparation methods used prior to classical chromatographic determination methods. These researchers report that high concentrations of ESA persist in water for several months after the last application of alachlor. Recent data for different alachlor ELISA kits have shown that at least one ELISA (Ohmicron RaPID Assay) has lower cross-reactivity with ESA (0.16) relative to alachlor than the Millipore EnviroGard® alachlor ELISA (1).

Both ELISA and GC/NPD appear to be properly calibrated. This assumption is supported by the raw calibration data for the ELISA determinations and the recovery of fortified surrogates, internal standards, and spiked compounds for the IHL GC/NPD determinations. IHL did not provide raw calibration standard data.

Although, the length of time that the water samples were stored prior to analysis by IHL is not easily ascertained from the IHL Report, I suspect that their results are valid. Future GC/NPD confirmatory or comparison analyses should include a few sample duplicates, one of which, is fortified with known amount(s) of the target analyte(s) at the time of collection for determination of with-in sample storage and handling effects. Samples collected in June and July may be microbially much richer and better able to degrade labile compound such as the targeted herbicides than water samples collected in April or May.

Cross-reactivities used to predict ELISA concentrations from IHL GC/NPD results were obtained from the ELISA kit manufacturer (Millipore) and are assumed valid.

J.L. Zajicek, USDI, NBS, MSC, 93ELISDA.LET

**Observations related to the 1992 ELISA data:**

1 Only a few samples from the ELISA triazines and chloroacetanilides analyses should have been diluted due to their low $B/B_0$s relative to that of the highest calibration standard.

2 Calibration curve-fit correlations (multiple r) were good, 0.98 - 0.99+.

3 Some samples had very high $B/B_0$s > 1.0 (i.e. greater than the zero calibration standard) and these samples should have been reanalyzed as-is or as a 1/2 dilution. These high $B/B_0$s suggest a sample matrix effect or an error in the ELISA procedure.

4 In the Millipore reports (based on the Millipore microplate ELISA software), all data printed in parenthesis should be considered suspect, since the calculated concentration is based on a $B/B_0$ response that is either below that of the highest calibration standard or above that of the lowest calibration standard. The parentheses generated by the Millipore software are flags to warn the analyst.

Analytical Recommendations:

1 **To minimize false negatives in class specific ELISAs, calibrate the ELISA using dilutions of a weak (CR = 0.01-0.05) to intermediate (CR = 0.10-0.25) cross-reacting compound.** This is contrary to the calibrations used for the triazine and chloroacetanilide ELISAs, where the compounds with the highest cross-reactivities (atrazine CR = 1.0 and alachlor CR = 1.0) were used for the respective calibrations. Water samples with high ELISA measured concentrations (e.g. >100 $\mu$g/L) would need to be confirmed by an alternative reference method, but relatively high concentrations (e.g. 20-50 $\mu$g/L) of compounds with low cross-reactivity (e.g. cyanazine CR = 0.01) would not be trivialized (i.e. measured as $\leq$0.5 $\mu$g atrazine/L). The calibration should also take into consideration the relative toxicity of the various cross-reacting compounds. In the case of triazines, if all members of this class of herbicides have similar toxicity, then the above discussion is valid. The above calibration recommendations are also valid when the relative toxicities of the class members are unknown (assumed similar). However, if the toxicity of atrazine is 50-fold greater than that of the other triazines then it would make sense to calibrate the ELISA with dilutions of atrazine, since cross-reactivity would parallel relative toxicity.

2 **That the ELISA manufacturers should be contacted to determine if their published cross-reactivities hold for mixtures of the individual herbicides.** Comparisons of ELISA vs GC/NPD determined herbicide concentrations in water

samples of the above Minnesota Wetland Management Districts suggests that, in some cases, the predicted-ELISA concentrations based on GC/NPD and published ELISA cross-reactivities are less than the actual ELISA measured concentrations. These results suggest that the reported cross-reactivities are negatively biased or that these cross-reactivities are not additive for mixtures of herbicides and their metabolites. I plan to investigate this possibility in the near future. Other unmeasured cross-reacting compounds, such as metabolites, may account for the observed differences, but this appears unlikely for the triazine screen.

3 **Concentrations based on ELISA responses ($B/B_0$s) that are outside the *calibration-range* of the respective assay, defined by the $B/B_0$s of the highest and the lowest standards, should be considered suspect. For high concentration samples, when dilution is not possible, the sample result should be reported as "≥ the measured concentration" (e.g. ≥ 2.2 μg atrazine/L water). For "Clean" water samples that can not be reanalyzed the sample result should be reported as a qualified not detected (e.g. ND, possible matrix interference). For low concentration water samples the sample result should be reported as ≤ the measured concentration for results between the ELISA detection limit and the concentration of the low calibration standard (e.g ≤0.09 μg alachlor/L of water).**

High Concentration Water Samples: For samples with $B/B_0$s < the highest calibration standard, dilute the water sample by 1/2 to 1/4th and reanalyze. If the ELISA concentration based on this first dilution (after correcting for the dilution) is similar to that of the original raw-water sample, then the result is assumed to be valid, and no additional dilutions are required. If the dilution corrected concentration of the first dilution is more than 10% to 25 % greater than the raw water concentration, then at least two more serial dilutions should be analyzed to establish a valid ELISA concentration.

"Clean" water samples: For samples with $B/B_0$s more than 2 standard deviations greater than that of the zero calibration standard, reanalyze the raw water sample together with a 1/2 dilution. If the $B/B_0$s for these two analyses are either both more than 2sd > that of the zero calibration standard or only the raw water sample is more than 2sd > the zero standard, the ELISA measurements are negatively biased by a matrix effect in this sample. If the $B/B_0$s for the two analyses are ≤ that of the zero calibration standard, then the original ELISA result was due to an assay error. For screening analyses it should be satisfactory to simply reanalyze the raw water from samples that have $B/B_0$'s significantly greater than 1.0.

Low Concentration Water Samples: Samples with $B/B_0$s more than 5% > that of the low calibration standard should be reanalyzed together with a 1/2 dilution of the low calibration standard or the sample should be concentrated 5- to 10-fold using solid phase extraction or by some other means recommended by the ELISA kit

manufacturer. Usually these low concentration samples need not be reanalyzed when doing class screening measurements.

4. **When ELISA calibrations for class specific assays are calibrated with compounds of high efficacy relative to other members, all positive samples should be diluted at least two- to four-fold to validate the measured concentration.** Refer to the discussion in section 2 of the observations for the 1993 triazine data.

5. **Comparison or confirmation analyses (GC, HPLC, or SPE/ELISA) used to validate ELISA class specific screening results should utilize extraction and fractionation methods that will measure all potentially cross-reacting compounds including unusual parent herbicides, breakdown products, and metabolites.** For example, the IHL GC/NPD determinative method probably could have detected the ESA metabolite of alachlor, had it been present in the hexane extract injected into the GC/NPD. Because the extraction was selective for the more hydrophobic triazine and chloroacetanilides, the hydrophilic ESA was not extracted.

6. **That duplicate samples collected for confirmatory analyses should be spiked in the field at the time of collection to determine the sample storage and handling effects that might occur prior to the actual reference method analysis.** Since some chemicals, such as herbicides, are rapidly metabolized by bacteria, it is important to directly fortify a few real world samples with the target analytes. This is especially important, if the water samples are to be stored without a bactericide for several days prior to extraction.

The above recommendations are related to analytical method and sample considerations. In addition, I would like to recommend several data analysis possibilities.

1. The obvious first choices are direct statistical comparisons of the **results** for the two methods of analysis. You could compare the means, standard deviations, etc. of the two data sets. You could, also do various regression analyses to compare the results of ELISA measurements with the IHL results. These statistical analyses are standard, but may not provide the type of evaluation that is needed when comparing screening method data.

2. Consider a binary comparison (not detected = 0 vs. detected above some rational threshold = 1, or even higher level comparisons (not detected = 1, ND < x > first threshold = 2, and first threshold < x = 3). Thresholds for ELISA should probably be scaled differently from those for the IHL GC/NPD results (i.e. the above three categories could be considered ND, low, and high, which are scaled accordingly). The benefit of this type of analysis is in comparing the frequency of

correct vs. incorrect decisions that are made concerning the degree of herbicide contamination. In these analyses the GC/NPD results would usually be considered the true results.

3. Additional data comparisons might include factors about known application rates and history (e.g. documented application of atrazine immediately prior to the sample collection = 1, documentation that no atrazine was applied = 2, unknown application history = 0, suspected application of atrazine based on common practice = 0.5, documented application of cyanazine application = 3, etc.) This type of analysis might assist in identifying ELISA or GC/NPD positive or negative biases such as ESA.

It may be difficult to define objective decision thresholds for each screening method, but even subjective criteria may be useful. These are just a few of the many data analysis possibilities. I feel that it is important to quantitatively compare the information content of each method, when possible. A cost and timeliness analysis might also be in order.

References:

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2 Baker, D.B., Bushway, R.J., Adams, S.A. and Macomber, C. (**1993**) Immunoassay screens for alachlor in rural wells: False positives and an alachlor soil metabolite, Environ. Sci. Technol. 27: 562-564.

3 Feng, P.C.C., Wratten, S.J., Horton, S.R., Sharp, C.R., and Logusch, E.W. (**1990**) Development of an enzyme-linked immunosorbent assay for alachlor and its application to the analysis of environmental water samples, J. Agric. Food Chem. 38: 159-163.

General References on Immunochemical Methods of Analysis:

1 Vanderlaan, M., Stanker, L., and Watkins, B. (**1991**) Immunochemical Techniques in Trace Residue Analysis, A Tutorial, In **Immunoassays for Trace Chemical Analysis, Monitoring Toxic Chemicals in Humans, Food and the Environment**, Eds: Vanderlaan, M., Stanker, L.H., Watkins, B.E., and Roberts, D., American Chemical Society (Symposium Series 451), from PACIFICHEM '89, December 17-21, 1989, Honolulu, HI.

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2 Gee, S.J., Hammock, B.D., and Van Emon, J.M. (1994) A Users Guide to Environmental Immunochemical Analysis, Environmental Monitoring Systems Laboratory-Las Vegas, Office of Research and Development, US EPA, Las Vegas, NV, EPA/540/R-94/509

I have enclosed copies of selected recent articles concerning ELISA determinations of triazines and alachlor, together with revised copies of tables 1 and 2. I hope that my review, recommendations, and the enclosed references are useful for your project report, and I wish you success in your future ELISA efforts. I would like to thank you for providing me the opportunity to review and comment on your ELISA field screening data. This exercise has given me new insight into the analytical considerations of ELISA and reference field screening methods for herbicides that will be useful in designing future ELISA research.

Sincerely yours,

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**Table 1. Comparison of ELISA and CGC/NPD Determinations of Triazines and Selected Atrazine Metabolites in Surface Water Samples from Selected Waterfowl Production Areas (WPA) of the Litchfield Wetland Management District, Minnesota**

ELISA determinations by Keren Ensor, USFWS Twin Cities Field Office, Bloomington, MN 55425
CGC/NPD determinations by Univ. of Iowa, Hygienic Laboratory, 102 Oakdale Campus, Iowa City, IA

**Triazines**
CR is cross reactivity from Millipore 50%Bo data.

| WPA Treatment | Dates | CR=1.0 Atrazine ng/mL | CR=0.0093 (Bladex) cyanazine ng/mL | CR=0.02 DE-Atrazine ng/mL | CR=0.02 DI-Atrazine ng/mL | Predicted ELISA Results* ng/mL | ELISA ng/mL | CGC/NPD Total ng/mL |
|---|---|---|---|---|---|---|---|---|
| Sunberg (LT1) | April | 1.2 | 3.5 | 0.37 | 0.31 | 1.25 | 2.22 | 5.38 |
| | May | | | | | | 2.29 | NA |
| | June | 0.71 | 2 | 0.3 | 0.32 | 0.74 | 1.8 | 3.33 |
| | July | 0.6 | 3.1 | 0.35 | 0.44 | 0.64 | 2.13 | 4.49 |
| Norway L. (LT2) | April | 0.11 | 0.05 | 0.05 | 0.05 | 0.11 | 0.25 | 0.11 |
| | May | | | | | | 0.93 | NA |
| | June | 0.13 | 0.11 | 0.05 | 0.05 | 0.13 | 0.42 | 0.24 |
| | July | 0.13 | 0.05 | 0.05 | 0.05 | 0.13 | 0.52 | 0.13 |
| Greenleaf (LT3) | April | | | | | | 0.05 | ND(0.1) |
| | May | | | | | | 0.11 | ND(0.1) |
| | June | | | | | | 0.19 | ND(0.1) |
| | July | 0.36 | 0.05 | 0.05 | 0.05 | 0.36 | 0.96 | 0.36 |
| Bjur (LT4) | April | | | | | | | NA |
| | May | | | | | | 0.29 | ND(0.1) |
| | June | 28 | 0.05 | 0.84 | 0.23 | 28.02 | 4.80 | 29.07 |
| | July | | | | | | 0.17 | ND(0.1) |
| Lindgren L. (LT5) | April | | | | | | | NA |
| | May | 0.37 | 0.05 | 0.17 | 0.05 | 0.37 | 1.15 | 0.54 |
| | June | 0.11 | 0.12 | 0.05 | 0.05 | 0.11 | 0.26 | 0.23 |
| | July | 3.1 | 0.14 | 0.75 | 0.31 | 3.12 | 4.00 | 4.3 |

CGC/NPD Triazine Totals = atrazine + cyanazine + desethyl-atrazine + desisopropyl-atrazine
*Based on the sum of: CR * [CGC/NPD] for all cross reacting compounds
ELISA this sample should have been diluted additionally to bring the response (A450) into the calibration range (A450 <= A450 of the 2.0 ng/mL high atrazine calibration standard).

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**Table 2. Comparison of ELISA and CGC/NPD Determinations of Chloroacetanilides, Alachlor and Metolachlor, in Surface Water Samples from Selected WPAs of the Litchfield Wetland Management District, Minnesota**

**Acetanilides (as Alachlor)**
CR is cross reactivity from Millipore 50%Bo data.

| WPA Treatment | Dates | CR=0.015 (Dual) Metolachlor ng/mL | CR=1.0 (Lasso) alachlor ng/mL | Predicted ELISA Results* ng/mL | ELISA ng/mL | CGC/NPD Total ng/mL |
|---|---|---|---|---|---|---|
| Sunberg | April | 0.05 | 0.05 | 0.05 | 0.45 | 0.05 |
| (LT1) | May | | | | 0.79 | NA |
| | June | 0.05 | 0.05 | 0.05 | 0.87 | 0.05 |
| | July | 0.05 | 0.05 | 0.05 | 1.16 | 0.05 |
| Norway L. | April | 0.05 | 0.05 | 0.05 | 0.2 | 0.05 |
| (LT2) | May | | | | 0.45 | NA |
| | June | 0.05 | 0.05 | 0.05 | 0.48 | 0.05 |
| | July | 0.05 | 0.05 | 0.05 | 0.31 | 0.05 |
| Greenleaf | April | 0.05 | 0.05 | 0.05 | 0.2 | 0.05 |
| (LT3) | May | 30 | 0.05 | 0.50 | 2.42 | 30 |
| | June | 2 | 0.05 | 0.08 | 0.58 | 2 |
| | July | 0.05 | 0.05 | 0.05 | 0.22 | 0.05 |
| Bjur | April | | | | | NA |
| (LT4) | May | 13 | 2.4 | 2.60 | 6.09 | 15.4 |
| | June | 19 | 0.89 | 1.18 | 5.31 | 19.89 |
| | July | 0.05 | 0.05 | 0.05 | 4.59 | 0.05 |
| Lindgren L. | April | | | | | NA |
| (LT5) | May | 0.05 | 4.2 | 4.20 | 6 | 4.2 |
| | June | 0.05 | 0.05 | 0.05 | 1.75 | 0.05 |
| | July | 0.05 | 0.05 | 0.05 | 3.08 | 0.05 |

ELISA and CGC/NPD ELISA measurements are detecting cross reacting compounds not detected by CGC/NPD ( i.e. metabolites, other chloroacetanilides, or cpds lost prior to CGC/NPD).

CGC/NPD Alachlor Totals = Dual (metolachlor) +Lasso (Alachlor)

ELISA this sample should have been diluted additionally to bring the response (A450) into the calibration range (A450 < = A450 of the 2.5 ng/mL high alachlor calibration standard).

*Based on the sum of: CR * [CGC/NPD] for all cross reacting compounds

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