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THE FATE AND IMPACT OF 2,4-D ON AN AQUATIC ECOSYSTEM
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- Environment Canada

TIE FATE AND IMPACT OF 2.4-D IN AN AQUATIC ECOSYSTEM B.F.Scott, E. Nagy and D.S. Painter EXECUTIVE SUMMARY

The recent invasion of Canadian waters by Eurasian watermilfoil has resulted in increased use of the herbicide 2,4-D and increased public concern with the safety of such chemical control measures. This study was an Environment Canada response to such concerns expressed in areas as diverse as the Trent-Rideau-Severn waterway and the Okanagan Lakes in British Columbia. Our study was designed to determine the fate and impact of the butoxyethyl ester and $N, N$-dimethylamine formulations of 2.4-D. The ester is the only approved formulation in Canada for aquatic use, and the amine was being considered by Agriculture Canada as a replacement.

Both formulations were effective in milfoil control at nominal concentrations of 1 ppm. The half-life of $2,4-D$ in the water varied from 6 to 8 weeks in the two year study. The herbicide and its degradation product, 2,4-dichlorophenol, were detected in the water, sediment and various components of the biota throughout the study. Some direct effects of the herbicide were observed on fish fry and clams. Secondary effects were increased bacteria populations and snail populations, enhanced growth of clams and a shift in the benthic community after the milfoil collapse and decay.

The experimental pond ecosystems were found to maximize effects by containment of the chemical and to facilitate a study of the chemical's impact on components of the biota.

DEVENIR ET REPERCUSSIONS DU 2,4-D DANS UN ECOSYSTEME D'ETANG

E. Nagy, D.S. Painter et B.F. Scott RESUME A L'INTENTION DE LA DIRECTION

Récemment, l'envahissement des eaux canadiennes par le myriophylle eurasien a provoqué une utilisation accrue de l'herbicide 2,4-D et a intensifié l'inquiétude manifestée par le public quant à lạ sécurité de telles mesures de limitation. La présente étude est la réponse d'Environnement Canada à l'inquiétude manifestée dans des régions aussi différentes que les voies navigables Rideau et Trent-Severn et les lacs de l'Okanagan en Colombie-Britannique. L'étude a été conçue de façon à déterminer le devenir et les répercussions des formulations à base d'ester butoxyéthylique et de sel de $N, N$-diméthylammonium du $2,4-D$. L'ester est la seule formulation approuvée au Canada pour usage dans le milieu aquatique; Agriculture Canada a envisagé la possibilité de le remplacer par le sel d'ammoníum.

A une con centration nominale de 1 ppm , les deux formulations limitent efficacement le myriophylle. Pendant l'étude d'une durée de 2 ans, le temps de demi-élimination du $2,4-D$ dans l'eau variait de 3 à 5 semaines. L'herbicide et son produit de dégradation, le 2,4-dichlorophénol, ont été décelés dans l'eau, les sédiments et diverses composantes du biote pendant toute la durée de l'étude. Certains effets directs de l'herbicide ont été observés sur le frai de poissons et sur les clams. Voici certains de ces effets secondaires : accroissement des populations de bactéries et

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de gastropodes, croissance accrue des clams et modification de la communauté benthique après la destruction du myriophylle et sa décomposition.

L'utilisation de ce type d'écosystèmes expérimentaux a permis de rendre maximum les effets des produits en les confinant, ce qui a facilité l'étude de leurs répercussions sur les composantes du biote.

The fate and impact of two formulations of 2.4-D were examined in an experimental pond ecosystem. The half-life of the 2.4-D in the water varied from 6 to 8 weeks in a two year study. The herbicide and its degradation product, 2,4-dichlorophenol, were detected in the water, sediment and various components of the biota throughout the study. At the application rate of 1 ppm in the water, no significant effects were observed on the phytoplankton and zooplankton communities. Populations of bacteria had increased after the collapse and decay of the milfoil. Fungal levels were observed to decline following treatment. The decaying milfoil also caused a shift in the zoobenthos from chironomid to tubificid oligochaete dominated communities, and increased snail populations. The growth of clams was initially inhibited, but significantly enhanced after the milfoil decay. White sucker fry exhibited some mortality the first week after treatment in the first year. No mortality was observed in the second year. The difference between the two treatment years was attributed to increasing pH and the subsequent predominance of the phenolate form of $2,4-\mathrm{DCP}$. Toxicity studies on clams also indicated that the non-dissociated form of 2,4-DCP was more toxic. Adult fish were not affected in either year.

## THE STUDY TEAM:

B.F. Scott - pond design, study planning, field sampling, overall coordination.
E. Nagy - study planning, field sampling, sediment analyses.
D.S. Painter - study planning, field sampling, milfoil study.
W.D. Taylor - field sampling, protozoa study.
B.J. Dutka and A. Kwan (NWRI) - bacteria study.
J. Sherry (NWRI) - the study on fungi.
A.J. Niimi (GLFRB) - 1980 fish toxicity experiments.
M.N. Charlton (NWRI) - participated in the diurnal DO experiment.
J.H. Hart (NWRI) - pond construction and maintenance, 2,4-D analyses.
J. Wood (NWRI) - milfoil study, sampling (Scuba diving).
J. Mackie and M. Stephenson (U. of Guelph) - conducted both laboratory and field experiments on clams.
M. Dickman (Brock U.) - supervised the phytoplankton study (C.E. Prescott, P. Hayes).

The authors wish to acknowledge the contributions of the above team members.

The experimental ponds were constructed on a site provided by the Hamilton Region Conservation Authority.

## INTRODUCTION

In Canada, hundreds of tonnes of 2,4-D are used annually for the control of terrestrial and aquatic weeds. In addition to the 2.4-D used in aquatic weed control, the chemical can enter the aquatic environment from terrestrial sources. The recent invasion into Canadian waters of Eurasian watermilfoil, Myriophyllum spicatum, the chemical control of which relies on the use of 2,4-D, has resulted in both an increased use and increased public concern with the safety of such chemical control measures. This study was conducted in response to such concerns expressed in areas as diverse as the Trent-Rideau-Severn waterway, and the Okanagan Lakes in British Columbia.

The chemical, 2,4-D, can be used in several formulations such as esters, acid salts and amines. The only commercial formulation approved in Canada for aquatic weed control is the butoxyethyl ester in a slow release formulation (Aquakleen). In the late 1970s, Agriculture Canada had banned the terrestrial use of the butylester formulation because of drift problems, was reviewing the use of butoxyethyl ester, and was considering the N, N-dimethylamine formulation for aquatic uses. Our study was designed to determine the fate and impact of the latter two formulations in an aquatic ecosystem.

Previous studies have found that $2,4-\mathrm{D}$ does not persist long


#### Abstract

in the aquatic environment (1). Although a laboratory study reported some effects on phytoplankton (2), field studies generally report no effect on phytoplankton, zooplankton, clams, or fish (3.4). For a perspective we may note that the 96-hour $L_{50}$ of the $2.4-\mathrm{D}$ acid to bluegill is 350 ppm, whereas the recommended treatment concentration is 1 ppm (5). A laboratory study inferred that the decomposing vegetation after 2,4-D treatment could produce anoxic conditions, presenting a secondary hazard to fish (6).


Previous ecosystem studies on $2,4-\mathrm{D}(3,7,8,9)$ have investigated several components of the food chain to determine uptake and persistence of the chemical in the biota, and possible toxic effects. Our approach utilized experimental ponds to study the impact of $2,4-D$ on the components of the ecosystem, and on the community structures. The ponds, as closed systems, were considered well suited for the study of the fate and persistence of the chemical. A critique of using such ponds is given elsewhere (10).

A preliminary report on the 1980 work (11) is partially incorporated into this manuscript to give a more complete overview of our 1980 and 1981 results.

## Site Preparation

The site was an isolated location in the Fifty Point Conservation Area managed by the Hamilton Conservation Authority, in the Niagara Penisula, near Winona, Ontario. In August of 1979, six large ponds were excavated to the specifications shown in Fig.l. The final ground level around the ponds was 50 cm higher than the original, to minimize water runoff from the surrounding land. Four layers of 6 mil black polyethylene liner were positioned over the surface of the excavation and sediment was added to a depth of 60 cm over the flat bottom and to a depth of 15 cm over the gently sloping ends, and 10 cm over the steeper sides of the ponds. Sediment material was taken from the excavated soil, which was once the bottom of a large lake which predated nearby Lake Ontario. Material from the surface layer of soil was not used.

Water was pumped into the six excavations from a nearby trout pond, with the water intake situated near the sediment so that planktonic and benthic organisms would be introduced. In September of 1979, 35 mature milfoil plants with their roots intact, were planted in the level sediment area of each pond. These plants were about 40 cm high on May 1, 1980. On May 9, 15 additional plants, each 100 cm high, were planted in three rows of five across each pond. The plants in the middle row were numbered
for plant growth measurements. Shortly thereafter, twenty common shiner Notropis cornutus fingerlings were added to each pond.

Pisidiid clams (Sphaerium rhomboideum) were collected from a natural pond in 1980 and transported live to the experimental area. Ten clams of known length were placed inside each of several plexiglass containers ( 11.5 cm i.d.) which were distributed in all six ponds. The containers had $60 \%$ of the sides removed and 40 um Nytex mesh netting covering the openings as well as the tops and bottoms of the plexiglass cylinders. Willow leaves were placed in each container as a food substrate which covered a sediment layer.

Sampling techniques and schedules

Samples were collected from mobile bridges constructed for this study. A diving platform was attached to each bridge so that a scuba diver lying on the platform could be moved about the pond for sediment sampling and plant growth measurements without stirring up the sediment.

Composite water samples were subsampled for bacteria, phytoplankton, protozoa, water column fungi, water chemistry, and particulate material. The composite samples were obtained from the mobile bridges, using a 2 liter clear plastic van Dorn bottle. Three samples were taken from the near surface waters, two from mid-depth and one from near the bottom. These were combined in a
large container, stirred and appropriate aliquots were placed in prepared sample bottles.

Samples were generally collected fortnightly during periods of open water and monthly during periods of ice cover, except immediately before and after the addition of the chemical. prior to the 1980 treatment, additional samples were collected one week prior, one day prior to, one day after, two days after, four days after, one and three weeks after treatment. In 1981, additional samples were collected but not as extensively as in the 1980 study. A summary of sampling dates and types of samples collected are given in Tables la,b,c.

Chemical treatment of ponds

On June 25, 1980, four ponds were treated with 2,4-D, two with the $N, N$-dimethyl amine formulation and two with the butoxyethyl ester formulation. The additions were calculated to produce nominal concentrations of about 1 ppm , the recommended dosage for milfoil control. The two remaining ponds were used as controls. The pond designations are shown in Fig.2. on July 5 , 1981, the two former control ponds were treated with 2,4-D, one with the amine and the other with the ester.

POND CHEMISTRY

## Water Chemistry

The water chemistry parameters of nitraté, nitrite, ammonia, TKN, particulate nitrogen, filtered, unfiltered and reactive phosphorus, dissolved organic carbon, particulate carbon, alkalinity, sulphate, chloride, calcium and magnesium ions were determined in the water samples, as described in the Standard Methods Manual (12). In 1980, the pH was determined by taking a water sample with the van Dorn sampler, transferring to a beaker, and measuring with a Beckman pH meter. In the same year, dissolved oxygen and water temperature were measured with a YSI Dometer, near the surface, mid-depth and near the bottom.

In 1981, the probes of YSI oxygen meters were positioned in the ponds with their leads connected to the meters secured in a nearby trailer. The output was connected to strip chart recorders. The probes were calibrated at least four times a week.

The plots for water column concentrations of ammonia, nitrate, TKN, particulate nitrogen, filtered and unfiltered phosphorus, dissolved organic carbon, alkalinity and particulate carbon are shown in Figs. 3 to 11 respectively. Both years' results are plotted on the same curve for an individual pond when that pond was used in both 1980 and 1981. The line before day 400 denotes the zero time for the second year's test. The results are difficult to interpret as the values are regulated by the
ecosystems in the ponds and by precipitation. It is important to note, however, that the trends were similar in all treated and untreated ponds.

Alkalinity behaved differently in the two years (Fig. 10). In 1980, there was no change associated with the 2,4-D addition. In 1981, the alkalinity increased substantially after the chemical treatment. The calcium ion concentrations for 1981 are plotted in Fig. 12. The observed increase was not followed by an increase in magnesium ion concentrations (Fig. 13). The alkalinity and calcium increases were attributed to the milfoil releasing calcium carbonate from its leaves into the water column as it succumbed to the 2,4-D. The plot for magnesium indicates that the increase in calcium did not originate from surface water runoff into the ponds. A similar increase in alkalinity was not noted in 1980 probably because there was less milfoil biomass in the ponds.

Neither the ammonia nor the nitrate concentrations showed any increase in the amine-treated ponds (Figures 3 and 4), although the nominal herbicide concentrations were about $1 \mathrm{mg} / \mathrm{L}$.

Dissolved organic carbon concentrations showed an immediate increase after treatment in the amine treated ponds, and slight increases in the ester ponds (Fig. 9). Long term trends are difficult to discern because of the variability of this parameter.
measured at the three depths, indicates that the ponds were at saturation level, despite the decomposition of the dead plant tissue.

The diurnal oxygen values in 1981, from five days before to twenty-one days after treatment, were measured at mid-depth in the ester pond, and are shown in Fig. 14 and 15. Breaks in the plot indicate malfunctions of the recording devices. The oxygen content of all ponds underwent normal diurnal variation. The oxygen generally decreased until July 21 , when minimum values where measured. After this time the oxygen content of the water increased. Generally, the oxygen was at saturation or supersaturation levels, except directly above the sediment, where the oxygen content was zero during the collapse and decomposition of milfoil.

Incident sunlight was measured at a point several miles away from the pond area, with the results plotted in Fig. 16. Minimum values in the incident radiation occurred in the same time period as the minimum was observed in the oxygen content of the water.

The last part of Fig. 15 illustrates the diurnal patterns of top and mid-depth levels of the oxygen content in the ester pond. The gross production, net production and respiration of the primary producers can be determined from the diurnal oxygen content curves. The net production values given in Table 2 are similar to those measured for freshwater bodies (13).

The bottom portions of the graphs in Figures 14 and 15 show the diurnal variation of pH in the ester treated pond. over the interval monitored, the values varied about pH 10. The maxima of the pH curve occurred during the dark period. The pH variations reflect the changes in oxygen content with a slight time lag between the curves for the two parameters. The most noticeable exceptions occurred between July 19 to 21 , at the time when the dissolved oxygen levels were lowest.

Secchi depth measurements, taken in 1980, show the same variation in all ponds (Fig. 1).
$E_{h}$ measurements, representing oxidizing or reducing conditions in the sediment, were made on August 19 and November 19. 1980, on cores removed from the ponds. The cores were 10 cm in diameter and 20 cm in length. The measurements were made at 5 cm intervals using a platinum $E_{h}$ electrode connected to a Radiometer pH Meter 29 (Copenhagen).

The results are recorded in Table 3. The cores from the dark sediment areas of one of the control ponds had lower values than those from the lighter or brown colored areas. The dark areas of sediment were under the Chara plants. The dark colour and reducing conditions may be attributed to the release of sulphur compounds by these plants (14). The sediments in the treated ponds were more reducing than in the control ponds on both
sampling dates. The sediments in November showed increased $E_{h}$ readings, i.e. more oxidizing regimes, in all ponds.

Sediment respiration (oxygen demand) increased in all treated ponds during milfoil collapse and decay. Sediment oxygen production, indicating algal growth, increased only about two months after treatment, by which time milfoil decay was well in progress (24).

FATE OF 2.4-D

## Experimental

1. Water Column: All samples were collected using a $2-L$ plexiglass van Dorn sampler from mid-depth of the ponds unless Otherwise stated. Approximately liter of water was poured into an amber-coloured glass bottle containing 2 g each of Rohm \& Haas Amberlite XAD-2 and XAD-7 ion exchange resin. The samples were acidified with 4 mL of concentrated sulphuric acid and stored at $4^{\circ} \mathrm{C}$ in the dark.

At the time of analysis, the bottle and contents were warmed to room temperature and shaken for one hour. The supernatant was poured off and retained. The resin was poured into a 60 mL chromatography column. Excess water was drained from the column until 1 cm of the water remained above the resin. Diethyl ether ( 25 ml ) was added, and the remaining water was drained from the column and combined with the decanted water to determine the sample volume. The ether was kept in contact with the resin for 15 minutes, then eluted with two 5 mL portions of diethyl ether. The eluates were combined with 15 mL of low-boiling petroleum ether, and dehydrated with 2 g of anhydrous sodium sulphate.

The extract was reduced to 5 mL on a Rotovap and was transferred to a 10 mL volumetric flask. About 2 mL of
diazomethane in methanol was added, allowed to react for several hours and then made up to volume with methanol prior to GC analysis.
2. Sediment: Sediment samples were obtained by using clear plastic tubes with a 3.6 cm ID. In 1980, scuba divers took core samples with a 10 cm tube, collecting 4 cm long cores. In 1981, long core tubes were used from the mobile bridges to retrieve the sediment samples. The samples were kept on ice until returned to the laboratory, where they were frozen. At the time of analysis, the samples were brought to room temperature and shaken with 50 mL of $0.1 \mathrm{M} \mathrm{Na}{ }_{3} \mathrm{PO}_{4}$ for one hour, then the mixture was
centrifuged for an hour and filtered. The filtrates were acidified to pH 2 with sulphuric acid. The acidified solution was extracted once with 50 mL and twice with 25 mL of diethyl ether. The combined extract was passed through a glass wool column to break the emulsion and produce a clear extract. This was treated in a similar manner to the extracts from the water samples.
3. Particulate Material: Approximately 800 mL of the pond water was filtered through a 0.45 um filter. The residue was treated in the same manner as the sediment samples, but there was no emulsion phase in the extracts and this eliminated the need for the glass wool treatment.
4.Clam Analysis: Clams were removed from the containers in the ponds at appropriate times, labelled and stored frozen. The
clams were removed from the cold, thawed, weighed and digested in 30 mL of reagent grade, concentrated HCl. The acid solution was extracted three times with 50 mL portions of pesticide grade benzene. The combined extracts were washed with 50 mL , organic free, distilled water and dehydrated with anhydrous sodium sulphate ( 2 g ). The extract was treated as above.
5. Fish: Common shiners were collected from the ponds using bait traps and fly fishing techniques. They were killed, wrapped in aluminum foil and labelled, then put on ice. On returning to the laboratories, they were stored frozen. Prior to analysis, the fish were thawed, weighed, measured and dissected. In particular, the liver, gonads, kidneys and spleen were separated from the main body tissue. The fish were treated in the same manner as the clams. With larger fish, the bodies were dissolved in 100 mL of of HCl , and the acid was extracted three times with 100 mL of benzene.
6. Milfoil: Apical tips and whole plants were collected and stored at $-20^{\circ} \mathrm{C}$. Once thawed, the plant or tip was weighed, placed in a Waring blender with 50 mL of solvent mixture (benzene - methanol, 60:40) and stirred for about 5 minutes. The contents were Soxhlet extracted with an additional 100 mL of the solvent mixture for 16 hours. The extract was reduced to 50 mL on a Rotovap, and washed with three 50 mL aliquots of 0.5 M NaOH . The aqueous phase was acidified to pH 2 with sulphuric acid and extracted with $3 \times 50 \mathrm{~mL}$ diethyl ether. The extract was handled in
the same way as the water extracts. Runs of this method with known amounts of 2,4-D, in the absence of milfoil, gave quantitative recoveries, indicating no loss of the chemical during the lengthy extraction prociess. The extraction efficiency from milfoil was then tested by a radiotracer method.

Five apical tips of milfoil were placed in 800 mL water containing 10 ucuries of $c^{14}$ - labelled 2,4-D. After five hours, the tips were extracted by the above method, the plant material was then burned in a Biological Material oxidizer. The evolving $\mathrm{CO}_{2}$ was trapped in a $\mathrm{K}_{2} \mathrm{CO}_{3}$ solution which was counted on a scintillation counter. The extraction efficiency, calculated from these counts, was about 848 (Table 4).
7. Instrumentation: Three different gas chromatographs were used during this study, all equipped with $\mathrm{Ni}^{63}$ electron capture detectors. Initially a Perkin-Elmer model 900B gas chromatograph was used with a $15 \mathrm{~m} \times 0.05 \mathrm{~cm}$ ov-225 support-coated-open-tubular column. Nitrogen was the carrier gas ( $15 \mathrm{~cm}^{3} / \mathrm{min}$. ) with argon/methane ( $95 / 5$ ) used as the make-up ( $50 \mathrm{~cm}^{3} / \mathrm{min}$. ). The initial temperature was $120^{\circ} \mathrm{C}$, and the oven was programmed to increase $12^{\circ} \mathrm{C} / \mathrm{min}$. until it reached $190^{\circ}$ which was held for 15 minutes. The injector and the detector were held at $260^{\circ} \mathrm{C}$ and $230^{\circ}$. respectively. The minimum detectable amounts were about 0.1 ng with the injection error being 28. All 1980 water and sediment samples were analysed with this instrument.

The second instrument was the Perkin-Elmer Sigma II gas Chromatograph with S.E. 30 J\&W fused silica capillary columns ( 30 m $x 0.2 \mathrm{~mm}$ I.D.). This machine was equipped with a split-splitiess injector. The injection volume was 1 uL. After 0.7 minutes the sample was split 60/1. The initial temperature was $55^{\circ} \mathrm{C}$ and this was maintained for 0.7 minutes followed by an increase at $30^{\circ} \mathrm{C} / \mathrm{min}$ until $90^{\circ} \mathrm{C}$ was reached. This was maintained for 14 minutes, then increased at $6^{\circ} \mathrm{C} / \mathrm{min}$. until the oven reached $260^{\circ} \mathrm{C}$ then the instrument returned to the initial starting conditions. The column flow was $1 \mathrm{ml} / \mathrm{min}$ hydrogen and $60 \mathrm{~mL} / \mathrm{min}$ make-up gas (argon-methane, 95/5). The injector temperature was $260^{\circ} \mathrm{C}$ and the E.C. detector was kept at $350^{\circ} \mathrm{C}$. This instrument was used to analyse the 1981 sediment samples, a few water samples and clam tissue.

Finally a Perkin-Elmer 2000 gas chromatograph was used equipped with a P.E AS-IOOB autosampler and a Durabond 5 J\&W 30m $x$ O.2mm I.D. capillary column. The initial temperature was $55^{\circ} \mathrm{C}$ which was held for 0.7 minutes after which time the sample was automatically split 60:1. The temperature increased by $30^{\circ} \mathrm{C} / \mathrm{min}$ until $90^{\circ} \mathrm{C}$ which was held for 14 minutes. Then the temperature was increased by $3^{\circ} \mathrm{C} / \mathrm{min}$. to $165^{\circ} \mathrm{C}$, when the program rate was changed to $30^{\circ} \mathrm{C} / \mathrm{min}$ until the temperature reached $280{ }^{\circ} \mathrm{C}$ which was held for 5 minutes before the automatic cooling cycle began. The injector was held at 280 ${ }^{\circ} \mathrm{C}$ and the E.C. detector at $300^{\circ} \mathrm{C}$. The column flow rates
were identical to those used for the Sigma II. This instrument was used for the fish and plant samples.

Results

1. Water Column

The concentrations of $2,4-\mathrm{D}$ in the water column are shown in Fig 18. The initial variation between the two amine curves arises from the different mode of introduction of the chemical. In Pond 2, the amine was sprayed on the water surface and the chemical had to pass through the air-water interface before it entered the water column. The chemical was introduced under the surface in pond 5, where it rapidly dispersed. The concentration curves for the ester exhibited two distinct patterns. After an initial maximum at day 16 the concentrations slowly decreased. After such initial variations, the 2,4-D concentrations followed similar patterns in all treated ponds.

As shown in Fig.19, the concentrations were found to follow first order kinetics with both formulations. The first few points from the amine pond were not considered in the kinetic analysis as the 2,4-D was not homogeneously distributed in the water column over the first few days following treatment. In the ester ponds, the rate constants were calculated for the data after the initial peak of $2,4-\mathrm{D}$ concentrations. The rate constant for the disappearance of the amine was 0.039 day-1 with a correlation
coefficient of 0.98. The corresponding values for the ester were $0.041 \mathrm{day}^{-1}$ and 0.99 , respectively. The half lives for the disappearance of 2,4-D from the water, calculated from these rate constants, were 18 and 17 days, respectively. Detectable concentrations were observed for the first 200 days.

2,4-dichlorophenol (2,4-DCP) was found in minor amounts in both formulations. The ester formulation contained 0.18\% 2,4-DCP, while the amine contained 5\%. A nominal 1 ppm 2,4-D concentration in the amine pond may therefore be accompanied a 2,4 -DCP concentration of 0.05 ppm . In amine Pond 5, the initial concentration was 0.1 ppm one hour after treatment (Fig. 20). This is in line with the observed initial 2.4-D concentrations of about 1.5 ppm , arising from incomplete mixing of the chemical in the water column. The initial results for amine pond 2 show a gradual increase of $2,4-$ DCP as a result of the surface application. However, after 24 hours, the concentrations were similar in both amine treated ponds. In the ester treated ponds there were low but measureable amounts of 2,4 -DCP in the water column. The concentrations of 2,4-DCP were variable and did not follow the smooth decay of the parent 2,4-D. The general trends for the increases and decreases are the same in all ponds and seem to follow the secchi depth values shown in Fig.l7, implicating suspension and settling of particulates as possible factors influencing the concentration of $2,4-D C P$ in the water column.
treated with 2,4-D, one with the N, N-dimethylamine and the other with the butoxyethyl ester. The 2,4-D concentrations in the water column are depicted in Fig. 21. The analyses of the first 48 hours are shown on the left hand side of the figure. The amine, released under the water surface, quickly reached a maximum concentration, whereas that of the ester formulation increased slowly. After the initial variation, first order kinetics produced similar rates of disappearance for the two formulations, with the rate constants of 0.0198 and $0.0196 \mathrm{day}^{-1}$, for the amine and the ester, respectively. The half life calculated for the disappearance of $2,4-\mathrm{D}$ from the water was 35 days. For the duration of the experiment, i.e. 120 days after treatment, 2,4-D concentrations remained at detectable levels.

The concentrations of $2,4-D C P$ in the water column are shown in Fig. 22. 2,4-DCP was detected for six weeks after treatment. Initial concentrations in the amine treated pond were very much lower than anticipated as the initial formulation had a $5 \%$ impurity of the phenol. The maximum concentrations were determined almost a week after treatment, when a level of 0.2 ppm was detected. However, this was a single observation.
2. Sediment

The concentrations of $2,4-\mathrm{D}$ in the sediment are depicted in Fig. 23. The points on the figure represent averages from four to six individual samples. Very few sets of samples from a given
pond had measureable amounts of the chemical in all samples indicating a patchy distribution of 2,4-D.

During the first thirty days after treatment, no 2,4-D was recovered from the sediment in the amine ponds, but there were measureable amounts in the ester ponds. As the ester ponds were dosed with pellets containing the $2,4-D$, the samples taken near $a$ pellet would result in a high value. About seven weeks after addition, some 2,4-D was detected in the sediment of all ponds and this decreased to near-detectable levels by day 82. Subsequently, there was a slight increase in all ponds.

The results of the sediment analyses are illustrated in Fig. 24, showing that after the initial variations, 2,4-DCP concentrations followed a similar pattern in all treated ponds. Measureable quantities of the 2,4 -DCP were observed in the sediment up to 145 days following treatment.

The concentrations of $2,4-D$ detected in the sediment during the 1981 study are shown in Fig.25. Initially those points for the ester treatment were slightly higher than those for the amine but both decreased to about $0.001 \mathrm{mg} / 10 \mathrm{~cm}^{2}$ within 42 days following treatment and continued to diminish.

Fig. 26 illustrates the concentrations of the phenol during the experiment. While $2,4-D C P$ was observed in the ester pond sediment on all but one of the sampling dates, it was undetected
in the amine pond during most of the study period.

## 3. Clams

Table 5 lists the 2,4-DCP and 2,4-D concentrations measured in clam tissues in the 1980 and 1981 field tests. Preliminary experiments with laboratory raised clams showed there were no interfering peaks present at the retention times that 2,4-D and 2,4-DCP were eluted on the gas chromatograph.

The initial clam tissue analyses (June 23), showed appreciable concentrations of 2.4-D and measurable amounts of 2,4-DCP in the clams from four of the ponds. The clams had been collected from a natural pond near residential dwellings and weed control agents used on the lawns may have contaminated them. However, the 2,4-D concentrations in the treated pond clams were higher than in the clams from the control ponds, throughout the experiment. The same trend was observed in the 2,4-DCP concentrations. The highest 2.4-D concentrations were found in the clams at day 55 after treatment. The phenol concentrations were variable, with at least one clam showing nondetectable 2,4-DCP in each pond. To ensure that the method was measuring 2,4-D and 2,4-DCP, a number of samples, denoted by asterisks, were spiked with the acid and phenol for subsequent analysis.

After the trouble encountered in 1980, the clams for the 1981 test were collected from an isolated area of the same natural pond
which had no chance of 2,4-D contamination prior to treatment. The clams were found to be free from both 2.4-D and 2.4-DCP prior to treatment. After treatment, both 2,4-D and 2,4-DCP were detected in the clams (Table 6). Samples taken five days after treatment contained the highest amounts of 2,4-D. The phenol was detected in only three clam samples, one from each pond, five days after treatment, with an additional positive value in the amine pond. Many of these samples were spiked with the chemical to confirm the chromatographic peaks.
4. Fish

Table 7 lists the concentrations of 2,4-D and 2,4-DCP in ug/g tissue of twenty-two complete fish samples, collected ten months after the 1980 treatment. Generally, most samples had 2,4-D in the body tissue which is that part of the fish exclusive of kidneys, gonads, liver, spleen and stomach. Least material was found in the kidneys, liver and spleen. In general, the fish from the treated ponds contained more of the chemical than those from the controls.

## 5. Milfoil

Results from the extraction of the milfoil are listed in Table 8. plants taken from the control ponds exhibited no detectable concentrations of 2,4-D until after day 41. In the treated pond, the plants showed the highest concentrations soon
after treatment. Plants collected on days 41 and 57 were removed from the sediment where they had collapsed earlier. Comparison of plant tips and mid sections indicated generally more 2,4-D in the former. 2,4-DCP was not detected in any of the plants. As the extraction recoveries were about $84 \%$, the reported concentrations are minimum values.

The 1981 milfoil analyses are shown in Table 9. plants collected from the ester pond (a 1980 control), prior to treatment, contained considerable 2,4-D. Since the plants appeared to be healthy, the high value may be considered an anomaly. The plants in the amine pond had no detectable amounts of 2,4-D before treatment but measureable amounts after the treatment. Again, no 2,4-DCP was detected in any plant tissue.

## 5. Particulates

Analysis of suspended particulate matter during the 1980 study found small amounts of 2,4-DCP, at the detection level of the analytical method.

Discussion

In both 1980 and 1981, the disappearance of the 2,4-D in the water column described a smooth curve after the initial mixing of the amine and the dissolution from the clay pellets of the ester. The rate constants for 2,4-D disappearance are consistent with
photochemical decomposition (15) rather than bacterial action.

Although the disappearance of 2.4-D in both years followed first-order kinetics, the rate constants were different in the two years of the study. The only water quality parameter consistently different in the two years was pH , averaging 7.3 in 1980 and 10.0 in 1981. The photochemical decomposition of the chemical may have thus been pH dependent $(16,17)$.

The parent compound, 2,4-D, was found in the various compartments of the system, which included the sediment, fish, clams and the plants. No detectable amounts of $2,4-\mathrm{D}$ were found initially in the sediment of the amine treated ponds in 1980, but there was a maximum about day 55 after treatment. This probably resulted from the decomposition of the milfoil and release of the 2,4-D into the sediment. The ester ponds' sediments in 1980 exhibited measureable concentrations of 2,4-D immediately after treatment undoubtedy resulting from the pellets on the sediment. These values gradually decreased, then increased again to concentrations similar to those in the amine ponds about day 55 , again, probably due to the collapse of the milfoil. In 1981, the sediment concentrations of 2,4-D were about a tenth of those found in 1980, and diminished to trace levels by day 42. During most of the experimental period in both years, the estimated amount of 2,4-D in the sediment was approximately 18 of the total $2,4-D$ in the system.

Suspended particulates, sampled during the first month after treatment, contained traces of both 2,4-D and 2,4-DCP. The settling of the particulate material is one mode of transport of the chemical to the sediment. Adsorption of 2,4-DCP on particulates may be the explanation for the observed correlation between 2,4-DCP concentrations in the water column and secchi depth.

The $2,4-$ DCP concentrations are a result both of impurities in the formulations and 2,4-D degradation(15). Measureable quantities of $2,4-$ DCP were found in all samples of the water column in the 1980 field season but only in a few water samples in 1981. This difference may be ascribed to the increasing pond pH during the two years (7.3 to 10). The $\mathrm{pK}_{\mathrm{a}}$ of 2,4-DCP is 7.3 (18), therefore at pH 7.3 the dominant form of the phenol is the undissociated molecule, while at pH 10 , the phenolate ion predominates. As the latter is more susceptible to photodecomposition, the lower DCP concentrations in 1981 would not be unexpected.

Concentrations of the 2,4-DCP in the sediment were an order of magnitude lower than those of the parent 2,4-D. In 1980, the maxima of $2,4-$ DCP after day 20 correspond to those exhibited by the 2,4-D. This indicates that the 2,4- DCP came from the same source as the 2,4-D or was derived from the 2,4-D. The common source of $2,4-\mathrm{D}$ in all ponds was the milfoil. Small quantities of the 2,4-D, picked up by the plants, may be released into the
sediment from the decomposing plants.

Other life forms, namely the clams and the fish, took up some of the 2,4-D and 2,4-DCP. The 2,4-D was detected in their tissues throughout the study period in both treated and untreated ponds, but the concentrations were higher in the former.

## TOXICITY STUDIES

Laboratory Study

Young Sphaerium rhomboideum were collected from a permanent pond ( $43^{\circ} 29^{\prime} 40^{\prime \prime} \mathrm{N}, 80^{\circ} 09^{\prime} 36^{\prime \prime} \mathrm{W}$ ) near Guelph, ontario and maintained for at least one month for acclimatization in pond water and sediment at $17^{\circ} \mathrm{C}$ and a sixteen hour photoperiod. Prior to the test, clams were placed in water-filled glass dishes at $17^{\circ} \mathrm{C}$ for twenty-four hours. This test water was $50 \%$ raw well water and $50 \%$ deionized water (total hardness $195 \mathrm{mg} / \mathrm{L}$ as $\mathrm{CaCO}_{3}$ and alkalinity of $140 \mathrm{mg} / \mathrm{L}$ as $\mathrm{CaCO}_{3}$ ) with a final pH of 7.9. Five 22.7 L tanks were used for the tests, one as a control containing only water, another containing test-water and methanol which was used as a solvent for the 2,4-D and 2,4-DCP and the test tanks. Known concentrations of stock solutions (Eastman-Kodak White Label 2,4-D or 2,4-DCP in methanol) were added to the tanks. Ten clams were placed in each tank thirty minutes after addition of the chemical. Clams were removed at definite intervals after treatment (3, 6 18 and 24 hours, then twice daily until the end of the test period of 120 hours), and examined for mortality with the survivors put back into the test tank. Signs of life were taken to be gaping valves, the animal actively moving or responding to gentle prodding, or an observable heartbeat. In doubtful cases, the animal was left in the dish for confirmation in the next observation period. The the survivors after the $24-\mathrm{hr}$ test were transferred into containers with clean
pond water and sediment, and delayed mortality was checked one week later.

In the test with $2,4-\mathrm{D}$, the pH was not controlled, but the tests with the phenol were conducted at pH 6.8 and 8.8 to compare the non-dissociated phenol and the phenolate ion, respectively. The pH was adjusted every 24 hours with $\mathrm{H}_{2} \mathrm{SO}_{4}$ or NaOH solutions. The 2,4-D and 2,4-DCP concentrations of the tank solutions were determined before and after the test.

> An $L_{50}$ was calculated each time the animals were examined using a probit analysis calculation (Institute of Computer Sciences, University of Guelph). Toxicity curves were constructed using the results of the computer analysis or estimated from a graph if the probit analysis could not be used since there were not at least two kills of the test animals.
2.4-D concentrations of up to $250 \mathrm{mg} / \mathrm{L}$ were not lethal to s . rhomboideum after 96 hours exposure. No delayed mortality was observed for two weeks after the test clams were transferred to uncontaminated growth dishes.
2.4-DCP was more toxic. Figures 27 and 28 show the toxicity curves for this chemical at pH 6.8 and pH 8.8. The $96 \mathrm{hr} \mathrm{LC}_{50}$ was $24.3 \mathrm{mg} / \mathrm{L}$ at pH 6.8 , and $65.9 \mathrm{mg} / \mathrm{L}$ at pH 8.8 . This indicates that the phenolate ion, existing at the higher pH , is significantly less toxic than the undissociated phenol.

The toxicity curves show no threshold concentrations of the toxicant, implying that even low 2,4-DCP concentrations could cause some clam mortality.

Field study

In 1980, white sucker fry (Catostomus cornutus), with a maximum length of 6 cm , were used in a short term toxicity study. Ten live fish were placed in each of 24 cylindrical mesh-covered tubes. Four of these containers were placed at mid-depth in each pond. One day prior to and up to one week after treatment, the fingerlings were replaced every twenty-four hours and the live fish remaining in the containers were counted.

In the 1981 field season, a minimum of two containers were used in each treated pond and in one of the controls (pond 3). Occasionally, fish were placed in the other control pond as well. Animals in the area often raided the fish containers.

The results for the 1980 series are presented as control corrected data, where values from the control pond are subtracted from those in the treated ponds. Figure 29 illustrates the toxicity to the fingerlings in 1980. After the first two days of the experiment, the observed mortalities gradually decreased. No significant mortality was observed one week after the treatment. These results did not correspond with the measured 2,4-D
concentrations in the water. The toxic agent was probably the 2,4-DCP, as in the laboratory clam study. In the 1981 experiment, variable mortalities were observed in the control ponds, but no significant mortality could be ascribed to the chemical treatment.

The 1980 mortalities did not appear to be related to 2,4-D concentrations in the ponds, and were ascribed to the presence of 2,4-DCP. A probable cause of the lower fish mortality in 1981 was the higher pond pH and the predominance of the phenolate form of 2,4-DCP in that year.

## BACTERIA

## Experimental

Samples for microbial densities were taken from the composite water sample and were placed in l-L sterile bottles on ice until they were returned to the laboratory. The samples were then tested for the following microbiological parameters (19):
a) heterotroph counts reported as colony forming units (CFU), estimated by spread plate procedures using a seven day incubation period at $20^{\circ} \mathrm{C}$;
b) epi-fluoresence microbial biomass estimates;
c) organic sulphur reducing bacteria densities determined by Most Probable Number (MPN) procedures; and
d) sulphur oxidizing bacteria densities determined by MPN procedures.

The sediment samples were stored on ice in sterile lock-top plastic bags. They were homogenized in a blender and 10 grams of the material was removed, and analysed for heterotrophs, organic sulphur reducing and sulphur oxidizing bacterial populations as described above.

## Results

Accurate determination of microbial populations or biomass is difficult. One of the more reliable techniques is the direct counting of bacteria using epi-fluorescence techniques. Bacterial water column data obtained with this technique are shown in Fig. 30, with the data plotted as the mean for each type of pond treatment. Total microbial populations were very similar in all ponds before and after $2,4-\mathrm{D}$ additions, varying from $1.6 \times 10^{5}$ to $4.5 \times 10^{6}$. From July 1, 1980, the total microbial populations are shown as a series of maxima and minima, with the treated ponds having the lowest minima, and the ester ponds exhibiting the greatest maxima. However, during the first seven days after treatment, the amine treated ponds had the lowest microbial densities. Total biomass as estimated by ATP measurements is shown in Fig. 31. These data indicate that for the period, from two days to approximately twenty-five days following treatment, the amine-treated ponds showed a definite increase in ATP content while the other ponds showed a decrease. After this twenty-two day period, all ponds showed similar ATP levels and patterns.

Plating and MPN procedures allow for the examination of components of the bacterial community. Heterotrophic bacterial densities in the water column are displayed in Fig. 32. No trends are apparent prior to treatment but from two weeks to sixty-nine days after treatment, the ester-treated ponds had the highest
densities. Sulphur oxidizing bacteria bacterial densities are illustrated in Fig. 33. No trends were evident before treatment. After treatment, the ester ponds generally showed higher densities than the controls. The amine pond densities were equal or greater than those of the controls but lower than those of the ester ponds. Organic sulphur reducing bacterial densities, shown in Fig. 34, were usually highest in the ester ponds. Control pond and amine pond densities were similar up to day 56 when all treated ponds had populations of over 1000 times greater than the control ponds. After this time, the treated ponds generally exhibited greater densities than the controls.

Mean heterotrophic bacterial densities, organic sulphur oxidizers and sulphur reducing bacteria densities recovered from sediment samples are shown in Figs. 35,36 and 37 , respectively. The heterotrophic data indicate there was little or no difference between the control and treated ponds. Organic sulphur reducing bacteria populations in the sediment were not significantly affected by the addition of 2,4-D. From day 7 to day 56 all ponds exhibited similar trends with the ester ponds having the highest populations, and the control ponds, the lowest. After day 56, there was an inconcert decrease of 1.5 log units in the amine and control pond densities, while the ester pond sediments maintained the high sulphur oxidizing populations until day 112. By day 147 . these populations decreased to levels found in the other pond sediments.

Water column and sediment fungal populations were quantified using membrane filtration and spread plate techniques, respectively. No consistent inter-pond differences were detected in water column fungal levels in the pre-treatment period. After the treatment, a short term ( 1 to 55 days) depression of geoaquatic fungal levels was observed in all treated ponds. Preliminary analysis of the data failed to reveal significant differences between any of the duplicate ponds before or after the 2,4-D application. Other mycological parameters, such as total yeasts, pink yeasts, or non-pink yeasts, showed no significant treatment effects. The limited data base did not reveal any treatment effect on sediment fungal levels. A complete account of the mycological study of the ponds is in preparation and will be presented as a separate report.

## PHYTOPLANKTON

Methods

Phytoplankton samples were collected using a 2-L clear plexiglass van Dorn sampler. For a representative sampling of the water column, 4, 3 and 2 liters of water were taken from the top, middle and bottom layers, respectively. They were stirred in a pail and a l-L aliquot was placed in a clear wide-mouth glass bottle containing 5 mL of modified Lugol's solution. The bottle was stored in the dark; at $4^{\circ} \mathrm{C}$. In the two treatment years, different methods of analysis were used. The work in 1980 formed the basis of a thesis at Brock University (20). In short, each sample was allowed to settle for a minimum of one week, after which time 5 mL was placed in an Utermohl counting chamber. A phase-contrast Leitz "diavert" inverted microscope was used with Nemarsky interference optics. Counting of the algae was performed at 500X magnification until at least 150 individuals were counted in three replicate samples. Standard techniques were used to calculate diversity indices (21).

In the 1981 field season, the algal concentrate was placed in a Sedgewick - Rafter counting chamber and a light microscope was used at 500X magnification. Again at least 150 individuals were counted in replicate samples. The biomass was estimated using the cell counts, an assumed cell density of 1.0 , and the cell volumes determined by other investigators (22,23). The average
value for the control pond, for a given sampling date, was subtracted from the treated ponds' values to give the control-corrected values.

Results

The 1980 work is reported in the Brock University thesis (20). Figure 38 shows the total algal populations in the ponds during 1980. No impact by the 2,4-D treatments could be discerned. The diversity indices of the phytoplankton populations were calculated using the Shannon-Weaver treatment. The analysis of variance of these indices indicate that the species diversity was not affected by the pond treatment.

The 1981 phytoplankton biomasses are shown in Figure 39. Once again, there was no apparent effect by the treatment. Although some of the species in the treated ponds showed some positive and negative variation from the control, both before and after treatment, no blooms were observed either in terms of total cell counts or dominance by a given species.

## (a) PROTOZOOPLANKTON

## Methods

Protozoan samples were removed from the composite sample used for water chemistry and phytoplankton analysis. This aliquot was placed in a $1-L$ bottle containing 5 mL of modified Lugol's solution, then stored in the dark at $4^{\circ} \mathrm{C}$. For the analysis, the bottle was shaken and a known volume was placed in a 50 mL Utermohl settling chamber placed over a counting chamber. This was left to settle for at least 24 hours. The counting chamber was then examined at 200X magnification with a Nikon phase contrast inverted microscope. Over 200 individuals were counted in each sample at the usual densities. In samples with a large number of small flagellates, extra transects were made at 400 x magnification. Occasionally, replicate samples were taken.

For each sample, the first ten specimens of each taxon were measured for the volume calculation. The volumes were pooled across ponds and sampling dates to calculate a mean for each taxon, which was then used to calculate the biomass, assuming a density of $1 \mathrm{~g} / \mathrm{cm}^{3}$. Identifications were made mostly to genus, and were based on a variety of sources, the most important being found in reference 31.

Results

The similarity between replicates (Table-10) and casual evaluation of the cross correlation between the ponds as well as the autocorrelation within the ponds, suggests that the time trends shown by the data are real, and not artifacts of sampling a spatially heterogeneous system.

The total ciliate populations over the time of the experiment are shown in Fig. 40. Very little, if any, effect can be discerned for the addition of the 2,4-D. The lowest portion of the figure represents the total ciliate populations about the time of treatment in 1980. A comparison of the values for the treated and the control ponds indicates no major initial effect on the ciliates. The total ciliate biomass also indicates that 2,4-D treatment did not affect total ciliate communities (Fig. 41). The biomasses of planktonic amoebae and zooflagellates, minor contributors to the total protozoan biomass, did not appear to be affected by the 2,4-D treatments.
since the total ciliate population was not influenced by the chemical, the major taxa in the ciliate community were examined. The populations of Urotricha, Halteria, Strobilidium, Strombidium, and Askenasia were not affected by the treatment. The populations of four major taxa were examined immediately before and after the treatment date. No apparent affect of the treatment could be observed.
(b) MESOZOOPLANKTON

Rotifers and crustaceans were the two major contributors to the mesozooplankton commnity. Their total biomass did not appear to be affected by the treatment as illustrated for the ester ponds in Fig.42. Examination of the populations immediately before and after treatment showed no affect. The relative abundance of rotifers and crustaceans was also unaffected by the treatment. The dominant species of the rotifer community varied through the year, but usually Keratella cochlearis was the most abundant. The crustacea Daphnia galeata and Bosmina longirostris were generally present in all samples.

## ZOOBENTHOS

The methods and results are given in detail elsewhere (24). In both 1980 and 1981 the growth of Pisidiid clams (Sphaerium rhomboideum) was inhibited immediately after 2,4-D treatment. Growth resumed after 7-13 days in 1980 and $20-27$ days in 1981. Subsequently, growth and survival in the treated ponds was greater than in the control ponds. The initial growth inhibition was tentatively attributed to a combination of the toxic effect of metabolically produced 2,4-DCP, and the metabolic cost of avoiding, excreting, or metabolizing 2,4-D and 2,4-DCP. Subsequent growth and survival of the clams was attributed to the increased supply of detritus and periphyton caused by the collapse
and decay of the milfoil beds. Clams reproduced successfully in all ponds. Examination of brood sacs produced on their gills showed no changes in the rate of formation or development of larvae.

The natural macro-benthic community was examined routinely in 1980 and 1981. No direct effects of the treatment were found. Secondary effects of the treatment were observed over a period of months. By day 388 after the 1980 treatment, cluster analysis of taxonomic presence-absence data showed that pond communities could be grouped according to the treatments. Ecological Community Analysis (25) indicated a shift from chironomid dominated communities in the controls to tubificid oligochaete dominated communities in the treated ponds.

## SNAILS

## Experimental

On September 19.1980, a Scuba diver counted the number of snails within a rectangular template measuring $26 \times 13 \mathrm{~cm}$. The template was placed on at least nine separate positions on the pond sediment, including the sloping sides. Only those snails that appeared to be alive, based on their appearance and colour, were counted.

Results

The snail counts in Table 11 were taken to quantify visual observations made during sampling. Snails were more abundant on the bottom sediments and sides of the treated ponds, with the numbers in the amine ponds being the highest. Since the snails could still be on the milfoil in the control ponds, individual plants were examined for snails, but only one or two were observed in each quadrant. The 2,4-D addition thus appears to have indirectiy stimulated the snail population through the presence of decaying milfoil. The lower populations in the ester ponds could be caused by high localized 2,4-D concentrations on the sediment produced by sunken pellets of the formulation.

## MACROPHYTES

## Experimental

Milfoil growth was determined by measuring the length of all stems and branches of the five designated plants. The "total" stem length was the average of the measurements of the five plants. Occasionally, the health of the plants was monitored by removing small fragments of the plants for net $\mathrm{CO}_{2}$ uptake measurements.

Recolonization by milfoil was attempted on August 19 and September 18, 1980. On August 19, apical tips from the control ponds were planted into the sediment in each pond, including the controls. On September 18, apical tips were planted in the sediment, suspended above the sediment, and planted in pots of clean sediment, in all ponds to separate $2,4-\mathrm{D}$ effects in the sediment or the water column.

Macrophyte $\mathrm{CO}_{2}$ uptake was measured in a infrared gas analyser (Series 225, Analytical Development Co., Ltd., Hoddeson, England) at $20^{\circ} \mathrm{C}$, and a light intensity of $400 \mathrm{uE} / \mathrm{m}^{2} / \mathrm{sec}$. $\mathrm{CO}_{2}$ uptake was measured and expressed relative to the dry weight.

At the time of treatment, the milfoil was established in all ponds. The plantings of September of 1979 had grown rapidly during the following May and June so that the weed bed was essentially uniform and equally spaced by late June. High turbidity in the ponds contributed to a low biomass of plants (10-50 g D.W.m ${ }^{-2}$ ) compared to that of a normal weed bed (100-300 g D.W.m ${ }^{-2}$ ). Fig. 43 illustrates the mean total stem length of the five center row plants in each pond. Prior to treatment, the mean total stem lengths ranged between 2.10 and 4.00 meters. The decreases recorded in two of the treated ponds before application were the result of fragmentation, a natural phenomenon associated with the propagation of the plants. July and August values for the plants in the control ponds show that the plants had stabilized, neither increasing nor decreasing their mean total stem lengths. Fourteen days after application, the treated plants were on the bottom and any disturbance resulted in disintegration of the stem.

Carbon dioxide uptake of apical stems is plotted against time in Fig. 44. In mid-June, there was a period of maximum uptake exhibited by all tips investigated. By day 16 after treatment, none of the plants in the treated ponds exhibited photosynthetic activity and were considered dead. plants in the control ponds had approximately constant levels of carbon dioxide uptake during this period.

Elodea canadensis, Potamogeton crispus, P. pectinatus, P. foliosus, Chara and Typha were minor contributors to the macrophyte flora in all ponds. After treatment, the Elodea and Potamogeton ssp. thrived in the control ponds. In the treated ponds, these macrophytes were inhibited with only P. crispus present in small amounts. In the absence of the milfoil, Chara formed large macrophyte beds in all the treated ponds, covering most of the pond bottoms. Chara was also present in the control ponds but to a lesser extent. Typha grew on the moist soil surrounding the ponds and spread into the water. The growth of Typha was stunted in the treated ponds. Young plants attempting to grow at or below the water line were not successful during the first 97 days after treatment. After this time, young Typha were observed growing in the shallows of the amine ponds.

Apical stems of the milfoil introduced into all treated pond sediments on August 19, 1980 (day 55) showed symptoms of $2,4-\mathrm{D}$ toxicity one week later. These included splayed-back leaves, white nodes which eventually turned brown, and dwarfed, stunted growth. Within two weeks after introduction, these apical stems were dead. The plants transplanted back into the control ponds on August 19 did well. The concentrations of $2,4-\mathrm{D}$ in the water column on August 19 were 0.7 ppm in the ester ponds and 0.25 ppm in the amine ponds.

The next transplant experiment was carried out on September 19, 1980, or 86 days after treatment. In the ester ponds, the
average concentration of the 2.4-D was about 0.1 ppm and in the amine ponds about 0.13 ppm . In this experiment, five apical stems were placed in the uncontaminated soil contained in porous clam chambers, to allow movement of the $2,4-\mathrm{D}$ between contaminated and uncontaminated soil. Five stems were placed in the contaminated soil adjacent to the chambers, and five stems were suspended in the water column above the chambers. Seven days after transplanting, all apical stems in the treated ponds had white nodes 1 or 2 cm from the tips. All plants had survived six weeks after introduction. However, fused leaves on new growth developed on the "rooted" apical tips in all treated ponds. The floating tips in one ester pond, with 2,4-D concentrations below 0.1 ppm , had normal growth. In the other ester pond, with over 0.1 ppm 2,4-D, no regrowth occurred. The rooted plants in and outside the "clean sediment" chamber in the first pond developed fused leaves indicating that 2,4-D travelled to the clean sediment and was translocated to the tip. The floating stems and potted plants in the amine ponds ( 0.1 ppm ) both exhibited fused leaves on the new growth. These observations suggest that concentrations of 0.1 ppm 2,4-D in the water can affect the growth of the target organism.

The effect of low concentrations of added material in the water colum and its effect was exhibited shortly after the original treatment. While one of the amine ponds was being sprayed, some of the vapours were blown over one of the control ponds. A water sample taken one hour after treatment showed there
was 0.075 ppm of $2,4-$ DCP in a near-surface sample from this control pond, but 2,4-DCP was undetectable by the four hour sampling time. One week later, while the divers were measuring stem lengths, a slight degree of fused leaf syndrome was observed on the milfoil in this pond but not in the other control.

By the time of the 1981 treatment the macrophyte communities of the previously treated ponds consisted of Elodea, Chara and potomageton. In the 1980 control ponds, the dominant macrophyte was M. spicatum but Potomageton crispus, Elodea, Chara and Typha were also present. In 1981, the M. spicatum biomass was ten times greater than in the previous year. These were mature plants which exhibited fruiting bodies in late June.

The milfoil succumbed to the 2,4-D treatment in about 15 days. Cessation of $\mathrm{CO}_{2}$ uptake was similar to the 1980 Observations. During the collapse of the milfoil bed, the leaves and stems of the plants were covered with epiphytic growth.

This report deals with two main subjects: the effectiveness and fate of the herbicide 2.4-D in a pond ecosystem, and the effects of the herbicide treatment on various components of the biota. Included are the results of laboratory and field toxicity studies, and a general statement of the usefulness of pond ecosystems in such studies.

Both herbicide formulations killed the milfoil in 2 to 3 weeks at nominal concentrations of 1 ppm in the water. Actual concentrations in the water column were significantly below 1 ppm during most of the treatment period. Transplant experiments indicated that 2,4-D concentrations above 0.1 ppm were effective in milfoil control confirming reported laboratory results (26). The nominal concentration recommended for milfoil control, ie. 1 ppm, is 10 times higher than the effective concentration , 0.1 ppm, to account for drift and dilution in natural water bodies.

The main difference between the two formulations was the immediate availability of 2.4-D in the water column when the amine was used compared to the slow release of 2,4 -D from the ester pellets. The spraying of the amine resulted in aerial drift of the chemical, causing sub-lethal effects on milfoil in an adjacent control pond in which 2.4-DCP was detected. Spraying of the amine under the water surface eliminated the drift and resulted in much more immediate distribution of the chemical in the water column.

The disappearance of 2,4-D in the water column followed first order kinetics with both formulations. The half-lifes for the two years varied between 17 and 35 days. The 2,4-D remained detectable in the water phase for about four months after application with the concentrations remaining above recommended drinking water standards of 0.1 ppm for most of this period.

Dichlorophenol (2,4-DCP) was present in the system both as an impurity in the formulations and as a degradation product of 2.4-D. Its presence was observed in the water column for most of the study period in 1980. In the following year, probably due to the higher water pH , it was present at lower concentrations and for a shorter period.

The 2,4-D appeared in the sediments of the ester ponds immediately after treatment, and in the sediments of the amine ponds after the milfoil's collapse. The release of 2,4-D from decomposing milfoil appeared to be a significant source of 2.4-D. The chemical persisted in the sediment for about as long as it was detected in the water column. The 2,4-DCP concentrations in the sediment were generally an order of magnitude lower than that of 2,4-D and were observed for as long as the $2,4-\mathrm{D}$ was present.

Fish and clam tissues all had 2,4-D and 2,4-DCP in all ponds but the concentrations were generally higher in the individuals from the treated ponds. The milfoil samples from the control ponds
did not contain the chemical while samples from the treated ponds contained only 2.4-D.

Laboratory toxicity tests showed that $2,4-D$ was not toxic to clams at concentrations of 250 ppm. The $2,4-$ DCP, on the other hand, was toxic with a $96 \mathrm{hr} \mathrm{LC}_{50}$ of 23.4 and 65.9 ppm at pH 6.8 and pH 8.8, respectively, indicating that the phenol was more toxic than the phenolate ion. White sucker fry exhibited limited mortality during the first six days in the treated ponds. On the first day only the amine ponds showed higher mortality than the ester ponds. Adult common shiners were not affected.

The various biological compartments of the pond ecosystems showed minimal or no treatment effect. The bacteria populations were unaffected following treatment but showed some increases in the treated ponds following the collapse of the milfoil beds. The phytoplankton abundance or species diversity were unaffected. The zooplankton populations were similarily unaffected. The zoobenthos were not directly affected by the treatment but a community shift occurred from chironomid to tubificid dominanted communities after the collapse of the milfoil beds. The number of snails were observed to increase as a secondary effect, ie after the milfoil collapse. The growth of clams was inhibited in the treated ponds during the first one to three weeks after application. Their subsequent growth, on the other hand, was enhanced by the increase in food supply. No effects on the clams' reproductive capacity were observed.

The collapse of the milfoil did not result in either dissolved oxygen depletion or nutrient enrichment in the water column. Calcium concentrations and alkalinity were observed to increase once milfoil decompostion began presumably due to dissolution of calcium carbonate on the surfaces of the milfoil leaves. Young plants of cattails (Typha) did not colonize the shoreline of the treated ponds until the 2,4-D concentrations had declined.

The pond ecosystems used in this study showed remarkably little variation from pond to pond with respect to water quality parameters and most of the biological components. Phytoplankton abundances, on the other hand, had high variability which precluded the detection of subtle effects of the treatment. The one year allowed for the stablization of the ponds appeared to be sufficient for the establishment of healthy ecosystems.

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Table la
1980 and 1981 Eampling Echedule

| Date | 23/01/80 | 20/02/80 | 18/03/80 | 16/04/80 | 06/05/80 | 14/05/80 | 02/06/80 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Days co/from ereatment | -154 | -126 | -99 | -70 | -50 | $-43$ | -29 |
|  |  |  | - |  |  | - |  |
| Water Chem. 2.4-D/DCP | I | x | \% | x | \% | 2 | \% |
| gediment |  |  |  |  |  |  |  |
| Phytoplankton | z | 8 | $z$ | I | z | $\dot{\text { x }}$ |  |
| Protosoa |  |  |  | - | 8 | $\underline{ }$ | $\underline{x}$ |
| tooplankton | $\Sigma$ | $\mathbf{z}$ | x | I | $\underset{ }{2}$ | $\underline{2}$ | $\underline{8}$ |
| Bacteria | $x$ | $\mathbf{z}$ | $\mathbf{x}$ | \% | - | 2 | \% |
| Fungi | I | $\underline{2}$ | x | 2 |  | x | ${ }^{\mathbf{x}}$ |
| $\begin{aligned} & \text { Clams ( exam.) } \\ & \text { Clams (anaí.) } \end{aligned}$ |  |  |  |  |  |  |  |
| Figh exch. |  |  |  |  |  |  |  |
| Milfoil |  |  |  |  |  |  |  |
| a) 2,4 D | : |  |  |  |  |  |  |
| b) $\mathrm{CO}_{2}$ |  |  |  |  |  |  |  |
| Snail |  |  |  |  |  |  |  |
| Macrobenthos | . |  |  |  |  |  |  |
| $E_{h}$ Ehdim Resp |  |  |  |  |  |  |  |


| Date | 10/06/80 | 17/06/80 | 23/06/80* | 25/06/80 | 26/06/80 | 27/06/80 | 28/06/80 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Days to/from treatment | -16 | -8 | -2 | 0 | 1 | 2 | 3 |
| Water Chem. | x | $\mathbf{z}$ | $\mathbf{x}$ |  |  |  |  |
| $2.4-D / D C P$ |  |  | $x$ | $\Sigma$ | $x$ | $\begin{aligned} & x \\ & x \end{aligned}$ | , |
| Sediment | x |  | $\mathbf{z}$ |  |  |  |  |
| Phytoplankton | 8 | $x$ | x |  | 'X | x |  |
| Protozaa | $x$ |  | $x$ |  | x | x |  |
| zooplankton | $\pm$ | $x$ | $\mathbf{x}$ |  | z | x |  |
| Bacteria | $\underline{\square}$ | $\mathbf{x}$. | x |  | x | X |  |
| Fungi | $\mathbf{x}$ | X | $x$ |  | $\underset{\chi}{x}$ | $\underline{x}$ |  |
| Clams (exam.) <br> Clame(anal.) |  |  | $\underline{8}$ |  |  |  |  |
| Fish exch. |  |  | $\pm$ | $\pm$ | $\Sigma$ | \% | x |
| Milfoil <br> a) 2.4 -D <br> b) $\mathrm{CO}_{2}$ Snails | z |  | $\mathbf{z}$ |  | $x$ | x |  |
| Macrobenthos |  |  | $\underline{1}$ |  |  |  | $\because$ |
| SAdim. Resip. |  |  | $\dot{x}$ |  |  |  |  |



Table ib

| Da | 16/07/80 | 22/07/80 | $\begin{aligned} & 1980 \text { and } \\ & 24 / 07 / 80 \end{aligned}$ | 81 sempl 05/08/80 | $\begin{aligned} & \text { schedule } \\ & \text { 19/08/80 } \end{aligned}$ | 27/08/80 | 03/09/80 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Days to/from treatment | 21 | 27 | 29 |  |  |  |  |



| Date | 16/09/80 | 19/09/80 | 30/09/80 | 15/10/80 | 28/10/80 | 19/11/80 | 17/12/80 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Days to/from treatment$\qquad$ | 83 | 86 | $97$ | $112$ | $125$ |  |  |
|  |  |  | 97 | 112 | 125 | 147 | 175 |
| Water Chem.$2.4-\mathrm{D} / \mathrm{DCP}$ |  |  |  |  |  |  |  |
|  | $\dot{x}$ |  | $\mathbf{x}$ | z | $x$ | * | x |
|  |  |  |  | x | $\mathbf{y}$ | $\dot{8}$ | z |
| Phytoplankton | $x$ |  |  | $x$ |  | $\underline{x}$ |  |
| Protozoa | $\underline{1}$ |  |  | + | x | $x$ | $\mathbf{x}$ |
| Zooplankton | $\mathbf{x}$ | - | \% | $\underline{x}$ | ${ }^{\mathbf{x}}$ | $x$ | x |
| Bacteria | $\underline{x}$ |  | $\underline{\mathbf{z}}$ | 2 | - ${ }^{\mathbf{x}}$ | $x$ | x |
| Fungi | $x$ |  | $\underline{z}$ | $\underline{8}$ | x | ${ }^{2}$ | $x$ |
| clams ( exami.) | $\mathbf{x}$ |  | $\pm$ | $\underline{2}$ | x | x | x |
| Clams (anal.) |  |  |  |  |  |  |  |
| Fish exch. |  |  |  |  | z | x |  |
| $\begin{aligned} & \text { Milfoil } \\ & \text { a) } 2,4-\mathrm{D} \\ & \text { b) } \mathrm{CO}_{2} \end{aligned}$ |  |  |  |  |  |  |  |
| Snail ${ }^{\text {c }}$ |  | x |  |  |  |  |  |
| Macrobenthos |  |  | z |  | $x$ |  |  |
| Stim.Resp. |  | x |  |  |  | x |  |



Table le
1980 and 1981 Sampling Schedule

| Date | 08/07/81 | 10/07/81 | 15/07/81 | 19/07/81 | 22/07/81 | 29/07/81 | 05/08/81 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Days to/ficam |  |  |  |  |  |  | -3/08/81 |
| treatiment <br> Days to/from | 379 | 381 | 386 | 390 | 393 | 400 | 407 |
| 'al treatsit. | 0 | 2 | 7 | 11 | 14 | 21 | 28 |
| mater Cham. 2.4-D/DCP |  |  | - $\mathbf{x}$ |  | \% | z | $\dot{\text { x }}$ |
| Pediment | z |  | $\pm$ | $\Sigma$ | $\underline{x}$ | $\boldsymbol{x}$ | I |
| Protozoa |  |  | E |  | $\pm$ | $x$ | 8 |
| Sopplankton |  |  | 8 |  | $\underline{8}$ | x | I |
| Bacteria <br> Fungi |  |  | E |  | x | $\mathbf{x}$ | $\dot{\mathbf{x}}$ |
| Clams(exam.) <br> Clame (anal.) |  |  | x |  | x |  |  |
| Rish exch. Milfoil | x | $\underset{x}{x}$ |  | I | x | $\ddot{x}$ | $x$ |
| $\begin{aligned} & \text { a) } 2,4-\mathrm{D} \\ & \text { b) } \mathrm{Co} \\ & \text { snail } \end{aligned}$ |  |  |  |  |  | x | z |
| Macrobenthos |  |  |  |  |  |  |  |
| dim.Resp. |  |  |  |  |  |  |  |

Bote: Four more days of fish exch. not ahow.

| Date | 12/08/81 | 19/08/81 | 02/09/81 | 16/09/81 | 30/09/81 | 14/10/81 | 28/10/81 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Days to/from treatment | 414 |  |  |  |  |  | 28/10/81 |
| Days to/from |  | 421 | 435 | 449 | 463 | 477 | 491 |
| - 81 treatmt. | 36 | 43 | 57 | 71 | 85 | 99 | 113 |
| Water Chem. |  | x |  |  | , |  |  |
| 2.4-D/DCP |  | $x$ | ${ }^{2}$ | $x$ | 2 | \% | $\mathbf{x}$ |
| Sediment | x | x | + | $x$ | x | $x$ | $x$ |
| Phytoplankton |  | $x$ | ${ }^{\mathbf{x}}$ | $x$ | $\pm$ | x | \% |
| Protozoa |  | x | $\underline{x}$ | \% | $x$ | $\mathbf{x}$ | $\dot{x}$ |
| Cooplankton |  | \% | $\pm$ | $x$ | $\underline{8}$ | x | x |
| Fungi |  |  |  |  |  | x | $\pm$ |
| Clams (exam.) |  | x | x |  |  |  |  |
| Clams (anal.) |  |  | $x$ | I |  | $x$ |  |
| Milfoil | z | $\mathbf{x}$ |  |  | . | x |  |
| a) 2.4 -D <br> b) $\mathrm{CO}_{2}$ <br> Enail |  | $\underline{x}$ |  |  |  |  | x |
| macrobenthos |  |  |  |  |  |  | . |
| $\mathrm{El}_{\text {gim.Resp. }}$ |  |  |  |  |  |  |  |

## Table 2

Productivity in pond 6 (1980)

| Time Interval (date in July) | Daily Respiration $\qquad$ | Net Productipn $\left(\mathrm{mg} \mathrm{~m}^{-2} \mathrm{day}-1\right) .$ | Gross Pyoductivity (mg m ${ }^{-2} \mathrm{day}^{-1}$ ) |
| :---: | :---: | :---: | :---: |
| 2- 3 | 300 | -25 | 1.90 |
| 10-11 | 624 | -27 | 4.31 |
| 11-12 | 641 | 9 | 4.11 |
| 12-13 | 718 | 82 | 5.52 |
| 13-14 | 260 | 37 | 2.05 |
| 14-15 | 441 | 108 | 3.79 |
| 15-16 | 490 | 116 | 4.18 |
| 16-17 | 437 | -79 | 2.47 |
| 17-18 | 431 | -40 | 2.70 |
| 18-19 | 330 | -149 | 1.25 |
| 19-20 | 325 | -137 | 1.30 |
| -20-21 | 425 | -215 | 1.45 |
| 21-22 | 356 | 242 | 4.13 |
| 22-23 | 389 | 55 | 3.06 |
| 23-24 | 460 | 4 | 3.13 |
| 24-25 | 394 | 38 | 2.98 |

## Table 3

## $\mathrm{E}_{\mathbf{h}}$ in Pond Sediments

(a) Readings taken August 19, 1980


| $0-5 \mathrm{~cm}$ |  | 95 | 50 | 35 | 130 | 80 | 170 | 25 |
| ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: |
| $5-10 \mathrm{~cm}$ |  | 60 | 30 | 20 | 145 | 50 | 130 | 10 |
| $10-15 \mathrm{~cm}$ |  | 10 | 140 | 10 |  | 60 |  | 10 |
| $15-20 \mathrm{~cm}$ |  |  |  | 5 |  | 130 |  |  |

(b) Readings taken November 19, 1980

| $0-5 \mathrm{~cm}$ | 123 | 109 | 228 | 301 | 277 | 351 |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| $5-10 \mathrm{~cm}$ | 198 | 153 | 240 | 291 |  | 361 |
| $10-15 \mathrm{~cm}$ | 170 |  | 253 |  |  |  |

## Table 4 <br> Recovery of 2,4-D from Milfoil

(Radiotracer technique)
(a) Counts from Plant Extract Counts from Extracted Plant Material

$$
0.0181 \times 10^{6}
$$

$$
0.0037 \times 10^{6}
$$

Total $0.0218 \times 10^{6}$
Efficiency ..... 83.2\%
(b) Counts from Plant Extract $0.0246 \times 10^{6}$ Courits from Extracted Plant Material $0.0047 \times 10^{6}$
Total $0.0293 \times 10^{6}$
Efficiency ..... 84.0\%
Extraction Efficiency ..... $83.6 \%$
Table 5

| Date | Concentrations of 2,4-D and 2,4-DCP in Clam Tissue (1980) |  |  |  |  |  |  |  |  |  | Pond 6 |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | Pond 1 |  | (micrograms per gram) |  |  |  |  |  |  |  |  |  |
|  |  |  | Pon | d 2 | Pon | 3 |  |  | Pon | d 5 |  |  |
| (1980) | 2,4-D | 2,4-DCP | 2,4-D | 2,4-DCP | 2,4-D | 2,4-DCP | 2,4-D | 2,4-DCP | 2,4-D | 2,4-DCP | 2,4-D | 2,4-DCP |
| 23/6 | 0.974 | 0.964 | 0.943 | 1.72 | 0.845 | 1.09 | 0.602 | 1.53 | 0.000 | 0.000 | 1.67 | 0.000 |
| 27/6 | 5.70 | 0.847 | 1.014 | 0.00 | 1.220 | 0.000 |  |  | 1.009 | 0.000 |  |  |
| 2/7 | 1.27 | 0.800 | 1.055 | 0.00 | 1.50 | 1.98 | 0.736 | 0.945 | 0.954 | 3.63 | 0.167 |  |
| 8/7 | 0.942 | 1.30 | $1.263 *$ | 1.62* | 1.102 | 0.000 | 0.730 | 0.502 | 0.157 | 0.000 | 0. | 0.00 |
| 15/7 | 0.244 | 0.000 | 0.226 | 0.000 | 0.125 | 0.000 | 0.522 | 0.000 | 0.122 | 0.0 | 0.1 | 0.00 |
| 21/7 |  |  | 1.69 | 2.54 |  |  | 0.159 | 1.14 | 1.31 | 2.54 | 0.851 | 0.971 |
| 22/7 | 0.773 | 0.000 |  |  | 0.816 | 0.000 |  |  |  |  | 0.00 | 0. |
| 5/8 | . 0.116 | 0.000 | 0.000 | 0.000 | 0.897 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.203 |
| 19/8 | $\begin{aligned} & 7.69 \\ & 5.74 \end{aligned}$ | $\begin{aligned} & 1.73 \\ & 2.79 \end{aligned}$ | 6.87 | 2.19 | 7.64 | 5.84 | 1.83 | 0.872 | 4.35 | 1.90 | 2.83 | 1.09 |
| 16/9 | 1.90 1.55 | $\begin{aligned} & 2.75 \\ & 2.16 \end{aligned}$ | 1.49 | $\begin{aligned} & 2.36 \\ & 2.33 \end{aligned}$ | 2.87 | 6.85 | 1.24 | 1.10 | 0.509 | 0.583 | 0.000* | $0.000{ }^{\circ}$ |
| 29/10 | 1.64 | 0.837 | 0.525 | 2.33 | 0.000 | 0.000 |  |  | 0.717 | 0.000 |  |  |

Table 6
Concentrations of 2,4-D and 2,4-DCP in Clam Tissue (1981)

| Date | $\frac{\text { Concentrations of 2,4-D and 2,4-DCP in Clam Tissue (1981) }}{\text { (micrograms per gram) }}$ |  |  |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  |  |  |  |  |  |  |  |
|  | Pond 3 | Pond 4 : |  | Pond 6 |  | Dåe | Pond 3 |  | Pond 4 |  |
| (1981) | 2,4-D 2,4-DCP | 2,4-D | 2,4-DCP | 2,4-D | 2,4-DCP | (1981) | 2,4-D | 2,4-DCP | 2,4-D |  |
| 28/5 | 0.000* 0.000* | $0.00{ }^{*}$ | 0.000* | 0.000* | 0.000* | 29/7 | 2, $\because$ | 2,4-DCP | . 325 | 4-DCP |
| 10/7 | 0.000* 0'.000* | 1.49 | 1.75 | 1.48 | 3.66 | 29/7 | 0. |  | 0.325 | 0.000 |
| 15/7 | 0.000* 0.000* | 0.161 | 0,000 |  |  |  | 0.20 | 0.0 | 0.190* | 0.000* |
|  |  | 0.161 | 0.000 | 0.151 | 0.000 | 2/9 | 0.774* | 1.08* |  |  |
| 22/7 |  | 0.100 | 0.000 | 0.538 | 0.000 | 13/10 | 0.277* | $0.00{ }^{*}$ | 0.242* | 0.000* |


| Sex | 2.4-D and 2.4-DCP in Common Shiners (1980) |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
|  | Weight | ```Body tissue (g)``` | Testes or ovaries (microgra | Kidneys <br> $s$ per gram) | Liver and spleen |
| - |  |  |  |  |  |
| (a) Pond 1 (ester treatment) |  |  |  |  |  |
| M | 70.29 | $(0.0 .007$ | $\text { (ND) } 0.291$ | $\mathbf{( N D}^{\text {ND }}$ | $\begin{gathered} \text { ND } \\ (0.273) \end{gathered}$ |
| M | 47.95 | (ND) | $(\mathrm{ND})^{0.286}$ | $(N D)^{N D}$ | $\begin{gathered} \text { ND } \\ (0.224) \end{gathered}$ |
| F (b) Pond 3 (ester treatment) |  |  |  |  |  |
| F | 15.469 | ND $(0.2)$ | ${ }_{(\mathrm{ND})}^{0.013}$ | $\begin{aligned} & \text { ND } \\ & \text { (ND) } \end{aligned}$ | $\begin{aligned} & \text { ND } \\ & \text { (ND) } \end{aligned}$ |
| F | 13.296 | $\begin{gathered} 0.17 \\ (0.22) \end{gathered}$ | $\begin{gathered} 0.1 \\ (\mathrm{ND}) \end{gathered}$ | $\begin{aligned} & \text { ND } \\ & \text { (ND) } \end{aligned}$ | $\begin{aligned} & \text { ND } \\ & \text { (ND) } \end{aligned}$ |
| F | 14.635 | $\begin{aligned} & 0.12 \\ & \text { (ND) } \end{aligned}$ | ${ }_{\text {(ND) }}^{0.106}$ | $\begin{gathered} \text { ND } \\ \text { (ND) } \end{gathered}$ | $\begin{aligned} & \text { ND } \\ & \text { (ND) } \end{aligned}$ |
| F | 10.761 | $\begin{gathered} 1.75 \\ (0.72) \end{gathered}$ | $\begin{aligned} & \text { ND } \\ & \text { (ND) } \end{aligned}$ | $\begin{aligned} & \text { ND } \\ & \text { (ND) } \end{aligned}$ | $\begin{aligned} & \text { ND } \\ & \text { (ND) } \end{aligned}$ |
| F | 14.856 | $\begin{gathered} 0.147 \\ (0.053) \end{gathered}$ | $\begin{aligned} & \text { ND } \\ & \text { (ND) } \end{aligned}$ | $\begin{aligned} & \text { ND } \\ & \text { (ND) } \end{aligned}$ | $\begin{aligned} & \text { ND } \\ & \text { (ND) } \end{aligned}$ |
| F | 16.556 | NA | ${ }_{\text {(ND) }}^{0.125}$ | $\begin{array}{r} 3.5 \\ \text { (ND) } \end{array}$ | $\begin{aligned} & \text { ND } \\ & \text { (ND) } \end{aligned}$ |
| M | 70.63 | $\begin{aligned} & 0.069 \\ & \text { (ND) } \end{aligned}$ | $\begin{aligned} & 0.259 \\ & \text { (ND) } \end{aligned}$ | $\begin{aligned} & 0.753 \\ & \text { (ND) } \end{aligned}$ | $\begin{aligned} & 0.325 \\ & \text { (ND) } \end{aligned}$ |
|  |  | Pond 2 (am | ne treatmen |  |  |
| F | 10.968 | $\begin{aligned} & 1.35 \\ & \text { (ND) } \end{aligned}$ | $\begin{aligned} & 0.121 \\ & (\mathrm{ND}) \end{aligned}$ | $\begin{aligned} & \text { ND } \\ & \text { (ND) } \end{aligned}$ | $\begin{aligned} & \text { ND } \\ & \text { (ND) } \end{aligned}$ |
| F | 13.616 | $\begin{gathered} 0.523 \\ (0.642) \end{gathered}$ | $\begin{gathered} \text { ND } \\ \text { (ND) } \end{gathered}$ | $\stackrel{\text { ND }}{\text { (ND) }}$ | $\begin{gathered} \text { ND } \\ \text { (ND) } \end{gathered}$ |
| $F$ | 12.731 | $\begin{gathered} 0.070 \\ (0.827) \end{gathered}$ | $\begin{aligned} & \text { ND } \\ & \text { (ND) } \end{aligned}$ | $\begin{gathered} \text { NA } \\ \text { (NA) } \end{gathered}$ | $\begin{aligned} & \text { ND } \\ & \text { (ND) } \end{aligned}$ |
| $F$ | 10.531 | $\begin{gathered} 0.082 \\ (0.409) \end{gathered}$ | ${ }_{(\mathrm{ND})}^{0.17}$ | $\begin{gathered} \text { ND } \\ \text { (ND) } \end{gathered}$ | $\begin{aligned} & 0.67 \\ & \text { (ND) } \end{aligned}$ |



Entries in parentheses are 2,4-DCP values
NA - not available
ND = not detected
TR - trace (below $0.001 \mathrm{ug} / \mathrm{g}$ )

## 2,4-D in Milfoil - 1980

## (micrograms/gram)

| Date | Pond 1 | Pond 2 | Pond 3 | Pond 4 | Pond 5 | Pond 6 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 26/6 | n.d. | 11.18 | 6.90 | n.d. | 17.92 | n.d. |
|  |  |  |  | n.d. | tr. |  |
| 27/6 | 106.1 | 15.60 | 8.02 | n.d. | 12.66 | n.d. |
| 2/7 | 16.78t | $11.80 t$ | $13.89{ }^{\text {t }}$ | n.d.t | $5.52{ }^{\text {t }}$ | n.d. |
|  | tr. ${ }^{17}$ | 10.98m | $11.30{ }^{\text {m }}$ | n.d.m | 8.15 ${ }^{\text {m }}$ |  |
| 3/7 | $37.1{ }^{\text {t }}$ | n.d.t | $8.00{ }^{\text {t }}$ | n.d.t | $12.13^{t}$ | n.d.t |
| 7/7 | $14.93{ }^{\text {tl }}$ |  |  |  |  |  |
|  | $7.92{ }^{\text {ml }}$ |  |  |  |  |  |
|  | n.d. $t 2$ |  |  |  |  |  |
|  | $16.42^{\text {m2 }}$ |  |  |  |  |  |
|  | $8.1^{\text {t3 }}$ |  |  |  |  |  |
|  | n.d. m3 |  |  |  |  |  |
|  | $9.73^{t 4}$ |  |  |  |  |  |
|  | n.d. ${ }^{\text {m }}$ |  |  |  |  |  |
|  | $\text { n.d. } \mathrm{t} 5$ |  |  |  |  |  |
|  | $\mathrm{tr}^{\mathrm{m} 5}$ |  |  |  |  |  |
| 8/7 |  | n.d.t | $10.55{ }^{\text {t }}$ | n.d. ${ }^{\text {t }}$ | $2.75{ }^{\text {t }}$ | n.d. ${ }^{\text {t }}$ |
| 5/8 |  | tr | 0.53 | 1.14 |  |  |
| 3/9 | 0.81 | 0.59 | 2.68 | 0.93 |  |  |

t - apical tip.
m $=$ mid-section of plant
tr-trace
numbers next to $t$ and $m$ indicate different plants

Table 9

## 2,4-D in Milfoil-1981

## (micrograms/gram)



Table 10

Counts of Protozooplankton
In Replicate Samples

|  | $\begin{aligned} & \text { Pon } \\ & \text { R } \end{aligned}$ | $\begin{gathered} 17 / 6 \\ 12 \end{gathered}$ | Pond ( 1 | $\begin{gathered} \hline 17 / 6 \\ 62 \end{gathered}$ |  | $\begin{gathered} \overline{6}, 6 \\ \$ 2 \end{gathered}$ |  | $\begin{gathered} \text { 3, Aug. } 5 \\ 2 \end{gathered}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Askenasia <br> Coleps <br> Cyclidium \#1 <br> Cyclidium \#3 <br> Halteria <br> Lagenophrya <br> Urotricha farcta <br> Urotricha \#2 <br> Others <br> Amoebae zooflagellates | 10 36 110 26 30 38 4 2 | $\begin{array}{r} 6 \\ 40 \\ 94 \\ 14 \\ 5 \\ 43 \\ 3 \\ 0 \end{array}$ | 34 <br> 51 <br> 43 <br> 69 <br> 34 <br> 5 <br> 8 | 34 <br> 51 <br> 33 <br> 69 <br> 38 <br> 7 <br> 1 | $\begin{aligned} & 14 \\ & 31 \\ & 83 \\ & 15 \\ & 18 \\ & 23 \\ & 11 \\ & 21 \\ & 18 \end{aligned}$ | $\begin{array}{r} 6 \\ 60 \\ 63 \\ 5 \\ 24 \\ 30 \\ 10 \\ 22 \\ 38 \end{array}$ | 8 147 28 8 9 15 0 | $\begin{array}{r} 2 \\ 146 \\ 27 \\ 2 \end{array}$ |
| Totals | 256 | 205 | 244 | 233 | 237 | 258 | 215 | 212 |
| $z$ error | -20\% |  | -4.5\% |  | +8.9\% |  | -1.4\% |  |

Table 11

## Snail Counts

| Sample | PO S | 1 <br> B |  | 2 |  | 38 | Po S | B | Po S | 5 B | Pond 6 | B |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 1 | Oe |  | 5 e |  | 5 e |  | 1 e |  | 9 e |  | Oe |  |
| 2 | Oe |  | 13 e |  | 4 e |  | Oe |  | 6 e |  | Oe |  |
| 3 | le |  | 8 e |  | 3 e |  | Oe |  | 3 e |  | Oe |  |
| 4 | 1 e |  | 8 e |  |  | 13 | Os |  | 78 |  | Oe |  |
| 5 |  | 13 |  | 33 |  | 12 |  | 2 |  | 27 |  | 3 |
| 6 |  | 14 |  | 29 | 3s |  | 18 |  |  | 41 |  | 1 |
| 7 | 38 |  | 58 |  | 48 |  | 28 |  |  | 28 |  | 0 |
| 8 | 25 |  | 148 |  | 3w |  | Ow |  | 12s |  | Is |  |
| 9 | Ow |  | 10w |  |  |  |  | 4 | 108 |  | Ow |  |
| 10 | 2w |  |  |  |  |  |  |  | 11w |  | On |  |

s - slope of pond
B - bottom
e,s,w,n indicate east, south, west and north slopes


Fig. 3. Ammonia concentrations in the water column (1980/1981).

?



Fig. 8. Total phosphorus concentrations in the water column





Fig. 12. Calcium ion concentrations in the water column, 1981. (left side shows the 1980 designations).

Ca+


Fig. 13. Magnesium ion concentrations in the water, 1981. (left side shows the 1981 designations).
$\mathrm{Mg}^{++}$








Fig. 19. Kinetics of 2,4-D disappearance in the water column.



Fig. 22. 2,4-DCP in the water column (1981).


Fig. 23. 2,4-D in pond sediment (1980).

Fig. 24. 2.4-DCP in pond sediment (1980).


Fig. 25. 2,4-D in pond sediment (1981). (mg•per $10 \mathrm{~cm}^{2}$ of bottom)


Fig. 26. 2,4-DCP in pond sediment (1981).
(mg per $10 \mathrm{~cm}^{2}$ of bottom)



Fig. 28.
Toxicity curve of $2,4-\mathrm{DCP}$ for S . rhomboideum at pH 8.8


Fig. 29. The mortality of common shiner fry. (1980, the first week after treatment)

Fig. 30. Microbial populations in the water column (1980).


Fig. 31. ATP results, 1980.

Fig. 33. Sulphur oxidizing bacteria
in the water column (1980).




Fig. 37. Sulphur reducing bacteria in the sediment (1980).




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Fig. 40. Total ciliate populations (1980).


Fig. 41. Ciliate biomasses (1980).




Fig. 43. Stem lengths of milfoil plants in 1981.
Captions left of vertical bar (treatment) show 1980 pond designations.


