R-4667

Study Title

Assessment of the Fate and Effects of Endosulfan on Aquatic Ecosystems Adjacent to Agricultural Fields Planted with Tomatoes

Data Requirement

Guidelines Reference Number
72-7

Authors

B. W. Cornaby, A. F. Maciorowski, M. G. Griffith
J. E. Navarro, S. E. Pomeroy, N. G. Reichenbach, J. S. Shuey and M. F. Yancey
(Battelle)
and S. Hickey (Hickey's Agri-Services)

Report Date

June 28, 1989

Performing Laboratories

Battelle
505 King Avenue
Columbus, Ohio 43201-2693

Hickey's Agri-Services
Laboratory, Inc.
P.O. Box 547
Camilla, Georgia 31730

Laboratory Project ID Numbers

N0954-5700

Study Submitted By

Hoechst Celanese Corporation
Route 202-206 North
Somerville, NJ 08876

Purpose of Submission

Data in response to the Re-registration Process of Endosulfan

Submission Volume

Volume 1 of 11

STATEMENT OF NO FIFRA SECTION 10 DATA CONFIDENTIALITY CLAIM

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Section 10 (d) (1) (A), (B), or (C).

The information contained herein is the property of Hoechst Celanese Corporation and although subject to release to nonmultinationals pursuant to FIFRA Section 10, such information is considered trade secret for all other purposes.

Company: Hoechst Celanese Corporation
Route 202-206 North
Somerville, NJ 08876

Company Agent: M. Volger
Dr. Bert Volger
Manager, Regulatory Affairs

Date: 6/28/89

STATEMENT OF GOOD LABORATORY PRACTICES

Statements of Good Laboratory Practices Compliance and the Study Director are contained in this report.

Refer to pages 0004 through 0010 of 2260 for the Quality Assurance Statements and to page 0011 of 2260 for the Study Director Statement. Based upon these statements, the submitter believes that this study meets the requirements of 40 CFR Part 160.

Submitter: B. Volger

Dr. Bert Volger
Regulatory Affairs

Date: 6-28-89

BATTELLE COLUMBUS QUALITY ASSURANCE STATEMENT

This study was inspected by the Quality Assurance Unit and reports were submitted to management and the study director as follows:

Phase	Date
Sampling-water, kicknet for biota, electrofishing, soil and sediment (baseline), water analysis (field, laboratory)	5/3/88, 5/4/88
Site visit to subcontracted biological/ecological facility, Aquatic Taxonomy Specialists (ATS)	5/12/88
Site visit to subcontracted analytical facility, Stilson Laboratories	5/24/88, 6/3/88
Site visit to subcontracted ecological/biological facility, Environmental Associates, Inc.	5/25/88
Laboratory Record Books audit	6/7/88, 6/9/88, 6/28/88, 6/29/88, 9/2/88, 9/22/88, 12/8/88
Field laboratory inspection	6/9/88
Application of pesticide, application card collection, drift card collection (pond and periphery of the field), soil and water spiking, sample log-in, sample packing, soil core collection	6/10/88, 6/28/88
Field set up prior to spraying, placement of application/drift cards, application of pesticide, application and drift card collection on the pond, leaf cutting and rinsing, rinsate collection	6/11/88, 6/27/88
Sample receipt and checking of Chain-of-Custody forms, sample storage, computer validation of sample custody transfer, standards composition for GC analysis	6/29/88
Sample concentration and evaporation for drift cards; sample extraction, spiking, concentration, and evaporation for pond water samples	6/30/88

Macroinvertebrate sampling, pond surveillance, collection of fish specimens, irrigation, ISCO sampling	6/29/88
Chain-of-Custody forms, computer output for dates 5/25/88-6/23/88	7/11/88
Facility inspection	7/20/88
Laboratory phases: water samples-extraction in methylene chloride, final concentration, transfer to GC vials, soil and sediment samples-computer Chain-of-Custody forms, transfer, sample agitation by shaker, centrifuging, decanting into funnel and extraction, concentration to 2 ml, gel permeation chromatography cleanup, analysis of standards, and sample storage	7/26/88-7/29/88
Unpack and dispense samples	8/10/88
Pond M-55-4: Soil sampling, decontamination between transects, water sampling, sealing and packing samples on ice, sediment sampling, sample log-in, packaging for storage, shipment, spiking of water samples	9/21/88
Preparation of containers for sediment cores	9/21/88
Pond M-55-4: Electroshocking; fish measurements, weight/identification; collection of fish specimens for residue determination	9/21/88
Packing truck for field sampling, kicknet sampling and sample preservation, phyto-and zooplankton sampling, Ekman sampling; pH, conductivity, DO, temperature, wind speed, and water level measurements, calibration of meters	9/22/88
Balance calibration records	9/22/88
Inspection of pond water sample extractions	11/29/88
Sample shipments (water quality, autotrophic index, residue)	12/20/88

Data audits-analytical data, biological/ecological data, raw data, sample logs, pond collection inventory sheets, QC data, Chain-of-Custody forms, sample transmittal forms, computer data base forms	10/25-26/88 12/16,19/88, 12/29/88, 1/6/89 1/13/89, 2/8/89 2/23/89, 2/28/89 3/1/89, 3/6/89, 3/13/89, 3/14/89 3/21/89, 3/24/89 3/29-30/89, 4/10/89, 4/18-19/89, 4/21/89, 4/25/89, 4/27/89 5/2-5/89, 5/8-9/89, 5/12/89 5/17/89, 5/24/89 5/31/89, 6/1/89, 6/5/89, 6/7/89
Final Report	6/19-28/89

Reports to study director and management: 5/13/88, 5/25/88, 5/26/88, 6/6/88, 6/14/88, 7/1/88, 7/5/88, 7/11/88, 7/20/88, 8/2/88, 9/2/88, 9/21/88, 9/22/88, 9/23/88, 10/25/88, 10/26/88, 11/29/88, 12/8/88, 12/16/88, 12/20/88, 1/6/89, 1/13/89, 2/8/89, 2/23/89, 2/28/89, 3/1/89, 3/6/89, 3/13/89, 3/14/89, 3/21/89, 3/24/89, 3/29/89, 3/30/89, 4/10/89, 4/18/89, 4/20/89, 4/21/89, 4/25/89, 4/27/89, 5/2/89, 5/3/89, 5/4/89, 5/5/89, 5/8/89, 5/9/89, 5/11/89, 5/12/89, 5/17/89, 5/24/89, 6/5/89, 6/7/89, 6/19-28/89

To the best of my knowledge the methods described were the methods followed and the data presented accurately represent data generated during the study.

Ramona Mayer 6/29/89
Ramona A. Mayer, Manager
Quality Assurance Unit
Health and Environment Group

Battelle
Ocean Sciences

QUALITY ASSURANCE STATEMENT

FOR

PROJECT NUMBER N-0954-5799

"ASSESSMENT OF THE FATE AND EFFECTS OF ENDOSULFAN ON AQUATIC SYSTEMS ADJACENT TO AGRICULTURAL FIELDS PLANTED IN TOMATOES"

In accordance with Good laboratory Practice standards (EPA/FIFRA, 40 CFR Part 160), this study has been monitored by Battelle Ocean Sciences' Quality Assurance Unit to determine conformance with the protocol and Standard Operating Procedures. Dates of study audits and when the audits were reported to the Study Director and management are listed in the attached table.

Patricia D. Royal

Patricia D. Royal
Manager, Quality Assurance Unit
Battelle Ocean Sciences Department

April 12, 1993
Date

BATTELLE - DUXBURY

QUALITY ASSURANCE AUDITS

CONDUCTED FOR PROJECT NUMBER N-0954-5799

"ASSESSMENT OF THE FATE AND EFFECTS OF ENDOSULFAN ON AQUATIC SYSTEMS ADJACENT TO AGRICULTURAL FIELDS PLANTED IN TOMATOES"

Date/Phase of Audit	Report to Study Director	Report to Management
06-10-88 / Sample preparation	06/10/88	07/19/88
06-28-88 / Sample analysis	06/30/88	07/19/88
08-11-88 / Initiation: Phase 3	08/11/88	08/29/88
08-26-88 / Sample preparation	08/29/88	09/15/88
09-02-88 / Sample analysis and Quality Control	09/07/88	09/15/88
09-15-88 / Initiation: Phase 4	09/15/88	09/26/88
10-04-88 / Sample analysis	10/06/88	03/15/89
12-09-88 / Data audit	12/09/88	12/13/88
12-19-88 / Chain-of-custody	12/29/88	03/15/89
12-27-88 / Sample preparation	12/29/88	03/15/89
04-12-89 / Final data review	04/12/88	04/12/88

QUALITY ASSURANCE STATEMENT

PROJECT: Battelle Georgia Pond Study

In compliance with the Good Laboratory Practice Regulations (40 CFR 160) this study was inspected by the Director of Quality Assurance. The Processes observed accurately reflect data collected during the conduct of the study. Dates of inspection, phase inspected, and reporting dates are as follows:

DATE OF INSPECTION	PHASE INSPECTED	DATE OF REPORT TO MANAGEMENT AND STUDY DIRECTOR
August 3, 1988	Sample documentation (receipt, examination, records and storage)	September 14, 1988
August 3, 1988	Sorting procedures, Quality Control, and record keeping	September 14, 1988

Director, Quality Assurance

QA Statement of : AQUATIC TAXONOMY SPECIALISTS
Malinta, OH 43535
13 June 1989

For: Battelle, Project Georgia Pond Study No. 954

The work was performed by Aquatic Taxonomy Specialists according to Good Laboratory Practices and was inspected by the quality assurance person in charge to insure that thefollowing analyses were conducted according to Standard Operating Procedures:

Taxonomic Verification of organisms in specific samples;
Phytoplankton-Mar 22, 88, May 10, 88
Zooplankton- Apr 4, 88, May 10,88, Jun 27,88.

Calculation of conversion factors (verified in recounts)
Phytoplankton- Mar 22, 88, May 10, 88,
Zooplankton- Apr 4,88, May 10, 88, June 27,88.

Correct Sample Preparation Methods;
Checked on June 27, 88.

QA inspections were made by William Cody

William Cody for
Aquatic Taxonomy Spec.

BATTELLE STUDY DIRECTOR STATEMENT

Phase I (site selection) and Phase II (pre-application) were conducted according to commonly-accepted scientific and professional standards, but were initiated prior to finalization of the EPA reviewed protocol. All Phase II data were subjected to data and sample-tracking review and audit, but were not field audited.

The field and laboratory aspects of Phase III (application) and Phase IV (post-application) of this study were conducted so as to conform with Good Laboratory Practices as published by the U.S. Environmental Protection Agency Good Laboratory Practice Standards, 40 CFR 160.

Battelle Study Director: Barney Cornaby
Dr. Barney Cornaby
Environmental Toxicology

Date: 6/29/89

SUMMARY

Hoechst Celanese Corporation (HCC) on behalf of FMC Corporation and Maktheshim-Agan, Inc. conducted a field study of the effects of endosulfan on farm pond ecosystems in southwestern Georgia. The study was conducted for HCC by Battelle and Hickey's Agri-Service Inc. in 1987 through 1988.

The endosulfan formulation used was Thiodan 3EC. Thiodan 3EC was applied to tomatoes under worst-case conditions: minimal buffer between tomatoes and pond edge; the highest application rates (highest amount of endosulfan per season) as recommended on the approved label; and forced irrigation after the last application. The rate per application was approximately 3.1 L/ha (1.12 kg endosulfan/ha or 1.00 lb endosulfan/acre).

Two watershed/pond systems served as treatment sites, with two additional watershed/pond systems as reference sites. Tomatoes planted in the reference watersheds received no endosulfan. Endosulfan entered the treatment ponds through two pathways: aerial drift and runoff. Runoff provided the primary exposure, and was induced by irrigation and/or natural rainfall following the third application. The achieved dose in the ponds represents a worst case situation due to drought conditions that resulted in a single major runoff event after the last application. The mean peak total endosulfan dose in pond water of one treatment pond (1.3 μg/L) was approximately twice that of the other treatment pond (0.6 μg/L). These endosulfan concentrations were 100 times lower than the endosulfan concentrations of runoff water at the flume.

As an endosulfan effects study, the investigation examined numerous physical, chemical and biological characteristics of the farm pond/watershed systems before, during, and after multiple endosulfan applications. Various comparisons examined the potential effects of endosulfan on phytoplankton, zooplankton, benthic macroinvertebrates (kicknet, emergent insects, Ekman dredge samples, S-samples), fish, pond metabolism, autotrophic index, and macrophytes. Several statistically significant differences between reference and treatment ponds occurred, but none was judged ecologically relevant.

Localized fish kills occurred at both treatment ponds following the major runoff event after the third application. No fish mortality occurred in either pond as a result of spray drift. Fish in the 10-60 mm size range accounted for over 90 percent of fish killed. The magnitude of the fish kill was greater in the higher dose treatment pond and is presumably due to dose and habitat differences at the points of runoff entry. The fish kills appeared to be localized, and resulted in no apparent effect on the fish population structure. The remaining structural and functional ecosystem parameters measured in the dosed farm ponds showed no changes attributable to endosulfan when compared to either the reference ponds or the baseline year.

TABLE OF CONTENTS

TABLE OF CONTENTS
(Continued)

TABLE OF CONTENTS
(Continued)

TABLE OF CONTENTS
(Continued)

TABLE OF CONTENTS
(Continued)

LIST OF FIGURES

LIST OF FIGURES
(Continued)

LIST OF FIGURES
(Continued)

LIST OF FIGURES
(Continued)

Page

LIST OF FIGURES
(Continued)

LIST OF FIGURES
(Continued)

LIST OF FIGURES
(Continued)

LIST OF TABLES

LIST OF TABLES
(Continued)

LIST OF TABLES
(Continued)

LIST OF TABLES
(Continued)

LIST OF APPENDICES

FINAL REPORT

on

ASSESSMENT OF THE FATE AND EFFECTS OF ENDOSULFAN ON AQUATIC ECOSYSTEMS ADJACENT TO AGRICULTURAL FIELDS PLANTED WITH TOMATOES

by

Battelle

and

Hickey's Agri-Services

to

Hoechst Celanese Corporation

I. INTRODUCTION

Endosulfan, a broad-spectrum insecticide, has been widely used since 1954. Numerous investigations on its properties, activities, behavior, and side effects have been performed throughout the world as cited in Goebel et al (1982). Laboratory studies have established that endosulfan is highly toxic to fish and invertebrates. However, when properly used, under field conditions, Gorbach et al (1971) demonstrated that endosulfan posed no risk to aquatic life. The study reported here is the first field trial with endosulfan conducted in the western hemisphere in which effects on non-target organisms are investigated. The study aims to strengthen the knowledge of endosulfan's distribution pattern and effects.

Hoechst Celanese Corporation (HCC) is in the process of reregistration of endosulfan with the United States Environmental Protection Agency (EPA). In April 1982 the U.S. EPA issued a Registration Standard for Endosulfan with the requirement for an aquatic field test being "reserved" at that time, dependent upon the results of the Agency's reviews of other related data and upon the finalization of the Agency's Guidelines. On October 25, 1985 the Agency requested the registrants to submit an appropriate study protocol for approval prior to initiation of field testing to simulate worst-case exposures.

The first protocol of January 29, 1986 was not acceptable to the Agency as specified in its review dated September 8, 1986. In this review the Agency recommended the development of a site specific protocol using a multiple farm pond system. Subsequently, meetings with representatives of Hoechst Celanese Corporation (HCC) and the Agency were held regarding the development of an acceptable protocol during the site selection process. The final protocol was accepted by the U.S. EPA on November 9, 1987 with the request of a few study modifications. HCC incorporated these changes and submitted the revised final protocol to the Agency on August 8, 1988.

Hoechst Celanese Corporation on behalf of FMC Corporation and Maktheshim-Agan, Inc. has conducted a field study of the effects of endosulfan on aquatic ecosystems when applied to adjacent agricultural fields. HCC has also added a fate study to the field investigations. To provide EPA with scientifically sound and environmentally realistic data to assess the potential risk associated with the use of endosulfan on all crops, a farm pond study was conducted under worst-case conditions using the highest application rates (highest amount of endosulfan per season) recommended on the approved label.

Battelle and Hickey's Agri-Services Laboratory Incorporated (HASLI) conducted the two-year farm pond study. Battelle was responsible for pond selection, all sampling, and subsequent sample and data analysis and reporting. HASLI was responsible for conducting and reporting all soils and agronomic work including the application of endosulfan. The study was conducted for HCC in southwestern Georgia in 1987 through 1988 using normal agricultural practices. This report summarizes the objective, methods, results, and other pertinent information about this field study.

II. TECHNICAL OBJECTIVE AND SCOPE

The overall technical objective of the farm pond study was to identify the biological effects to aquatic populations and ecosystems associated with the use of endosulfan. The field study provides empirical information regarding: (1) the temporal and spatial distribution of endosulfan into an adjacent aquatic environment resulting from ground application of endosulfan on tomatoes, (2) the effects of endosulfan residues on indigenous aquatic populations, and (3) the long-term stability and viability of exposed populations.

An aquatic ecosystem may receive a pesticide by direct application, drift, runoff (including water, and pesticide particles), or contaminated rain. The current EPA-registered Thiodan 3EC (endosulfan is the active ingredient at 3 pounds/gallon) label clearly and extensively warns against the direct use of endosulfan in surface waters. Accordingly, contamination of surface water via direct application is not representative of normal use. In designing the study, drift and runoff were considered the most likely pathways for the contamination of surface waters with endosulfan. Although the 91-m (300-ft) buffer zone required by the U.S. label should minimize aquatic exposure, a worst-case exposure was deemed prudent for this study. Application was made to surrounding fields as close as 4.6 m (15 ft) from the pond's edge. The study was designed to determine the impact of endosulfan through drift and runoff on plankton, macroinvertebrates, fish and emergent plants as well as selected functional measures in an aquatic ecosystem. The environmental fate of the substance in the watershed system was also assessed.

This field study identifies the consequences of endosulfan exposure to aquatic populations and ecosystems. Quantification of the physical, chemical, and biological characteristics of the experimental sites occurred prior to, during, and subsequent to endosulfan application. Effects are identified by (1) comparing the biological, chemical, and physical components of the treatment and reference ponds (untreated with endosulfan) and (2) comparing biological, chemical and physical data for the treatment ponds before and after the pesticide application.

The basic features of the study plan include:

- The agricultural fields, adjacent treatment ponds, and reference ponds were characterized in terms of baseline physical, chemical, and biological properties for one year prior to endosulfan application.

- The fields were planted with tomatoes.

- Thiodan 3EC at 1.33 qt thiodan/acre; this is equivalent to 1 lb endosulfan/acre or 1.12 kg endosulfan/ha. The pesticide was ground applied to the fields three times, with approximately two weeks between each application, beginning in May 1988 and ending in June 1988. Although a number of different endosulfan formulations (e.g., 50 WP) are available, Thiodan 3EC was chosen because it is the most widely used and is representative of the other formulations. The environmental fate and behavior, as well as the application rate of Thiodan 3EC is similar to the 50WP formulation.

- Treatment pond sampling to establish baseline conditions was initiated in the summer of 1987. Baseline sampling continued through December 1987, began again in March 1988, and continued until the endosulfan applications (May through June, 1988). Comparable reference pond sampling was initiated in May 1987, at times coincident with the treatment pond sampling. For the period July 1988 to December 1988, additional sampling was conducted.

- Chemical sampling and analysis quantified endosulfan residues in the finished spray mix, application cards, drift cards, plant foliage wash-off, agricultural field soil, field runoff water, pond water, pond sediment, and representative fish (bluegill and bass). This was done during the application period and subsequently per a set schedule during 180 days after the last spraying.

- Biological sampling and analysis included phytoplankton, zooplankton, macroinvertebrates, fish, macrophytes. Measures of fish reproduction, community metabolism, and periphyton production (autotrophic index), were also recorded and evaluated.

- Reporting the results and conclusions of the study followed as shown in the project study report.

Samples from the treatment and reference sites were collected and analyzed to assess relevant trends in aquatic populations and chemical residues. The study consisted of four distinct phases. These phases were:

Phase I. Site selection phase -- Treatment, reference, and contingency sites were selected for biological, chemical and physical measurements (winter 1986-summer 1987).

Phase II. Pre-application (baseline) phase -- This was the period (i.e., summer and fall 1987 and spring 1988) prior to the first endosulfan application, during which sampling and analysis established baseline biotic and abiotic conditions. Results of the baseline phase were used to select the final four sites and to identify ecological conditions in the treatment and reference ponds prior to endosulfan applications.

Phase III. Application phase -- This was the period (May through June, 1988) during which endosulfan was applied to the treatment fields three times at approximately two-week intervals as part of a normal agricultural program. Results of the application phase documented the presence and absence of immediate adverse effects on the aquatic populations and system.

Phase IV. Post-application phase -- The period (July through December, 1988) following the last application of endosulfan. Viability of aquatic populations was evaluated and the absence and presence of long-term effects were recorded.

III. SITE SELECTION

Site selection resulted in the location and qualification of four ponds for definitive study. Several hundred sites were considered in the southeastern United States where tomato production is relatively high. Tomatoes were chosen as the target crop for the endosulfan field study because they receive the highest application rate of endosulfan and because of the availability of dissipation information on endosulfan in tomatoes. The selected farm ponds and field systems are located in Colquitt, Mitchell, and Thomas Counties in southwestern Georgia. A more detailed version of the material summarized below is available in Appendix A.

A. Site Identification Criteria

Selection of the farm pond and field systems was based upon the following criteria:

- Ponds 0.8 to 2.0 ha (2 - 5 acres) in size
- Field to pond surface area ratio of 10:1
- Fields with a 3 percent to 8 percent slope to facilitate runoff into the aquatic system
- Healthy fish populations
- Benthos and phytoplankton present in sufficient numbers to demonstrate biological activity
- Water quality measurements that further support the concept of a relatively stable and active pond
- Cooperative owners
- An irrigation source to supplement naturally occurring precipitation in the event of insufficient rain.

B. Sources of Information

Information for the site selection phase was gathered from a variety of sources. Tomato production information was obtained from the U.S. Agricultural Statistics records, which list row and vegetable crop production by state. Individual county production was obtained by telephone conversations with the local county agricultural extension agents. The presence or absence of ponds in a given tomato-growing county was determined from United States Geological Survey (USGS) maps. USGS quadrangle maps showed the presence of ponds and the vegetation surrounding the ponds. County soil survey maps made from aerial photographs were also used to locate candidate ponds. Information on pond size, general type of surrounding vegetation, slope, soil type, and field size was determined from the maps. The maps, in conjunction with the information from the county extension agents, yielded a concise depiction of potential study ponds in many counties.

C. Information Analysis

The analysis of the information resulted in identification of several counties in which to search further for candidate farm field and pond system. The agricultural statistics for 1984 indicated the top 10 cotton-, tomato-, and potato-producing states. Florida and South Carolina were initially selected for the study. It was decided to expand the search. The 11th through 21st states, ranked in terms of tomato production, showed Alabama, Georgia, Louisiana, and North Carolina as additional states in the southeastern U.S. (USDA 1984). Within these six states, 15 counties were considered because of their high tomato production, i.e., they were the highest in the particular states. Counties with many ponds were considered more desirable than counties with few ponds. Counties within 80 to 160 km (50 to 100 mi) of a coast were eliminated because of: (1) the ephemeral nature of most coastal ponds, (2) flat topography unsuitable for runoff, and (3) light soils, less conducive to run-off. Through this process of elimination, Geneva and Houston counties in Alabama; Colquitt, Decatur, Grady, Mitchell, and Thomas counties in Georgia; and Gadsden County in Florida were selected for further consideration. The location of these counties is illustrated in Figure 1.

Mitchell County, Georgia, was judged to be the best candidate for the first part of more exhaustive analysis. Further discrimination was made with information from the soil survey maps. Locations of past and present tomato farms were determined based on written and verbal information from county extension agents.

Approximately 400 ponds were screened in southwestern Georgia, northeastern Florida, and southeastern Alabama. Following site reconnaissance trips and preliminary site investigations, eight candidate ponds were chosen for more detailed study in Mitchell and adjacent Colquitt, Grady, and Thomas counties in Georgia, all near the town of Pelham in Mitchell County.

D. General Site Description

Unsuitable pond ecosystems and unwilling land owners resulted in elimination of four of the eight candidate ponds during the pre-application/baseline phase (Phase II). Locations of the selected study sites are shown in Figure 2. Their identification codes and general locations are presented in Table 1. General soil characteristics show the four pond area to be dominated by sandy loam and loamy sand soils (Table 2).

Climatological data for the area is available from the closest weather station in Moultrie, Georgia, located approximately 19 km (12 mi) east of Pelham. Thus, weather reports are available for a location within a few kilometers (miles) of the candidate ponds. Climatological data for the period 1951 to 1975 are presented in Table 3. At Moultrie the mean monthly temperature is 19.3°C (66.7°F) with a mean daily minimum of 7.9°C (39.9°F) in January and a mean daily maximum of 33.1°C (91.6°F) in August. The mean annual precipitation is 129.1 cm (50.83 in.) with July being the month of highest rainfall.

E. Specific Pond Descriptions

This section provides a brief description of the four ponds. A topographic base map, aerial photograph, and concise descriptive text are provided for each pond.

1. Pond C-27-1

Pond C-27-1 is located in Colquitt County, Georgia. This pond was later selected as one of two treatment ponds. Figure 3 is an aerial photograph of the pond and adjacent agricultural fields. Pond C-27-1 has 1.4 hectares (3.5 acres) and a surrounding watershed of 20.4 ha (50.5 acres) (Figure 4). The water to land ratio is about 1:15. The greatest depth of 1.8 m (5 ft 5 in.) occurs in the southwest part of the pond near the dam. Approximate depth contours are shown in Figure 5. The pond was formed around 1960 by damming a small intermittent stream. The pond is roughly triangular, with the two longer sides surrounded by fields that slope toward the pond. The slope is from an arbitrary 30.5 m (100 ft) at the pond surface to 39 m (128 ft) at the top of the watershed. Soil textures were sandy clay loam, sandy loam and loamy sand and additional soil information is found in Table 2. Crops in the three years preceding the study included cotton, peanuts, soybeans, black-eyed peas, and garden vegetables. The most recently planted crops were peanuts, cotton and vegetables. No tomatoes have been planted at the C-27-1 study site in the recent past. However, the landowner consented to having tomatoes planted there for the duration of the study. Cotton was plowed under in 1987 per negotiations with the landowner. Based on landowner information, the following pesticides were applied to the fields during normal agricultural operations: Cotoran, DSMA, Galecron, Lannate, MSMA, Parathion, Prowl, Pydrin, Sencor, and Temik. According to the land owner endosulfan had not been used for at least 3 years before the application phase of the study. Preliminary analyses of the soil showed that no pesticide residues remained (Appendix B). Fertilizer (9-18-27 or 5-10-21) has been applied at 500 to 700 lb/acre. Side dressing of 50 to 100 lb/acre of nitrogen has also been applied. In addition, foliar fertilizer has been used on cotton. The pond is not used as an irrigation source.

Water quality data were typical of a soft water aquatic system and suggest no conditions acutely restrictive to most aquatic biota.

Biological sampling of the pond in 1987 revealed that the bluegill sunfish was the most abundant fish species, followed by largemouth bass. The largemouth bass population was found to be fairly stable. However, sampling suggested low reproduction and recruitment of largemouth bass. The largemouth bass-bluegill balance was judged to be fair-to-good.

The benthic macroinvertebrate assemblage was considered typical, with organism densities being low to moderate. Zooplankton densities in spring were also moderate as expected with relatively low phytoplankton densities. The lower trophic-level organisms appeared to adequately support the relatively high fish biomass. Strong predation pressure may have contributed to the relatively low secondary production.

2. Pond M-55-4

Another farm pond selected as a study site is pond M-55-4 in Mitchell County, Georgia. This pond was later selected as one of two reference ponds. Figure 6 is an aerial photograph of the pond and environs. M-55-4 has 0.8 surface hectares (2.1 acres) and an associated watershed of 9.6 ha (23.6 acres) (Figure 7). The ratio of water to land is about 1:11. The greatest measured depth was about 2.8 m (9 ft) in the eastern part of the pond near the middle of the dam. Approximate depth measurements are shown in Figure 8. The pond was created around 1962 and was renovated in 1977 (deepened and the edges dug). M-55-4 has farm fields of 3.8 and 5.8 ha (9.3 and 14.3 acres) that slope into the pond on each of the longer sides. The slope is from an arbitrary 30.5 m (100 ft) at the pond surface to 37.2 m (122 ft) to the west and 36 m (118 ft) to the east. At the upper end of the pond are 0.8 to 1.2 ha (2 to 3 acres) of woods. Soil textures were sandy loam and loamy sand and additional soil information is found in Table 2. Crops in the last 3 years preceding this study have been peanuts, corn, tomatoes, and snap beans. The most recent crops were corn and peanuts. Based on landowner information, the following pesticides have been used on the fields for normal agronomic operations: atrazine, 2-,4-DB, Balan, Basagran, Bravo, Dinitro, Dyanap, Lannate, Lasso, Maneb, Manzate, Nudrin, parathion, Prowl, Pydrin, pyrethroids, Temik, Thiodan, Treflan, and Vernam. According to the landowner endosulfan had not been used for at least two years preceding the application phase of the study. Preliminary analyses of the soil showed no pesticide residues remained (Appendix B). Fertilizers (5-10-15) have been applied at 500 lb/acre for peanuts and corn, 700 lb/acre for snap beans, 1,000 lb/acre for cucumbers, and 1.5 tons/acre for tomatoes. Also, sulfur has been added to snap beans. The pond has been used as an irrigation source for the adjoining fields, but it was not used for irrigation during this endosulfan study.

Water quality data were typical of soft water aquatic systems and suggest no conditions acutely restrictive to most aquatic biota.

A moderately dense largemouth bass population was characterized by large individuals, with no smaller-sized individuals collected. While this indicated possible imbalance in the top predator population, the bluegills showed a desirable size structure, suggesting good predatory control. A fair-to-poor largemouth bass to bluegill balance was indicated. A rich benthic fauna consisting of species indicative of well-oxygenated bottom conditions was observed in the center and littoral zones. While zooplankton densities in spring, 1987, were somewhat low, a more moderate phytoplankton population density suggested that productivity was not highly limiting.

3. Pond M-55-8

Another farm pond selected as a study site is pond M-55-8, also in Mitchell County, Georgia. This pond was later selected as one of two treatment ponds; the other treatment pond was C-27-1. Figure 9 is an aerial photograph of the pond and environs. This pond surface has 0.9 surface hectares (2.23 acres) and a watershed of 9.9 ha (24.1 acres) (Figure 10). The ratio of water to land is about 1:11. The greatest measured depth was about 1.5 m (5 ft), near the southern part of the dam. Approximate depth measurements are shown in Figure 11. The pond was created around 1960 by damming the watershed drainage. M-55-8 is triangular in shape, with fields of 3.8 and 5.8 ha (9.3 and 14.3 acres) on each of the longer sides that slope into the pond. The slope is from an arbitrary 30.5 m (100 ft) elevation at the pond's surface to 40.2 m (132 ft) to the northwest and 34.1 m (112 ft) to the east. A small wetland area is located at the upper end of the pond. Soil textures were sandy loam and loamy sand and additional soil information is found in Table 2. Crops in the last three years preceding the study have been limited to corn. Based on landowner information, the following pesticides have been used during the last three years: atrazine, Basagran, Dyanap, Lannate, Manzate, Nudrin, parathion, Prowl, Pydrin, Temik, and Vernam. According to the landowner, endosulfan had not been used for at least 3 years preceding the application phase of the study. Preliminary analyses of the soil showed no pesticide residues remained. Fertilizers have been applied at 500-700 lb/acre for corn. The pond is not an irrigation source for the adjoining fields.

Water quality data were typical of soft water aquatic systems and suggest no conditions acutely restrictive to most aquatic biota.

A moderately dense largemouth bass population was characterized by large individuals, with no smaller-sized individuals collected. While this indicates a possible imbalance in the top predator population, the bluegills exhibited a desirable size structure, suggesting good predatory control. A fair-to-poor largemouth bass-bluegill balance was indicated.

A rich benthic fauna consisting of species indicative of well-oxygenated bottom conditions was observed in the center and littoral zones. While zooplankton densities were somewhat low in the spring, a moderate phytoplankton density suggests that productivity was not limiting.

4. Pond T-4-1

Pond T-4-1 was another site for the field study of aquatic effects of endosulfan (Figure 12). This pond was later selected as one of two reference ponds; the other reference pond was M-55-4. This pond is in Thomas County, Georgia, immediately southeast of Mitchell County (see Figure 1). The pond surface at T-4-1 has 1.0 hectares (2.5 acres) and a watershed of 15.1 ha (37.4 acres) (Figure 13). The ratio of water to land is about 1:14. The greatest depth is about 1.2 m (4 ft), in front of the dam. Approximate depth measurements for pond T-4-1 are shown in Figure 14. T-4-1 was created about 1965 by damming a small intermittent stream. This pond is triangular in shape, with fields that slope into the pond on each side. One field is 6.9 ha (17.0 acres), and the other is 5.0 ha (12.3 acres), with a fence separating it from a third field of 3.3 ha (8.1 acres), for a total of 15.2 ha (37.4 acres). The layout of the pond and fields are shown in Figure 13. The slope is from an assumed 30.5 m (100 ft) at the pond surface to 36.6 m (120 ft) in the far western and eastern ends of the watershed. Soil textures were sandy loam and loamy sand and additional soil information is found in Table 2. Crops in the past three years for the fields joining the pond have been soybeans and corn. The landowner used atrazine, Balan, Bravo, Dinitro, Gramoxone, Lasso, Sencor, Temik, Treflan, and 2,4-DB on these crops. According to the landowner, endosulfan had not been used for at least three years preceding the application phase of the study. Preliminary soil analyses showed toxaphene present in the northeast portions of the area for study but no other pesticides were found. The pond is not used for irrigation.

Soft water conditions were indicated by the water quality parameters measured. Preliminary data suggested some instability in the largemouth bass population, with no evidence of smaller individuals. Measured bluegill density was high and individual sizes were small because of the weak cropping pressure from largemouth bass. A poor largemouth bass-bluegill balance was indicated.

A rich benthic fauna consisting of species indicative of well-oxygenated bottom conditions was observed in the center and littoral zones. High densities of phyto- and zooplankton were encountered, reflecting high production. These results may be partly the result of reconnaissance sampling being conducted later in the season than at most of the other ponds.

5. Summary

Each pond was briefly characterized in the site selection phase and more exhaustively characterized in subsequent phases. C-27-1 and M-55-8 were selected as the treatment ponds, with M-55-4 and T-4-1 serving as the reference ponds. This decision was based on such criteria as pond similarity, watershed topography conducive to runoff, and reliability of auxiliary water sources.

IV. SITE PREPARATION

After the leases for the four ponds and adjoining land were secured, several pond improvements were made. Some ponds required the removal of vegetation and reinforcement of the earthen dam spillways. Flumes were installed at the two treatment ponds (C-27-1 and M-55-8). Before the tomatoes were planted, an irrigation trial run verified that water runoff was achievable. About 41 ha (102 acres) of tomatoes were planted in the four watersheds following normal agronomic practices. Finally, permanent sampling locations were installed at M-55-8 and at C-27-1, as well as a weather station at each treatment site. This section provides more details on these site preparations.

A. Land Improvements

Removal of undesirable vegetation improved the watershed. Initial preparations included mowing and burning around each pond, as well as removing trees, brush, and other debris. These modifications were conducted to improve runoff into the ponds and to promote the movement of drift to the pond surface. Care was used to ensure that normal runoff was maintained except for the flume areas as discussed in Section B.

Modifications to the dam and spillway at M-55-8 improved water retention in the pond. The dam required reinforcement because it was determined that the dam's pond spillway might not withstand the excessive rainfall often encountered with heavy rains. The dam was strengthened by adding soil to weak areas and packing it with a bulldozer. The spillway was strengthened by placing cement-filled burlap bags behind the existing concrete structure. In neither case was the soil or cement placed in contact with the water in the pond, nor was the water in the pond disturbed by the process.

B. Flume Installations

Flumes at the two treatment sites permitted runoff monitoring and sampling runoff for endosulfan. A topographic reconnaissance was conducted to determine what size flumes would be necessary to handle runoff from the M-55-8 and C-27-1 locations. General recommendations included the location of the flumes and methods of diverting runoff into the flumes. Runoff from 3.6 ha (9 acres) at C-27-1 and from 0.9 ha (2.3 acres) at M-55-8 were diverted into the flumes. Diversions were achieved by cutting trenches sloping to the flumes. The depths of the trenches were adjusted as soil terrain changed. The location of the flumes and drainage areas are indicated on the survey map shown in Figures 19 and 21.

L. M. Leathers & Sons of Athens, GA, constructed a 0.45-m (1.5-ft) stainless steel H-flume with a stainless steel stilling well and approach section for the M-55-8 site and a 0.61-m (2-ft) stainless steel H-flume with a stainless steel stilling well and approach section for the C-27-1 site.

The flume was installed at M-55-8 in March 1988. A hole was dug at the flume site and the flume was leveled in the hole to allow natural flow of the water through the flume. The flume was then reinforced with posts set in concrete to avoid movement during runoff. In front of the flume, at the approach section, a concrete slab was poured to prevent the water from washing under the flume. Two 2.4-m by 1.2-m (8-ft by 4-ft) metal sheets 6.3 mm (0.25 in.) thick were placed at the mouth of the trenches and connected to the approach section of the flume to divert the water into it. Small rocks were placed in front of the concrete slab to impede sediment deposition in the flume.

At the rear of the flume, a trench leading to the pond was dug. Three 6-m (20-ft) sections of 0.45-m (18-in.) galvanized pipe were placed in the trench to divert the water into the pond. Approximately 3 m (10 ft) from the end of the last section of pipe and approximately 6 m (20 ft) from the pond, a triangular baffle consisting of two 1.2-m (4-ft) long "5 cm by 10 cm" (2 in. by 4 in.) boards was installed to spread the flow of water before it entered the pond. This was done to more closely approximate natural runoff into the pond. A 1.2-m by 1.2-m (4-ft by 4-ft) platform was erected near the stilling well to house an Isco automatic sampler used to collect samples of runoff water. A water level recorder was mounted on the stilling well to measure water level in the flume during runoff events. Pictures showing various aspects of the flume installation are shown in Figures 15 and 16.

A flume similar to that at M-55-8 was installed at the C-27-1 site in mid-April, 1988. This flume also included an Isco water sampler and a water level recorder. Only one 6-m (20-ft) section of 0.45-m (18-in.) galvanized pipe was placed at the rear of the flume because the flume was only 12 m (40 ft) from the pond. Also, instead of a wooden baffle like that at M-55-8, trenches were cut behind the flume to divert the water into the pond. As at M-55-8, the larger rocks were used in the flume approach area at C-27-1.

C. Trial Run

A "trial run" established that water would pass through the flume, but also pointed to the need for drainage modifications. On March 15, 1988, (week 63), a trial at the M-55-8 site demonstrated that runoff could be achieved with irrigation and that the flume complex was satisfactory. However, sediment deposition in the flume interfered with water flow and operation of the water level recorders on the stilling well, as well as sampling apparatus of the automatic sampler. Discussions among project members and subsequent consultation with Dr. Earl Grissinger (USDA Sedimentation Laboratory in Oxford, Mississippi), resulted in the placement of more and larger rocks at the approach to allow the flume to function. Also, the length of the boards on the baffle at the rear of the flume was increased from 1.2 m to 3.0 m (4 ft to 10 ft). These drainage modifications were found to be effective after other runoffs occurred. Because the trial run was successful at M-55-8, it was assumed that the flume would work at C-27-1 and this assumption proved correct.

D. Tomato Production

Tomatoes were planted in the four fields described above. General production practices for bare-ground, non-bedded tomatoes grown in southwest Georgia were used for all four locations. The soil was turned to an approximate depth of 25 cm (10 in.) with a moldboard plow in February. Other activities followed as described in this section.

Applications of herbicides and fertilizers constituted additional normal tomato management activities. One to two weeks prior to planting, fields were treated with 2.3 Liters of Treflan and 0.37 kg of Lexone DF per ha (one quart and 0.33 lb/acre) preplant. The chemical supplements were incorporated with two-disc harrowing to a depth of 5 to 8 cm (2 to 3 in.) for weed control. Manufacturers' sprayers were calibrated as outlined in HASLI Standard Operating Procedure (SOP) No. 88-3 (Appendix C). Application dates were:

C-27-1	April 4, 1988	Week 65
M-55-4	March 29, 1988	Week 65
M-55-8	March 28, 1988	Week 65
T -4-1	April 2, 1988	Week 65

Just prior to or at planting, 1500 lb per acre of fertilizer (5-10-15 or equivalent) was applied. An additional 500 lb per acre was applied to the tomatoes as a layby application three to four weeks after planting. The application dates for the at-planting fertilization were the same as the planting dates shown below. The dates for the lay-by application were:

C-27-1	May 2, 1988	Week 70
M-55-4	April 28, 1988	Week 69
M-55-8	April 25, 1988	Week 69
T-4-1	May 4, 1988	Week 70

The Floragard variety of tomatoes was planted in early April. The Floragard variety of tomatoes used in this study were grown by the Mobley Plant Company in Moultrie, Georgia. This variety is well adapted for growth in southwestern Georgia. Being somewhat resistant to various leaf diseases of the region, they require minimal fungicide applications. No fungicide applications were actually required during the study. Suggested planting dates in the protocol were from April 1, 1988 to April 15, 1988, depending upon the weather. Actual planting dates were:

C-27-1	April 5-10, 1988	Week 66
M-55-4	April 5-6, 1988	Week 66
M-55-8	April 4-5, 1988	Week 66
T-4-1	April 4-6, 1988	Week 66

Forty-one hectares (102 acres) of tomatoes were planted (Figure 17). The two treatment watersheds consisted of 14 ha (35 acres) at C-27-1 and 8.5 ha (21 acres) at M-55-8. The two reference watersheds consisted of 8.5 ha (21 acres) at M-55-4 and 10 ha (25 acres) at T-4-1. The tomato field: pond area ratios (based on maximum pond area) were 10.6:1 for C-27-1, 10.0:1 for M-55-4, 9.4:1 for M-55-8, and 9.6:1 for T-4-1.

Tomatoes were planted on both sides of all ponds. Some tomatoes were planted approximately 5 m (15 ft) from the ponds to satisfy the worst case conditions. Although the endosulfan label for tomatoes specifies a buffer of 91 m (300 ft), HCC and U.S. EPA agreed that a worst-case situation would be used. (The pond edge was that of mid-April 1988.)

Planting of 16 additional rows of tomatoes occurred on the south side of M-55-8 on May 10, 1988. When the majority of the tomatoes were planted on April 4-5, 1988, the soil near the pond was too wet to allow planting. The 16 rows, representing 37 m (120 ft) of tomatoes, decreased the distance between the tomatoes and the pond's edge to represent a worst-case situation. The distances between the edge of the tomato field and the edge of the pond are provided in Table 4.

Plant and row spacing followed normal agricultural practices. Tomatoes were planted in 1.8-m (6-ft) rows, with a spacing of 61 cm (24 in.) within each row, for a total of approximately 8900 plants per hectare (3600 plants/acre) (Figure 18). To allow for conventional ground-spraying of the tomatoes, every fifth row was not planted. This is standard practice for tomato production in the southeastern U.S.A. Water was applied at in the tomato rows at the rate of 3700 to 4700 L/ha (400 - 500 gal/acre) at the time of planting to ensure a good stand. Tomato rows were parallel to the edges of ponds, with one exception at the C-27-1 site, where tomatoes planted on the far southeastern side were planted with rows perpendicular to that edge of the pond. C-27-1 was planted in this manner using standard cultural practices to provide sufficient row length for cultivation and spraying by the farm equipment used.

Figures 19 through 22 show the location and extent of the tomato fields for each pond site. Note the proximity of the tomato fields to the edge of the pond.

Additional agricultural practices were followed during the growing season. Tomatoes were cultivated twice to facilitate weed control. A lay-by application of fertilizer was made at each location with the first cultivation. Cultivation dates were:

Site	1st Cult.	Week	2nd Cult.	Week
C-27-1	May 2, 1988	70	May 21, 1988	72
M-55-4	April 28, 1988	69	May 9, 1988	71
M-55-8	April 25, 1988	69	May 9, 1988	71
T-4-1	May 4, 1988	70	May 9, 1988	71

The second cultivation at C-27-1 was delayed because of wet fields from the May 10, 1988, storm. No cultivation occurred after the applications.

E. Permanent Sampling Locations

This section presents facts about three types of permanent sampling locations: (1) six pond zones numbered 1 through 6 were established for each pond; (2) six field transects also numbered 1 through 6 were established for the field surrounding the ponds; and, (3) 10 to 20 (depending on the size of the tomato fields) additional stations were established for various sample collection purposes. The following narration briefly explain each type of sampling location. Details about the nature and schedule of activities at each location are presented in the study methods section.

The six pond sampling zones established were marked by floats for biological sampling devices. Sampling for water, sediment, and biological purposes occurred at the sampling zones shown in Figure 23.

Markers designated six soil and foliage field sampling transects for the agricultural fields. Fields on each side of the ponds were divided into three zones. Transect markers were established from opposite corners across each transect as to create a zig-zag sampling pattern in the fields along with the application and drift card stations (Figures 24-29). Collection of foliage and soil samples occurred along these transects.

Application card platforms placed on the soil in the tomato fields collected endosulfan spray. Ten stations, each consisting of three platforms, were placed in the fields for each tank mix necessary for the endosulfan application. Ten stations (one tank mix) were located in M-55-8 fields; however, 20 stations (two tank mixes) were necessary for C-27-1. Stations were distributed throughout the fields (see Figures 24 and 27) and marked with wire flags. At each station, one platform was located in the middle of a row, one at the edge of a row, and one midway between two rows.

Thirty-five platforms to collect endosulfan drift were placed at the field and pond perimeters and pond surface. Fifteen monitoring stations for endosulfan drift were located along the outer perimeter of the treated fields (Figures 25 and 28). At each station, one stake for a horizontal drift card platform and another stake for a vertical drift card platform were driven into the ground. Ten stations were also located at the pond edge. Each station consisted of three adjacent stakes, each with a horizontal platform for attachment of absorbent cards. The cards collected endosulfan drift from the fields.

Ten additional drift stations were located in the middle of each treated pond, with posts supporting horizontal platforms located just above the water's surface (Figures 25 through 28). Each horizontal platform contained three absorbent cards for monitoring endosulfan drift onto the pond surfaces.

F. Weather Stations

A rain gauge mounted on a steel post was placed in the agricultural field to record precipitation at each study site. A wind speed and direction recorder at each treatment site measured wind conditions continuously during endosulfan application. A 10 cm by 10 cm (4 in. by 4 in.) wooden post extending 3 m (10 ft) above the ground was erected at C-27-1 and M-55-8 for attachment of the wind speed and direction recording instruments. No wind recording stations were present at M-55-4 and T-4-1, but as stated above, rain guages were present at these two sites.

G. Depth Markers

A permanent depth marker was installed in each pond. The depth marker was used to sight the level of the water to the nearest centimeter.

V. STUDY METHODS

The study methods section is divided into five subsections. Subsection A focuses on the application procedures for Thiodan 3EC, with emphasis on calibration, cleaning, tank mixing, and actual field application of approximately 3.1 L/ha (1.33 qt/acre). Irrigation equipment and its set-up and operation are covered in subsection B. Subsection C covers endosulfan sampling and analysis methods. Subsection D provides a concise digest of ecological methods, with presentations on sample collection, sample analysis, data analysis, and any technical constraints. Subsection E presents the statistical models used in data analysis. Subsection F deals with quality assurance activities. For scheduling and communication purposes, weeks were numbered sequentially beginning with the week of January 4, 1987 being week 1.

A. Endosulfan Applications

Endosulfan, as the active ingredient (a.i) of Thiodan 3EC manufactured by FMC Corporation, was used in the application. The EPA registration number is 279-2924 for FMC Corporation. Thiodan 3EC contains three pounds of endosulfan per gallon of product (3.0 lb endosulfan/gal based on 33.7 percent a.i. per gallon Thiodan 3EC at 8.896 lb/gal. The certificate of analysis and additional information are provided in Appendix D.

The endosulfan formulation was applied three times to the non-bedded tomatoes in a methodical manner. The calibration of the nozzles and other equipment followed rigorous procedures, as did cleaning and tank mixing. Careful computations were made of the amount of endosulfan in the tank mix at each of the two treatment sites.

Endosulfan was ground-applied using a tractor-pulled sprayer. A new pull-type vegetable crop sprayer equipped with hydraulic booms, bypass agitation, a Hydro 1500 series roller pump, and nozzles on 1.8-m (6-ft) spacings was used for all endosulfan applications. The sprayer was equipped with three hollow-cone TX12 nozzles directed to each row, with two of the nozzles extended as drops and one nozzle directly over the row. Demarcations were made in 10-gallon increments on the tank using a Fil-rite meter.

1. <u>Calibration, Cleaning and Tank Mixing</u>

Calibration of equipment and mixing of Thiodan 3EC for application followed HASLI SOP No. 88-3 (Appendix C), and was based upon the 1/128 acre method of calibration (North Carolina State University, 1975). This procedure considers distance, time, and quantity of sprayed materials. Prior to each endosulfan application, the time in seconds required to drive the distance determined by the formulae described in the SOP was established. The average of three trial times was used. Pressure settings on the sprayer were adjusted to collect the amount of liquid required to deliver the desired gallons per acre. Each nozzle was checked individually. If deviations of greater than 5 percent were found, the nozzle, strainer, and line were checked or exchanged until uniformity was established. A 20-second sample collection time was used to validate calibration. HASLI SOP titled "Specific calibration procedures for Southern AG Fiberglass and equipment...." is in Appendix C.

The spray tank lines and nozzles were cleaned after each application according to HASLI SOP No. 88-6 (Appendix C).

Tank mixing of endosulfan occurred immediately prior to application. HASLI SOP No. 88-11 (Appendix C) for tank-mixing liquid pesticides was followed for all endosulfan applications. After calibration of the sprayer with water, 760 to 950 L (200-250 gal) of water was added to the sprayer tank, and the pump and bypass agitation mechanisms on the sprayer were engaged. The 2.5-gal containers of Thiodan 3EC, which had been stored at Hickey Agri-Services Laboratory, Inc. chemical storeroom at 19° C to 33° C (66° F to 91° F) were vigorously shaken and poured into the sprayer tank. The containers were rinsed three times and the rinsate was poured into the tank. The amount of Thiodan 3EC and total volume of spray prepared for each location including an extra 10 percent to ensure adequate volume, were:

Location	Gallons of Thiodan 3EC	Gallons of Spray
C-27-1 W	6.25	375
C-27-1 E	6.25	375
M-55-8	7.5	450

Water was added to bring the sprayer to desired volume. The spray tank was agitated for at least 15 minutes before application and agitation was maintained until application was complete.

2. Field Spraying

Dose control followed a rigorous procedure. A tractor was used to pull the sprayer through the fields and power the sprayer's pump. The tractor (Figure 30) was operated at a ground speed of approximately 7.1 km/hr (4.4 mph), while operating at the manufacturer's suggested engine speed of 1500 rpm. At 1500 rpm, 7.1 km/ha (4.4 mph), and a sprayer pump pressure of approximately 690 kPa (100 PSI), the sprayer delivered approximately 187 L/ha (20 gal/acre) of Thiodan 3EC. The sprayer was set up for row applications. To account for the skip row, 187 liters of spray were applied to a given hectare of land (20 gal/acre). For example, at the M-55-8 location, there were 8.5 ha (21 acres) of land planted in tomatoes in the two fields surrounding the pond. Approximately 1590 L (420 gal) of solution containing 26.2 L (27.9 qt) of Thiodan 3EC was applied at each application on this 8.5 ha (21 acres) of land.

Thiodan was applied three times to each of two study sites (Figure 31). For the first application, C-27-1 and M-55-8 were both sprayed on May 27, 1988. The second application was conducted on June 10 and 11 for C-27-1 and M-55-8, respectively. The third application was on June 23 at M-55-8 and on June 27 at C-27-1. (The need at M-55-8 to set up irrigation equipment, irrigate for field runoff, disassemble the equipment and move it to C-27-1 for irrigation there required three days, preventing endosulfan application on consecutive days at the two sites). After each application, the approximate volume of spray mix left in the sprayer was recorded, and the remaining spray mix was applied to adjoining agricultural land outside the watershed area of the test ponds. The dates of application, total spray mix volume prepared, number of acres sprayed, and spray mix remaining in the sprayer after application are shown in Table 5.

Thiodan was applied at approximately 3.1 L/ha (1.33 qt/acre) as specified by the protocol. The endosulfan application rate was calculated by dividing the amount of material actually sprayed (total spray volume minus spray remaining in the tank) by the acres sprayed. This computation provides the gallons of spray applied per acre (gpa). The gpa applied, multiplied by the endosulfan concentration per gallon (1.33 qts divided by 20 gal = 0.0665) gives the actual amount of endosulfan applied per acre. For example, at M-55-8 on May 27, 1988, 415 gal were sprayed (450 - 35 = 415) on the 21 acres, for an application rate of 19.76 gal/acre or 184.8 L/ha. 19.76 gal/acre times the endosulfan concentration per gallon factor of 0.0665 yields an actual endosulfan rate of 1.31 quarts per acre.

Only minor travel time and equipment adjustments occurred during the field spraying. At the C-27-1 site, there was a natural drainage area that had eroded during the rainstorm of May 10, 1988. The tractor speed was reduced in order to safely cross it. When approaching this area, the sprayer was turned off for a distance of 3 m (10 ft) on both sides of the wash. This practice was also conducted when crossing the trenches cut to divert water into the flumes at the C-27-1 site and some areas of M-55-8.

On three occasions, minor problems were experienced with nozzle clogging or hose clamps slipping during the endosulfan application. When the problem was encountered, a new calibrated nozzle and/or drop nozzle replaced the defective one, and spraying was continued with less than two minutes of lost time. The only problem that resulted in a longer down-time during spraying was at the C-27-1 site on the last application date, June 27, 1988. The power take-off hose from the pump to the spray tank developed a leak. The sprayer and all valves were immediately shut off, the sprayer was moved to the end of the field, and repairs were made. Entire down-time was approximately 20 minutes. Such problems typically occur during spraying operations, and had no relevant effect on the study.

B. Irrigation Systems

The potential for drought conditions existing during the application phase of the study and this situation necessitated access to reliable water sources for irrigation. Two ponds near the M-55-8 site were used as water sources. One was the small pond located across the road, approximately 90 m (300 ft) west of the tomato field. The second pond was located approximately 0.4 km (0.25 mi) north of the tomatoes on the north side of the site. At the C-27-1 site, a pond located approximately 0.8 km (0.5 mi) from the tomatoes was reserved to irrigate both sides of the site. Neither known runoff nor known drift of other pesticides occurred in the irrigation ponds during the study and land adjacent to the irrigation ponds was leased to ensure no crops were grown requiring pesticide applications.

1. Irrigation Equipment

Irrigation equipment included pumping units, irrigation pipes, flexible hoses, and traveling irrigation guns. Two pumping units, 2130 m (7000 ft) of 15-cm (6-in.) aluminum irrigation pipe, and two traveler irrigation units, each equipped with 200 m (660 ft) of flexible hose and a sprinkler unit, were used to carry water for irrigation to force runoff into the ponds (Figure 32). These irrigation units were large enough to deliver sufficient water (up to 7.6 cm, or 3 in. per acre) to induce runoff into the ponds.

2. Equipment Set-up and Field Irrigation

At 8:20 a.m. on June 24, 1988 (week 77) one day following the third endosulfan application at the M-55-8 site (June 1988), irrigation of the north side of the site was initiated. The two irrigation units were operated simultaneously in order to cover the entire area with one pass. The 1500 rpm setting, a pump pressure of 760 kPa (110 psi) on the power unit, and a gear setting of 1 on the traveling gun should have delivered 2.5 cm (1 in.) of water per acre. However, because of the distance the water had to travel through the pipes, final delivery was found to be approximately 1.3 cm (0.5 in.), as verified by rain gauges placed throughout the field. Settings were changed at 10:00 a.m. on the units to deliver 2.54 cm (1 in.) per acre. Upon rechecking with rain gauges, the rate of irrigation was found to be 2.4 to 3.0 cm (0.95 to 1.2 in). Adjustments were made to the units to deliver more water (gear setting on the traveler changed to 1.5), but runoff into the pond still did not occur because of terraces in the field which retained the water and diverted some runoff away from the pond. Despite this, the ground was saturated, and a rainstorm (described below) during the night following irrigation resulted in runoff into that side of the pond. Irrigation was completed to 5:00 p.m on June 24, 1988 (week 77). The irrigation units were then moved to the south side of M-55-8 and prepared for irrigation the following morning (June 25, 1988; week 77). The units were geared down to increase irrigation. Irrigation was unnecessary, however, as sufficient rainfall (3.81 cm, or 1.5 in.) occurred overnight to produce a natural runoff event. Thus, runoff was brought about by a rainstorm after the third endosulfan application at M-55-8.

Irrigation from the nearby pond produced runoff after the third endosulfan application at C-27-1 (Figure 33). Irrigation of the east side of C-27-1 began at 9:30 a.m. on June 28, 1988 (week 78). Irrigation began approximately 20 hours after the third endosulfan application was completed. Problems were encountered with leaking pipes and engine problems with one of the power sources. Irrigation was stopped at 9:35 a.m. and reinitiated at 5:50 p.m. Both irrigation units were operated simultaneously to obtain maximum coverage. However, approximately one acre of tomatoes on the extreme eastern side received no irrigation because of the shape of the field and the water pattern restrictions of the irrigation units (straight line pulls only). Settings of 2100 rpm, pump pressure of 760 to 860 kPa (110 to 125 psi), and a gear setting of 2.5 on the traveler unit gave 2.0 to 3.0 cm (0.80 to 1.20 in.) of irrigation water, as verified by rain gauges placed throughout the field. The variation in amounts of irrigation was caused by the traveling guns gaining speed on the downward slopes of the field. The higher speeds yielded lower amounts of water. However, once initiated, runoff continued for several hours. Irrigation of this site was completed at 1:50 a.m. on June 29, 1988 (week 78). Runoff into the pond was still evident at daybreak.

The irrigation system was also set up on the west side of C-27-1 on June 29, 1988. Because of the configuration of the field, only 13 of the 17.5 acres could be irrigated. Major emphasis was placed on obtaining runoff from within the 4.7 ha (11.5-acre) flume area. Irrigation was initiated at 12:40 p.m. The speed and pressure settings on the power units and the gear setting on the traveling guns were the same as those used on the east side. Measured amounts of irrigation varied from 1.9 to 2.8 cm (0.75 to 1.1 in.). During the irrigation process, approximately one-half of the water from the flume area overflowed the trench that had been constructed to direct the water into the flume. The water that overflowed the trench flowed into the pond by way of a natural drainage ditch in the field located 4.5 to 6 m (15 to 20 ft) downslope from the trench. Irrigation of the site was completed at 7:00 p.m.

C. Endosulfan Sampling and Analysis Methods

Various types of residue samples were collected to characterize the endosulfan isomers and metabolites present at various locations in the watershed system. Samples were taken following ground applications of the commercially-formulated product. These included mixed pesticide spray before and after application, sprayed endosulfan, endosulfan drift, dislodgeable residue from sprayed tomato leaves and residue in soil, field runoff water, pond water, pond sediment, and fish.

Endosulfan sampling followed a prescribed schedule. Sample collection occurred one or two days before each application, usually within 24 hours following each application, and approximately 7 ± 1, 14 ± 3, 28 ± 3, 60 ± 3, 90 ± 3, and 180 ± 3 days following the final (third) application as prescribed in the protocol. A few days of leeway was incorporated into the post-application schedule to accommodate inclement weather or other sampling conflicts. For data presentaion purposes and to minimize confusion, endosulfan residue sample intervals were numbered from the time of the first application, which is referred to as Day 0.

Stringent safety rules were followed to protect workers from exposure to the endosulfan. Battelle workers entering the sprayed area within 24 hours of spraying were required to wear disposable Tyvek(R) suits, impermeable boots and gloves, and full-face respirators. Battelle workers on the pond and at the field perimeter wore respirators, rubber boots, and coveralls on the day of spraying. In addition, rubber boots were required in the fields for a period of five days after each application. After each exposure, boots and respirators were washed with methanol, coveralls were laundered and reused, and Tyvek suits were disposed. Details are found in Appendix C, SOP EEF-C-43-01.

1. Quality Control

Various types of field blank samples accompanied selected field samples. Field blank samples of soil, foliage, application/drift cards, sediment, and fish to be analyzed were obtained prior to the first endosulfan application (cards, soil, pond water, sediment, fish) or from untreated areas (soil, foliage, pond water, sediment, and fish) and submitted to the analytical laboratory along with residue samples and field spiked samples. In addition, rinsate blanks (samples of rinse water from the field equipment cleaning process) and trip blanks (blank media samples that accompanied sample containers into the field and back to the laboratory) were utilized occasionally.

Field spike samples also accompanied all field samples. Ampules of technical grade endosulfan with known concentrations of α-endosulfan β-endosulfan and endosulfan sulfate (hereafter referred to as endosulfan) were supplied by Hoechst AG Frankfurt to spike blank field samples. The ampules were broken into measured amounts of blank samples, and the spiked samples were shipped, stored, and analyzed with field residue samples. Ampules were available for the following matrices: pond water, run-off water, soil, sediment, fish, drift and application cards, and foliage rinsate. Endosulfan recoveries were used to assess pesticide residue stability in the various matrices. Details on preparation of spiked field samples are found in Appendix C, SOP EEF-C-45-02.

Both field blanks and spikes and laboratory blanks and spikes were analyzed with the same procedures used for the residue samples. The analytical method for each matrix is described in the sections that follow. Alpha, beta, and sulfate forms of endosulfan were analyzed and used to compute a total endosulfan concentration.

Each sample was logged into a computerized sample tracking system upon receipt at the Columbus laboratory. Chain of custody was maintained for each sample throughout the sampling, shipping, and analysis stages.

Aluminum and glass containers used to hold field samples were washed with detergent, followed by a methanol rinse, then with rinses of distilled water. For glass containers, the methanol rinse was preceded by 10 to 20 percent hydrochloric acid rinse. Aluminum foil envelopes were double-rinsed with methanol. Only aluminum sample containers were reused. These were rewashed as stated above and further described in Appendix C, SOP EEF-C-55-01.

2. Tank Mix and Sprayate Samples

Tank mix and sprayate samples were taken from each tank mix. For pond M-55-8 there was one tank for each of three application. For pond C-27-1 there were two tanks for each of three applications. The tank mix and sprayate samples were broken during transport to Hoechst AG in Frankfurt, Germany. Although analyses were attempted (see method for tank mix and sprayate in Appendix C), the results were highly variable and considered invalid. The application card and soil samples analyses confirmed that overall spraying and application rates were acceptable.

3. Application Cards

Absorbent cards of Whatman filter paper pinned to ground-level stations in the fields collected direct endosulfan sprayate during the applications. For each tank mix of spray, 10 sampling stations were placed in the fields (i.e., 10 stations at M-55-8 and 20 at C-27-1, where two tanks were needed; see Figures 24 and 27); three sampling platforms were placed at each sampling station. Each horizontal platform surface was approximately 4 in. above the soil surface and covered with aluminum-covered foam. At each station, one platform was placed on the center of the tomato row, a second was placed at the edge of the row, and a third platform was located between two rows. All platforms were within 2 m (7 ft) of each other. Immediately before each application, all platforms were covered with an additional layer of methanol-washed and fresh aluminum foil and a 10 cm by 10 cm (3.9 in. by

3.9 in.) absorbent card was pinned to the washed surface with stainless steel pins. Upon completion of spraying, the cards were removed (Figure 34). All cards at a station were combined into an aluminum foil envelope that was placed in a plastic bag and then placed on dry ice. Samples were transported frozen to the field laboratory in Pelham, Georgia, placed in freezers, and shipped frozen to the analytical laboratory. Details are found in Appendix C, SOP EEF-C-47-02.

The application cards from each station were analyzed with a detection limit of 0.1 μg total endosulfan (extrapolated to $\mu g/m^2$). In the analytical laboratory, each set of three combined cards was placed in 0.9-L (1-qt) wide-mouth bottles and extracted by tumbling with 400 mL of methylene chloride for 30 minutes. A portion of the methylene chloride extract was concentrated solvent exchanged to hexane, and analyzed by capillary column gas chromatography using an electron capture detector. Specific details are presented in Appendix C, SOP 50-113-01.

4. Drift Cards

Endosulfan spray drift was monitored using absorbent cards at the field edges, pond edges, and pond surfaces. Fifteen stations were located at the outer edge of the fields, 10 stations were located at the pond edges, and 10 were located at the pond surfaces (see Figures 25 and 28). Each field perimeter drift station consisted of a 30 cm by 30 cm (12 in. by 12 in.) vertical and horizontal platform located approximately 36 in. above the ground. The horizontal and vertical platforms were 30 to 60 cm (1 to 2 ft) apart, and approximately 6 m (20 ft) from the field edge. Each pond-surface and pond-edge drift station consisted of three 30 cm by 30 cm (12 in. x 12 in.) horizontal platforms. Platforms at the pond edge were 60 cm (2 ft) above the ground, whereas those in the pond were about 16 cm (6 in.) above the water's surface when initially installed. Any one of the three horizontal platforms was approximately 90 cm (3 ft) from the other two platforms. Immediately before endosulfan applications, the platforms were covered with an additional layer of methanol-washed and fresh aluminum foil and 20 cm by 25 cm (8 in. x 10 in.) absorbent cards were pinned to each platform. Beginning 30 minutes after completion of spraying, the cards from each station were folded with tongs, inserted into a single aluminum foil envelope, placed in plastic bags, and placed on dry ice in the field. The samples were transported frozen to the laboratory in Pelham, Georgia, placed in a freezer, and shipped frozen to the analytical laboratory. Details are found in Appendix C, SOP EEF-C-47-01.

The drift cards from each station were analyzed together to a detection limit of 0.1 μg total endosulfan (extrapolated to $\mu g/m^2$). They were placed in a 1-quart wide-mouth bottle and extracted by tumbling with 400 ml of methylene chloride for 30 minutes. A portion of the methylene chloride extract was concentrated, solvent exchanged to hexane, and analyzed for endosulfans by capillary column gas chromatography using an electron capture detector. Specific details are given in Appendix C, SOP 50-113-01.

5. Foliage

Collection of tomato foliage samples for dislodgeable residue followed each application. Forty leaf discs, each 2.5 cm^2 in area, were collected from each of six transects across the fields (see Figures 24 and 27), for a total of 240 leaf discs. Collections were made directly into a container attached to a leaf punch. The leaf discs were rinsed with a standard surfactant accepted generally for determination of dislodgeable residues. The rinsate was poured into aluminum bottles for later analysis. Samples were kept frozen for transport and storage until analysis. Details are provided in Appendix C, SOP EEF-C-52-02.

Foliage rinsate was analyzed to a detection limit of 1.0 μg total endosulfan (reported as $\mu g/m^2$). Approximately 300 ml of the foliage rinsate was extracted by tumbling with hexane in a bottle for 30 minutes. A portion of the hexane extract was dried over sodium sulfate and analyzed for endosulfans by capillary column gas chromatography using an electron capture detector. Specific details are given in Appendix C, SOP 50-116-02.

6. Soil

Soil samples were collected one to two days before and one to two days following each endosulfan application and approximately 7 ± 1, 14 ± 3, 28 ± 3, 60 ± 3, 90 ± 3, and 180 ± 3 days following the final application. A variance of several days was incorporated into the sampling schedule to accommodate inclement weather or possible sampling conflicts. Samples were collected from three transects (1, 3, and 5) at the reference ponds and the six transects at the treatment ponds (see Figures 24, 26, 27, and 29). Core samples 5 cm deep were taken using a 1-in. diameter stainless steel coring device. Ten samples were taken at approximately equally spaced points along each transect and composited. They were frozen in washed aluminum containers and kept frozen for transport and storage until analysis. All sampling equipment was cleaned between sample transects by rinsing with methanol and water. Details are presented in Appendix C, SOP EEF-C-49-02.

Composite soil samples were analyzed to a detection limit of 10 µg/kg. Approximately 50 g of soil was serially extracted by tumbling with acetone. The acetone extract was partitioned against methylene chloride after addition of ten percent aqeous sodium chloride. Extract clean up was accomplished by silica gel absorption chromatography. The final extract was concentrated, solvent exchanged with hexane, and analyzed for endosulfans by capillary column gas chromatography using an electron capture detector. Specific details and examples are given in Appendix C, SOP 50-117-02.

7. Pond Water

Samples of pond water were collected one or two days before and the day of the pesticide applications and following a schedule of approximately 3 ± 1, 7 ± 3, 14 ± 3, 28 ± 3, 60 ± 3, 90 ± 3, and 180 ± 3 days after the last application (through December 1988). Samples were collected from zones 2, 4, and 5 in the reference ponds and all six zones in the treatment ponds (see Figure 23). Integrated water column samples were collected from the sampling zones using a pump with a stainless steel impeller and casing and Teflon tubing. The apparatus was cleaned only between each pond for the first application. The apparatus and tubing were cleaned with methanol, distilled water, and again with methanol between each of the six zones and between each pond for second and third applications. Water was transferred to aluminum containers containing phosphate buffer and frozen for transport and storage. Details are presented in Appendix C, SOP EEF-C-48-02.

One-liter pond water samples were analyzed to a detection limit of 5 ng/L. Water was extracted with methylene chloride using a separatory funnel, and the extract was concentrated to 1 ml after solvent exchange with hexane. The extract was analyzed for endosulfans by capillary column gas chromatography using an electron capture detector. Specific details and examples are given in Appendix C, SOP 50-115-01.

8. Runoff Water

Field runoff samples were collected by automatic samplers placed in the treatment pond flumes. Whenever rain was sufficient to cause runoff into the flume, samples were collected automatically. Isco water samplers were positioned above the sampling point in the flumes. When runoff water flowed through the flume, an actuator turned on the Isco sampler and water was pumped from the sampling point. The sampler program was set to collect sequential samples every 20 to 30 minutes. Additional details are provided in Appendix C, SOP EEF-C-40-01.

One-liter samples of runoff water were analyzed to a detection limit of 5 ng/L. The runoff samples were extracted with methylene chloride using an automatic separatory funnel shaker, and the extract was concentrated to 1 ml after solvent substitution with hexane. The extract was analyzed for endosulfans by capillary column gas chromatography using an electron capture detector. Specific details are given in Appendix C, SOP 50-115-01.

9. Sediment

Sediment (hydrosoil) cores for residue analysis were obtained (Figure 35) at the same scheduled intervals and locations as pond water. Cores were taken at zones 2, 4, and 5 in the reference ponds and at all six zones in the treatment ponds (see Figure 23). A 5-cm (2-in.) butyrate plastic cylinder was forced into the sediment and then carefully removed with the core intact. Water above the core was siphoned off and the top 5 cm (2 in.) of sediment placed in an aluminum container and frozen for later separation and analysis. Details are found in Appendix C, SOP EEF-C-44-01.

Sediment cores were analyzed for endosulfans with a detection limit of 5 µg/kg. Approximately 50 g of sediment was serially extracted by tumbling with acetone. The acetone was combined with salted water and extracted with three portions of methylene chloride. The extract was cleaned up by silica gel adsorption chromatography. The final extract was concentrated, solvent exchanged to hexane, and analyzed for endosulfans by capillary column gas chromatography using an electron capture detector. Specific details and examples are given in Appendix C, SOP 50-117-01. Absence of endosulfan was confirmed in selected sediment samples by performing the GC analysis using a column of different polarity from the primary column.

10. Fish

Regular electrofishing events yielded fish for residue analysis. They were collected on an approximate bi-weekly to monthly basis. Approximately 20 to 25 g whole bluegill and largemouth bass were retained from each pond when possible, frozen intact, and shipped to the analytical laboratory for analysis. On some electrofishing events, it was not possible to obtain both species of fish and fish of desired size (see report of deviation in Appendix E).

Fish were analyzed for endosulfan residue. Approximately 10 to 20 g of chopped fish tissue was macerated, combined with 50 g anhydrous magnesium sulfate, and extracted twice with methylene chloride using a polytron tissue homogenizer. The fish sample was extracted a third time by shaking the sample by hand with additional methylene chloride. The extracts were passed through an alumina column, concentrated, and passed through a gel permeation column prior to analysis by gas chromatography using an electron capture detector. Specific details and examples are presented in Appendix C, SOP 50-114-02.

11. Data Analysis

Data from each chromatogram were automatically collected by computer. Peak heights or peak areas referenced against an internal standard were used for quantification of the endosulfans. Analysis reports were generated by the computer, reporting concentrations of each endosulfan in the proper units (e.g., ng/L, µg/kg,). Each chromatogram and report were manually inspected for proper baseline placement, correct identification of the endosulfan peaks, and proper factors used for the calculation. These factors include weight of sample, dilution factor, and volume of final extract. Final data tables were generated by electronic transfer of data from the Laboratory Information Management System (LIMS) to spreadsheet and word processor for final formatting. Examples of the output are provided with the appropriate SOP in Appendix C.

D. Ecological Sample Collection, Sample Analysis and Data Analysis

Collections and analyses of samples and data followed standardized and accepted methods. Written standard operating procedures were consistently followed for field and laboratory procedures to ensure samples and data obtained were processed correctly. Standard operating procedures used are found in Appendix C,. When variations from the written procedures were necessary, they were described with explanation or documentation.

Ecological and water quality sample collection generally occurred on a biweekly schedule. Samples were collected every two weeks for the following parameters: phytoplankton, zooplankton, macroinvertebrates grab samples (Ekman dredge), macroinvertebrate artificial substrates, macroinvertebrate net sweeps, emergent insects, and the periphyton autotrophic index. In situ determinations for the pond metabolism and observations of macrophyte growth occurred on the same schedule, as did in situ pond water quality determination (pH, temperature, conductivity, and dissolved oxygen) and water quality determinations for alkalinity, acidity, hardness, turbidity, total nitrate nitrogen, ortho-phosphate, total organic carbon, and total suspended solids. For scheduling purposes, sample weeks were numbered sequentially beginning with the week of January 4, 1987 being week 1. For the baseline year (1987) samples were collected for the weeks of: May 3 (week 18), 17 (week 20) and 31 (week 22); June 21 (week 25); July 5 (week 27) and 19 (week 29); August 2 (week 31), 16 (week 33) and 30 (week 35); September 13 (week 37); October 1 (week 40) and 18 (week 42); and November 1 (week 44) and 15 (week 46); and December 6 (week 49). Treatment pond M-55-8 was not selected for study until July, 1987 and, was not sampled prior to the week of July 19 (week 29).

Sample collection for the treatment year (1988) occurred in the weeks of March 20 (week 64); April 17 (week 68); May 1 (week 70), 15 (week 72), and 29 (week 74); June 12 (week 76) and 26 (week 78); July 10 (week 80) and 24 (week 82); August 7 (week 84) and 21 (week 86); September 4 (week 88) and 18 (week 86); September 4 (week 88) and 18 (week 90); October 2 (week 92), 16 (week 94) and 30 (week 96); November 13 (week 98) and 27 (week 100), and December 11 (week 102). Although samples were collected for all sampling weeks, several samples collected were not analyzed (see report of deviation, Appendix). Of the thousands of samples, a few leaked, were broken, were lost or went unpreserved (see reports of deviation in Appendix E).

The following discussion presents, sample or data collection methods, sample processing methods, data analysis methods, and constraints. As was the case with methods for residue chemistry, there are more details on ecological methods in the respective SOPs.

1. Weather

Precipitation, temperature and cloud cover were recorded whenever sampling or related activity occurred. The time of day was recorded in military notation. The air temperature was directly measured with a thermometer. Cloud cover was noted and estimated as a percent. Precipitation was described by presence (i.e., yes or no) and intensity. Any precipitation in the rain gauge was recorded and the rain gauge emptied. Wind direction was determined using a compass. Wind speed was measured using a portable wind gauge or estimated (e.g., light, steady, gusting). Details are in Appendix C, SOP C-40-01.

Wind conditions during endosulfan applications were mechanically recorded. Wind speed and direction recorders at the study sites were activated at least 24 hours prior to scheduled applications and continued recording for at least 24 hours after completion of the applications. Precipitation and wind data were then tabulated. The data were plotted on an x, y coordinate basis with time and amount being the axes. National Weather Service data on precipitation, temperature, and solar insolation supplemented site specific data. Daily precipitation and temperature records for 1987 and 1988 were obtained from the Moultrie, Georgia reporting station. Moultrie is located east of the ponds, 8 km (5 mi) from the nearest pond (C-27-1) and 32 km (20 mi) from the farthest pond (T-4-1).

2. Runoff

Precipitation runoff volumes from the flume drainage areas on C-27-1 and M-55-8 were continually monitored with water level recorders installed on the stilling wells of the flumes. Recorders were set up for 1-week recording periods during the application and post-application phases and charts were routinely changed weekly May-December 1988.

The volume of water discharged through a flume during runoff events was derived by examining and interpreting the strip charts from water level recorders. The charts continuously recorded the head, or height of water, in the flume overtime. The runoff volume is a function of the head and the period of time over which it occurs.

Total runoff volume was calculated by dividing the runoff into segments of approximately constant slope for which the average head, in feet, and duration, in minutes, of each segment was determined. The average head was converted to units of cubic feet per second (cfs) using flume rating tables in US Department of Agriculture Agricultural Handbook 224 (USDA, 1979). The table for a flume 0.46 m (1.5 ft) deep was used for the M-55-8 Site data, and the table for a 0.61-m (2-ft-deep) flume was used for the C-27-1 site data. The cfs was multiplied by the duration in seconds (minutes x 60) of the time interval over which it occurred to give a volume in cubic feet for that time interval. The sum of discharge volumes for all of the curve segments is the volume of runoff discharged in the event.

3. Sedimentation

Sediment traps collected sediment for measurement of deposition rates in all ponds from May 5, 1988 through December 6, 1988. Sediment traps consisted of a grouping of three vertical PVC cylinders (16 cm diameter, 36 cm height) welded to a platform. Sediment traps were placed on the bottom of the pond in the vicinity of the six zones (see Figure 23) for periods ranging from two to ten weeks. The sediment collection periods in 1988 were:

1	May 21-June 3	to	June 16-17	weeks 72-74 to 75
2	June 16-17	to	July 5	weeks 76 to 79
3	July 8	to	August 10	weeks 79 to 84
4	August 11	to	October 26	weeks 84 to 95
5	October 27	to	December 6	weeks 95 to 101

To be effective the top of the sediment traps had to remain below the pond surface. Because of this common-sense restriction, some alteration (movement to deeper water) of sampling locations occurred due to pond level reductions.

Lowering a PVC plate over the trap before removal reduced sample loss. Sediment traps were gently retrieved and placed on shore for at least four hours to allow for settling (Appendix C, SOP EEF-C-50-02). Sediment was then pumped out of each of the three cylinders and composited for each station and sediment traps replaced on the ponds. Samples were later dried in an oven and weighed to determine the amount of sediment collected (Appendix C, SOPs EEF-56-01 and EEF-F-5-01).

4. Pond Level

Pond water levels were measured regularly when sampling occurred. Pond levels were read to the nearest 1 cm from a reference gauge in each pond.

Pond bottom profiles were mapped using a sonar device in April, 1988 when the ponds were full. Transects parallel and perpendicular to the dam were established and pond depths were recorded along each transect to the nearest 6 in. (15 cm). The transects measurements were converted to x, y, z coordinates in metric units. Using the survey maps, x and y coordinates were calculated for each pond perimeter. Approximate bottom contours were established using the x, y, z converted transect data.

5. Water Quality

Twelve water quality characteristics were routinely determined for each pond. The characteristics were determined once in each two-week sampling period in 1987 and 1988. Grab samples of pond water, often runoff from treatment ponds, were not analyzed for nitrates, orthophospates and total suspended soilds per the protocol (see Report of Deviation in Appendix E). Determinations were made according to established standard operating procedures (Appendix C).

Four *in situ* determinations at each pond were pH, temperature, conductivity (also known as specific conductance), and dissolved oxygen. The pond surface water pH was determined using a portable pH meter. Surface temperature was determined by a calibrated glass thermometer. Specific conductance was determined by a portable field meter. Dissolved oxygen content of surface water was determined by the membrane electrode method. Calibration of instruments was conducted before starting a series of pond measurements and instruments were rechecked upon completion of the series at each pond. Details of these procedures are presented in Appendix C, SOPs EEF-C-28-01, EEF-C-33-01, EEF-C-34-02, and EEF-C-58-02.

Water quality determinations included eight additional parameters. These parameters were alkalinity, acidity, hardness, turbidity, total nitrates, ortho-phosphate, total organic carbon and total suspended solids. Samples were collected in appropriate containers, preserved (see Appendix C, SOP EEF-C-38-01), placed on ice, and shipped overnight to the analytical laboratory.

Water quality determinations were made by Battelle's laboratories and by Stilson Laboratory using the following procedures: alkalinity by titration and reported in mg/L (as $CaCO_3$), acidity by titration and reported in mg/L (as $CaCO_3$), hardness by titration and reported as mg/L ($CaCO_3$), turbidity through use of a turbidimeter and reported in nephelometric turbidity units (NTU), total nitrates through use of ion chromatography (HPLC) or automated CD reduction reported as mg/L nitrate nitrogen, ortho-phosphate by the automated ascorbic acid method and reported as mg/L, total organic carbon by use of an organic carbon analyzer and reported as mg/L, and non-filterable residue (total suspended solids) by filtration and reported as mg/L. Procedures for these methods are presented in Appendix C, SOPs EEF-D-01-02, EEF-D-45-01, EEF-D-02-02, EEF-F-64-01, ASCC-50-103-02, EEF-D-46-01, EEF-D-15-1, EEF-D-44-01, and Stilson Laboratory Procedures.

Summary statistics and graphs provided temporal and spatial comparisons of the water quality parameters. Means, minimums and maximums were used to summarize the water quality data for each pond for each collection period. The summarized data (means) for each pond were then graphed over time to provide qualitative comparisons of water quality among the ponds.

6. Phytoplankton

Phytoplankton was collected from the water column using both water sampling bottles and pumps. From April to mid August 1987, samples consisted of a total of three 2.2-liter Kemmerer bottle samples from the surface, middle, and bottom depths of each zone. The three grabs were composited and a total of 6.6 L filtered through a 64-μm mesh net, and the residue preserved in formalin. Because these provided insufficient sample volume, a larger 8.2-liter alpha bottle was used beginning in late August of 1987 to sample the three depths. The samples were filtered through 20-μm mesh and the residue preserved in formalin. The different sample methods were overlapped to allow comparisons. In 1988 a pump was used to collect phytoplankton during the entire sampling session. The two methods of the previous season were also repeated occasionally for comparative purposes. The inlet of the pump intake hose was raised and lowered through the water column until 20 L (5.3 gal) were collected. The discharge was filtered through a 20 μm mesh plankton net. All 1988 samples were rinsed into sample bottles and preserved with Lugol's solution. Specific sampling procedures are presented in Appendix C, SOP EEF-C-16-01.

Major phytoplankton genera were identified and densities were calculated by volumetric estimation. Phytoplankton were generally identified to genus. If generic determinations could not be made, the specimens were identified to the next highest practical taxonomic level. Appendix C contains the SOP for phytoplankton sample analysis.

Graphs (X-Y plots and kite diagrams), summary statistics presence-absence and a diversity index were used to analyze spatial and temporal trends of phytoplankton taxa and relative abundance. The summary statistics were calculated from densities transformed using natural logs (see below). For the diversity index, the data were not transformed. The means densities of the major taxa and the mean diversity indices were then graphed over time to provide temporal comparisons of phytoplankton densities and diversity between the ponds. Presence-absence determinations were conducted on each taxa collected and noted whether the taxon was present or absent, and if present the number of samples containing the taxon, during each of the three time periods evaluated (pre-spray, post-spray and year-end; refer to Section E, General Statistical Procedures).

Nested ANOVA assessed spatial and temporal trends in phytoplankton densities and diversity of the (x number of) major phytoplankton taxa. The analysis followed a sequential pattern starting with (1) transformation of the density data using natural logs because count data (numbers per samples) are commonly log-normally distributed and the variances increased with increasing mean densities (Johnson and Leone, 1964) and (2) nested ANOVA to test for differences between each of the ponds for each period.

Data from different sampling methods in 1987 and 1988 were not converged. In 1988 data used in the analyses were from only one method, whereas in 1987 data came from several methods (see report of deviation Appendix E). Because the sampling method used for a particular sampling period was the same for all ponds, the comparisons using the ANOVA models would not be adversely affected by the several sampling methods. That is, in the ANOVA models, comparisons between the ponds were essentially done on a week-by-week basis; hence comparisons between ponds would always be done on data collected from the same sampling technique. Thus, comparability was achieved.

7. Zooplankton

Sampling bottles and pumps were used to collect zooplankton in the water column. From April to mid-August, 1987, 2.2-liter Kemmerer bottle samples from the surface, middle, and bottom depths of each zone were composited and filtered through 65 μm mesh net. Because these provided insufficient sample volume, a larger 8.2-liter alpha bottle was used, beginning in late August 1987, to sample the three depths. The samples were filtered through a 80 μm mesh net. The different methods overlapped for comparisons. All 1987 zooplankton samples were preserved with alcohol. In 1988 a pump was subsequently used to collect zooplankton during the entire sampling session. The methods of the previous season were also used for comparative purposes. The inlet of the pump intake hose was raised and lowered through the water column until 20 liters of water were collected. The discharge was filtered through a 64 μm mesh plankton net. The alcohol-preserved specimen from 1987 were not suitable for zooplankton analysis, but formalin-preserved phytoplankton were collected in the same manner around the same time. Accordingly, zooplankton were analyzed by using the formalin-preserved phytoplankton samples. All 1988 zooplankton samples were preserved with buffered sugar formalin. Specific sampling procedures are found in Appendix C, SOP EEF-C-16-01.

Major zooplankton species were identified and population densities were calculated by volumetric estimation. Zooplankton were generally identified to genus. If generic determination could not be made, the specimens were identified to the lowest practical taxonomic level. The SOP for zooplankton sample handling is in Appendix C.

Graphs (X-Y plots and kite diagrams) and summary statistics, presence-absence, and a diversity index were used to analyze the zooplankton taxa and spatial/temporal trends in a fashion similar to the phytoplankton. Similarly, the assessment of the spatial and temporal trends using the tested ANOVA model was done as noted under phytoplankton. Data from different zooplankton sampling methods were not converged (see report of deviation, Appendix E). For details see the previous section on phytoplankton.

8. Benthos

Four sampling methods were used to examine benthic macroinvertebrate populations. These methods were Ekman dredge sampling, artificial substrates to supplement the Ekmans, kick nets, and emergent insect traps. Samples were later processed and the data analyzed.

Ekman dredge samples collected sediment-dwelling organisms. Three replicate grabs were taken at the two zones (zones 2 and 5) located near the center of the ponds. A 15 cm by 15 cm (6 in. x 6 in.) Ekman dredge was dropped through the water column, driving the open end of the box into the sediment. A brass messenger or plunger or a post was used to trigger the spring-powered jaws to close and grab a quantitative sample of the benthic macroinvertebrates. The dredge was then raised and the collected substrate was seived with a 40-mesh wash frame. The contents were rinsed with water and transferred to a labelled wide-mouth plastic bottle. The sample was preserved with 4 to 10 percent formalin. Specific procedures are found in Appendix C, SOPs EEF-C-15-01 and EEF-C-17-01.

The artificial substrate sampler, or S-sampler (= surface enhancer), consists of 14 plastic cylinders tied together with nylon cable ties (Figure 36). The surface enhancers were 5 cm in diameter and 5 cm high and contained an internal framework of 12 radiating struts. At each zone, an S-sampler unit was tied to a rope and lowered to the bottom of the pond. After a 4-week colonization period, the sampler was slowly raised, enclosed in a submersed plastic bucket, and the bucket and unit lifted out of the water. The S-sampler was transferred to an empty bucket, where the organisms were removed by hosing and gentle agitation. Both buckets were rinsed into a 80-mesh sieve. The sieved contents were washed into a labelled sample bottle and preserved with 4 to 10 percent formalin. An incorrect set of s-samplers was sampled one time and one adjustment was made (see Report of Deviation in Appendix E). Specific procedures are found in Appendix C, SOP EEF-C-22-01.

Aquatic kicknet sampling collected littoral macro-invertebrates. This qualitative sampling method collected organisms that may have been missed by selectivity of certain organisms for certain substrate types. Kicknet samples were collected at the four littoral zones (1, 3, 4, 6) of each pond. The net was positioned with the straight edge of the D-frame placed on the substrate. The collector moved the sampler along the bottom for 60 seconds in the habitat area. All representative substrates and vegetation were sampled. The contents from the net at each sampling area were placed in a labeled wide-mouth plastic bottle containing 10 percent formalin. Specific procedures are found in Appendix C, SOP EEF-C-22-01.

Emergent insect traps captured emerging aquatic insects. The emergence trap is a pyramidal vinyl-sided structure with an open bottom and a collection jar at the top (Figure 37). The trap was suspended in the water column at three zones in the ponds. The traps collected aquatic insects emerging from the water column over a several-day sampling period. In 1987, emergent insects were collected by agitating the sides of the trap to force live insects into the collection jar at the trap apex. An acetone soaked cotton ball was placed on the jar lid and slipped under the inverted sample jar at the top of the trap. Once insects were narcotized, a wash bottle with alcohol was used to flush the sample into a prelabled sample container. Insects that died in the trap during the collection percent were collected in the trap baffle. These insects were flushed into a sieve and then added to the rest of the sample. In 1988, the emergence trap was redesigned so that emergent insects were collected in an alcohol filled jar at the apex of the trap. (See Report of Deviation in Appendix E.) The alcohol filled jar eliminated the need for the baffle and the insects were taken directly from the alcohol bath and placed into a pre-labled sample jar. The sampling periods were for 2 and 7 days, in 1987 and 1988, respectively.

This sampling method provides a quantitative measure (number of insects collected/unit area/unit time) of aquatic insects undergoing the final molting stage from an aquatic immature form to an aerial aquatic form. This measure can be used to compare emergent insects from different locations with a consistent sampling effort.

Sampled organisms from Ekman, S-samples, kick nets and emergent traps were sorted, identified, and enumerated. After organisms were separated from debris and by species, they were identified to the lowest practical taxonomic group and enumerated. SOPs for each technique are found in Appendix C.

Graphs, summary statistics and a presence-absence evaluation illustrated spatial and temporal trends and major benthic taxa collected using Ekman dredges, S-samplers, kick nets and emergence traps. Bar graphs of the relative abundance of each taxa or taxa groupings collected by each method were used to identify the major taxa. For Ekman dredges, S-samples, and emergence traps, the densities recorded for each of the major taxa were summarized using summary statistics (means and standard deviations). Prior to calculation of the summary statistics, the count data (numbers of organisms) were transformed using natural logs. The summarized data (means) for major taxa were then graphed over time to provide temporal comparisons of benthos taxa between the ponds. Nested ANOVA procedures described in Section E General Statistical Procedures, were then used to make the four-pond comparisons.

The emergence trap data were converted to create a unit measure of number of insects emerging per day per m^2. Twenty-two pair of samples were available from 1988 where the 1987 and 1988 sampling techniques were run simultaneously (see report of deviation in Appendix E). The evaluation included (1) adjusting the data for each taxa to numbers per day the trap was in the field, (2) identifying which taxa were commonly found in the 22 pairs of samples in order for the statistical test to be meaningful, (3) calculation of a delta value for each pair of samples (insects/day 1988 method to insects/day 1987 method), and (4) calculation of the t statistic from the data to determine whether "delta" deviates significantly from 0.

9. Fish

Examination of fish populations included several methods. These are: qualitative fish observations, electrofishing, seining, and mark-and-recapture. Methods for sample collection, sample analysis, and data analysis for each technique are explained below. Initially, three 1 x 1 x 1 meter cages were set into each pond and stocked with adult bluegill and sunfish to monitor spawning during the course of the study. Disease problems in the caged fish and captive broodstock could not be replaced prior to application. Accordingly, the caging study was terminated, and replaced with supplemental seining to capture young of the year to provide observation of recruitment.

Fish Observations. Directed searches for dead fish occurred daily in 1988 for two to four days after each spraying event at all four ponds. Searches were conducted by walking the entire pond perimeter or visually surveying the pond perimeter by boat. From July through October, 1988, fish observations were made every Monday, Wednesday, and Friday, and after every runoff event. In November and December, 1988, fish observations occurred twice a week, usually on Tuesday and Friday and after every runoff event. All dead or dying fish observed in the pond were collected by dip net. Collected specimens were frozen or were placed in wide-mouth plastic bottles and preserved with 10 percent formalin. Field observations (including general fish activity, general condition of dead or dying fish, location of kill area(s), and weather conditions associated with any dead fish) were recorded in a field log book.

Preserved specimens were identified and the total number of each species was recorded. Length measurements were performed to the nearest 1 mm. Weight measurements were recorded to the nearest 0.01 g if decomposition had not begun; decomposing fish were not weighed. When minnows or young-of-the-year were so numerous that it was impractical to weigh and measure all specimens, a subsample of that species was taken for measurement. Details are found in Appendix C, SOP EEF-C-13-01.

Data on the numbers of dead fish collected were examined graphically. The data were plotted on a time line for each of the ponds and for treatment ponds. Runoff events were also indicated on the graphs.

<u>Electrofishing</u>. Electrofishing was used in 1987 and 1988 to quantitatively sample fish populations. Electrofishing was conducted with a standard 10-ft barge using pulsed DC current and electroshocking equipment (Figure 38). Electrofishing was conducted at night to reduce stress on fish and to more accurately sample those fish that remained in deeper water during the day. Electrofishing followed the shore of the entire pond perimeter and included selected habitats (e.g., stumps, brush). Shocking times ranging from 148 to 1238 seconds. Stunned fish were netted and placed in an oxygenated holding tank. Upon completion of electrofishing, all netted fish were identified, measured, weighed, and, in 1988 checked for tags. All fish were returned to the pond except individuals kept for reference or analytical purposes. Electrofishing was conducted every two weeks except during the hottest portion of the summer when it was conducted monthly to reduce stress on the fish population. Specific procedures are found in Appendix C, SOPs EEF-C-09-01 and EEF-C-10-01.

Bar graphs and histograms were analyzed for information on species composition and size classes. Species composition and relative abundance for each pond were illustrated using bar graphs that displayed data on numbers of fish for each taxa collected during each sampling event.

Length and weight data were plotted and evaluated using the ANCOVA model (described in Section E General Statistical Procedures) to examine spatial and temporal trends for large mouth bass and bluegill collected using electrofishing. Two-dimensional plots of weight and length data transformed using natural logs were examined to identify whether the length and weight relationships were consistent through time and between ponds. Outlier data (statistically speaking, the few data points substantially different from the remaining data) were also identified with the length and weight plots (outliers were not used in the ANCOVA procedures). The data were then evaluated using ANCOVA procedures. The ANCOVA procedures were applied to comparisons of the length and weight relationsnips for 1987 to the 1988 data collected during the same time period (mid-June to December).

Condition factors in the length/weight relationships part were calculated for large mouth bass and bluegill and were used to examine spatial and temporal trends. The relative condition factors were calculated for each fish (Neilsen and Johnson, 1983) and were evaluated using the 2-way ANOVA model in a fashion similar to those detailed above for phytoplankton. The condition factors were also used in a 1-way ANOVA evaluation similar to those described for the ANCOVA model.

Seining. Seining of small fish occurred every two weeks following the pesticide application in 1988 to examine qualitatively the reproductive success of sunfish species. Fry and other small fish could not be sampled by the electrofishing method. Seines of 3 mm (0.125 in.) mesh were pulled along the shore line for approximately 30 ft and brought to shore.

Thirty of the smallest sunfish species were retained for laboratory determinations of weight to the nearest 0.1 g and length to 1.0 mm. Specific procedures are found in Appendix C, SOPs EEF-C-14-01 and EEF-C-10-01.

Summary statistics provided average and minimum-maximum lengths and weights of sunfish collected with seines. These data were then tabularized and evaluated qualitatively to determine the reproductive success of sunfish.

Mark-and-Recapture. Mark-and-recapture studies were accomplished via fish tagging. Healthy adult bluegill and largemouth bass ≥100 mm obtained during electrofishing in the spring of 1988 were selected for tagging. Selected fish were weighed, measured, and tagged with commercially-available, individually-numbered abdominal anchor tags. Fish were tagged during the three spring 1988 electrofishing events prior to the application of endosulfan in May. Each recaptured, tagged fish was weighed, measured, and its tag number recorded before release back into the pond. Specific procedures are found in Appendix C, SOPs EEF-C-11-01 and EEF-C-10-01.

Population sizes of largemouth bass and bluegill for each pond in 1988 were estimated with the Lincoln Index Reference. Population estimates were conducted using the following steps: (1) only largemouth bass and bluegill greater than 100 mm were used, because the minimum size required for tagging was greater than 100 mm; (2) the data were segregated in two different ways. The first way was a marking and then a recapture time period, March to mid-June and June to August, respectively. The second way used the same marking period and each recapture period builds on the previous ones; (3) populations were then estimated using the Lincoln Index and the variance was estimated using Bailey's formula (Poole, 1974).

10. Pond Metabolism

Diel changes in dissolved oxygen (DO) concentrations were measured to determine production conditions in the ponds. The increase in pond DO from dawn to dusk reflects an estimate of net production while the decrease in DO in a pond from dusk to dawn reflects a fraction of the day's respiration. A DO meter was used to measure DO concentrations at 0.5-m depth intervals in the pond, assuming that water depth at the sampling zone accommodated serial depth determinations. As the summer drought progressed, pond water levels dropped, and some zones that were initially 1.5 m deep in May were dry in December. The DO measurements were taken at dawn, dusk, and the following dawn. Ideally, calm conditions would persist throughout the sampling period. If inclement weather (e.g., a thunderstorm) occurred during the measurement period, the preceding values were disregarded and the series was started again. Details are presented in Appendix C, SOP EEF-C-53-01. The daily metabolism and gross photosynthesis of the ponds were estimated from the DO concentrations of dawn and dusk.

Summary statistics and graphs illustrated spatial/temporal trends in pond metabolism. Means and standard deviations were used to summarize the pond metabolism for each pond during each collection period. The summarized data (means) for each pond were then graphed over time to provide temporal comparisons of pond metabolism between the ponds. The nested ANOVA model was used to examine spatial and temporal trends in pond metabolism. The procedures used followed those described in the section on phytoplankton with the exception that the 1987 data for the pre-spray time period were only evaluated qualitatively. Only one zone per pond was evaluated in 1987, therefore the nested ANOVA model could not be applied to these data.

Most of the measurements were complete in terms of adequate and matched depths, but there were a few exceptions (see report of deviation in Appendix E). Depth measurements had to be matched occasionally. The procedure used to match depths was as follows. A complete set of measurements for metabolism includes all D.O. measurements at the same depth for each of the three time periods (Dawn 1, Dusk 1, and Dawn 2). Incomplete sets were sometimes encountered when either extra D.O. measurements were taken for one time period relative to the other two time periods or when the bottom depths varied slightly between time periods when the D.O. was measured. When extra measurements were encountered, Rule 1 (below) was applied. When bottom depths varied slightly, Rule 2 was applied.

Rule 1. (Deleting a D.O. measurement for a particular depth.) If extra measurements were encountered the measurement associated with the depth which deviated to the greatest degree was dropped from the data base.

Rule 2. (Adjustment of bottom depths.) Where bottom depths for a complete data set were not identical, the bottom depths were averaged to yield the depth entered into the data base.

11. Autotrophic Index

An autotrophic index (AI) was determined by examining periphyton populations. A periphytometer, a sampling device consisting of a clear plastic frame containing microscope slides, was attached to floats that suspend the unit near or at the water's surface. The microscope slides were colonized by periphyton. The slides were retrieved after two weeks and returned to the laboratory for the determination of the autotrophic index. New slides were exposed every two weeks during the study. Details are found in Appendix C, SOP EEF-C-23-01.

The autotrophic index is determined from the ratio of biomass to chlorophyll *a*. The AI measurement reflects the amount of autotrophic chlorophyllous organisms relative to the amount of heterotrophic non-chlorophyllous organisms. Determination of AI required two procedures. First, periphyton was scraped from the slides and macerated, and then chlorophyll *a* was extracted from the periphyton sample with an aqueous acetone solution and quantified by fluorometry. Ash-free biomass of the periphyton sample was then quantified by weighing the dried chlorophyll solution, heating the dried sample for 1 hour at 500°C, and then subtracting the ash weight from the dry weight. Details are found in Appendix C, SOP EEF-C-39-01.

Graphs and summary statistics illustrated spatial and temporal trends in the autotrophic index. The data were first screened to eliminate data that were improperly handled during processing in the laboratory as evidenced by negative AI values (see Report of Deviation in Appendix E). The data were then evaluated using procedures described under phytoplankton.

12. Macrophytes

The distribution and abundance of aquatic macrophytes at the pond edges were mapped qualitatively. The perimeter of the pond was staked at 15-meter intervals. Symbols representing macrophyte genera were drawn on the map according to location. Representative specimens of each macrophyte genus present were collected and preserved. Large plants were preserved by drying them in a plant press. Small plants were preserved whole in 5 percent buffered formalin. Details are found in Appendix C, SOPs EEF-C-18-01 and EEF-C-19-01.

Data analysis consisted of examining the macrophyte at every third 15-m (47 ft) interval of the pond perimeter. The selected intervals were organized into three zones: unexposed pond edge, exposed pond bottom, and littoral area along the pond edge. The macrophytes found in each of these three zones were identified, and the abundance of each species was estimated.

Temporal trends in macrophyte abundance were examined graphically. The data for the major groups of macrophytes were plotted for each pond over time. These plots were then used to identify temporal trends for each pond and to visually compare the reference and treatment ponds.

E. General Statistical Procedures

Four statistical models were used to quantitatively analyze the data. These models included three analyses of variance models (ANOVA) and one analysis of covariance model (ANCOVA). The first two models, nested and two-way ANOVA models, was used to analyze the majority of the data on a per sampling basis. The other two models, one-way ANOVA and ANCOVA were used to analyze the bass and bluegill data on a per pond basis, comparing 1987 to 1988. These models, their application and assumptions are described below.

1. Nested ANOVA Model

A fixed-effects nested ANOVA (nested ANOVA) was used to compare the four ponds when data were available for different zones within a pond (e.g. chironomid emergence). The model is of the following form:

$$y_{ijkl} = u + p_i + z_{ij} + t_k + pt_{ik} + E_{ijkl}$$

where:

y = endpoint measured (e.g., chironomids emerging per day per square meter)
u = grand mean
p = pond
z = zone nested in pond
t = time
pt = pond by time interaction
E = error or the zp interaction.

The model assumptions included homogeneity of the variance and normality of the residuals. These were evaluated for each endpoint using residual plots and normality plots (Neter and Wasserman, 1974).

The generalized model was applied to each of three time periods:

1) PRE-SPRAY -- data following week 33 (August 16, 1987-- when pond M-55-8 sampling began) to week 72 (May 15, 1988 -- the week before the first application of the 1988 data). The pre-spray period is used to determine which reference ponds are most similar to each of the two treatment ponds.

2) POST-SPRAY -- week 73 (May 22, 1988 --the week of the first application) to approximately week 87 (August 28, 1988 -- the week when seasonal declines begin). The post-spray period is to evaluate whether the ponds have been impacted by endosulfan immediately after application of endosulfan.

3) YEAR-END -- The seasonal decline at the end of the year from approximately week 88 (September 4, 1988 -- the week when seasonal declines begin) to week 103 (December 11, 1988 -- end of study). The year-end period is to determine whether recovery has occurred.

The analyses conducted for each of these three time periods were as follows:

1) PRE-SPRAY -- ANOVA model specified above where the "t" variable is a 4-week period. That is, when there are multiple sampling events per 4-week period, each sampling event would be considered a replicate for that interval.

2) POST-SPRAY -- ANOVA model specified above where the "t" variable is one week. Each week would correspond exactly to the biweekly sampling events conducted in the field.

3) YEAR-END -- ANOVA model specified above where the "t" variable is one week. Each week would correspond exactly to the biweekly sampling events conducted in the field.

The following multiple comparisons were conducted. If pond by time interactions were found to be significant, then multiple comparisons were conducted for each time period "t", specified in the model.

1) PRE-SPRAY -- Four multiple comparisons were conducted (each reference pond to each treatment pond): (A) T-4-1 to C-27-1, (B) T-4-1 to M-55-8, (C) M-55-4 to C-27-1, and (D) M-55-4 to M-55-8. The output from these comparisons was the selection of two pairs of ponds, including (1) C-27-1 and the reference pond that was most similar to it (i.e. the reference pond that has the least number of significant differences in the multiple comparisons of each reference pond to C-27-1, and (2) M-55-8 and the reference pond that was most similar to it based on the criteria noted above.

2) POST-SPRAY -- Conduct multiple comparisons on the pairs of ponds (C-27-1 and a reference pond and M-55-8 and a reference pond) determined in the PRE-SPRAY ANOVA/multiple comparison evaluation.

3) YEAR-END -- Perform analyses as specified under the POST-SPRAY time period.

Bonferroni procedures were used for the multiple comparisons because they can be used with different sample sizes and because they have a well-defined experimentwise error rate (ERR) (Neter and Wasserman, 1974). The ERR was set at 0.05 to provide an overall Type I error rate of 0.05 for the multiple comparisons. A simple example of the application of this model can be found in the discussion of phytoplankton densities.

2. Two-Way ANOVA Model

A reduced version of the nested ANOVA (two-way ANOVA) was used to evaluate fish condition factors because fish were collected for the entire pond and not from specified zones. The model is of the following form:

$$c_{ikl} = u + p_i + t_k + pt_{ik} + E_{ikl}$$

where:

c = condition factor of fish [$y=w/(al^b)$] where: c=condition factor, w=weight of the fish, l=length of the fish, a and b are constants from the weight length relation (Nielsen and Johnson, 1983)
u = grand mean
p = pond
t = time
pt = pond by time interaction
E = error.

The procedures described above were then applied to determine (1) which set(s) of ponds to compare to the treatment ponds using the

PRE-SPRAY data, and then (2) whether there were differences in the condition factors for bass and bluegill during the POST-SPRAY and YEAR-END time period. An example of the application of this model can be found in the discussion of Fish:length/weight relationships.

3. One-Way ANOVA Model

The condition factor for fish was also used in a one-way ANOVA model to evaluate, on a per-pond basis, whether the condition of the fish collected in 1988 was similar to those collected in 1987. The months used in both years included mid-June through December as the months to represent the time following application of endosulfan in 1988. The model is of the following form:

$$c_{ij} = u + y_i + E_{ij}$$

where:

c = condition factor (see two-way ANOVA model)
u = grand mean
y = year (1987 or 1988)
E = error.

Residual and normality plots as described above were also conducted on the one-way ANOVA model during the POST-SPRAY and YEAR-END time period. An example of the application of this model can be found in the discussion of fish: length/weight relationships.

4. ANCOVA Model

The final model used for the quantitative analysis was an ANCOVA model. This model was used to evaluate the weights of bass and bluegill because weight was significantly influenced by the length of the fish. This model was used to compare, on a per-pond basis, the weight-length relationship for fish collected in 1987 to those collected in 1988. The months used in both years included mid-June through

December because these months represented the time following application of endosulfan in 1988. The model is of the following form:

$$w_{ij} = y_i + l_j + yl_{ij} + E_{ij}$$

where:

w = weight of the fish in g
y = year (1987 or 1988)
l = length of the fish in mm
yl = interaction between year and length
E = error.

Residual and normality plots were also conducted on the ANCOVA model. The assumption that the slopes were similar between the years was also tested with the "yw" interaction. If the yw_{ij} were not significant, a reduced model of the following form was fit to the data:

$$w_{ij} = y_i + l_j + E_{ij}$$

An example of the application of this model can be found in the discussion of fish: weight/length relationships.

In summary, three ANOVA models and one ANCOVA model were applied to the data. For the nested ANOVA and the two-way ANOVA models, the models were used in the four-pond comparison for each of three time periods. The three time periods included: 1) PRE-SPRAY (week 33 to 72); 2) POST-SPRAY (week 73 to approximately week 87); and YEAR-END (approximately week 88 to 102). The nested and two-way ANOVA models were used to show how the ponds were behaving relative to one another before spraying and the output for this time period was the selection of the individual reference pond that was most similar to each treatment pond. The two pairs of ponds were then tracked through the remainder of the study to determine if the treatment ponds differed from the reference ponds.

For the one-way ANOVA and the ANCOVA model, the model was used for comparsions, on a per-pond basis, of the 1987 and 1988 data. The months used in both years included June through December as these months represented the time following application of endosulfan in 1988. The one-way ANOVA model was used to evaluate the condition factors and the ANCOVA model was used to evaluate the length-weight relationships for bass and bluegill.

F. Quality Assurance

1. Standard Operating Procedures

Written standard operating procedures (SOPs) were followed for field and laboratory activities. Existing SOPs applicable to the study were followed or new SOPs or modifications of existing SOPs were prepared and followed. Battelle SOPs were peer-reviewed and signed by the preparer; this was followed by approvals by research management and the Quality Assurance unit. SOPs included all phases of data collecting, sample collecting and processing, sample handling and shipment, sample analysis, and instrument maintenance and use.

2. Quality Assurance Audits

Observations of field and laboratory procedures were made by staff of the Quality Assurance unit. These observations were made to ensure Good Laboratory Practice guidelines and SOPs, as well as health and safety guidelines, were followed to ensure data quality and a safe work environment. Field audits were conducted during endosulfan application period and in the post-spray period. Laboratory audits of Battelle facilities were routinely conducted and site visits were made to subcontractors' facilities (EA Engineering, Stilson Laboratories, and Cody and Associates). Data audits of field records, laboratory data, computer-generated computations and results, and reports were conducted to ensure data consistency and accuracy.

VI. RESULTS

This chapter provides the results of the fate and effects study on aquatic populations and ecosystems adjacent to agricultural fields planted in tomatoes. As primarily an endosulfan effects study, the present investigation examined numerous physical, chemical, and biological characteristics of the farm pond/agronomic field test systems before, during, and after multiple applications. Because of the number of individual measurements made, it is possible to overlook the fact that the test systems and endpoints represent a dynamic, interactive system which functions as an integrated whole. Accordingly, physical and chemical conditions collected during the study represent the condition of the aquatic habitat during the study period. Endosulfan residues provide an examination of the dosing, translocation and fate of the applied endosulfan within the farm pond/agronomic field system. The biological measures represent a subset of structural and functional endpoints that could potentially be affected by endosulfan. Nevertheless, no one endpoint nor measurement represents the system as a whole.

For investigative and reporting purposes, the results were compartmentalized into three major areas. (1) physical and chemical conditions consisting of weather observations, field runoff, pond level changes, sedimentation patterns, and 12 water quality characteristics (Part A), (2) endosulfan residues in separate matrices (Part B), (3) and ecological measures consisting of various structural and functional endpoints (Part C). Each of the parameters under each major category is discussed separately below. Each is discussed separately with little consideration of interactive effect between parameters. The integration of cause and effects within the dynamic and holistic context will be presented in Chapter VII.

A. Physical and Chemical Conditions

Physical and chemical conditions are described for five environmental features: weather, field runoff, pond levels, sedimentation and water quality. Weather conditions for 1988 and the 25-year mean are described, along with wind direction and speed conditions during each of the six endosulfan applications. Seventeen field runoff events, changes in pond levels, and sedimentation patterns during 1988 are also described. Twelve water quality parameters for each pond are described during the pre-spray, post-spray, and year-end periods. Appendix F contains the weather and water quality data which are summarized in this part.

1. Weather

The weather in 1987-1988 in southwestern Georgia followed the general regional pattern of mild winters and hot, humid summers with thunderstorms (refer to Section III.D. on General Site Description for additional information).

During 1988, rainfall was below the annual average of 129 cm (50.8 in.) (NOAA, 1978) at Moultrie, GA, 8 km (5 mi) to the east of the closest pond (C-27-1). In 1988 approximately 104 cm (41 in.) of rain fell at Moultrie (Figure 39). Thus, precipitation was below average by 25 cm (10 in.) in 1988.

Rainfall amounts during the 1988 sampling period of March 15 to December 15 were 49.8 cm (19.6 in.) at C-27-1, 43.9 cm (17.3 in.) at M-55-4, 52.3 cm (20.6 in.) at M-55-8, and 47.2 (18.8 in.) at T-4-1 (Figure 40). During the same interval, 61 cm (24 in.) fell at nearby Moultrie compared to approximately 95 cm (37.5 in.) in an average year. Precipitation was below average during the 1988 sampling period, with drought conditions existing throughout the region. Rainfall events occurred infrequently as showed by the plateau during the May-June growing season (Figure 40), which coincided with endosulfan applications. On May 10, prior to the first application, an intense storm yielded 9.18 cm (3.62 in.) of rain at C-27-1, resulting in substantial surface erosion from the adjacent fields into the pond, producing a muddy appearance in the pond that remained for several weeks. Rainfall occurred more frequently after applications had been completed, and resulted in numerous runoff events.

Prevailing winds are generally from a northerly direction (northeast--northwest). Winds ranged from light in the summer, averaging 5 to 10 km/hr (3 to 6 mph) (NOAA, 1988a,b), to gusty when thunderstorms occurred.

Five of the six endosulfan applications occurred during morning hours when winds were usually at a minimum. All applications at C-27-1 occurred in the morning. The second and third applications at M-55-8 occurred in the morning, while the first application took place during the late afternoon. Wind speeds at each application averaged 4.8 km/hr (3 mph) or less, except for the second tank mix of the first application at C-27-1, when wind speeds increased to an average of 8.4 km/hr (5.2 mph) (Tables 6 and 7).

2. Field Runoff

Monitoring of runoff occurred at the flume drainage areas of C-27-1 and M-55-8. Monitoring began at the time of the first endosulfan application (May 27, 1988) and continued until mid-December 1988. Runoff flow was monitored to determine volume using the water level recorders in the flume stilling wells and was sampled for endosulfan using ISCO samplers installed at the flumes. Seventeen runoff events were sampled for endosulfan at the two ponds. Ten runoffs occurred at C-27-1 and seven runoffs at M-55-8. Events were numbered sequentially in order of occurrence, regardless of location.

Monitoring of ten runoff events occurred at C-27-1 (Table 8). This included an induced runoff event following the third endosulfan application when runoff from the non-flume side of the field was also sampled. Storms resulting in runoff occurred in June, July, August, September, October, and November. The minimum precipitation (measured at the flume) resulting in runoff was 0.99 cm (0.39 in.). Lesser amounts resulted in insufficient runoff to activate the water sampler or result in discernible readings on the water level recorder chart. Mechanical failures of the water level recorder prevented runoff volume calculations for three events. The average precipitation resulting in runoff was 2.8 cm (1.15 in.); the average recorded runoff from the drainage area was 7.25 m^3 per event.

Seven runoff events were monitored at M-55-8; only one occurred prior to the final endosulfan application and was caused by a natural rainfall event. Storms resulting in runoff occurred in June, August, September, October, and November. The least amount of precipitation (as measured at the flume) resulting in a runoff event was 1.32 cm (0.52 in.) (Table 9). Mechanical failures of the water level recorder prevented runoff calculations for one event. The average precipitation resulting in runoff was 2.75 cm (1.08 in.); the runoff from the drainage area averaged 17.18 m^3 per event.

In summary, a total of 17 runoff events were monitored at C-27-1 and M-55-8 to collect samples for endosulfan analysis and to determine runoff volume.

3. Pond Level

Water levels in the two treatment ponds (C-27-1 and M-55-8) dropped about 60 cm (2 ft) during 1988 (Figure 41); levels in the reference ponds (M-55-4 and T-4-1) dropped approximately 110 cm (3.5 ft). Water loss by evaporation began in early May and continued throughout the year.

4. Sedimentation

Sediment traps set in the vicinity of the six stations collected settling particles from as early as May 21 to December 6, 1988. Sampling intervals ranged from 13 to 76 days (Table 10).

Sedimentation rates for individual sampling intervals ranged from 1 $g/m^2/day$ (T-4-1, C-27-1) to 165 $g/m^2/day$ (C-27-1) (Table 10).

Three ponds exhibited sedimentation rates between 19 and 24 $g/m^2/day$. T-4-1 (reference) had a sedimentation rate 1/2 this value (Table 10). M-55-4 (reference) exhibited the highest average sedimentation rate in 1988 (24 $g/m^2/day$) followed by the two treatment ponds C-27-1 (23 $g/m^2/day$) and M-55-8 (19 $g/m^2/day$). The reference pond T-4-1 had the lowest sedimentation rate with 10 $g/m^2/day$. Sedimenation rates also differed within a given pond. The August 12 to October 26 period for C-27-1 showed a mean of 39 $g/m^2/day$. The highest rate for M-55-8, was observed during the same period. By contrast, June 17 to July 5 exhibited the highest rate of 50 $g/m_2/day$ at M-55-4. T-4-1 showed a an overall low sedimentation rate, but it showed 27 $g/m_2/day$ during the October 27 to December 6 period.

5. Water Quality

Results of routine water quality monitoring conducted every two weeks during the 1987 and 1988 sampling seasons are presented below. The water quality measurements consisted of 12 parameters: pH, temperature, conductivity, dissolved oxygen, alkalinity, acidity, hardness, turbidity, nitrates, orthophosphate, total organic carbon, and total suspended solids. Generally, the water quality of the four ponds was typical of Southeastern U.S. soft water ponds. The ponds were similar with respect to water quality parameters which are discussed separately below.

pH. The mean pH of all four ponds was similar during the 2-year study period with mean pH ranging from 7.2 to 7.7 (Table 11). The recorded values were strongly influenced by the time of day, photosynthesis, and the pond specific metabolic conditions when measurements were taken. Mean pH values for each sample date in 1987 and 1988 for each pond are provided in Figure 42.

Temperature. Mean temperatures of the four ponds over the 1987-1988 study period ranged from 24.2 to 26.0°C (Table 11). Temperatures reflected ambient seasonal trends. The lowest observed temperature was 7°C in late December. The highest temperature was near 34° in late July to early August. Mean temperatures for each sample date in 1987 and 1988 for each pond are provided in Figure 43.

Conductivity. Mean conductivity of the four ponds over the 1987-1988 study period ranged from 66 to 88 μmhos/cm (Table 11). Conductivity near 50 μmhos/cm were typical of early spring and late fall, with peak conductivity of 100 to 150 μmhos/cm occurring in late summer. The highest observed conductivity of 260 μmhos/cm occurred in M-55-4 in the spring of 1987 and may be related to spring run-off. Mean conductivity for each sampling period for each pond are shown in Figure 44.

Dissolved Oxygen. Mean dissolved oxygen of the four ponds during the 1987 - 1988 study period ranged from 8.0 to 8.8 mg/L (Table 11). Considerable variation occurred, reflecting differences in time of day when measurements were made, and diurnal changes in pond oxygen levels as influenced by photosynthesis and respiration (see also Section VI on Pond Production). Mean dissolved oxygen concentrations for each sampling date in 1987 and 1988 for each pond are provided in Figure 45.

Alkalinity. Mean alkalinity of the four ponds during the 1987-1988 study period ranged from 11 to 19 mg/L (as $CACO_3$) (Table 11). Alkalinities for all ponds were similar. Mean alkalinities for each sampling date for each pond are shown in Figure 46.

Acidity. Mean acidity of the four ponds during the 1987-1988 study period ranged from 4.14 to 5.11 mg/L (Table 11). Mean acidities for each sampling period for each pond are shown in Figure 47. Several coincident peak acidities occurred in all ponds in both 1987 and 1988, particularly in weeks 72 and 82 (mid-May and late-July, 1988), when increases of 5 to 15 mg/L were measured.

Hardness. Mean hardness for the four ponds during the 1987-1988 sampling period ranged from 12 to 16 mg/L (as $CaCO_3$) (Table 11). All hardness determinations spanned a range from 4-33 mg/L, indicative of soft water. Mean hardness values for each pond for each sampling date are provided in Figure 48.

Turbidity. Mean turbidity for the four ponds during the 1987 - 1988 sampling period ranged from 12.5 to 63.6 NTU (Table 11). Turbidity was generally low (5 to 10 NTU) throughout the study, except for the spring of 1988, when turbidity levels in all ponds were elevated. Mean turbidity values for each pond for each sampling date are provided in Figure 49. At the beginning of the 1988 sampling season (week 64), turbidity in M-55-8 was approximately 125 NTU. The remaining three ponds ranged 400 to 600 NTU. Turbidity stabilized near 10 NTU by week 70 with the exception of C-27-1. An intense storm at C-27-1 on May 10, 1988 (week 71), resulted in considerable soil erosion from adjacent fields into the pond. Turbidity was approximately 850 NTU at C-27-1 for week 72, but returned to approximately 10 NTU by week 82.

Nitrates. Mean total nitrate concentrations for the four ponds during the 1987 - 1988 sampling period ranged from 0.28 to 0.43 mg/L (Table 11). Mean total nitrate for each pond for each sampling date are shown in Figure 50. Nitrate concentrations in 1987 were generally near detection limits for all four ponds (0.03 mg/L). In contrast, total nitrate were higher and more variable in 1988 for all ponds (Figure 50). Additionally, the watersheds surrounding all ponds were cleared and tilled in early 1988 to accommodate tomato planting. Application of fertilizer and the removal of vegetation and clearing resulted in increased run-off and erosion which may account for the increased variability in 1988.

Orthophosphate. Mean orthophosphate concentrations for the four ponds during the 1987-1988 study period ranged from 0.03 to 0.04 mg/L (Table 11). Mean orthophosphate values for each sampling date for each pond are shown in Figure 51. Generally, orthophosphate was higher and more variable in 1988 than 1987. This difference may be attributable to increased nutrient loading from run-off attributable to clearing of the pond edge vegetation in 1988. Orthophosphate concentrations stabilized near 0.03 mg/L for much of 1987 and 1988. In July 1988 after week 80, orthophosphate increased in all four ponds. In the fall--around week 100--of 1988 orthophosphate increased again in all four ponds.

Total Organic Carbon. Mean total organic carbon (TOC) for the four ponds during the 1987-1988 study period ranged from 8.0 to 11.3 mg/L (Table 11). Mean total organic carbon concentrations for each sampling data for each pond are depicted in Figure 52. During 1987, TOC concentrations decreased in the summer and increased in the fall. Average 1988 TOC levels increased slightly through the year until November when concentrations began decreasing especially in M-55-8. All mean TOC concentrations were in the 4 to 15 mg/L range, with the exception of one sampling period in M-55-8 when concentrations averaged 28 mg/L due to one sample with an unusually high TOC concentration.

Total Suspended Solids. Mean total suspended solids for the four ponds during 1988 (TSS) ranged from 7.9 to 24.3 mg/L (Table 11). (Analyses for Total Suspended Solids were not initiated until the 1988 sampling season.) Mean total suspended solids for each sampling date for each pond are depicted in Figure 53. An intense storm occurred at C-27-1 during week 71, resulting in considerable soil erosion into the pond from the adjacent fields. Average TSS levels in C-27-1 sharply increased to 143 mg/L following the storm and remained elevated for approximately two months. Thereafter, TSS levels at C-27-1 were similar to the other three ponds.

Summary. Generally, the water quality characteristics of the four ponds were similar, and typical of soft water ponds in the southeastern U.S. There were few remarkable differences in pH, temperature, dissolved oxygen, alkalinity, or hardness between ponds and years (1987, 1988) of the study. The spring increase in turbidity evidenced in 1988, but not in 1987, was because seasonal sampling in 1988 began in March, earlier than in 1987 when sampling began in May. Pond C-27-1 exhibited a dramatic increase in turbidity due to a localized thunderstorm that struck the pond and watershed on May 10, 1988 (week 71) of the study, resulting in extensive soil erosion from adjacent fields to the pond. Total suspended solids were monitored during 1988.

Several parameters -- conductivity, acidity, nitrate, orthophosphate and total organic carbon -- exhibited midsummer peaks in 1988 that did not occur in 1987. These peaks are attributable to increased runoff and erosion into the ponds due to the removal of pond edge vegetation in preparation for planting.

B. Endosulfan Concentrations

Concentrations of endosulfan were determined in various media before, during and/or following the endosulfan application period. Sampling for endosulfan residues was conducted over an 8-month period (from pre-spray in May 1988 through post-spray and year-end to 180 days after the third application, ending in December 1988). Determinations included alpha-endosulfan, beta-endosulfan, and endosulfan sulfate concentrations from the Thiodan 3EC (see certificate of analysis in Appendix D) for application cards, foliage rinsate, drift cards from three areas for each pond site, soil, runoff water, pond water, sediment, and fish. Also, blank and spike information from the field and method validation information are provided. Total endosulfan was calculated from the individual components using the following rules: (1) the total equals the sum of the concentrations of alpha-endosulfan, beta-endosulfan, and 0.962 times the concentration of endosulfan sulfate, and (2) if the individual endosulfan concentration was less than the detection limit, zero was used for the calculation of the total. The amount of endosulfan sulfate was adjusted to account for the difference in molecular weight of the metabolite. Three significant digits were recognized for means and totals.

Information about the line of reasoning for the average and total calculations are shown below. This example is for pond water from C-27-1 prior to the first application.

	Alpha	Beta	Sulfate	Total
	< 5	< 5	7	6.7
	< 5	< 5	< 5	0.0
	< 5	< 5	6	5.8
	< 5	< 5	7	6.7
	< 5	< 5	7	6.7
	< 5	12	8	19.7
Actual mean:	0	2.0	5.8	7.6
Reported value:	< DL	< DL	5.8	7.6

The individual totals are shown at the right side per the above-mentioned formula. Averages for alpha- and beta-endosulfan and endosulfan sulfate are at the bottom of each column. Note that the average for beta is 2.0, but is reported as < DL because it is less than the detection limit of 5 ng/L. The average total reflects the presence of beta in the one sample out of six. Therefore, means which were less than the detection limit are reported as < DL, but were used in the calculation of total endosulfan.

The detection limits for each matrix are listed below.

Matrix	Detection Limit
Application cards*	3 $\mu g/m^2$
Foliage rinsates*	10 $\mu g/m^2$
Drift cards*	0.6 (3 cards) $\mu g/m^2$ or 0.8 (2 cards) $\mu g/m^2$ or 1.7 (1 card) $\mu g/m^2$
Soil	10 ug/kg
Runoff water	5 ng/L
Pond water	5 ng/L
Sediment	5 ug/kg
Fish	10 ug/kg

*These detection limits are dependent on a post-analysis validation which will be appended to this report as soon as it is available.

The following discussion presents the findings from analyses of the media listed above. When more detail is needed regarding field or analysis methods see the appropriation section in the study methods and also the SOPs (Appendix C). A few samples were either not taken or taken but not analyzed and these are identified in the report of deviations in Appendix E. Data about measured endosulfan concentrations are provided in Appendix G.

1. Thiodan 3EC

According to the certificate of analysis, Thiodan 3EC contained 33.7% endosulfan (Appendix D).

2. Application Cards

Application cards were placed in the fields immediately before spraying and were retrieved beginning 30 minutes after spraying ceased on a given part of the watershed. All cards were collected within one hour of the termination of spraying. Each application card station consisted of three cards evenly spaced from directly in the middle of the planted row to the middle between the two planted rows. Mean total concentration of endosulfan on application cards in the two fields ranged from 53,400 to 91,000 $\mu g/m^2$. The average of all six applications was approximately 71,800 $\mu g/m^2$. This means that an average of 0.80 lb/acre of total endosulfan was measured on the application cards. Assuming a portion of the intended dose of 1.0 lb/acre was present on the foliage and a minor portion was in drift, there is relatively close agreement between the measured dose on the cards and the planned dose on the foliage. The 0.80 lb/acre conversion was determined with the following line of reasoning:

$$1\ \mu g = 10^{-9}\ kg$$

$$1\ ha = 10^{4}\ m^2$$

$$1\ \mu g/m^2 = 10^{-5}\ kg/ha$$

$72{,}000\ \mu g/m^2 = 0.72$ kg/ha
corrected for field spike recovery of 80%
(see Table 30) = 0.90

kg/ha x 0.89 = lb/acre

0.90 kg/ha x 0.89 = 0.80 lb/acre.

C-27-1. Total endosulfan concentrations at the first application on May 27 averaged 91,000 $\mu g/m^2$ for the 20 application card stations. The range of total endosulfan concentrations was 58,000 to 132,000 $\mu g/m^2$ (Table 12). The alpha:beta isomer ratio was 69:31.

Total endosulfan concentration on application cards following the second application on June 10 averaged 68,500 $\mu g/m^2$. The alpha:beta isomer ratio was 68:32. Total endosulfan concentrations ranged from 37,000 to 117,000 $\mu g/m^2$ (Table 12).

Average concentrations of total endosulfan on June 27, following the third application, were 78,200 $\mu g/m^2$. The alpha:beta isomer ratio was 64:36. Total endosulfan concentrations ranged from 53,000 to 182,000 $\mu g/m^2$ (Table 12).

M-55-8. Total endosulfan concentrations at the first application on May 27 averaged 69,000 $\mu g/m^2$ for the 10 application card stations (Table 13). The alpha:beta isomer ratio was 69:31. Total endosulfan concentrations ranged from 14,300 to 92,000 $\mu g/m^2$.

Total endosulfan concentration on application cards on June 11, following the second application averaged 53,400 $\mu g/m^2$ (Table 13). Concentrations ranged from 26,200 to 79,000 $\mu g/m^2$ for total endosulfan. The alpha:beta isomer ratio was 69:31.

On June 23, average concentrations of total endosulfan following the third application were 70,800 $\mu g/m^2$ (Table 13); the alpha:beta ratio was 65:35. Total endosulfan concentrations ranged from 48,000 to 116,000 $\mu g/m^2$.

Summary. Total endosulfan concentrations on application cards were found to be similar following the three treatments at each of the two study ponds. Concentrations following the second application were lower than the first and third applications. Total endosulfan concentrations at M-55-8 averaged 64,400 $\mu g/m^2$, compared to the average of 79,200 $\mu g/m^2$ at C-27-1. The ratio of alpha:beta was 67:33.

3. Foliage

Foliage or leaf rinsate samples were collected for three of the six applications beginning three to four hours after ground application of Thiodan 3EC was completed and required up to two hours to collect. The other three were collected the morning following the application. Foliage samples were collected from six transects. Concentrations of total endosulfan in foliage rinsate from the two fields averaged 7,090 $\mu g/m^2$. The alpha:beta ratio of isomers averaged 37:61, with an average of two percent endosulfan sulfate present. The ratio observed on foliage (37:61:2) was different from the ratio observed for the application cards (67:33:0). Alpha-endosulfan is more volatile than is beta-endosulfan. The foliage samples were taken up to six hours later than the application cards. Sufficient time may have elapsed for some of the alpha to be volatilized from the sample accounting for the difference.

C-27-1. Foliage rinsate following the first application on May 27 contained an average of approximately 10,000 μg total endosulfan per m^2 of tomato leaf. Total endosulfan consisted of 36, 61, and 3 percent alpha- and beta-endosulfan and endosulfan sulfate, respectively. Foliage rinsate concentrations ranged from 4,260 to 13,700 $\mu g/m^2$ total endosulfan on the tomato leaves (Table 14).

Following the second application on June 10, the rinsate concentration of total endosulfan averaged approximately 2,530 $\mu g/m^2$ consisting of 31 percent alpha-endosulfan, 68 percent beta-endosulfan, and 1 percent endosulfan sulfate. Total endosulfan concentrations ranged from 1,480 to 5,550 $\mu g/m^2$ (Table 14), somewhat lower than the ranges after the first application.

Rinsate concentration following the third application on June 27 averaged approximately 8,810 $\mu g/m^2$ total endosulfan. This was composed of 44 percent alpha-endosulfan, 55 percent beta-endosulfan, and 1 percent endosulfan sulfate. The range of the total endosulfan concentrations was from 5,640 to 10,600 $\mu g/m^2$ (Table 14), more similar to the first than the second application.

M-55-8. Foliage rinsate on May 28, following the first application, showed average concentrations of approximately 5,750 $\mu g/m^2$ consisting of 40 and 60 percent alpha- and beta-endosulfan, respectively. The range of total concentrations was 3,600 to 8,350 $\mu g/m^2$ (Table 15).

Following the second application on June 11, the average rinsate concentration of total endosulfan was approximately 8,770 $\mu g/m^2$, consisting of 46, 54, and 1 percent alpha- and beta-endosulfan and endosulfan sulfate, respectively. Concentrations of total endosulfan ranged from 3,040 to 18,000 $\mu g/m^2$ on the tomato leaves (Table 15). The mean and maximum values were higher than for the first application.

Rinsate concentrations on June 23, following the third application, averaged 6,660 $\mu g/m^2$ total endosulfan, composed of 41 percent alpha-endosulfan 58 percent beta-endosulfan, and 1 percent endosulfan sulfate (Table 15). The range of total endosulfan values was from 4,270 to 10,400 $\mu g/m^2$, with the mean being more similar to the first than the second application.

Summary. Mean concentrations were similar for the first and third applications at C-27-1 and at M-55-8. The lowest mean value occurred at C-27-1 after the second spraying. However, the second application provided the highest mean at M-55-8. The mean total concentration for all applications was approximately 7,110 $\mu g/m^2$ at C-27-1, while the overall mean was 7,060 $\mu g/m^2$ at M-55-8. Thus, the two means were similar.

4. Drift Cards

Drift cards were emplaced on the stations around the field, around the pond, and on the pond surface. This was done immediately before endosulfan spraying began and cards were retrieved beginning 30 minutes after spraying ceased. Drift card retrieval took up to 2.5 hours. Thus, drift cards were retrieved within 3 hours of the cessation of spraying. Concentrations of total endosulfan on drift cards varied considerably with location and wind condition. These concentrations ranged from below detection level to 1,170 $\mu g/m^2$ on drift cards located on field perimeter, pond perimeter, and pond surface. Compared to mean endosulfan concentrations on application cards (range from 53,400 to 91,000 $\mu g/m^2$), the concentrations on drift cards represented only a small part of the total endosulfan application to the field.

C-27-1. Concentrations of total endosulfan at the field edges ranged from below the detection limit to 560 $\mu g/m^2$, below the detection limit to 750 $\mu g/m^2$, and below the detection limit to 990 $\mu g/m^2$ for applications 1, 2, and 3, respectively (Table 16). Concentration ranges over the three spray periods were influenced by local winds (Figures 54-56) that transported endosulfan aerosols from the field during application. Additional wind direction and speed information are provided in Table 7. Drift cards on the upwind sides of the fields usually had low or undetectable quantities of endosulfan. Drift cards in close proximity to the field edge on the downwind side received larger quantities of drift. The alpha:beta isomers for all the three applications dominated the mix with endosulfan sulfate representing a small part of the total.

Total endosulfan concentrations at the pond edge ranged from 13.8 to 760, 94.0 to 610, and 10.3 to 230 $\mu g/m^2$ for applications 1, 2, and 3, respectively (Table 16). Individual site values (Figures 57-59) illustrate the effect of wind on drift card concentrations. Cards located along the northwest edge proximate to the downwind side of the upper field received the higher concentrations of endosulfan. Endosulfan components for all three applications were highest for alpha- and beta-endosulfan and low for endosulfan sulfate.

Concentrations of total endosulfan at the pond surface averaged 166, 218, and 28.1 $\mu g/m^2$ for applications 1, 2, and 3, respectively (Table 17). The concentrations at individual drift card stations ranged from 5.3 to 358 $\mu g/m^2$. Pond surface drift was similar for applications 1 and 2, but mean total concentrations at application 3 were 1/6 to 1/8 of those observed for the first two applications. The alpha- and beta-endosulfan isomers averaged 75 and 25 percent, respectively, of the total endosulfan over the three applications.

M-55-8. Concentrations of total endosulfan at the field edges ranged from below the detection limit to 300, below the detection limit to 1170, and 8.7 to 407 $\mu g/m^2$ for applications 1, 2, and 3, respectively (Table 18). Concentrations ranging from below detection limits to 1,170 $\mu g/m^2$ over the three spray periods were influenced by local winds (Figures 60-62) that transported endosulfan aerosol from the field during application. Drift cards on the upwind sides of the fields usually had low or undetectable quantities of endosulfan, while those in close proximity to the field edge on the downwind side received larger quantities of drift. However, following the third application, some upwind drift cards contained unexpectedly high concentrations of endosulfan because the spray boom/tractor pivoted near them. The concentrations of alpha- and beta-isomers for the three applications were all high. Endosulfan sulfate constituted only a small part of the total.

Total endosulfan drift concentration at the pond edge ranged from below the detection limit to 14.4, below the detection limit to 43.8 and 33.5 to 170 $\mu g/m^2$ for applications 1, 2, and 3, respectively (Table 18). Pond edge drift concentrations following the third application were higher than the two previous applications. Concentrations ranged from below detection limits to 170 $\mu g/m^2$ over the three applications. Individual site values along with wind direction are shown in Figures 63-65. Additional wind direction and wind speed information are provided in Table 7. Total endosulfan components were high for alpha-endosulfan and beta-endosulfan, and low for endosulfan sulfate.

Concentrations of total endosulfan at the pond surface averaged 2.0, 2.2, and 99.3 $\mu g/m^2$ for applications 1, 2, and 3, respectively (Table 19). The concentrations at the drift card stations ranged from below the detection limit to 145 $\mu g/m^2$ (Table 19; Figures 63-65). Mean endosulfan measured at the pond surface following the third application was approximately 45 times the concentrations detected at the two prior applications. This was due to the wind-driven transport of endosulfan from the north field at M-55-8 toward the pond surface (Figure 65). The alpha- and beta-endosulfan isomers were 77 and 23 percent, respectively, for the third application; endosulfan sulfate was not detected (Table 19).

Summary. Drift cards located around the field edges, pond edges, and on the pond surface provide information on the amount of endosulfan that drifted beyond the sprayed fields and onto the ponds.

At the pond edges, concentrations for the applications 1 and 2 were higher, at C-27-1 than at M-55-8, but were similar for application 3. Endosulfan concentrations at the pond surface showed the same pattern as at the pond edge. Based on drift estimates, mean concentrations of endosulfan on the pond surface of C-27-1 were 83 and 99 times greater than the mean concentrations calculated at M-55-8 for applications 1 and 2, but changed at application 3, where they were about 1/3 the concentration of M-55-8. The total input from all three applications of endosulfan measured at the pond surface of C-27-1 was 412 $\mu g/m^2$, while at M-55-8 the total was 104 $\mu g/m^2$.

5. Soil

The top 5 cm of soil was sampled from six transects prior to and within 24 hours after each endosulfan field application and at sequential intervals (approximately 7, 14, 28, 60, 90, and 180 days) following the third application. The soil from fields adjacent to the two treatment ponds contained an average of approximately 2,400 $\mu g/kg$ total endosulfan after the third application. The average alpha:beta:sulfate ratio of isomers and sulfate after the third application was 42:52:6.

By December, 180 days after the last application, the soil averaged approximately 330 $\mu g/kg$ total endosulfan in a alpha:beta:sulfate ratio of 5:54:41, indicating that most of the endosulfan had been dissipated from the soil, and that most of the alpha-isomer was lost.

C-27-1. Total endosulfan concentrations accumulated during each of the spray periods to a peak of 2,280 $\mu g/kg$ on June 28 (Table 20). A runoff event on June 29 transported a fraction of the total endosulfan from the soil. On July 5 total endosulfan was 1,640 $\mu g/kg$. Mean total endosulfan ranged from 733 $\mu g/kg$ on July 11 to a value of 1,630 $\mu g/kg$ on August 23. This variation may be due in part to the field vegetation dying down and releasing additional beta-endosulfan to the soil. By December 17, mean total endosulfan had decreased to 322 μg/kg (Table 20; Figure 66).

The alpha:beta:sulfate ratio of endosulfan averaged 38:55:7 immediately after the three applications. By December 17, the ratio was 1:54:45.

M-55-8. Mean total endosulfan concentrations peaked at 2,510 μg/kg on June 23 (Table 21). A runoff event on June 25 transported a fraction (see Table 23) of the total endosulfan out of the soil. On June 29, mean total endosulfan was 1,070 μg/kg. The mean total endosulfan ranged from 958 μg/kg on July 7 to 1460 μg/kg on July 21. By December 13, total endosulfan had decreased to 347 μg/kg (Table 20; Figure 67).

The alpha:beta:sulfate ratio of endosulfan averaged 45:49:6 immediately after the third application. By December 13, the ratio was 9:54:37.

M-55-4 and T-4-1 (Reference). At M-55-4, one of twenty-one samples of soil had detectable beta-endosulfan at 11 μg/kg. At T-4-1, six of twenty-one samples had detectable beta-endosulfan, and one had detectable alpha-endosulfan. Five of the samples with detectable beta-endosulfan were all from transect 5, an area on the east side of the pond where toxaphene was found (Appendix B). However, the average for each field at the ponds was less than or equal to the detection limit of 10 μg/kg. It should be noted that when working at or near the detection limit, it is expected that a small number of false positives will occur. As indicated by the absence of endosulfan sulfate in these samples, the expected degradation product of the parent compounds, the peaks identified as alpha- and beta-endosulfan in the chromatogram at that retention time are likely interfering coextractants. Even if real, the endosulfan concentrations detected at the reference ponds were two to four orders of magnitude less than those detected at the treatment ponds and are not relevant.

Summary. Total endosulfan concentrations were similar in fields adjacent to the two treatment ponds. Soil concentrations of total endosulfan peaked after the third application, but decreased by 86 percent by December, 180 days after the last application. Decreases were due to degradation and volatilization. The ratio of alpha, beta, and sulfate forms of endosulfan were also similar at the two treatment ponds. Initial ratios (after the first application) averaged 63:33:5 (total does not equal 100 due to rounding), but by December had shifted to 5:54:41.

6. Runoff Water

Runoff water from the two treatment fields was sampled by ISCO samplers at each flume following initiation of endosulfan applications to the fields. It was observed that runoff entered each pond not only through the flume, but at other entry points. Natural runoff occurred only at M-55-8 prior to the final endosulfan application. After that, it was necessary to irrigate to produce runoff to the two treatment ponds. However, after July 1 there was no more irrigation and natural runoff events were measured. Runoff events were numbered as they occurred, regardless of whether the runoff occurred at C-27-1 or M-55-8.

C-27-1. There were two induced and eight natural runoff events at C-27-1. No natural runoff occurred from the time endosulfan application was initiated through the third application. Runoff was induced beginning the day following the third application by overhead irrigation (see Section on Irrigation Systems). All subsequent runoff events were the result of natural precipitation.

In addition to runoff samples at the flume, runoff samples were also taken on June 28 and 29, 1988 from runoff channels in the watershed at C-27-1. On the side of C-27-1 where there was no flume, irrigation began at 1750 hours on June 28, 1988, and was completed at 0150 hours on June 29, 1989. Three runoff channel samples from this side yielded the following results:

Sample Number	Military Time	Value (ng/L)
7837	2030	92,200
7838	2125	133,000
7839	2135	198,000

The concentration was 202,000 ng/L for a sample collected in the runoff channel above the flume (sample 7836) at 1641 on June 29. Thus, in-field runoff exhibited total endosulfan concentrations ranging from 92,200 ng/L to 202,000 ng/L beginning within a few hours after irrigation started.

Endosulfan concentrations at the flume, as taken by the ISCO sampler, varied through time. Samples were collected every other hour, starting with hour 1. Irrigation on the flume side began at 1240 hours on June 29, 1988, and was completed at 1900 hours. On June 29, 1989, at the C-27-1 flume, the following patterns were observed for runoff number 4 (Table 22).

Sample Number	Military Time	Hour	Value (ng/L)
7844	1703	(hr. 1)	203,000
7846	1903	(hr. 3)	191,000
7848	2103	(hr. 5)	127,000
7850	2302	(hr. 7)	81,400

This pattern follows the general observation that concentrations were highest in the first few hours than the later hours of a storm's runoff. A sample (number 7836) collected above the gravel at the flume showed 202,000 ng/L of total endosulfan compared to 203,000 ng/L in the flume; thus, the gravel did not appear to impede endosulfan-ladened particles.

On June 30 at 1258 hours a pond edge sample (No. 7840) was taken to assess endosulfan concentrations in the pond after runoff. This sample was taken close to the runoff channel coming from the nearby flume. Endosulfan concentrations were 360 ng/L, 570 ng/L, and 360 ng/L for alpha- and beta-endosulfan and endosulfan sulfate, respectively for a total endosulfan concentration of 1,280 ng/L.

Concentrations at hour 1 decreased to 45,100 ng/L later (July 5) and further to 12,600 ng/L on July 12 (Figure 68). By November concentrations of total endosulfan at hour one had decreased to less than 5,360 ng/L (Table 22).

Beta-endosulfan was the major constituent of the runoff residue through Day 75. Thereafter, endosulfan sulfate was the major component (Table 22).

M-55-8. There were seven natural runoff events at M-55-8. This runoff followed partial field irrigation augmented by natural rainfall. All subsequent runoff events were the result of natural rainfall. One small natural runoff event (number 1, June 10) occurred prior to the second application and contained 26,600 ng/L (hour 2) total endosulfan.

Total endosulfan concentrations were at 4,800 ng/L (August 3), 8,990 ng/L (August 9) and 8,450 ng/L in late September (Table 23). Runoff concentrations decreased to 326 ng/L (runoff number 17) by late November (Figure 69).

Beta-endosulfan was the major constituent of the endosulfan runoff through day 74 (Table 23). Afterwards, endosulfan sulfate was the dominant constituent.

Summary. Runoff from the C-27-1 fields contained a maximum mean total endosulfan of 203,000 ng/L endosulfan two days after the final application. The maximum mean concentration at M-55-8 was 79,600 ng/L at hour 3 after the third spray. Maximum concentrations for C-27-1 were more than twice the maximum levels detected at M-55-8, due in part to the larger watershed area. Endosulfan continued to be present at all rainfall events for the duration of the study.

7. Pond Water

Pond water was sampled before and started within 3 hours after the cessation of each endosulfan field application and at sequential intervals (approximately 3, 7, 14, 28, 60, 90, and 180 days) following the third application. Integrated water column samples were collected at the six zones in the pond. Endosulfan entered the ponds via aerial drift during applications to adjacent tomato fields and from field runoff after rains or irrigations.

C-27-1. Mean concentration of total endosulfan was 81.8 ng/L on May 27, immediately following the first application (Table 24; Figure 70). On June 8, before the second application, mean total endosulfan was measured at 123 ng/L. These concentrations rose to 257 ng/L (June 10) immediately following the second application and declined to 10.5 ng/L on June 24 prior to the third application on June 27. Water samples collected on June 30, three days following the third application and the day of forced runoff via irrigation, contained 1,110 ng/L endosulfan; concentrations peaked two days later on July 2 (five days after spraying, three days after irrigation) at 1,310 ng/L. Mean total concentration in pond water dropped sharply to 319 ng/L three days later (July 5), was at 195 ng/L (July 25) and continued to decline thereafter. Only small quantities of endosulfan sulfate (range of 11.5 to 14.4 ng/L) were detectable six months (December 17) following the final application.

The alpha- and beta-isomers, which were the dominant forms of endosulfan through July 2, were present in varying proportions throughout the study. Beginning with the July 5 samples, endosulfan sulfate became the major component of the endosulfan found in the pond water and remained so for the duration of the study.

M-55-8. Mean concentration of total endosulfan was 124 ng/L on May 27, the day of the first application (Table 25; Figure 71). Concentrations declined to below the detection limit of 5 ng/L prior to the second application. Mean total endosulfan rose to 53.7 ng/L after the second application. The final endosulfan application was on June 23. Irrigation and natural rainfall occurred June 24. On June 25, following field runoff, mean concentrations of total endosulfan peaked at 583 ng/L. Mean total concentrations declined to 30.7 ng/L by June 30. Small quantities were detected after July 21, over three weeks after the last application. The proportions of alpha- and beta-endosulfan and endosulfan sulfate varied throughout the study period, but endosulfan sulfate became the dominant form by mid-July.

M-55-4 and T-4-1 (Reference). At M-55-4, nine of twenty-one samples had apparent detectable endosulfan. The primary constituent was endosulfan sulfate. At T-4-1, seven of twenty-two samples had detectable endosulfan. Of these, one had alpha-, beta- and endosulfan sulfate. Otherwise, there was no dicernable pattern. The average level of total endosulfan was below the detection limit for both ponds. All levels detected were less than three times the detection limit, with two exceptions. These concentrations are an order of magnitude less than the maximum concentrations detected in the treatment ponds.

Summary. Entry of endosulfan into the ponds followed two routes. Endosulfan was detected in water from both C-27-1 and M-55-8 following application of the pesticide to adjacent fields. Spray droplets were transported by air currents to the pond. Concentrations of total endosulfan reached 257 ng/L in C-27-1 and 53.6 ng/L in M-55-8 following the second application. Concentrations tended to decrease between applications. Irrigation and precipation transported endosulfan from the fields via runoff to the ponds following the third application. Concentrations in the ponds following field runoff reached an average of 1,310 ng/L of total endosulfan in C-27-1 and 583 ng/L in M-55-8. Concentrations of endosulfan in pond water declined to background concentrations in six months at C-27-1 and three months at M-55-8 following the final application. This difference may be attributed to the greater amount in C-27-1 pond water of total suspended solids (see

Figure 53) to which endosulfan may have been bound. Throughout the sampling period, pond M-55-8 had lower concentrations of endosulfan than did C-27-1.

8. Sediment

Pond sediment was sampled one or two days before and within one or two days after each endosulfan field application and at sequential intervals (approximately 7, 14, 28, 60, 90, and 180 days) following the third application. Sediment was collected from each of six zones. Endosulfan entered the sediments mainly via runoff through pond water and sedimentation of suspended particles to the pond bottom. Total endosulfans in the sediments peaked shortly after the first runoff events (Figures 72 and 73), and declined gradually thereafter.

C-27-1. Endosulfan was not present prior to June 25 and then increased to 49.2 μg/kg on July 5, 8 days after the third application and following a runoff on June 29 (Table 26). Mean total endosulfan concentrations rose from below the detection level to 25 μg/kg between mid-July and late August, and then declined to less than the detection limit by December 17. This trend (Figure 72) is similar to that seen in the soil (Figure 66).

The alpha- and beta-isomers, which were the dominant forms of endosulfan through July 5, were present in varying proportions throughout the study. Beginning with the July 25 samples, endosulfan sulfate became the major component of the endosulfan found in the pond sediment and remained so for the duration of the study.

M-55-8. The mean concentration of total endosulfan in the pond sediments increased from less than the detection limit of 5 μg/kg to 99.4 μg/kg on June 25, 2 days after the third application and after a runoff event on June 25 (Table 27). Mean total endosulfan concentration dropped to 15.1 μg/kg by July 7, then rose to 29.1 μg/kg by late August.

By December 13, concentrations had decreased to below the detection level (Figure 73; Table 27).

The alpha- and beta-isomers, which were the dominant forms of endosulfan through June 25, were present in varying proportions throughout the study. Beginning with the July 7 samples, endosulfan sulfate became the major component of the endosulfan found in the pond water and remained so for the duration of the study.

M-55-4 and T-4-1 (Reference). No endosulfan was detected in any of twenty-one sediment samples from T-4-1. Ten of twenty-two sediment samples from M-55-4 contained apparent detectable levels of endosulfan. All ten were found to contain endosulfan sulfate and one sample contained alpha- and beta- endosulfan. It should be noted that when working at or near the detection limit, it is expected that a small number of false positives will be found.

Summary. Endosulfan concentrations in the sediments were influenced by runoff events. Total endosulfan concentrations in sediments at both ponds peaked immediately following the first major runoff events at both ponds. Mean concentrations of total endosulfan peaked at twice the level in the sediments of M-55-8 (99.4 μg/kg) than at C-27-1 (43.5 μg/kg). However, by late August, both ponds had similar amounts of total endosulfan. Concentrations fluctuated similarly at both ponds and declined to less than or near the detection level by December.

9. Fish

Fish for tissue analysis were collected from both treatment ponds beginning on May 18 (9 days before first application) and continuing through mid-December. Only endosulfan sulfate was found in fish collected from C-27-1 (Table 28). Alpha- and beta-endosulfan were never detected in fish from the treatment ponds.

Concentrations of total endosulfan in fish tissue ranged from below the detection limit to 21.6 μg/kg one, two, and three months after the third endosulfan application. Endosulfan sulfate was present in both bluegill and largemouth bass from C-27-1, in similar concentrations. No endosulfan was detected in fish from M-55-8 (Table 29).

Of 14 fish analyzed from M-55-4, one had apparent endosulfan sulfate (13 μg/kg) (Appendix G). Of 14 fish from T-4-1, one had apparent beta-endosulfan (11 μg/kg). The average of endosulfan concentrations detected in fish from the reference ponds were less than the detection limit 10 μg/kg.

10. Field and Trip Blanks

Three types of blanks were analyzed from the field: 1) field blanks, 2) equipment rinsates, and 3) trip blanks. Field blanks were unspiked matrices collected prior to endosulfan applications at the treatment ponds, or from reference ponds and fields. Equipment rinsates were used to evaluate the cleaning procedures for the sampling equipment. Trip blanks (empty containers shipped along with samples) were used to check for contamination arising from shipping the samples from the field to the laboratory. The following accounts summarize the field blanks and equipment rinsates (Appendix G) for each sample type.

Application Cards. Of five field blanks and rinsates, one had apparent detectable levels of endosulfan. This sample contained apparent very low levels of alpha- and beta-endosulfan and endosulfan sulfate. The detected endosulfan was three orders of magnitude less than the amounts typically encountered on application cards and is not relevant.

Foliage. Of eleven field blanks and equipment rinsates, five had apparent detectable endosulfan. Of these five samples, three contained apparent alpha-endosulfan, and two contained apparent beta-endosulfan, all at a level of less than twice the detection limit. The detected concentrations were two orders of magnitude less than the amounts of endosulfan typically encountered on foliage samples and are not relevant.

Drift Cards. Of nine field blanks, one had apparent detectable endosulfan. This sample contained apparent alpha- and beta-endosulfan at a level two orders of magnitude less than the maximum concentrations encountered on drift cards and are not relevant.

Soil. Of 54 field blanks and equipment rinsates, three had apparent detectable endosulfan, two of these samples were trip blanks which accompanied the December samples into the field and back to the laboratory and contained apparent beta-endosulfan and endosulfan sulfate. The remaining sample was an equipment rinsate which contained apparent alpha- and beta-endosulfan. The detected concentrations of endosulfan were an order of magnitude less than the concentrations found in the soil in December and are not relevant.

Pond Water. Of 55 field blanks and equipment rinsates, 15 had apparent detectable endosulfan. Of these 15 samples, 12 contained apparent endosulfan sulfate. The detected endosulfan was one to two orders of magnitude less than typical concentrations encountered in pond water for comparable time periods and are not relevant.

Runoff Water. Of 14 field blanks, seven had apparent detectable concentrations of endosulfan. Two of these samples contained apparent alpha- and beta-endosulfan and endosulfan sulfate. Three additional samples contained only alpha- and beta-endosulfan. The detected endosulfan was three to four orders of magnitude less than concentrations encountered in runoff for comparable time periods and are not relevant.

Sediment. Of 33 field blanks and equipment rinsates, four had apparent detectable concentrations of endosulfan. The detected endosulfan was an order of magnitude less than the concentrations encountered in sediment for comparable time periods and are not relevant.

Fish. Of 10 field blanks, one had detectable concentrations of endosulfan. The concentrations detected were an order of magnitude higher than the concentrations used to prepare endosulfan spiked fish tissue, suggesting that a technical problem influenced this sample, and the result is not relevant.

Summary. With the exception of the single high concentration found in fish, all field blanks were found to be free of endosulfan or at a concentration that would not interfere with analytical results for the samples. In addition, many samples had apparent residues of only alpha- or beta-endosulfan or endosulfan sulfate suggesting these peaks were actually interfering co-extractants.

11. Field Spikes

Field spikes were generated by adding a known amount of each of the endosulfans to each matrix collected from reference ponds and fields. These samples accompanied the samples shipped to the lab and were analyzed using the same methods as the other samples. The following accounts summarize the field spikes for each sample type.

Application and Drift Cards. Recovery from field spiked application and drift cards averaged 78, 83, and 80 percent for alpha- and beta-endosulfan and endosulfan sulfate, respectively (Table 30). Standard deviations were 14, 19, and 21, respectively.

Foliage. Average recovery from field spiked tomato foliage was 116, 119, and 132 percent for alpha- and beta-endosulfan and endosulfan sulfate, respectively (Table 31). Standard deviations were 33, 32, and 39, respectively.

Soil. Soil was spiked at both 5 and 50 μg/kg. Average recovery from soil spiked at 5 μg/kg was 82, 97, and 94 percent, respectively (Table 32). Standard deviations were 5, 6, and 5, respectively. Average recovery from field spiked soil at 50 μg/kg was 70, 86, and 71 percent for alpha- and beta-endosulfan and endosulfan sulfate with standard deviations of 18, 22, and 17, respectively.

Pond Water. Pond water was spiked at 25, 50 and 500 ng/L. Average recovery from field spiked pond water at 25 and 50 ng/L was 176, 209, and 459 percent for alpha- and beta-endosulfan and endosulfan sulfate, respectively. Average recovery from the water spiked at 500 ng/L was 85, 90, and 100 percent with standard deviations of 19, 21, and 31, respectively (Table 33).

Seven field spiked samples were found to contain consistently high levels of endosulfan and were not used in the average recovery calculations. Laboratory spiked solvent samples yielded acceptable recoveries at the same extraction times indicating good method performance. It is possible that these seven samples were improperly spiked.

Runoff Water. Runoff water was spiked at two levels, 125,000 ng/L and 250,000 ng/L. Average recovery for the runoff water spiked at 125,000 ng/L was 88, 94, and 95 percent with standard deviations of 41, 43, and 39, for alpha- and beta-endosulfan sulfate, respectively (Table 34). Average recovery for the runoff water spiked at 250,000 ng/L was 84, 87, and 84 percent with standard deviations of 15, 16, and 18, respectively.

Sediment. Sediment was spiked at two levels, 5 and 50 μg/kg. Average recovery for the sediment spiked at 5 μg/kg was 64, 76, and 65 percent, with standard deviations of 9, 11, and 8, for the alpha- and beta-endosulfan and endosulfan sulfate, respectively (Table 35). Average recovery for sediment spiked at 50 μg/kg was 77, 87, and 68 percent with standard deviations of 21, 28, and 24, respectively.

Fish. Recovery from field spiked fish tissue averaged 26, 31, and 31 percent for alpha- and beta-endosulfan and endosulfan sulfate, respectively* (Table 36). These low recoveries resulted from leaks in the sample containers and are not representative of the method performance.

12. Method Validation

The methods for the analysis of endosulfan were validated prior to sample extraction and data collection. Application and drift card* and leaf rinsate methods were not validated prior to study. Validation consisted of analysis of unspiked matrix and matrix spiked at the detection limit and at 5 to 10 times the detection limit. The following accounts summarize the validation results.

Pond Water and Runoff Water. The results for the method validation for water at the detection limit of 5 ng/L were 131, 66, and 70 percent recovery for alpha- and beta-endosulfan and endosulfan sulfate, with standard deviations of 15, 18, and 6, respectively. The method was also validated at 25 ng/L with results of 96, 83, and 92 percent recovery for alpha- and beta-endosulfan and endosulfan sulfate, with standard deviations of 9, 13, and 37, respectively.

* Validation will be performed and data will be appended to this report as soon as it is available.

Soil. The results for the method validation for soil at the detection limit of 10 ug/kg were 132, 104, and 92 percent recovery for alpha- and beta-endosulfan and endosulfan sulfate, with standard deviations of 23, 17, and 12, respectively.

Sediment. The results for the method validation for sediment at 1 ug/kg were 72, 95, and 48 percent recovery for alpha- and beta-endosulfan and endosulfan sulfate, with standard deviations of 15, 14, and 34, respectively. As a result, the limit of detection of the method was raised to 5 μg/kg (see report of deviation in Appendix E). A method detection limit was based on the field spikes at 5 ug/kg. Recovery of the field spikes at 5 ug/kg are 64, 76, and 65 percent recovery for alpha- and beta-endosulfan and endosulfan sulfate, with standard deviations of 9, 11, and 8, respectively (Table 35).

Fish. The results for the method validation for fish at the detection limit of 10 ug/kg were 78, 101, and 7.3 percent recovery for alpha- and beta-endosulfan and endosulfan sulfate, with standard deviations of 7.6, 8.6, and 4.9, respectively.* The endosulfan sulfate recovery was low due to the high apparent level of endosulfan sulfate in the "clean" fish matrix used for the validation; this difference was subtracted out of the spiked samples. The method was also validated at 100 ug/kg with results of 84, 95, and 86 percent recovery for alpha- and beta-endosulfan and endosulfan sulfate, with standard deviations of 6.6, 4.8, and 4.1, respectively.

* This detection limit is dependent on a post-analysis validation which will be appended to this report as soon as it is available.

C. Ecological Measures

This section covers the ecological measures. These measures are presented in the following order:

- Phytoplankton
- Zooplankton
- Benthic Macroinvertebrates
  - Kick-net
  - Emergent Insects
  - Ekman Dredge Samples
  - S-Samplers
- Fish
- Pond Metabolism
- Autotrophic index
- Macrophytes

In each group, a discussion of qualitative biological patterns precedes results of comparative and quantitative statistical analysis. The generalized statistical model was applied to each of three time periods:

(1) PRE-SPRAY -- data following week 33 (when treatment pond M-55-8 sampling began August 16, 1987) to week 72 (May 15, 1988) of the 1988 data. The pre-spray period was used to determine which reference ponds were most similar to each of the two treatment ponds.

(2) POST-SPRAY -- week 73 (May 22, 1987) to approximately week 87 (August 28, 1988). The post-spray period was to evaluate whether the ponds have been impacted by endosulfan immediately after application of endosulfan.

(3) YEAR-END -- The seasonal decline at the end of the year from approximately week 88 (September 4, 1988) to week 103 (December 12, 1988). The year-end period was to determine whether recovery has occurred.

More details and discussion on statistical procedures are available in Section E of the Study Methods Chapter. For data presentation purposes, only the treatment and reference pond pairs selected are discussed in the main body of the report. The non-selected treatment and reference pond pairs for all endpoints are provided in Appendix H. Raw data for ecological measure are provided in Appendices I through O. A summary closes each group and serves to ready the reader for the integration chapter (VII).

1. Phytoplankton

Qualitative Observations. Over the two-year study period, the four test ponds yielded 98 separate phytoplankton taxa, representing seven algal divisions (Table 37). Raw phytoplankton data are provided in Appendix I. The green algae (Chlorophyta) were the most diverse group in the collection, consisting of 53 taxa, followed by diatoms (Bacillariophyta) and blue-greens (Cyanobacteria), each represented by 14 taxa. The yellow-green algae (Chrysophyta) were represented by nine taxa, whereas the euglenoids (Euglenophyta) consisted of four taxa. The cryptophytes (Cryptophyta) and the yellow-brown algae (Pyrrophyta) were each represented by three taxa.

The total number of taxa in each pond over the two-year study period was similar, ranging from 82 to 84. C-27-1 (treatment pond) and M-55-8 (treatment pond) contained 84 total taxa. M-55-4 (reference pond) yielded a total of 83 individual taxa, and, T-4-1 (reference pond) contained 82 total taxa.

The four ponds had 65 taxa in common, with 10 taxa found in only three ponds. An additional 13 taxa were found in only two ponds. Each pond had certain taxa unique to that pond. Thirteen taxa occurred only in 1987 (an unidentified chloroflagellate, Sorastrum, Didymocystis, Arthrodesmus, Onychonema, Zygnema, Ulothrix, Bulbochaete, Pinnularia, Amphora, Spondyliosum, Chroococcus, and an unidentified euglenophyte). Eleven taxa (Spermatozoopsis, Franceia, Nephrocytium, Actinastrum, Microlegna, Salpingoeca, Fragillaria, an unidentified cryptophyte, a coccoid cyanobacterium, Raphidiopsis, and Calothrix) occurred only in 1988.

The relative abundance of the major phytoplankton groups in 1987 and 1988 is depicted graphically for C-27-1 (treatment pond), M-55-4 (reference pond), M-55-8 (treatment pond), and T-4-1 (reference pond) in kite diagrams (Figures 74, 75, 76, and 77, respectively). The width of the individual kites represents the percent relative abundance of the specific taxa for the individual collection dates. The wider or narrower the vertical space between any two points, the greater or smaller the percent relative abundance. For example, cyanobacteria were the most abundant taxon between weeks 70 and 80 (Figure 74). Because of the species richness for green algae, individual taxa were grouped by order (Chlorococcales, Tetrasporales, Volvocales, Ulotrichales, Oedogoniales, and Zygnematales), while the remaining taxa were grouped by division (Bacillariophyta, Chrysophyta, Pyrrophyta, Euglenophyta, Cryptophyta, and Cyanobacteria = Cyanophyta).

Comparison of phytoplankton mean relative abundance in C-27-1 for 1987 and 1988 (Figure 74) indicated increased abundance of the Cyanobacteria and Chlorcoccales beginning week 76 (June 12, 1988), with a subsequent increase of the Chlorococcales from week 78 (June 26, 1988). The Cyanobacteria bloom subsided by week 84 (August 7, 1988), with a second bloom of the Chlorococcales beginning the same week. The Volvocales, Tetrasporales, Pyrrophyta, and Euglenophyta were also more abundant in 1988 than in 1987. This algal bloom coincided with the application period, which occurred between weeks 73 (May 22, 1988) and 78 (June 26, 1988). In contrast, the 1987 midsummer blooms of the Chrysophyta and Zygnematales did not reoccur in 1988.

Because of the magnitude of the Cyanobacteria and Chlorococcales blooms in C-27-1, the mean relative abundance of the individual taxa comprising these two groups was plotted (Figures 78 and 79, respectively). The Cyanobacteria bloom from weeks 74 (May 29, 1988) to 84 (August 7, 1988) consisted primarily of Anabaena, Raphidiopsis, Anabaenopsis, and Microcystis. The secondary cyanobacteria bloom from weeks 90 (September 18, 1988) to 102 (December 1988) was composed primarily of Lyngbya and Microcystis. The Chlorococcales bloom from weeks 78 (June 26, 1988) to 84 (August 7, 1988) consisted primarily of Coelastrum, Pediastrum, Scenedesmus, and Ankistrodesmus (Figure 79). After week 88, Coelastrum and Kirchneriella predominated, with Dictyosphaerium, Ankistrodesmus, Pediastrum, Oocystis, Scenedesmus, and Crucigenia playing a secondary contributing role.

Comparison of the mean relative abundance of the major phytoplankton groups for M-55-4 (reference pond) in 1987 and 1988 (Figure 75) indicated a general increase for the Chlorococcales, Tetrasporales, Pyrrophytes, Euglenophyta and the Cyanobacteria in 1988 relative to 1987. While a decrease in relative abundance between 1987 and 1988 was evident for the Zygnematales, the Chrysophyta exhibited the most evident decrease in relative abundance. The Cyanobacteria generally increased in 1988, particularly during weeks 76 (June 12, 1988) to 82 (July 24, 1988). The decrease in Chrysophyta and Zygnematales in 1988, coupled with an increase in Cyanophyta, was a trend that occurred in both a treatment (C-27-1) and a reference (M-55-4) pond.

For M-55-8 (treatment pond), mean relative abundance of several groups increased in 1988 relative to 1987. Increased relative abundance was noted for the Chlorococcales, diatoms, Tetrasporales, Volvocales, and Cyanobacteria (Figure 76). Decreases were observed in the relative abundance of the Zygnematales and Chrysophyta. This trend was consistent with the previously discussed reference (M-55-4) and treatment pond.

Qualitative comparison of phytoplankton mean relative abundance between 1987 and 1988 for T-4-1 (reference pond) indicated a slight decline of some groups, with a slight increase for others (Figure 77). Reduced relative abundance was noted for the Ulotrichales, and euglenoids in 1988. Increases were evident for the Tetrasporales, Oedogoniales, Chrysophyta, and Cyanophyta in 1988. The Chlorococcales exhibited an increase in relative abundance for the spring and summer of 1988 when compared to 1987.

In summary, general relative abundance trends were similar for all ponds. Cyanobacteria and Chlorococcales were generally more abundant in all ponds in 1988 when compared to 1987. The Zygnematales and Chrysophyta were less abundant in 1988 than 1987 for the two treatment (C-27-1, M-55-8) and one reference (M-55-4) ponds, with relative abundance of these two groups similar in T-4-1 (reference) for 1987 and 1988.

Density. Mean phytoplankton densities (log of the total number per liter) of the four ponds over the entire study period ranged from 7.11 to 14.87. The wide range of densities reflects seasonal growth trends, as well as blooms of individual taxa as discussed above. M-55-4 was selected as the reference pond for a quantitative comparison of phytoplankton densities for both treatment ponds: C-27-1 and M-55-8. The preference of one control pond over the other for comparison to a particular treatment pond was based on pairwise comparisons of the reference ponds with the treatment ponds during the pre-spray time period. The results that guided this selection were as follows:

1. Applications of the ANOVA model to the pre-spray data for all ponds indicated a significant interaction between pond and week (model $R^2 = 0.6$; pond-by-week interaction $F = 10.11$, df = 18,288, PR>F = 0.0001).

2. Follow-up Bonferroni multiple comparison procedures indicated significant differences in 4 of 7 weeks in the pre-spray period for T-4-1 and C-27-1, and M-55-4 and C-27-1. Significant differences occurred in 5 of 7 weeks for M-55-4 and M-55-8 during the pre-spray period, and in 5 of 7 weeks for T-4-1 and M-55-8 (Experiment-wise error rate = 0.05).

3. Based on the multiple comparison procedure, no clear preference for a reference/treatment pond

pairs emerged. Therefore, the two pond pairs were selected to coincide with later zooplankton pond pairs. The two pairs of ponds for quantitative comparisons of phytoplankton densities were M-55-4 and M-55-8, and M-55-4 and C-27-1.

Phytoplankton densities for the two pairs of ponds differed significantly during the post-spray (weeks 74-87; May 29 - August 28, 1988) time period (model R^2 = 0.7; pond-by-week interaction F = 8.06, df = 15,100, PR>F = 0.0001). Multiple comparisons between M-55-4 (reference pond) and M-55-8 (treatment pond) indicated significant differences in 5 of 6 weeks during the post-spray period (Figure 80), whereas significant differences occurred in 4 of 6 sampling weeks for pond M-55-4 (reference pond) and C-27-1 (treatment pond) (Figure 81).

During the year-end time period, phytoplankton densities remained significantly different for the two pairs of ponds. There was a pond-by-week interaction (model R^2 = 0.9; pond-by-week interaction F = 30.64, df = 15,96, PR>F = 0.0001). Phytoplankton densities were significantly greater in treatment pond M-55-8 for 5 sampling weeks in the year-end period when compared to reference pond M-55-4 (Figure 80). For treatment pond C-27-1, phytoplankton densities were significantly greater than in reference pond M-55-4 in all six sampling weeks (Figure 81).

In summary, phytoplankton densities reflected seasonal trends, with highest densities in mid to late summer. The statistical comparisons between the two pairs of ponds, M-55-4 and M-55-8, and M-55-4 and C-27-1, indicated that phytoplankton densities were similar to or significantly higher in the treatment ponds than in the control ponds for the post-spray and year-end time periods. Relative to M-55-4, a significant decrease in phytoplankton density occurred in C-27-1 (treatment pond) during weeks 74 and 76 following the first and second applications of endosulfan (Figure 81). A similar decline was not observed after the third application (June 27, 1988) in C-27-1 relative to reference pond M-55-4. From week 80 (July 10, 1988), phytoplankton density in C-27-1 was similar to, or greater than, that in M-55-4 for the remainder of the study. Further, it should be emphasized significant differences between C-27-1 and M-55-4 were noted in the pre-spray period (Figure 81). For treatment pond M-55-8, phytoplankton density was consistently greater than, or similar to, that of control pond M-55-4 for the pre-spray, post-spray and year-end periods with the exception of the last collection in December 1988 (week 102).

Diversity. The mean Shannon-Weaver diversity index for each collection period in 1987 and 1988 for the two treatment ponds (C-27-1, M-55-8) and the two reference ponds (M-55-4, T-4-1) ranged from 0.48 to 2.63 (Figures 82 and 83). Selected treatment and reference ponds were used for quantitative comparison of Shannon-Weaver diversity indices for phytoplankton. The selection of one control pond over the other for comparison to a particular treatment pond was based on pairwise comparisons of the reference ponds with the treatment ponds during the pre-spray time period. The results that guided this selection were as follows:

1. Application of the ANOVA model to the pre-application data for all ponds indicated a significant interaction between pond and week (model $R^2 = 0.6$; pond-by-week interaction $F = 10.48$; df = 18,288; PR>F = 0.0001).

2. Follow-up Bonferroni multiple comparison procedures indicated significant differences between the treatment and reference ponds in the pre-spray application period. The Shannon-Weaver diversity index in T-4-1 was significantly different from C-27-1 in 3 of 7 weeks (weeks 39, 43, 70). The Shannon-Weaver index of T-4-1 was significantly different from M-55-8 in 4 of 7 weeks (weeks 35, 43, 64, 70). In contrast, the Shannon-Weaver index of M-55-4 was significantly different in 1 of 7 weeks for C-27-1 (week 39) and in 2 of 7 weeks for M-55-8 (weeks 43 and 70) (experiment-wise error rate = 0.05).

3. M-55-4 was selected as the reference pond for both the C-27-1 and M-55-8 treatment ponds because there were fewer significant differences in the pre-application period than

were observed for T-4-1. The two pairs of ponds for quantitative comparisons of phytoplankton Shannon-Weaver diversity indices are C-27-1 and M-55-4, and M-55-8 and M-55-4.

Phytoplankton diversity for the two pairs of ponds differed significantly during the post-spray time period (model R^2 = 0.9; pond-by-week; F = 39.70, df = 15,100, PR>F = 0.0001). Multiple comparisons between C-27-1 and M-55-4 indicated there were significant differences in phytoplankton diversity for three of six weeks (Figure 82), whereas there were significant differences in two of six weeks between M-55-8 and M-55-4 (Figure 83).

During the year-end time period, phytoplankton diversity for the two pond pairs remained significantly different. There was a pond-by-week interaction (model R^2 = 0.9; pond-by-week interaction F = 64.1, df = 15,96, PR>F = 0.0001). Multiple comparisons between C-27-1 and M-55-4, and M-55-8 and M-55-4, revealed significant differences in four of six year-end sampling weeks (Figures 82 and 83).

Visual inspection of the rise and fall of diversity indices for C-27-1 and M-55-4 (Figure 82) between weeks 72 (May 15, 1988) and 92 (October 2, 1988) revealed a similar trend for both ponds. The reference and treatment ponds exhibit a series of three rapid declines and increases in phytoplankton diversity. However, the declines in M-55-4 (reference) lagged behind those in C-27-1 (treatment) by 2 to 5 weeks. In the year-end phase (Figure 82), the lag phenomenon appeared to account for the significant differences in diversity between the two ponds for the weeks of August 21 (week 86), September 4 (week 88), September 18 (week 90), and October 16 (week 94), 1988. For the last two collection periods of the year, phytoplankton diversity was not significantly different between C-27-1 (treatment) and M-55-4 (reference). In any event, significant differences were noted in phytoplankton diversity in the pre-spray, post-spray, and year-end phases for C-27-1 and M-55-4.

The phytoplankton diversity index pattern of M-55-8 and M-55-4 was similar throughout the pre- and post-spray periods (Figure 83).

Significant differences in diversity occurred for the weeks of October 25, 1987 (week 43), May 1, 1988 (week 70), June 26, 1988 (week 78), and July 10, 1988 (week 80), with phytoplankton diversity in M-55-8 (treatment pond) greater than that of M-55-4 (reference pond). In the year-end phase, a reversal of the trend toward greater phytoplankton in diversity occurred. Although phytoplankton diversity was significantly greater in M-55-8 than M-55-4 for the weeks of September 4, 1988 (week 88) and September 18, 1988 (week 90), diversity was greater in M-55-4 (control pond) for the weeks of November 13, 1988 (week 98) and December 11, 1988 (week 102). Significant differences were noted in phytoplankton diversity in the pre-spray, post-spray, and year-end phases for M-55-8 and M-55-4.

Summary. Qualitatively, trends in relative abundance for the major phytoplankton groups were similar in both the treatment and reference ponds. Quantitatively, phytoplankton densities reflected seasonal trends, with highest densities in mid to late summer. Generally, phytoplankton densities in the treatment ponds were similar to, or significantly higher, than the control ponds after pesticide application. Exceptions include a significant decrease in phytoplankton density in C-27-1 relative to M-55-4 after the first and second applications of endosulfan (weeks 74 and 76; May 29 and June 12, 1988). However, C-27-1 phytoplankton diversities were greater than or similar to those of M-55-8 following the third endosulfan application, and the remainder of the study. Phytoplankton diversity in C-27-1 (treatment pond) was significantly different than M-55-4 (reference pond) for the post-spray and year-end sampling periods. However, the differences appear to be due to a similar series of rapid rises and falls in diversity that occur in both C-27-1 and M-55-4, but are 2-5 weeks out of phase. In contrast, M-55-8 (treatment pond) generally had a higher phytoplankton diversity than M-55-4 (reference pond) after endosulfan application, with the exception of the last two year-end sampling periods which reflect the onset of the winter seasonal decline. Both treatment ponds exhibited significant differences in phytoplankton diversity from the reference pond in the pre-spray, post-spray, and year-end phases.

2. Zooplankton

Qualitative Observations. Over the two-year study period, the four test ponds yielded 54 separate zooplankton taxa representing three phyla (Table 38). Raw zooplankton data are provided in Appendix J. A few samples from one collection time were not preserved as discussed in the report of deviation in Appendix E. Two classes of protozoa (Rhizopoda and Ciliata) were collected, containing a total of 17 (4 and 13, respectively) taxa. The Rotifera were the most diverse zooplankton group in the collection, consisting of 25 taxa distributed between two orders (Ploima and Flosculariacea represented by 19 and six genera, respectively). Crustacean zooplankton consisted of eight genera of Cladocera and two genera of Copepoda. Copepod nauplius larvae and copepodite larvae were also present in the collections. Ostracoda were present in all ponds, but were not identified below the ordinal level of taxonomic resolution.

The total number of zooplankton taxa in each pond over the two-year study period was similar, ranging from 51 to 54. C-27-1 (treatment pond), M-55-8 (treatment pond), and M-55-4 (reference pond) each yielded 51 individual taxa. T-4-1 (reference pond) contained 54 taxa. The four ponds had 43 taxa in common, with three taxa (Synchaeta, Colorella, Notommata) found in only three ponds. An additional five taxa (Euchlanis, Testudinella, Tintinnidiom, Trichotria and Campanella) were found in only two ponds, and four taxa (Mytilina, Pleuroxus, unidentified keronid rotifer, and Epistylis) were found in only one of the ponds. Four genera (Mytilina, Synchaeta, Tintinnidium, and an unidentified keronid rotifer) were found only in 1987. Three genera (Dileptus, Campanella, and Epistylis) were found only in 1988 (Table 38).

The relative abundance of the major zooplankton groups in 1987 and 1988 are depicted qualitatively in kite diagrams for C-27-1 (treatment pond), M-55-4 (reference pond), M-55-8 (treatment pond), and T-4-1 (reference pond) (Figures 84, 85, 86, and 87, respectively). The width of the individual kites represent the percent relative abundance of the specific taxa on the individual collection dates. Thus, the wider or narrower the vertical distance between two points, the greater or smaller the relative abundance. Rotifera were consistently the most abundant and most diverse zooplankton group.

Qualitative comparison of zooplankton mean relative abundance in C-27-1 (treatment pond) (Figure 84) indicated increased abundance of rotifers, cladocerans, and protozoa for 1988 when compared to 1987. The relative abundance of ostracods and copepods was similar between 1987 and 1988. The predominant rotifer taxa in 1987 were Keratella, Conochilus, and Conochiloides (Figure 88). The mean relative abundance of rotifers shifted in 1988. Although Keratella, Conochilus, and Conochiloides, represented a significant fraction, additional genera (Brachionus, Asplanchna, Aneuropsis, Kellicotia, Hexarthra, Filinia, Pompholyx, Polyarthra, and Trichocerca) constituted a major fraction of the rotifer fauna in 1988.

Comparison of the mean relative abundance of the major zooplankton groups for M-55-4 (reference pond) also indicated a slight decrease in copepods between 1987 and 1988, with a slight increase in ostracods (Figure 85). Cladocerans abundance was greater in 1988 than 1987. Rotifer mean relative abundance was also greater in 1988 than 1987. Predominant taxa were similar for both years with Keratella, Brachionus, Filinia, and Pompholyx more abundant in 1988 than 1987 (Figure 89). Polyarthra and Kellicotia were less abundant in 1988 than 1987.

For M-55-8 (treatment pond), the mean relative abundance of copepods declined somewhat between 1987 and 1988, whereas cladocerans, rotifers, protozoans and ostracods increased in mean relative abundance in 1988 (Figure 86). Although rotifers exhibited a general increase in mean relative abundance in 1988 for the spring and summer seasons, the autumnal increase observed in 1987 did not occur in 1988. The autumnal increase of mean relative abundance for rotifers in 1987 was caused by increases in Concochilus and Keratella (Figure 90).

Qualitative comparison of zooplankton mean relative abundance for T-4-1 (reference pond) between 1987 and 1988 indicated increased abundance of cladocerans, protozoans, rotifers, and ostracods, with little change in copepods (Figure 87). Relative abundance of rotifers exhibited an increase throughout most of 1988. The large peak in relative abundance for week 34 in 1987 was caused by an increase in Conochiloides that did not occur in 1988 (Figures 87 and 91). Rotifer genera that increased in abundance in 1988 included Brachionus, Asplanchna, Conochilus, Filinia, Keratella, and Polyarthra (Figure 91).

In summary, general trends in relative abundance of major zooplankton groups were similar for all ponds. In both the treatment and reference ponds, the relative abundance of cladocerans, rotifers, and protozoans increased in 1988 relative to 1987.

Density. Mean total zooplankton densities (log of the total number per liter) of the four ponds over the entire study period ranged from 5.49 to 9.41 organisms per liter (Figures 92 and 93). The range of densities reflect seasonal growth trends, as well as increased abundance of individual taxa as presented in the qualitative observation section above. M-55-4 was selected as the reference pond for a quantitative comparison of zooplankton densities in both treatment ponds, C-27-1 and M-55-8. The preference of one control pond over the other for a comparison to a particular treatment pond was based on pairwise comparisons of the reference ponds with the treatment ponds during the pre-spray time period. The results that guided this selection were as follows:

1. Applications of the ANOVA model to the pre-spray data for all ponds indicated a significant interaction between pond and week (model R^2 = 0.6; pond-by-week interaction F = 7.21, df = 18,288, PR>F = 0.0001).

2. Follow-up Bonferroni multiple-comparison procedures indicated significant differences in

2 of 7 weeks in the pre-spray period for T-4-1 and C-27-1, and in 1 of 7 weeks for M-55-4 and C-27-1. Significant differences occurred in 1 of 7 weeks for M-55-4 and M-55-8 during the pre-spray period, and in 2 of 7 weeks for T-4-1 and M-55-8 (experiment-wise error rate = 0.05).

3. M-55-4 was selected as the reference pond for both M-55-8 and C-27-1 because of fewer significant differences during the pre-spray period. The two pairs of ponds for quantitative comparisons of total zooplankton densities were M-55-4 and M-55-8, and M-55-4 and C-27-1.

Zooplankton densities for the two pairs of ponds differed significantly during the post-spray (weeks 74-87; May 29 to August 28, 1988) time period (model R^2 = 0.8; pond-by-week interaction F = 13.08, df = 15,100, PR>F = 0.0001). Multiple comparisons between M-55-4 (reference pond) and M-55-8 (treatment pond) indicated significant differences in 2 of 6 weeks during the post-spray period, whereas significant differences occurred in 3 of 6 sampling weeks for pond M-55-4 (reference pond) and C-27-1 (treatment pond).

During the year-end time period, zooplankton density remained significantly different for the two pairs of ponds. There was a pond-by-week interaction (model R^2 = 0.8; pond-by-week interaction F = 19.03, df = 15,96, PR>F = 0.0001). The two pairs of ponds were significantly different during the year-end time period, with total zooplankton densities generally higher in the treatment ponds (Figure 92 and 93). Multiple comparisons between M-55-4 (reference pond) and M-55-8 (treatment pond) indicated significant differences in 5 of 6 weeks during the year-end phase. Significant differences also occurred in 5 of 6 weeks for M-55-4 (reference pond) and C-27-1 (treatment pond).

Zooplankton densities reflected seasonal trends, with highest densities in mid- to late-summer. The statistical comparisons between the two pairs of ponds, M-55-4 and M-55-8, and M-55-4 and C-27-1, indicated zooplankton densities similar to or significantly higher in the treatment ponds than in the control ponds for the post-spray and year-end time periods. A significant decrease in zooplankton density occurred when treatment ponds C-27-1 and M-55-8 were compared to control pond M-55-4 during weeks 76 and 102 (Figures 92 and 93).

Diversity. The mean Shannon-Weaver diversity index for zooplankton for the four ponds ranged from 0.78 to 2.19 over the entire study period (Figures 94 and 95). M-55-4 was selected as the reference pond for C-27-1, whereas T-4-1 was selected as the reference pond for the treatment pond M-55-8 for quantitative comparison of the zooplankton Shannon-Weaver diversity indices. The preference of one control pond over the other for comparison to a particular treatment pond was based on pairwise comparisons of the reference ponds with the treatment ponds during the pre-spray time period. The results that guided this selection were as follows:

1. Application of the ANOVA model to the pre-spray data for all ponds indicated a significant interaction between pond and week (model $R^2 = 0.4$; pond-by-week interaction $F = 3.85$; df = 18,288; PR>F = 0.0001).

2. Follow-up Bonferroni multiple comparison procedures indicated significant differences between C-27-1 and the reference ponds in the pre-spray application period (experiment-wise error rate = 0.05). The Shannon-Weaver index of T-4-1 and M-55-4 was significantly different from C-27-1 in 2 of 7 weeks (weeks 39, 43). In contrast, the Shannon-Weaver index for T-4-1 was not significantly different from M-55-8 for the 7 periods sampled in the pre-application phase. Treatment pond M-55-8 differed from M-55-4 in 1 of 7 weeks for the pre-application phase (experiment-wise error rate = 0.05).

3. T-4-1 was selected as the reference pond for M-55-8 because there were fewer significant differences than for M-55-4. M-55-4 was selected as the reference pond for C-27-1 to include both reference ponds in the overall analysis of treatment ponds. The two pairs of ponds for quantitative comparisons of zooplankton Shannon-Weaver diversity indices are C-27-1 and M-55-4, and M-55-8 and T-4-1.

The zooplankton Shannon-Weaver diversity indices for the two pairs of ponds differed significantly during the post-spray time period (model R^2 = 0.7; pond by week interaction F = 6.18; df = 15,100, PR>F = 0.0001). Multiple comparisons between C-27-1 and M-55-4 indicated significant differences in 1 of 6 sampling weeks in the post-spray time period (Figure 94). Zooplankton diversity was also significantly different in 1 of 6 sampling weeks for M-55-8 and T-4-1.

During the year-end time period, zooplankton diversity for the two pond pairs remained significantly different. There was a pond-by-week interaction (model R^2 = 0.7; pond-by-week interaction F = 5.78, df = 15,96, PR>F = 0.0001). Multiple comparisons between M-55-4 (reference pond) and C-27-1 (treatment pond) revealed significant differences in 2 of 6 sampling weeks (Figure 94), whereas T-4-1 (reference pond) differed from M-55-8 (treatment pond) in 1 of 6 weeks (Figure 95).

Summary. Qualitatively, zooplankton relative abundance exhibited similar trends in the reference and treatment ponds. All ponds exhibited increased zooplankton abundance in 1988 over 1987, which may be the result of greater phytoplankton abundance and a concurrent increase in the available food supply for zooplankton. Rotifers were the most abundant group in all ponds and generally increased in all ponds in 1988 when compared to 1987. Cladocera increased in the two treatment ponds as well as the two reference ponds in 1988 over 1987, as did Protozoa and Ostracoda. Copepoda remained similar in all ponds in 1987 and 1988.

Zooplankton density and diversity were examined quantitatively. Zooplankton densities reflected seasonal trends, and statistical comparisons showed zooplankton densities between the two pairs of ponds (M-55-4 and M-55-8; M-55-4 and C-27-1) were generally higher in the treatment ponds than in the control ponds for both the post-spray and year-end time periods. Although zooplankton densities in both treatment ponds M-55-8 and C-27-1 were higher than or equal to reference pond M-55-4 for most of the post-spray and year-end periods, significant decreases were observed in both M-55-8 and C-27-1 during weeks 76 and 102, which reflect the application period and winter seasonal decline respectively.

Total zooplankton diversity was significantly different in treatment pond C-27-1 when compared to reference pond M-55-4 in 1 of 6 weeks (week 80) for the post-spray period, and two of six sampling weeks (weeks 86 and 90) for the year-end phase. These differences appear to be related to a rotifer and protozoa bloom coincident with these two sampling weeks. Total zooplankton diversity was significantly lower in M-55-8 (treatment) than T-4-1 during 1 of 6 weeks (week 78) for the post-spray period, and the last week (102) of the year end phase (weeks 82 to 102). The lowest diversity in M-55-8 is apparently associated with rotifer blooms (Figures 86 and 90). Interestingly, zooplankton were generally more abundant in the two treatment ponds in the application year (1988) than in the baseline year (1987).

3. Benthic Macroinvertebrates

Sample types for benthic macroinvertebrates included kick nets, emergence traps, Ekman dredge samples, and S-samples (artificial substrates). The kick net samples were qualitative and were collected only from the four littoral zones nearest the pond edges. Emergence traps were set at three pond zones, whereas the Ekman dredge samples were collected only from the two mid-pond zones. S-samples were used at all pond zones for examinations of taxonomic composition and biomass of the benthic community. Because of the redundancy in benthic sample types, only a subset of S-samples were analyzed (see report of deviation in Appendix E). Each of the sample types is discussed separately below following the general pattern of qualitative biological results followed, where applicable, by quantitative statistical analyses. A summary of benthic macroinvertebrates covers both qualitative and quantitative trends. Raw benthic macroinvertebrate data are provided in Appendix K.

Kick-Net. Seventy-nine taxa of macroinvertebrates were collected by kick-net in the near shore area of the ponds over the entire study period (Table 39). M-55-4 exhibited the highest number of taxa (66), followed by T-4-1 (63), M-55-8 (49), and C-27-1 (48) (Appendix H). Thirty-eight of the 79 taxa collected over the two-year study period were common to all ponds. As qualitative samples, the kick-net results were only examined qualitatively, and were not subjected to quantitative statistical analysis.

The taxa collected in the kick-net samples were combined into 4 higher taxonomic groups to examine relative abundance of the major groups. Chironomidae were the dominant macroinvertebrate organisms, representing 53 percent of the total collection. Oligochaeta were the next most dominant taxa and comprised 10 percent of the total collection. The remaining groups each represented less than 10 percent of the total collection and were combined into miscellaneous invertebrates.

Relative abundance of the major macroinvertebrate groups (Figures 96, 97, 98, and 99) revealed community shifts for the two reference ponds for the pre- and post-spray study phases. The relative abundance of oligochaetes decreased in M-55-4 (Figure 97) during the period coincident with pesticide application in the treatment ponds, while chironomids increased. Relative abundance of miscellaneous invertebrates appeared to be similar between 1987 and 1988. Oligochaetes also decreased in T-4-1 over the same time period, whereas miscellaneous invertebrates increased (Figure 98).

For the treatment ponds, the relative abundance of macroinvertebrates changed between the pre-treatment and post-treatment phases. In C-27-1, oligochaetes decreased after application, while chironomids increased (Figure 96). In M-55-8, the relative abundance of chironomids decreased for weeks 74 - 76 after application, but increased in abundance for the remainder of the year (Figure 98). Miscellaneous invertebrates increased in 1988 over 1987.

Macroinvertebrate community structure as measured by relative abundance exhibited greater stability in the treatment ponds than in the reference ponds between 1987 and 1988. Except for a decrease in chironomid abundance in M-55-8, shifts in macroinvertebrate relative abundance observed in treatment ponds also occurred in at least one of the reference ponds.

Both C-27-1 (treatment pond) and M-55-4 (reference pond) exhibited a decrease in the relative abundance of oligocheates and an increase in chironomids in the year-end or post-treatment phase. Increased relative abundance of miscellaneous insects (especially coleoptera) was exhibited in both M-55-8 (treatment pond) and M-55-4 (reference pond) in the post-treatment phase.

Based on presence/absence data (Table 39) several macroinvertebrate taxa in treatment ponds were less common with respect to reference ponds after application. Physid snails and Coenagnionidae were less common in the year-end phase in M-55-8 (treatment), but no apparent differences were evident for the remaining three ponds. Neither _Peltodytes_ nor Turbellarians were collected in the year-end phase in C-27-1, but were collected in the other three ponds.

Similar to the treatment ponds described above, other taxa become less common in the reference ponds in the same time interval coincident with the post-application period. In M-55-4 (reference), no corixidae were collected after application and no _Caenis_ were collected in the year-end phase (Table 39). However, both taxa were collected in all study phases in both the treatment ponds. Accordingly, no clear changes in relative abundance of macroinvertebrates in the littoral zones of the ponds, the point of entry of runoff, were directly attributable to endosulfan.

Emergent Insects. The four test ponds contained 47 taxa of emergent insects. Of these taxa, 95 percent of the insects sampled were chironomids. The remaining taxa were minor contributors, each consisting of less than two percent of the total collection. The minor taxa consisted primarily of Ephemeroptera and Trichoptera, which were most common in ponds M-55-4 and T-4-1 (Figures 100, 101, 102, and 103). Other frequently encountered insects included coleoptera, hemiptera, and odonata. Because the emphasis of this ecological endpoint was emergent rate, no further qualitative analysis was performed on species composition.

Emergence rates followed a marked unimodal seasonal trend. The emergence rate was highest in mid-summer (weeks 80-85) and lower in spring and autumn (Figures 104 and 105). For example, 1988 emergence rates (numbers/m^2/day) for chironomids for all ponds in April, August, and November, 1988 were 10.8, 25.6, and 3.1, respectively.

Because chironomids represented 95 percent of the total emergent collection, chironomids were the only taxon selected for quantitative analysis. The reference pond M-55-4 was selected for quantitative comparison to the treatment pond C-27-1 for chironomid emergence rates. Reference pond T-4-1 was compared to treatment pond M-55-8. Nested ANOVA results indicated that these were the most similar reference/treatment pond pairs during the pre-spray period. The results that guided this selection were as follows:

1. Application of the nested ANOVA model to the pre-spray data for all ponds indicated a significant interaction between pond and week (model $R^2 = 0.60$; pond-by-week interaction $F = 4.48$, df = 18,269, PR>F = 0.0001)

2. Follow-up Bonferroni multiple comparison procedures indicated no significant differences between reference/treatment ponds pairs M-55-4 and C-27-1. There were significant differences in 3 out of 7 weeks for M-55-8 and T-4-1, 2 out of 7 weeks for M-55-8 and M-55-4, and 1 out of 7 weeks for C-27-1 and T-4-1 (experiment-wise error rate = 0.05).

3. M-55-4 was selected as the reference pond for C-27-1 because there were no significant differences between the ponds during the pre-spray time period. M-55-4 was also selected as the reference pond for M-55-8 because it differed during fewer sampling weeks as compared to the other reference pond, T-4-1. The pairs of ponds for the quantitative analysis were M-55-4 and C-27-1, and M-55-4 and M-55-8.

Emergence rates for the two pairs of ponds differed significantly during the pre-spray time period (for details, see pond selection). Multiple comparisons between M-55-4 (reference pond) and C-27-1 (treatment pond) indicated no significant differences during the pre-spray period, whereas significant differences occurred in 2 of 7 sampling weeks for pond M-55-8 (reference pond) and M-55-4 (treatment pond) (Figures 104 and 105).

Emergence rates for the two pairs of ponds differed significantly during the post-spray time period (model R^2 = 0.70; pond-by-week interaction F = 3.98; df = 21,63, PR>F = 0.0001). Multiple comparisons between M-55-4 and C-27-1 indicated there were significant differences in 1 of 8 weeks, whereas there were no significant differences for M-55-4 and M-55-8 (Figures 104 and 105).

During the year-end time period, emergence rates for one of the pond pairs remained significantly different (Figures 104 and 105). The pond-by-week interaction was not significant and only the main pond effect was used in the multiple comparisons (model R^2 = 0.83, pond main effect F = 14.63, df = 3,64, PR>F = 0.0001). Multiple comparisons between M-55-4 and C-27-1 revealed no significant differences while for M-55-4 and M-55-8 there were significant differences throughout the year-end time period with an average emergence rate of 10 and 5 individuals/m^2/day for M-55-4 and M-55-3, respectively.

Chironomid emergence rate was significantly lower in C-27-1 (treatment) than in M-55-4 (reference) (Figure 104) only during the week of June 5 (week 75). During the post-spray period (May 22 [week 73] through September 3 [week 87]), C-27-1 had a significantly lower chironomid emergence rate than the reference pond M-55-4. The week of June 5, emergence for C-27-1 and M-55-4 was 4 and 14 individuals/m^2/day, respectively, which was the one sampling week during the post-spray time period when the ponds differed. One endosulfan application preceded this sample, with pond C-27-1 receiving total endosulfan through drift. Drift from subsequent applications at C-27-1 deposited addition total endosulfan in the pond, with no noticeable impact on chironomid emergence rates. No statistically significant differences were found between M-55-4 and M-55-8 during the post-spray period.

For the year-end period (September 4 [week 88] through December 10 [week 101]), there were no statistically significant differences in chironomid emergence in one of the reference/treatment pond pairs (C-27-1 and M-55-4), while in the other pond pair (M-55-4 and M-55-8), M-55-8 had significantly lower emergence rates (Figure 105).

In summary, these data suggest that the influence of endosulfan on chironomid emergence in C-27-1 was non-existent or short-lived, and did not persist beyond the last application (June 27, week 78). For the other pond pair, because no differences in emergence rates were noted in the post-spray time period, reduced emergence rate noted in M-55-8 during the year-end time period appears to be due to an earlier seasonal decline in emergence for M-55-8 relative to M-55-4. This conclusion is supported by the absence of an effect on emergence in C-27-1 which received a greater dose of endosulfan over the study period.

Ekman Dredge Samples. Ekman samples contained 40 taxa of benthic macroinvertebrates from the four test ponds. Although T-4-1 exhibited the lowest macroinvertebrate densities, it had the highest number of taxa (32); followed by M-55-4 (30), C-27-1 (27), and M-55-8 (18). Dominant taxa for 1987 and 1988 varied slightly among ponds. C-27-1 and M-55-8 (treatment ponds) were dominated by Chaoboridae (28 and 42 percent, respectively) and Chironomidae (26 and 38 percent, respectively). Chaoborids (46 percent) and Oligochaeta (23 percent) dominated M-55-4 (reference pond), while chironomids (41 percent) and oligochaetes (15 percent) dominated T-4-1 (reference pond).

In 1988, all the ponds exhibited higher densities of benthic macroinvertebrates in the spring (weeks 64-72), with lower densities in the summer (weeks 74-80) (Figures 106, 107, 108, and 109). C-27-1 (treatment) exhibited an increase in abundance of benthic macroinvertebrates in autumn (weeks 82-102), which was the trend noted in all the ponds in 1987. Increased abundance was also observed to a lesser extent in the other ponds. Emergence of aquatic insects from the sediment is seasonal, with higher rates during the summer. Spring and autumn are periods of lower emergence; consequently, the sediment would contain more insect larvae in the spring and autumn versus the summer. Because this ecological measure focused on dominant taxa and on densities of macroinvertebrates in sediment, no further qualitative analysis was performed on species compositions.

Two pairs of treatment and reference ponds were selected for use in a quantitative comparison of chironomid, chaoborid, and oligochaete densities. These selections on a taxon-by-taxon basis were based on the following logic.

For chironomid densities, T-4-1 was the reference pond selected for a quantitative comparison with each of the treatment ponds. The results that guided this selection were as follows:

(1) Application of the nested ANOVA model to the pre-application data for all ponds for chironomidae indicated no significant interaction between pond and week and a significant pond main effect (model $R^2 = 0.66$; pond main effect $F = 7.05$; $dF = 3,80$; $PR>F = 0.0003$).

(2) Follow-up Bonferroni multiple comparison procedures for the pond main effect indicated no significant differences between treatment ponds (C-27-1 and M-55-8) and the reference pond T-4-1, and a significant difference between the treatment ponds and the other reference pond, M-55-4 for chironomid densities during the pre-spray period (experiment-rate error rate = 0.05).

(3) T-4-1 was selected as the reference pond for C-27-1 and M-55-8, because T-4-1 was not significantly different from the treatment ponds during the pre-application time period (Figures 110 and 111). The two pairs of ponds for quantitative comparisons of chironomid densities were C-27-1 and T-4-1, and M-55-8 and T-4-1.

There were no significant differences in the two pairs of ponds, C-27-1 and T-4-1, and M-55-4 and T-4-1, during the pre-spray time period. During the post-spray time period, the pond by week interaction was not significant and only the main effect means were evaluated in the multiple comparisons (model R^2 = 0.86; pond main effect F = 15.66; df = 3,20; PR>F = 0.0001). C-27-1 (treatment pond) had significantly lower densities of chironomidae relative to T-4-1, while M-55-8 and T-4-1 had similar densities (experiment-wise error rate = 0.05) for the post-spray time period. The average densities for C-27-1, M-55-8 and T-4-1 were 30, 232, and 117/m^2, respectively.

For the year-end time period, again only the pond main effect was significant (model R^2 = 0.80; pond main effect F = 3.66, df = 3,19, PR>F = 0.03). M-55-8 had significantly lower densities of chironomids relative to T-4-1, while chironomid densities for the pond pair C-27-1 and T-4-1 did not differ significantly (Figures 110 and 111). The average densities for C-27-1, M-55-8 and T-4-1 were 188, 133, and 376 individuals/m^2, respectively.

In summary, the reference pond T-4-1 was selected for quantitative comparisons of chironomid densities to the treatment ponds C-27-1 and M-55-8 (Figures 110 and 111). For the post-spray period (May 22 through September 3), chironomid densities were higher in T-4-1 than in C-27-1 (densities = 117, 30/m^2, respectively). There were no differences between T-4-1 and M-55-8 during this period. During the year-end period (September 4 through December 10), no significant differences in chiromomid densities were observed between T-4-1 and C-27-1 while for T-4-1 and M-55-8, the densities were lower in M-55-8 (376 and 133/m^2, respectively).

For chaoborid densities, the reference pond, M-55-4 was selected for a quantitative comparison with M-55-8, and T-4-1 was selected as the reference pond for C-27-1. The results that guided this selection were as follows:

(1) Application of the nested ANOVA model to the pre-application data for all ponds for chaoboridae indicated a significant interaction

between pond and week (model R^2 = 0.83; pond by week interaction F = 3.37, dF = 18,80, PR>F = 0.0001).

(2) Follow-up Bonferroni multiple comparison procedures indicated that C-27-1 differed from both reference ponds, T-4-1 and M-55-4, 2 of 7 sampling weeks. M-55-8 differed from T-4-1 and M-55-4 6 of 7 and 1 of 7 sampling weeks, respectively (experiment-wise error rate = 0.05).

(3) M-55-4 was selected over T-4-1 as the reference pond for M-55-8, because of the fewer number of significant differences during pre-application. Either T-4-1 or M-55-4 could have been selected as the reference pond for C-27-1 since they both differed from C-27-1in 2 of 7 sampling weeks. T-4-1 was selected as the reference pond so that both reference ponds would be used in the subsequent analyses. **The two pairs of ponds for quantitative comparisons of chaoborid densities are C-27-1 and T-4-1, and M-55-8 and M-55-4.**

Chaoborid density for the two pairs of ponds differed significantly during the pre-spray time period (for details, see pond selection) (Figures 112 and 113). Multiple comparisons between M-55-8 and M-55-4 indicated there were significant differences in 1 of 7 sampling weeks (Figure 113), whereas there were significant differences for C-27-1 and T-4-1 in 2 of 7 sampling weeks during the pre-spray period (Figure 112).

Chaoborid densities for the two pairs of ponds did not differ significantly during the post-spray time period. The pond by week interaction was not significant and the pond main effect was significant for the post-spray time period (model R^2 = 0.80, pond main effect F = 10.06, df = 3,20, PR>F = 0.0003). While the pond main effect was significant, the differences noted were not between the pond pairs selected (C-27-1 and T-4-1, and M-55-8 and M-55-4).

During the year-end time period, chaoborid densities for the two pond pairs were significantly different. The pond-by-week interaction was not significant and only the pond main effect was evaluated for the year-end period (model R^2 = 0.88; pond main effect F = 15.92, df = 3,19, PR>F = 0.0001). Comparisons between M-55-8 and M-55-4, and C-27-1 and T-4-1, revealed significant differences in both pairs of ponds during the year-end sampling period (Figures 112 and 113). The average densities of chaoborids for M-55-8 and M-55-4 were 86 and 625 individuals/m^2, respectively, and for C-27-1 and T-4-1, the densities were 179 and 38 individuals/m^2, respectively.

In summary, the reference pond M-55-4 was selected for quantitative comparison of chaoborid densities to the treatment pond M-55-8. Reference pond T-4-1 was compared to treatment pond C-27-1 (Figures 112 and 113). During the post-spray period (May 22 through September 3) chaoborid densities were not significant in either of the pond pairs. For the year-end period, chaoborid densities became significantly lower in T-4-1 (reference pond) than in C-27-1 (density = 38 and 179 individuals/m^2, respectively). Chaoborid densities were higher in M-55-4 (reference pond) than M-55-8 (density = 625 and 86 individuals/m^2, respectively).

For oligochaeta densities, reference pond M-55-4 was selected for a quantitative comparison with C-27-1. Pond T-4-1 (reference pond) was selected for a comparison with M-55-8). The results that guided this selection were as follows:

(1) Application of the nested ANOVA model to the pre-application data for all ponds for oligochaeta indicated a significant interaction between pond and week (model R^2 = 0.67; pond by week interaction F = 2.24; dF = 18,80; PR>F = 0.008).

(2) Follow-up Bonferroni multiple comparison procedures indicated no significant differences between C-27-1 and either of the reference ponds M-55-4 and T-4-1 (experiment-wise error rate = 0.05). Significant differences were observed between M-55-8 and T-4-1 during 1 of 7 sampling weeks. For M-55-8 and M-55-4 there were significant differences in 2 of 7 sampling weeks.

(3) T-4-1 was selected over M-55-4 as a reference pond for M-55-8, because T-4-1 had fewer

significant differences with M-55-8. No significant differences were observed between C-27-1 and both reference ponds so either reference pond could have been selected. M-55-4 was selected so that both reference ponds would be used for the analysis. The two pairs of ponds for quantitative comparisons of oligochaete densities are C-27-1 and M-55-4 and M-55-8 and T-4-1.

Oligochaete density for the two pairs of ponds differed significantly during the pre-spray time period (for details, see pond selection) (Figures 114 and 115). Multiple comparisons between M-55-8 and T-4-1 indicated there were significant differences in 1 of 7 sampling weeks, whereas there were no significant differences between C-27-1 and M-55-4.

Oligochaete density for the two pairs of ponds differed significantly during the post-spray time period. The pond-by-week interaction was not significant while the pond main effect was (model R^2 = 0.85; pond main effect F = 14.19, df = 3,20, PR>F = 0.0001). There were significant differences between C-27-1 and M-55-4 during the post-spray time period with the average densities being 16 and 286 individuals/m^2, respectively. There were no significant differences between M-55-8 and T-4-1.

During the year-end time period, the pond-by-week interaction was not significant while the pond main effect was (model R^2 = 0.85; pond main effect F = 13.37, df = 3,19, PR>F = 0.0001). Comparisons between C-27-1 and M-55-4, revealed significant differences in the ponds during the year-end time period. The oligochaeta densities for the pond pair, M-55-8 and T-4-1 remained similar during the year-end time period (Figures 114 and 115).

The reference pond M-55-4 was selected for quantitative comparison of oligochaeta densities to treatment pond C-27-1. Reference pond T-4-1 was compared to M-55-8 (Figures 114 and 115). During the post-spray period, M-55-4 exhibited significantly higher oligochaeta densities than the treatment pond C-27-1 (density = 286 and 16/m^2, respectively). No differences were observed between the oligochaeta densities in T-4-1 and M-55-8. During the year-end period, no differences in oligochaeta densities were observed between M-55-8 and T-4-1, while for C-27-1 and M-55-4, the densities were lower in the treatment pond (20 and 205/m^2, respectively).

In summary, the treatment pond C-27-1 exhibited more significant differences from the control in macroinvertebrate densities than did treatment pond M-55-8. Chironomid and oligocheata densities were generally lower in C-27-1 than the reference ponds (T-4-1 and M-55-4) (Figures 110, 111, 114, and 115). C-27-1 exhibited chaoborid densities similar to T-4-1 during the post-treatment time period while during year-end periods, the densities were higher in C-27-1 (Figures 112 and 113). M-55-8 exhibited significantly lower chaoborid and chironomid densities than M-55-4 and T-4-1, respectively, during the year-end period. These were the two significant differences in macroinvertebrate densities collected by Ekman dredges in M-55-8.

S-Samplers. S-samplers from the four test ponds all yielded benthic macroinvertebrate communities dominated by oligochaetes and chironomids (Figures 116-123). Other commonly encountered taxa included dragonflies and damselflies, caddisflies, mayflies, beetles, true bugs, other miscellaneous true flies, leeches, and snails. Because of the reduced dataset used for this sample type (see Report of Deviation in Appendix E), S-sample data is only analyzed qualitatively.

In 1987, C-27-1 (treatment pond) was dominated by oligocheates, chironomids, and miscellaneous insects (Figure 116). In 1988, chironomids became numerically dominant while all other taxa became minor contributors. During both 1987 and 1988, M-55-8 (treatment pond) was dominated by chironomids (Figure 118). During both years, oligocheates and miscellaneous insects were consistent but minor contributors to total density.

In early 1987, M-55-4 (reference pond) was dominated by oligocheates. The community became dominated by chironomids in October 1987, which persisted through 1988 (Figure 117). T-4-1 (reference pond) had the most balanced macroinvertebrate community as determined by S-samplers (Figure 119). Chironomids and miscellaneous insects were the major contributors to density throughout most of the study. However, Chironomids completely dominated pond T-4-1 on week 86 (August 21, 1988).

In 1987, the major contributors to macroinvertebrate biomass in C-27-1 (treatment pond) were leeches and gastropods, miscellaneous insects, and chironomids (Figure 120). In 1988, chironomids became the dominate taxa with respect to biomass. At M-55-8 (treatment pond), chironomids were the primary contributors to macroinvertebrate biomass in 1987 (Figure 122). Leeches and gastropods became dominant in 1988.

Biomass distribution at the two reference ponds were fairly consistent throughout the study. M-55-4 biomass was composed primarily of midges (chrionomids, ceratopognidae, and chaoboridae, with chironomids predominating) and miscellaneous (non-midge) insects (Figure 121). At T-4-1, miscellaneous insects were the primary contributors to macroinvertebrate biomass except for week 86 when midges dominated (Figure 123).

Benthic Macroinvertebrate Summary. Qualitative inspection of the kick net sample data indicated that relative abundance of oligochaetes and chironomids shifted in both the treatment and reference ponds. Based solely on relative abundance of the kick-net sample data, macroinvertebrate community structure exhibited greater stability in the treatment ponds than in the reference ponds over the 1987-1988 study period. S-samples were used to examine select macroinvertebrate biomass and density characteristics of the benthic community on a qualitative basis. All ponds generally exhibited an increase in chironomid biomass from 1987 to 1988 with the exception of M-55-8, where chironomids were similar or less abundant in 1988 when compared to 1987. Chironomids dominated the benthic community of the pond in terms of relative abundance and biomass in C-27-1 (treatment) M-55-4 (reference). However, leeches and snails dominated the biomass in M-55-8 (treatment), whereas insects (other than chironomids) played a dominant role in T-4-1 (reference), except for week 86 (August 21, 1988) when chironomids also dominated biomass.

Of 47 taxa of emergent insects collected over the study period, 95 percent were chironomids with any one of the remaining taxa comprising less than 2 percent of the total collection. Because of their dominance, only chironomids were selected for quantitative analysis of insect emergence. The nested ANOVA model for chironomid emergence indicated a single significant difference between C-27-1 and its reference pond during the post-spray period (1 of 8 collections), and none for M-55-8. For the year-end period, there were no significant differences in chironomid emergence for C-27-1 and its reference pond while M-55-8 had significantly lower emergence rates relative to its reference pond for the entire year-end time period.

The Ekman dredge sample contained a total of 40 taxa of benthic macroinvertebrate with chironomids, chaoborids, and oligochaetes comprising the dominant taxa. Accordingly, only the latter three taxa were selected for quantitative analysis. The C-27-1 treatment pond exhibited a greater number of significant differences in macroinvertebrate densities than the M-55-8 treatment pond when compared to references. Chironomid and oligochaete densities were significantly lower in C-27-1 than in the reference ponds for the post-spray period. During the year-end time period, only oligochaetes remained significantly lower in C-27-1 relative to its reference pond. C-27-1 exhibited significantly higher chaoborid densities in both the post-spray and year-end periods, but this same trend occurred throughout the pre-spray period as well.

4. Fish

Species Composition. The four ponds contained 15 fish species, with eight species in common (Table 40). Raw data on fish are provided in Appendix L. Largemouth bass (*Micropterus salmoides*) and sunfish (*Lepomis* sp.) constituted the main predator-prey component. Other important species included black crappie (*Pomoxis nigromaculatus*), golden shiner (Notemigonus crysoleucas), and mosquito fish (Gambusia affinis). Chain pickerel (Esox niger) and adult black crappie are carnivores, whereas the other fishes are prey.

The ponds varied in their species composition. The two main differences occurred in the treatment ponds. M-55-8 contained a high number of black crappie and C-27-1 contained a high number of warmouth (Lepomis gulosus). The eight remaining fish species (Table 40) were less dominant.

Fish Kill. Observed fish kills in the treatment ponds were associated with the first runoff at C-27-1 after the third application of endosulfan. The total number of dead fish collected in 1988 totaled 447 in C-27-1, and 227 in M-55-8 (Figure 124 through 126). Eighty-eight percent of the dead fish counted in C-27-1, and 73 percent in M-55-8 were observed in the three-day period following this runoff event. Subsequent runoff events caused no significant fish deaths. No dead fish were observed in T-4-1 (reference pond), while three dead fish were collected in M-55-4 (reference pond) during this three-day period (Figure 125). Two dead fish were found in M-55-8 before this time, while one was found prior to runoff in C-27-1 (Figure 124). These three fish were the only deaths observed after the first and second endosulfan applications, prior to runoff after the third application.

Fish in the 10 to 60 mm size range accounted for over 91 percent of the fish killed in C-27-1 and M-55-8 (Figures 127, 128, 129, and 130). Eighty-seven percent of the dead fish in C-27-1 were in the 10 to 40 mm size range, whereas 85 percent of the dead fish in M-55-8 were in the 10 to 60 mm size range. The eight larger fish (> 110 mm) that died in M-55-8 (4 percent) were bluegill and crappie. No dead fish collected from C-27-1 were larger than 110 mm.

Near-shore areas contained a majority of the dead fish. Small fish utilize these areas for feeding and predator avoidance. Pond edges, the first areas to receive the field runoff containing endosulfan were also the areas with the highest water temperatures. High temperatures the day of the runoff events may have had a synergistic effect with endosulfan.

Dead fish in C-27-1 (treatment pond) consisted of eight species (Figure 126). Mosquito fish (61 percent) and sunfish (warmouth, bluegill, and sunfish spp.) (27 percent) comprised 88 percent of the total fish kill. The other five fish species constituted a smaller proportion of the fish kill in C-27-1 (treatment pond). Mortality in M-55-8 consisted of four fish species. Black crappie (30 percent) and sunfish sp. and bluegill (44 percent) comprised 74 percent of the fish kill in M-55-8. Mosquito fish and largemouth bass accounted for < 15 percent of the remaining identifiable dead fish from M-55-8.

The number of dead fish observed in the reference ponds, M-55-4 and T-4-1, for the 1988 sampling season was 17 and 75 individuals, respectively (Figure 125 and 126). The ponds had different morphologies, with T-4-1 being shallower; therefore, it was more susceptible to water loss by evaporation, higher temperatures and possible overcrowding of the fish. The low water level and higher temperatures may account for the higher numbers of dead fish observed in T-4-1 (69) compared to M-55-4 (9) between August and October (Figure 125). After October, fewer dead fish were observed.

<u>Other Qualitative Observations</u>. Pond perimeter tours for fish kills were conducted during the application and post-application phases of the study. Even during the peak fish kill period following the induced and natural runoff events at the treatment ponds, live fish were observed undergoing normal behavior. At both the dawn and dusk observations, feeding activity of fishes was evident by surface activity and swirling water typical of crepuscular feeding activity. During the perimeter tours, fish of various sizes were observed, and typically exhibited normal escape behavior as observers approached. On the whole, typical fish behavior was noted after the major runoff induced fish kill (after the third application), suggesting the fish kill represented a small fraction of the total fish population inhabiting the ponds. Despite the fact that endosulfan induced fish kills were largely

restricted to the smaller size classes, the number of small fish observed actively swimming along the pond perimeter was not appreciably different before, during or after the kill.

Black-spot disease (<u>Neascus</u> sp.), an external parasite, was also observed during electroshocking collections in 1988. Black-spot disease is caused by the attachment of a larval fluke that is transported by fish-eating birds to snails, and then to fish. This external parasite is common in fish populations and is not lethal. Black-spot disease occurred in C-27-1 (treatment) and T-4-1 (reference pond). Only warmouth and bluegill sunfish appeared to be affected. The parasite infections appeared to be more common in C-27-1 than T-4-1. The parasite was not observed on fish collected in M-55-4 or M-55-8.

<u>Community Structure</u>. The number of young-of-year (YOY) largemouth bass collected by electroshocking was low prior to endosulfan applications in C-27-1 (treatment) and M-55-4 (reference) (Table 41). The high number of largemouth bass collected on week 70 is partially due to electroshocking of pockets of schooling YOY. This phenomenon was observed in both C-27-1 and M-55-4. Largemouth bass cease schooling behavior as they grow older. Increased turbidity from the thunderstorm at C-27-1 (treatment) in May may have contributed to a drop in numbers. The absence of YOY largemouth bass in C-27-1 late in 1988 contrasts with 1987. A decline in the number of largemouth bass was also observed to a lesser extent in T-4-1 (reference) and M-55-8 (treatment). The number of YOY largemouth bass collected from T-4-1 decreased after early May (week 70) from 44 fish to no more than 16 on any subsequent sampling date in 1988. The number of YOY largemouth bass collected from M-55-8 also declined from 49 fish in late May (week 74) to no more than 9 on any subsequent sampling date in 1988. Recruitment into a larger size class does not explain these decreases. Largemouth bass YOY captured in M-55-4 (reference) were low in number after May, but more YOY were collected in M-55-4 than the other ponds through the end of 1988 (Table 41).

Adult (> 200 mm) largemouth bass numbers decreased in all ponds as the 1988 season progressed (Table 41). C-27-1 (treatment) and M-55-4 (reference) had more largemouth bass adults in 1987 than for comparable dates in 1988. Numbers of adult largemouth bass in M-55-8 were approximately the same for both years. T-4-1 (reference) had more largemouth bass adults in 1988 than for comparable dates in 1987, which can be attributed to the growth of stocked fish into the larger size group. YOY largemouth bass were stocked in T-4-1 to supplement the existing population. By October of 1987 (week 42), stocked fish had grown larger than 200 mm.

The sunfish (Lepomis sp.) populations remained relatively stable during 1988 in all ponds, except for a shift in the bluegill/warmouth structure in C-27-1 (treatment) (Figures 131, 132, 133, and 134). In C-27-1, YOY warmouth became a large part of the sunfish population in August (week 86), when bluegill and warmouth exhibited a ratio of approximately 1:2. The previous sampling period (week 82) showed a bluegill/warmouth ratio of about 10:1. This shift was temporary, because by the middle of November (week 98), the ratio shifted back in favor of bluegill (4:1). Bluegill and warmouth in C-27-1 had similar ratios (3:1) in October and November of 1987. The increase in the number of small warmouth indicate that in C-27-1, from August through October 1989, conditions must have favored warmouth reproduction and survival. The sunfish populations in the other ponds remained stable, with no major fluctuations (Figures 132, 133, and 134).

Reproduction and Recruitment. Tables 41 and 42 show recruitment information. Biweekly seining established that Lepomis spp. (bluegill and warmouth) reproduced successfully in all ponds from mid-June through August of 1988 (post-treatment phase). Lepomis spp. are multiple spawners that reproduce several times a year at temperatures ranging from 17 to 31°C (Auer 1982). Lepomis spp. larvae measure about 3 mm after hatching and can grow up to 1 mm a day (Auer 1982).

An approximate hatching date was calculated by using a hatching length of 3 mm and a growth rate of 1 mm/day. By subtracting

1 mm a day down to the hatching length (3 mm) from the smallest YOY *Lepomis* spp. captured on June 19, 1988 (Table 42); approximate hatching dates were calculated to be the following: June 10 (C-27-1); June 4 (M-55-4); and June 6 (T-4-1). All these dates occurred during the application phase. By using the smallest YOY *Lepomis* spp. specimen captured in 1988 to represent the last hatching period, the same line of reasoning as above was used to determine the extent of reproductive success in the post-treatment phase. Using August 14 for C-27-1 (treatment) and T-4-1 (reference), July 17 for M-55-8 (treatment), and October 9 for M-55-4 (reference), the following hatching dates were calculated: August 6 (C-27-1); July 11 (M-55-8); October 2 (M-55-4); and August 7 (T-4-1). This verifies that *Lepomis* sp. were reproducing at least six weeks after the last application of endosulfan.

Largemouth bass recruitment also occurred in all ponds. Largemouth bass spawn once a year, at temperatures ranging from 16 to 24°C (Auer 1982). Largemouth bass larvae measure about 5 mm after hatching (Auer 1982). Young largemouth bass were first collected by electroshocking in early May (week 70) in three of the ponds and mid-April (week 68) in T-4-1 (treatment), which indicated that largemouth bass had successfully reproduced in all ponds prior to May (pre-treatment phase) (Table 41).

Population Estimates. Mark-recapture studies resulted in a low number of recaptures. Accordingly, the calculated population estimates were highly variable and subject to cautious interpretation. Bailey population estimates for 1988 indicated that bluegill numbers were similar in three of the ponds, with lower numbers in T-4-1 (reference). Estimates for June and July (weeks 74-82), the period with the highest number of recaptures, revealed that M-55-4 (reference) had the highest number of bluegill, with approximately 2,600 fish, followed by M-55-8 (treatment) (≈1800), C-27-1 (treatment) (≈1500), and T-4-1 (reference) (≈160) (Table 43). Some standard deviations were as high as 57 percent of the estimate (M-55-4, week 72) because of the low frequency of recaptures (5 percent). C-27-1 had only one bluegill recapture for all of 1988 (Table 44).

The Bailey population estimates provides no observable trends in the size of the bluegill populations through the 1988 sampling season (Table 44). Because of the large standard deviations associated with the population estimates and the fluctuation of the estimates for 1988, it could not be established whether the bluegill populations were increasing or decreasing in size.

The size of the largemouth bass populations in 1988 was similar in three of the ponds, and lower in C-27-1 (treatment). Estimates for June and July (weeks 74-82) revealed that M-55-4 (reference) had the highest number of largemouth bass with approximately 370 fish, followed by M-55-8 (treatment) (≈270), T-4-1 (reference) (≈260), and C-27-1 (treatment) (≈60) (Table 43). The recapture rate was better for largemouth bass (38 percent) than bluegill, but some standard deviations were still as high as 55 percent of the estimate (T-4-1, week 94) (Table 45). T-4-1 (reference) is the only pond that had an observable trend, with an apparent increase in largemouth bass numbers through 1988, although the high standard deviations make definitive assessment difficult (Table 45).

The low number of recaptures make it difficult to interpret the mark-recapture data. No definite conclusions could be made as to the effect of endosulfan on the numbers of largemouth bass and bluegill.

Length/Weight Relationships. Condition factor (k), a length-weight relationship, was used for statistical analysis of bass and bluegill by the ANOVA model. T-4-1 was selected as the reference pond for C-27-1 (treatment) and M-55-4 was selected as the reference pond for M-55-8 (treatment) for quantitative comparison of condition factors (k) for bluegill and largemouth bass. The results that guided this selection were as follows:

Bluegill condition factor (k) for pre-spray evaluation:

(1) Application of the 2-way ANOVA model to the pre-spray data for all ponds indicated a significant interaction

between pond and week (model R^2 = 0.09; pond-by-week interaction, F = 6.31, df = 12,3966. PR>F = 0.0001)

(2) Follow-up Bonferroni multiple comparison procedures indicated significant differences during four out of five weeks in the pre-spray time period between C-27-1 and both control ponds. Significant differences were found during two out of five weeks between M-55-8 and T-4-1 and during one out of five weeks between M-55-8 and M-55-4 (experiment-wise error rate = 0.05).

(3) M-55-4 was selected as the reference pond for M-55-8 because it was significantly different from M-55-8 during the pre-spray period for only 1 week (Figure 135). Both reference ponds were significantly different from C-27-1 for the same number of weeks for the pre-spray time period so T-4-1 was selected so that both reference ponds would be incorporated in the analysis (Figure 136). **Two pairs of ponds for quantitative comparisons of bluegill condition factors are C-27-1 and T-4-1, and M-55-8 and M-55-4.**

Largemouth bass condition factor (k) for pre-spray evaluation:

(1) Application of the 2-way ANOVA model to the pre-spray data indicated that neither the pond-by-week or pond main effect were significant (model R^2 = 0.02)

(2) Follow-up Bonferroni multiple comparison procedures were not necessary for comparison of the ponds because of the results from the 2-way ANOVA model.

(3) M-55-4 was selected as the reference pond for M-55-8 and T-4-1 was selected as the reference pond for C-27-1 because they were not significantly different from the treatment ponds during the pre-spray time period (Figures 137 and 138). These ponds were also selected because they matched the ponds used for analysis of bluegill condition factors. **Two pairs of ponds for quantitative comparisons of largemouth bass condition factors are C-27-1 and T-4-1, and M-55-8 and M-55-4.**

Bluegill condition factors for the two pairs of ponds differed significantly during the pre-spray time period (for details, see pond selection above). Multiple comparisons between C-27-1 and T-4-1 indicated there were significant differences in four of five weeks (Figure 136), whereas there was one significant difference for M-55-8 and M-55-4 (Figure 135).

Bluegill condition factors for the two pairs of ponds differed significantly during the post-spray time period (model R^2 = 0.06; pond-by-week interaction F = 12.61, df = 9,1915, PR>F = 0.0001), while during the year-end time period, bluegill condition factors for the two pond pairs were not significantly different (model R^2 = 0.005). Multiple comparisons between both pond pairs during the post-spray time period indicated there were significant differences in one of four weeks (Figures 135 and 136).

Largemouth bass condition factors were not significantly different between either pond pair for 2 of the 3 phases. During the pre- and post-spray time periods there were no significant differences (model R^2 = 0.02 and 0.03, respectively). During the year-end time period there were significant differences between the ponds (model R^2 = 0.15; pond main effect F = 10.46, df = 3,222, PR>F = 0.0001). C-27-1 had a significantly higher condition factor relative to T-4-1 (1.09 and 0.99, respectively) while M-55-4 and M-55-8 were not significantly different (Figures 137 and 138).

In addition to the ANOVA model described above, a separate analysis of covariance (ANCOVA) was developed for comparisons of bluegill and largemouth bass from 1987 to 1988 (Table 46). The condition of bluegill was not significantly different in C-27-1 (treatment) and decreased in all other ponds from 1987 to 1988 (post-application months). Weight of bluegill (least square mean [LSM]) and condition (k-factor), a measure of plumpness, were not significantly different from 1987 to 1988 in C-27-1 as determined by analysis of covariance (ANCOVA) and 1-way analysis of variance (ANOVA), respectively (Tables 46 and 47). The weight and condition of bluegill was significantly less in 1988 relative to 1987 in the other three ponds (α = 0.05). The degree of change was relatively small, yet still statistically significant; LSM had a maximum change of 0.5g in M-55-4, while M-55-4 had a maximum change in the k-factor of 0.11 (Tables 46 and 47).

The condition of largemouth bass was not significantly different from 1987 to 1988 (post-spray period) for all ponds

(Table 46). As determined by ANCOVA, the weight (LSM) of largemouth bass was also not significantly different for all ponds (Tables 46 and 47).

Summary. Species compositions of the four ponds varied slightly, but were dominated by largemouth bass and bluegill sunfish as the primary predator-prey relationship. M-55-8 (treatment pond) contained a higher number of black crappie than the remaining ponds, whereas C-27-1 contained a greater number of warmouth.

Observed fish kills in the treatment ponds were associated with the natural and induced runoffs for the watersheds at M-55-8 and C-27-1, respectively, immediately following the third endosulfan application. Fish in the 10-60 mm size range accounted for over 91 percent of the fish killed in C-27-1 and M-55-8. Mosquito fish (61 percent) and two sunfish (warmouth and bluegill sunfish) species (27 percent) comprised 88 percent of the total number of fish killed in C-27-1. Black crappie (30 percent) and sunfish species and bluegill (44 percent) comprised 74 percent of the fish killed in M-55-8. Near shore areas contained the largest number of dead fish, and fish kill were largely restricted to the three-day period following the aforementioned runoff events.

Regarding community structure, young of the year (YOY) largemouth declined in C-27-1, but appeared to begin decreasing prior to endosulfan application. A decreased number of largemouth bass was also observed in a reference (T-4-1) and the second treatment (M-55-8) pond, but occurred to a lesser degree than in C-27-1. Sunfish populations (Lepomis spp.) remained stable during 1988, except for a shift in the bluegill pond warmouth structure in C-27-1. YOY warmouth constituted a larger part fraction of the sunfish population in August 1988. The shift was temporary and there was a return to bluegill dominance (November 1988).

Lepomis spp. (bluegill and warmouth) successfully reproduced in all ponds from mid-June through August of 1988 and later, as evidenced by the presence of newly recruited fish captured by seining.

Largemouth bass spawned in all ponds prior to the application period, but recruitment was limited to May with no apparent recruitment in reference and treatment ponds after the early spring spawning .

Bailey population estimates were calculated for all ponds. However, the low number of recapture yielded highly variable data that could not be used to ascertain endosulfan effects on either largemouth bass population or bluegill population in the treatment ponds.

Bluegill and largemouth bass condition factors were examined by an ANOVA model. Bluegill in C-27-1 were not significantly different from T-4-1 (treatment) pond between 1987 and 1988. However, bluegill condition was significantly lower in the remaining three ponds in 1988 than 1987. There was no significant difference in largemouth bass condition between 1987 and 1988. The ANCOVA analyses of bluegill and largemouth mass length weight relationship provides the following results. The bluegill weights were significantly lower in 1988 than 1987 for treatment (M-55-8) and reference (T-4-1, M-55-4) ponds and not C-27-1. There were no significant differences for largemouth bass weights for any of the ponds in 1987 and 1988.

5. Pond Metabolism

Pond metabolism was examined by monitoring temperature and dissolved oxygen over a consecutive dawn-dusk-dawn interval. Raw data are provided in Appendix M. The data from the three analyses were used to estimate gross and net primary production. Gross production includes both photosynthesis and respiration, whereas net production consists of gross production minus respiration. Gross primary production in the four test ponds ranged from 0 to 7 gC/m^3 (Figures 139 and 140). For net production, the range was 0 to 3 gC/m^3 (Figures 141 and 142) for the four test ponds. The wide range of gross and net production reflects the seasonal trends. Production is low in the spring, peaks in the summer, and then declines in the autumn. In the spring and autumn, the gross and net production ranged from 0 to 4 gC/m^3 and 0 to 2 gC/m^3, respectively. In the summer, they ranged from 1 to 7 gC/m^3 and 0.5 to 3 gC/m^3, respectively.

M-55-4 was selected as the reference pond for a quantitative comparison of gross and net production to the two treatment ponds. The preference of M-55-4 to T-4-1 as the reference pond was based on (1) pairwise comparisons of the reference ponds with the treatment ponds during the pre-spray time period and (2) lack of production measurements from T-4-1 during four sampling periods after endosulfan application. The lack of certain values resulted from the loss of certain stations due to the pond drying out over the course of the drought year. Some data were also lost due to sampling logistics (see report of deviation, Appendix E). The results that guided this selection were as follows:

Gross production pre-spray evaluation:

(1) Application of the nested ANOVA model to the pre-spray data for all ponds indicated a significant interaction between pond and week (model $R^2 = 0.73$; pond-by-week interaction $F = 2.80$, $df = 6,16$, $PR>F = 0.047$)

(2) Follow-up Bonferroni multiple-comparison procedures indicated no significant differences between treatment ponds and the reference ponds for gross production (experiment-wise error rate = 0.05)

(3) M-55-4 was selected as the reference pond for M-55-8 and C-27-1 because it (a) was not significantly different from the treatment ponds during the pre-spray time period (Figures 139 and 140) and (b) had a complete set of gross production measurements comparable to the treatment ponds while T-4-1 did not due to one station drying out over the course of the year. Two pairs of ponds for quantitative comparisons of gross production are C-27-1 and M-55-4, and M-55-8 and M-55-4.

Net production pre-spray evaluation:

(1) Application of the nested ANOVA model to the pre-spray data for all ponds indicated a significant interaction between pond and week

(model R^2 = 0.89; pond-by-week interaction F = 9.80, df = 6,16, PR>F = 0.0001)

(2) Follow-up Bonferroni multiple comparison procedures indicated significant differences during 1 out of 3 weeks in the pre-spray time period between C-27-1 and T-4-1, M-55-8 and T-4-1, and M-55-8 and M-55-4. No significant differences were noted between C-27-1 and M-55-4. (experiment-wise error rate = 0.05)

(3) M-55-4 was selected as the reference pond for M-55-8 and C-27-1 because it (a) was not significantly different from C-27-1 during the pre-spray time period and differed during only 1 week from M-55-8 (Figures 141 and 142) and (b) had a complete set of gross production measurements comparable to the treatment ponds while T-4-1 did not due to one station drying out over the course of the year. **Two pairs of ponds for quantitative comparisons of net production are C-27-1 and M-55-4, and M-55-8 and M-55-4.**

During the pre-spray time period, the gross production for C-27-1 was generally lower than for M-55-4, while M-55-8 and M-55-4 had similar gross production. The 1987 data (pre-spray) were evaluated qualitatively since replicates for production were not collected. For C-27-1, gross production was lower in the treatment pond than in the M-55-4 reference pond (Figure 139). In 1988 (pre-spray), there were no statistically significant differences between C-27-1 and M-55-4. The results for M-55-8 and M-55-4 showed that gross production was visually similar in 1987 and no statistical differences were noted in 1988 pre-spray period. (For details, see pond selection and Figures 139 and 140.)

The gross production for the two pairs of ponds differed significantly during the post-spray time period (model R^2 = 0.9; pond-by-week interaction F = 5.62, df = 20,52, PR>F = 0.0001). Multiple comparisons between C-27-1 and M-55-4, and M-55-8 and M-55-4 indicated that there were significant differences in gross production in five out of the eight weeks of sampling in the post-spray time period (experiment-wise error rate = 0.05) (weeks 74 to 88) (see Figures 139 and 140).

During the year-end time period (weeks 88 to 101), gross production for the two pairs of ponds remained significantly different. The pond-by-week interaction was not significant and only the main effects for ponds were used in the multiple comparisons (model $R^2 = 0.91$; pond main effect F = 20.38, df = 3,30, PR>F = 0.0001). Both C-27-1 and M-55-8 had significantly lower gross production than M-55-4 (experiment-wise error rate = 0.05) (see Figures 139 and 140).

During the pre-spray time period, net production in M-55-8 and M-55-4 were qualitatively similar in 1987 and began to diverge at the end of the pre-spray time period in 1988 (Figure 142). For C-27-1 and M-55-4 (Figure 141), the treatment pond (C-27-1) had a lower net production relative to the reference pond in 1987 (qualitative evaluation). In 1988 (pre-spray), there were no differences between the ponds (for details, see pond selection).

The net production for the two pairs of ponds differed significantly during the post-spray time period (model $R^2 = 0.9$; pond-by-week interaction F = 7.37, df = 20,52, PR>F = 0.0001). Multiple comparisons between M-55-8 and C-27-1 with the reference pond, M-55-4, indicated that there were significant differences in net production in five out of the eight weeks of sampling in the post-spray time period (see Figures 141 and 142).

During the year-end time period, net production for the two pairs of ponds converged. There was a pond-by-week interaction (model $R^2 = 0.92$; pond-by-week interaction F = 3.86, df = 14,30, PR>F = 0.0009). The two pairs of ponds were significantly different during the first two sampling periods of the year-end time period. For the rest of this time period, the ponds had similar net production (Figures 141 and 142).

In summary, gross and net pond production showed seasonal trends, with the highest values being recorded in the summer. For quantitative comparisons of production, reference pond M-55-4 was selected as the most appropriate pond to compare to the two treatment ponds. The statistical comparisons between the two pairs of ponds, C-27-1 and M-55-4, and M-55-8 and M-55-4, indicated that gross and net production during the post-spray time periods were, in general, significantly lower in the two treatment ponds as compared to M-55-4. In the year-end time period, the gross production remained significantly lower for the treatment ponds as compared to M-55-4 based on a main (i.e., pond) effect. For net production, pond production values converged in the year-end period.

6. Autotrophic Index

The autotrophic index (AI) is a ratio based on biomass and chlorophyll a that can be used to evaluate the trophic nature of the periphyton community. Low AI values indicate a high relative abundance of photosynthetic (autotrophic) organisms to consuming (heterotrophic) organisms. As waters become organically enriched, the proportion of heterotrophic, nonchlorophyllous organisms, such as filamentous bacteria and stalked protozoa, increases. Conversely, as waters become less organically enriched, the proportion of these organisms decreases. Larger values indicate heterotrophic conditions that may indicate poor water quality or eutrophic conditions. AI is calculated as follows:

$$AI = \frac{\text{Biomass (mg/m}^2\text{)}}{\text{Chlorophyll (mg/m}^2\text{)}}$$

Thus, AI provides another measure about the baseline condition and any potential change to baseline conditions caused by a perturbation.

The mean AI for the four ponds ranges from 33 (C-27-1) (treatment) to 5432 (T-4-1) (reference). Raw data are provided in Appendix N. These AI values which are all generally above those normally encountered in streams and rivers, indicating that all four ponds have heterotrophic periphyton communities. Indeed, farm ponds are generally eutrophic to dystrophic and results for the study ponds are consistent with expectations. Some of the AI samples thawed during shipping and are described in a report of deviation (Appendix E).

The reference pond T-4-1 was selected for quantitative comparison to treatment pond C-27-1; reference pond M-55-4 was compared to M-55-8. Nested ANOVA results indicated that these were the most similar reference/treatment pond pairs during the pre-spray period. Statistical comparison of the reference treatment pond pairs selected time periods is discussed below. The results that guided this selection were as follows:

(1) Application of nested ANOVA model to the pre-spray data for all ponds indicated a significant interaction between pond and week (model $R^2 = 0.54$; pond-by-week interaction $F = 4.15$; $dF = 18,281$; $PR>F = 0.0001$).

(2) Follow-up Bonferroni multiple-comparison procedures indicated no significant differences between M-55-4 and M-55-8. C-27-1 differed from the two reference ponds during only one week 64 (T-4-1 also differed from M-55-8 during one week (experiment-wise error rate = 0.05)

(3) M-55-4 was selected as the reference pond for M-55-8 since there were no significant differences between the two ponds, while between T-4-1 and M-55-8 there was one significant difference. Either reference pond could have been paired with C-27-1 since they both differed from the treatment pond during one sampling week. T-4-1 was selected to compare to C-27-1 in order to include both reference ponds in the subsequent analyses. **The pond pairs are C-27-1 and T-4-1, and M-55-8 and M-55-4.**

The autotrophic index for the two pairs of ponds (Figures 143 and 144) differed significantly during the pre-spray time period (for details, see pond selection). Multiple comparisons between M-55-4 and M-55-8 indicated no significant differences during the pre-spray period (Figure 144), whereas significant differences occurred in 1 of 7 sampling weeks for ponds T-4-1 and C-27-1 (Figure 143).

The autotrophic index for the two pairs of ponds differed significantly during the post-spray time period (model $R^2 = 0.58$; pond-by-week interaction F = 2.81, df = 15,98, PR>F = 0.001). Multiple comparisons between both pairs of ponds indicated there were significant differences in 1 of 6 weeks (Figures 143 and 144).

During the year-end time period, the autotrophic index for the two pond pairs remained significantly different. There was a pond by week interaction (model R^2 = 0.72; pond-by-week interaction F = 6.82, df = 15,95, PR>F = 0.0001). Multiple comparisons between M-55-4 and M-55-8 revealed significant differences in 2 of 6 year-end sampling weeks while for T-4-1 and C-27-1 there were no significant differences (Figures 143 and 144).

Five significant differences between AIs for the pond pairs occurred sporadically in the pre-spray, post-spray, and year-end time periods. When the differences occurred, the reference ponds had higher AI values. High AI values are generally indicative of heterotrophic communities.

In summary, AI ranges from approximately 33 to 5,432 and these values are consistent with expectations for small ponds. Differences between the reference ponds (AI, usually higher) and treatment ponds (AI, usually lower), are not attributable to endosulfan.

7. Macrophytes

Twenty-four aquatic macrophyte and two macroalgal taxa occurred in the near-shore areas of the ponds. Raw data are provided in Appendix O. These aquatic plants included submergent, emergent, and floating species (Table 48). C-27-1 (treatment) and T-4-1 (reference) contained 18 species each, followed by M-55-4 (reference) with 14 species, and M-55-8 (treatment) with 13 species. The four test ponds contained five emergent macrophyte species in common (Table 48), consisting of the sedge (*Cyperus* sp.), spike rush (*Eleocharis aciculas* and *E. obtuso*), bulrush (*Scirpus* sp.), St. John's-wort (*Hypericum boreal*); and one floating algal species (filamentous green algae Chlorophyceae). Because the sampling technique was qualitative, quantitative analyses were not performed on macrophytes, rather they were examined qualitatively.

Lowered pond water levels caused by evaporation accounted for a majority of the changes in the abundance of aquatic macrophytes. Loss of water by evaporation affected the reference ponds more than the treatment ponds. T-4-1 (reference) decreased approximately 106 cm in depth from early April (week 66) to mid-December (week 103), 1988. M-55-4 (reference) decreased approximately 116 cm in the same time period. C-27-1 and M-55-8 (treatment ponds) decreased 67 and 51 cm, respectively, from early April to mid-December, 1988. The decreased abundance of bulrush (Scirpus sp.) in all four ponds exemplifies the loss of aquatic macrophytes attributable to the loss of edge habitat associated with decreased pond water levels.

Spike rush (Eleocharis aciculas) and big duckweed (Spirodela punchtata) exhibited larger declines in M-55-8 (treatment) than in reference ponds during the post-treatment phase (Figures 145 and 146). Spike rush (emergent) abundance declined in M-55-8 (treatment) after early August (week 84) compared to the other ponds (Figure 145). The other three ponds maintained similar levels of abundance of spike rush until early December (week 101). Big duckweed (floating) also declined in M-55-8 (treatment) after early-June (week 75) 1988, but a similar decline was also observed to a lesser extent in T-4-1 (reference) (Figure 146).

In summary, various changes occurred in macrophytes, but no causal effect between aquatic macrophyte abundance and endosulfan exposure could be verified from the available data.

VII. DISCUSSION

As an endosulfan effects study, the present investigation examined numerous physical, chemical and biological characteristics of the farm pond/agronomic field test systems before, during, and after multiple endosulfan applications. Various measures were gathered during the study, and are separately discussed in the preceding results section of this report. Due to the number of individual measures and endpoints, it is easy to lose sight of the fact that the ponds represent dynamic, interactive ecosystems. Accordingly, endosulfan residue data provide information regarding the dosing, translocation and fate of the applied endosulfan within the farm pond/agronomic field systems. Physical and chemical characteristics of the ponds represent the abiotic condition of the aquatic habitat during the study period. The biological measures represent a subset of structural and functional endpoints that could potentially be affected by endosulfan. Nevertheless, no single endpoint measurement represents the ecosystem as a whole.

This section summarizes the results observed in the study, and discusses chemical, biological, and ecological interactions which may have influenced the observed results.

A. Test Systems

Four farm ponds located in Colquitt, Mitchell, and Thomas counties in southwestern Georgia were selected as test systems. The ponds met the following criteria: 0.8 to 2.0 ha (2 to 5 acres) in size; a field-to-pond surface area ratio of 10:1; surrounding fields with a 3 to 8 percent slope to facilitate runoff into the aquatic systems; healthy fish populations; stable benthos, zooplankton, and phytoplankton communities representative for this type of pond ecosystems; water quality supportive of a stable and active pond; cooperative owners; and an irrigation source to supplement naturally occurring precipitation in the event of drought conditions. The four ponds ultimately selected for study were designated as C-27-1 (treatment pond), M-55-4 (reference pond), M-55--8 (treatment pond), and T-4-1 (reference pond).

The fields surrounding each pond were planted with tomatoes using standard agronomic practices common to southwestern Georgia. Despite the fact that the label for the endosulfan formulation (Thiodan 3EC) used in the present study specifies a buffer of 91 m (300 ft) from water bodies, some tomatoes were planted as close as 5 m (15 ft) of the pond edges. The study was designed without the buffer to simulate a worst-case situation as mutually agreed upon by the sponsor and the U.S. EPA. Agronomic practices used at the designated treatment ponds (C-27-1, M-55-8) and reference ponds (M-55-4, T-4-1) were similar, but the tomatoes planted in the reference watersheds received no endosulfan.

B. Dosing

The endosulfan formulation used was Thiodan 3EC. Thiodan 3EC is manufactured by FMC Corporation, and contains 3 pounds of endosulfan per gallon of product (3.0 lb endosulfan/gallon based on 33.7 percent active ingredient per gallon of Thiodan 3EC which weighs 8.896 pounds per gallon, per certificate of analysis; Appendix D). Thiodan 3EC was applied to the treatment fields surrounding C-27-1 and M-55-8 three times at a rate of approximately 3.1 L/ha (1.12 kg endosulfan/ha or 1.00 lb/endosulfan acre). The application dates were May 27, June 10, and June 27, 1988 for C-27-1; and May 27, June 11 and June 23, 1988 for M-55-8.

Endosulfan entered the ponds through two pathways, aerial drift and runoff. However, the latter route of entry provided the primary dose for the study. Based on endosulfan residue analyses, the achieved endosulfan dose in C-27-1 (1.3 μg/L) was approximately twice that of M-55-8 (0.6 μg/L). The entry and degradation of endosulfan from drift and runoff, and the actual endosulfan concentrations measured in pond water and sediment for C-27-1 and M-55-8 are discussed separately below.

The aerial drift contribution to pond water in C-27-1 was demonstrated by the presence of endosulfan after the first and second endosulfan applications, even though no rainfall or runoff occurred in the C-27-1 test system until after the third application. Mean total endosulfan in C-27-1 pond water was 81.8 ng/L on May 27, 1988, immediately following the first application. On June 8, 1988 (before the second application) mean total endosulfan was 123 ng/L. Immediately following the second application, mean total endosulfan was 257 ng/L on June 10, 1988, and declined to 10.5 ng/L on June 24, 1988, prior to the third application. Mean total endosulfan in pond water at C-27-1 peaked after the first runoff event induced via irrigation. Pond water from C-27-1 averaged 1,110 ng/L total endosulfan on June 30, 1988 (three days post-application and the day after the forced runoff event) and peaked two days later (July 2, 1988) at 1,310 ng/L. These concentrations decreased sharply to 319 ng/L three days later, steadily declined thereafter, and only small quantities of endosulfan sulfate were detectable in C-27-1 pond water six months after the final application (December 17, 1988).

Endosulfan in pond sediment entered via runoff through pond water and sedimentation of suspended particles to the pond bottom. Mean total endosulfan concentrations in C-27-1 sediment increased to a maximum of 43.5 μg/kg on July 5, 1988 (8 days after the third application) following a forced run off event on June 29, 1988. One explanation for the lower peak sediment concentrations in C-27-1 was the high turbidity caused by the May 10, 1988 storm event at this watershed. Fine particulate sediments remained suspended in C-27-1 for approximately two months, giving C-27-1 pond water the color and appearance of coffee with cream. Endosulfan may have selectively adsorbed to the fine suspended sediment in C-27-1, and remained suspended in the water column rather than settling to the pond bottom as for M-55-8. Total endosulfan concentrations in C-27-1 sediment ranged from below the detection limits (<5 μg/kg) to 25 μg/kg between mid July and late-August, declining to below detection limits by December 17, 1988.

Treatment pond M-55-8 received approximately half the endosulfan dose observed for treatment pond C-27-1 pond water. Mean total endosulfan in M-55-8 pond water was 124 ng/L on May 27, 1988 (the day of the first application) and is attributable wholly to aerial drift. Mean total endosulfan decreased to below detection limits (5 ng/L) prior to the second application, and rose to 53.7 ng/L on June 11, 1988. This measured mean concentration cannot be wholly attributed to aerial drift. A natural runoff event occurred at M-55-8 on June 10, 1988. On June 22, 1988 (prior to the third application) mean total endosulfan in M-55-8 was 16.5 ng/L. The final endosulfan application occurred on June 23, 1988. A combination of irrigation and actual rainfall occurred on June 24, 1988 driving a runoff event. On June 25, mean total endosulfan in M-55-8 pond water peaked at 583 ng/L, and declined to 30.7 ng/L on June 30, 1988. On July 21, 1988 (24 days after the last application) only minute quantities (<10 ng/L) of endosulfan were detected in M-55-8. Thereafter, endosulfan declined to below detection limit by December 13, 1988.

The mean total endosulfan concentration in M-55-8 pond sediments increased from less than detection limits (<5μg/kg) before the first application to a maximum of 99.4 μg/kg on June 25, 2 days after the third application and after a runoff event on June 25, 1988. Mean total endosulfan dropped to 29.1 μg/kg by late August. By December 13, 1988, sediment concentrations in M-55-8 were less than detection limits.

In summary, the mean peak dose of endosulfan in pond water was approximately 1.3 ug/L in C-27-1, with a concomitant value of approximately 0.6 μg/L for M-55-8. Concentrations of endosulfan in pond water declined to background concentration in six months at C-27-1 and three months in M-55-8. Endosulfan concentrations in pond sediments were influenced by runoff events. Endosulfan sediment concentration in C-27-1 and M-55-8 peaked immediately following the first major runoff events after the third application at both ponds. Mean total endosulfan concentrations in sediment in M-55-8 (99.4 μg/kg) peaked at approximately twice those encountered in C-27-1 (43.5 μg/kg). However, by late summer, sediment in both ponds had similar amounts of endosulfan (10-30 $\mu g/kg$) which declined to less than the detection limit by December.

The dose regime described above represents a worst-case exposure scenario. Thiodan 3EC was applied contrary to the direction on the label. Drought conditions during the application period resulted in little dilution of the applied pesticide between applications. In addition, forced runoff through irrigation (C-27-1) and rainfall (M-55-8) resulted in driving endosulfan into the ponds within 24 hours after the final application.

Mean total soil concentrations exhibited an 86 percent reduction from the observed peak concentrations (following the third application) to the end of the study (December 1988).

Like the soil, endosulfan in pond water and sediment declined substantially between the peak mean total endosulfan concentration and the end of the study period. Pond water total endosulfan residues in C-27-1 declined by 75 percent within a week of the last application and by 99 percent at study termination in December. In M-55-8, endosulfan residues in pond water declined by 95 percent within a week of the third application, and were below detection limits after 3 months. Endosulfan residue data from both treatment ponds indicated that the runoff events immediately following the third pesticide application provided the critical dose to the ponds. Following the peak dose, endosulfan residues in runoff slurry, pond water, and sediment declined, despite the occurrence of 8 and 5 natural runoff events in C-27-1 and M-55-8, respectively, between the last application and study termination.

C. Water Quality

Generally, the water quality characteristics of the four ponds were similar, and typical of soft water ponds in the southeastern U.S. There were few remarkable differences in pH, temperature, dissolved oxygen, alkalinity, or hardness between ponds for years 1987 and 1988.

Several parameters (conductivity, acidity, nitrate, orthophosphate, and total organic carbon) exhibited midsummer peaks in 1988 that did not occur in 1987. These peaks were presumably attributable to increased runoff and erosion into the four test ponds due to the removal of pond edge vegetation and preparation of the fields for planting. Treatment pond C-27-1 exhibited a dramatic increase in turbidity, nitrate, and phosphate due to a localized thunderstorm that struck the pond/agronomic field test system on May 10, 1988 (week 71), resulting in extensive soil erosion from the fields into this pond. The remaining treatment (M-55-8) and reference ponds (M-55-4, T-4-1) were unaffected by this storm event.

D. Ecological Measures

As discussed in preceding sections of this chapter, the mean peak dose of total endosulfan in pond water was approximately 1.3 µg/L in C-27-1 and 0.6 µg/L in M-55-8. Peak sediment concentrations of endosulfan were 99.4 and 43.5 µg/kg, respectively, for M-55-8 and C-27-1.

The clearest impact of endosulfan on pond biota was the fish kills that occurred at both treatment ponds. Fish in the 10-60 mm size range accounted for over 90 percent of fish killed. The magnitude of the fish kill was greater in C-27-1 than M-55-8, and was presumably attributable to the higher dose in pond water, as well as habitat differences in the two ponds at the points of runoff entry. A total of 447 dead fish were collected in C-27-1 in 1988, with 88 percent (393 fish) of these observed in the 3-day period following the first runoff event after the third application. In M-55-8, a total of 227 dead fish were collected in 1988, with 73 percent (165 fish) collected within three days of the first runoff event after the third application.

The fish kills appeared to be localized, primarily affecting shallow areas near the points of runoff entry. Although a total of 14 runoff events occurred during the post-spray and year-end study phases, only the initial runoff events immediately following the third endosulfan application at each pond were responsible fish kills.

Despite the fish kills at both ponds, no adverse effects to the farm pond fish populations were evident for the remainder of the study. Species composition of fishes in the four ponds varied slightly, but were dominated by largemouth bass and bluegill sunfish as the primary predator-prey relationship. Treatment pond M-55-8 contained a higher number of black crappie than the remaining ponds, whereas C-27-1 contained a greater number of warmouth. Young of the year largemouth bass declined in C-27-1, but appeared to begin decreasing prior to endosulfan application. Additionally, largemouth bass decreased in a reference pond (T-4-1), and the second treatment pond (M-55-8). Sunfish populations remained stable during 1988, except for a shift in the bluegill and warmouth structure in C-27-1. Young of the year warmouth constituted a larger portion of the sunfish population in 1988. The shift was temporary, however, and a return to bluegill dominance occurred by November 1988. No evaluation of the biological consequences of this shift for the pond ecosystem can be given. However, it is believed that if there was an effect at all, the impact of the change in composition of closely related species to the ecosystem was insignificant. Lepomis spp. (bluegill sunfish and warmouth) successfully reproduced in all ponds from mid-June through August of 1988, as evidenced by the presence of newly recruited fish. Largemouth bass spawned in all ponds prior to the endosulfan application period. However, recruitment was limited to May with no apparent largemouth bass recruitment in either the treatment or reference ponds after the early spring spawning period.

Bluegill and largemouth bass condition factors were compared statistically for selected treatment/reference pond pairs, and between the baseline and application years for each individual pond. Bluegill condition factors were different for the compared treatment/reference ponds in the pre-spray phase and in one week of the post-spray period. Condition factors and weight of bluegill were not significantly different between 1987 and 1988 in the C-27-1 treatment pond receiving the highest dose of endosulfan, but were significantly lower in all the other ponds. Here too, the degree of change was so small (0.5 g for weight and 0.11 for condition factor) that the biological significance and the ecological consequences of these changes is questionable.

In largemouth bass the condition factor was greater in treatment pond C-27-1 when compared to the reference pond T-4-1 for the year-end phase. However, as neither weight nor condition factors were different between 1987 and 1988 in any of the ponds, the above observation can be regarded as biologically and ecologically insignificant.

Fish are good indicators of long-term effects of perturbation, and they tend to integrate effects of lower trophic levels. As such, fish population and community structure is reflective of integrated environmental health. Endosulfan was responsible for localized fish kills in the present study. However, no biologically significant effects to the fish populations or communities were observed over the study period. The fish community exhibited no apparent adverse long-term effects due to endosulfan, suggesting no negative impact in the trophic levels. Nevertheless, the lower trophic levels were examined separately and are discussed below.

The macroinvertebrate communities of the farm pond test systems were examined by kick-net samples, artificial substrates (S-samples), emergent insect traps, and Ekman dredge samples. Each sample type provides data about different segments of the macrobenthic fauna. Qualitatively, the kick net sample data indicated that in terms of relative abundance oligochaetes decreased and chironomids increased in both treatment and reference ponds. Macroinvertebrate community structure in the treatment ponds was more stable over the entire study period than in the reference ponds based on percent relative abundance. Certain pollution-sensitive species such as the mayfly Caenis were more prevalent in the treatment ponds than the reference ponds after spraying.

S-sample data were also used to examine macroinvertebrate biomass and density characteristics of the benthic community on a qualitative basis. All ponds generally exhibited an increase in chironomid biomass from 1987 to 1988. Chironomids dominated the benthic community of the pond in terms of relative abundance and biomass in C-27-1 (treatment) and M-55-4 (reference). However, leeches and snails dominated the biomass in M-55-8 (treatment), whereas insects (other than chironomids) played a dominant role in T-4-1 (reference). For week 86 (August 21, 1988), chironomids also dominated invertebrate biomass at T-4-1.

The Ekman dredge samples contained 40 taxa of benthic macroinvertebrate, with chironomids, chaoborids, and oligochaetes comprising the dominant taxa. Only the latter three taxa were selected for quantitative analysis. The C-27-1 treatment pond exhibited a greater number of significant differences in macroinvertebrate densities than the M-55-8 treatment pond when compared to reference ponds. Chironomid and oligochaete densities were significantly lower in C-27-1 than in the reference ponds for the post-spray period. During the year-end time period, only oligochaetes remained significantly lower in C-27-1 relative to its reference pond. C-27-1 exhibited significantly higher chaoborid densities in both the post-spray and year-end periods, but this same trend occurred throughout the pre-spray period as well. Regarding Ekman samples, M-55-8 was not statistically different from the reference ponds M-55-4 or T-4-1 in the post-spray period and not different from C-27-1 and M-55-4 for the year-end period. It is therefore demonstrated that in at least one reference pond comparable declines to the one indicated in M-55-8 in chironomid densities in the sediment can be observed. These observations are corroborated by the emergence data.

Although 47 taxa of emergent insects were collected over the study period, chironomids constituted 95 percent of the specimens, with the remaining taxa each comprising less than 2 percent of the total collection. Because of their dominance, only chironomids were selected for quantitative analysis of insect emergence. The nested ANOVA model for chironomid emergence indicated a single significant difference between C-27-1 and its reference pond during the post-spray period (1 of

8 sampling weeks), and none for M-55-8. For the year-end period, there were no significant differences in chironomid emergence for C-27-1 and its reference pond while M-55-8 had significantly lower emergence rates relative to its reference pond for the entire year-end period.

The statistically significant differences between C-27-1 and the selected reference ponds indicated: a decrease in chironomid emergence on week 75 (June 5, 1988); a reduction in chironomid densities during the post-spray period; a reduction in oligocheate densities for the post-spray and year-end periods; and an increase in chaoborid densities for the year-end phase.

Although these statistically significant results may be attributed to endosulfan effects, it is important to note that the endosulfan dose events in C-27-1 consisted of aerial drift into the ponds after the first and second applications on weeks 73 and 75 (May 27, 1988 and June 10, 1988), respectively. The major dose, however, occurred with the run-off event after the third application on week 78 (June 29, 1988). For all of the macroinvertebrate parameters discussed above, the statistically significant reduction occurs after the first or second application. They are not maintained or exacerbated by the third application and its associated high endosulfan dose from run-off. Instead, the macroinvertebrate parameters generally showed an increasing trend suggesting a perturbation other than toxicity. The high turbidity in treatment pond C-27-1 after the May 10, 1988, storm also must be considered as contributing to the decreased chironomid density. It was likely that sedimentation loadings sufficiently disrupted habitat to cause the statistically significant decreased densities of chironomids detected by the analysis. This hypothesis is supported by the increase in chaoborids which are planktonic rather than sediment dwellers, and thereby less sensitive to physical disruption due to sedimentation. The sedimentation hypothesis is further supported by comparing the same parameters for treatment pond M-55-8 with its selected reference pond.

In M-55-8, emergence rate was similar to that of the reference pond in the post-spray period, and showed a reduction over the reference pond for the year-end period. When M-55-8 is compared with T-4-1 (not selected for statistical analysis) emergence rate from M-55-8 is almost identical with the one from T-4-1. Again, the occurrence of a reduction on emergence -- as to be expected by a reduction in densities -- is not necessarily attributable to a chemical impact. Chironomid and oligochaete densities followed the same general trends for the post-spray period, and also showed a reduction for the year-end period. Although a large decrease in chironomid and oligochaete densities occurred in M-55-8 two weeks following the first run-off event after the third application, similar reductions occurred in treatment pond T-4-1, negating a causal relationship to endosulfan.

Like macroinvertebrates, the zooplankton community represents the secondary production trophic level of aquatic ecosystems and should be sensitive to pesticides developed for use on terrestrial insects. Yet, qualitative trends in the zooplankton communities of the farm ponds provide little evidence for toxic effects to zooplankton. The major zooplankton groups increased in relative abundance for both the treatment (C-27-1, M-55-8) and reference ponds (T-4-1, M-55-4). The most dramatic increases were for the rotifers and protozoans, but the relative abundance of cladocerans also increased from 1987 to 1988. Ostracods relative abundance in 1988 was similar to or greater than that for 1987. Concomitant with the increase in relative abundance, zooplankton densities were significantly greater in both treatment ponds when compared to reference pond M-55-4, with the exceptions of weeks 76 (June 12, 1988) and 102 (December 12, 1988). If the mean total zooplankton density pattern for each pond are compared qualitatively, T-4-1 (treatment pond) exhibited a rapid decline and rapid rise between weeks 74-76. The two treatment ponds (C-27-1 and M-55-8) exhibited a similarly rapid decline and rise between weeks 76 and 78, whereas a decline of this nature did not occur in M-55-4. As such, the decline in density on week 76 in the treatment ponds occurred near the same time in one of the reference ponds (T-4-1). Interestingly, the week 76 decline in the treatment pond occurred after the first two applications. However, neither treatment pond exhibited a significant reduction in

zooplankton densities after the major dose of endosulfan through run-off
following the third application. The differences between treatment pond and reference pond M-55-4 for the last sampling week (December 12, 1988) reflected a typical year-end decline caused by seasonal decrease in temperature and the phytoplankton food base.

Regarding zooplankton diversity, several significant differences occurred between the two treatment ponds and the selected reference pond (M-55-4). Decreases in diversity can be traced to increased relative abundance of one or more zooplankton taxa and vice versa.

Like zooplankton, phytoplankton in all ponds exhibited a general increase in relative abundance between 1987 and 1988. Indeed, the increased zooplankton abundance in 1988 was presumably due to an increased phytoplankton forage base in the treatment year. One explanation for increased phytoplankton relative abundance in 1988 was agronomic preparation of the fields to accommodate planting. The watersheds surrounding all ponds were cleared, tilled, and fertilized in the early spring of 1988 prior to planting tomatoes. These practices may be wholly responsible for the increased phytoplankton due to increased nutrient loading during early spring rains.

Increased nutrient loading through run-off prior to endosulfan application would increase phytoplankton production through organic enrichment. This trend was evidenced by a greater relative abundance of phytoplankton in all ponds for 1988. The Cyanobacteria (bluegreens) and Chlorococcales (green algae) increased in all ponds in 1988, with bloom conditions particularly evident in C-27-1. The Cyanobacteria bloom in C-27-1 started the week of June 12, 1988 (week 76) which was after the first two endosulfan applications, but prior to the primary endosulfan input into the pond associated with the induced run-off events of June 28 and 29, 1988.

Phytoplankton densities in the two treatment ponds (C-27-1, M-55-8) were generally higher than the two reference ponds (T-4-1, M-55-4) in the post-spray and year-end study phases, suggesting no direct phytotoxic effect. As the increase in relative abundances and densities in zooplankton showed, endosulfan had no impact on this group of organisms feeding on phytoplankton. Therefore an indirect effect of endosulfan via primary consumers on the phytoplankton community can be rejected. The phytoplankton bloom in C-27-1 was more likely associated with the localized thunderstorm that affected the C-27-1 watershed/pond systems.

Phytoplankton diversity in C-27-1 (treatment pond) was generally lower than that of M-55-4 (reference pond). In contrast, M-55-8 (treatment pond) generally had a higher phytoplankton diversity than reference pond M-55-4. Visual inspection of the rise and fall of diversity indices for C-27-1 and M-55-4 revealed a similar trend for both ponds. The two ponds exhibited a series of three rapid declines and increases in phytoplankton diversity. However, the declines in M-55-4 lagged behind those in C-27-1 by 2 to 5 weeks. In the year-end phase, the lag phenomenon appeared to account for the significant differences in diversity between the two ponds.

Pond metabolism showed significant differences between treatment and reference ponds in 1988. However, these differences were attributable to the behavior of the ponds independent of the endosulfan dose in the pond water. Gross production patterns for both M-55-8 and M-55-4 were similar, but differ in amplitude. The different amplitudes at the start of the season reflected individual characteristics of each test system. Despite amplitude differences, the pattern of increases and decreases was similar in the paired ponds. This phenomenon was also true of pond pair C-27-1 and M-55-4. Net production was likewise variable, but followed the general pattern of gross production. Reference pond M-55-4 showed increased net production in the post-spray period as did treatment pond C-27-1. In the year-end period, both declined. The same pattern exists for M-55-8 and M-55-4.

The autotrophic index (AI) showed five significant differences for the pond pairs. The differences occurred sporadically in the pre-spray, post-spray, and year-end periods. When the differences occurred, the reference ponds usually had slightly higher AI values indicative of heterotrophic communities. There was no readily apparent association between endosulfan and any of these changes. However, a trend toward increasing heterotrophy is consistent with organic enrichment that may have occurred due to agronomic practices at the pond watersheds.

Changes in macrophytes were attributed to decreases in pond water levels. No causal effect between aquatic macrophyte abundance and endosulfan exposure could be verified from the available data.

As an overall summary, one can conclude that endosulfan applied as Thiodan 3 EC in a worst case scenario to fields surrounding established aquatic ecosystems (farm ponds) and transported into the ponds via drift and run-off did only cause acute effects to some small fish. No effects on the ecosystem as a whole could be established. None of the farm pond ecosystems changed structure or function in a way that they did not resemble their status in the year prior to treatment or the respective reference ponds at the end of the study period.

VIII. REFERENCES

Auer, N.A. ed. Identification of Larval Fishes of the Great Lakes Basin with Emphasis on the Lake Michigan Drainage. Great Lakes Fishery Commission Spec. Pub. 82-3. Univ. of Michigan, Ann Arbor, 1982.

Goebel H., S. Gorbach, W. Knauf, R. Rimpau, H. Huttenbach. Properties, Effects, Residues and Analytics of the Insecticide Endosulfan. Res. Rev. 83, 1-165, 1982.

Gorbach S., R. Haaring, W. Knauf, H.J. Werner. Residue analyses in the water system of East Java (River Brantas, Ponds, Sea Water) after continued large-scale application of Thiodan in Rice. Bull. Environ. Contam. Toxicol. 6, 40-47, 1971.

Green, J.D. Certificate of Analysis. FMC Corporation, Jacksonville FL, March 9, 1988.

Johnson, N.L., F.C. Leone. Statistics and Experimental Design in Engineering and the Physical Sciences, Vol. II, 2nd edition. John Wiley & Sons, New York, 1964.

National Oceanic and Atmospheric Administration. Climatography of the United States, No. 60, Climate of Georgia. National Climatic Data Center, Asheville NC, 1978.

Murty, A.S. Toxicity of Pesticides to Fish, vol II. CRC Press, Inc., Boca Raton FL, 1986.

National Oceanic and Atmospheric Administration. 1987 Local Climatological Data--Columbus, Georgia. National Climatic Data Center, Asheville NC, 1988a.

National Oceanic and Atmospheric Administration. 1987 Local Climatological Data--Tallahassee FL. National Climatic Data Center, Asheville NC, 1988b.

Neter, J., W. Wasserman. Applied Linear Statistical Models. Richard D. Irwin, Inc., Homewood IL, 1974.

Nielson, L.A., D.L. Johnson. Fisheries Techniques. American Fisheries Society, Bethesda MD, 1983.

North Carolina State University. 1975 North Carolina Agricultural Chemicals Manual. Raleigh NC, 1975.

Poole, R.W. An Introduction to Quantitative Ecology. McGraw-Hill, New York, 1974.

U.S. Department of Agriculture. Field Manual for Research in Agricultural Hydrology. U.S. Department of Agriculture, Washington DC, Agriculture Handbook No. 224, 1979.

U.S. Department of Agriculture. Agricultural Statistics--1984. US Government Printing Office, Washington DC, 1985.

LIST OF PRINCIPAL PARTICIPANTS
ON ENDOSULFAN POND STUDY
FROM BATTELLE AND HICKEY'S AGRI-SERVICES

Name	Discipline	Role in program
Barney W. Cornaby, Ph.D.	Ecological Toxicology	Study Director
Anthony F. Maciorowski, Ph.D.	Ecological Toxicology	Deputy Director
Kenneth M. Duke, Ph.D.	Ecological Toxicology	Senior Reviewer
Sandra M. Anderson	Quality Assurance	Chemistry Quality Assurance
Scott R. Brown	Quality Assurance	Environmental Quality Assurance
Michael Griffith	Benthic Ecology	Field Coordinator
Steve Hickey, Ph.D.	Agronomy	Agronomy/Application
Ramona Mayer	Quality Assurance	Quality Assurance
John E. Navarro	Fisheries	Field Coordinator
Steven E. Pomeroy	Environmental Assessment	Field Coordinator
Thomas R. Powell	Analytical Chemistry/Sampling	Field Chemist
Norman G. Reichenbach, Ph.D.	Biological Statistics	Bio-Statistician
John A. Shuey, Ph.D.	Environmental Toxicology	Bio-Statistician
Mark F. Yancey, Ph.D.	Analytical Chemistry	Analytical Chemist

HICKEY'S AGRI-SERVICES LABORATORY
P. O. Box 547
CAMILLA, GEORGIA 31730-0547

June 16, 1989

My firm held the contract directly with Hoechst for the land improvement, flume installation, trial run, and tomatoe production. Also, we were responsible for endosulfan applications and irrigation. I originated the data and language in those sections of the report. Quality assurance activities for my part of the work was handled by Hoechst. Barney Cornaby assisted me on the report relative to editorial matters.

Steve Hickey	Barney Cornaby
Steve Hickey, Ph.D Hickey's Agri-Services Laboratory	Barney Cornaby, Ph.D. Battelle
06/18/89 Date	June 22, 1989 Date

ACCURATE ANALYSIS SUPPORTED BY TOTAL AGRONOMICS

ARCHIVAL STATEMENT

All original raw data, documentation, records, protocols, and final report generated as a result of this study shall be stored in the Archival Center of the Health and Environment Group at Battelle Memorial Institute, Columbus, Ohio.

Biological samples shall be stored at Battelle Memorial Institute, Columbus, Ohio until the Sponsor decides by December, 1989 on a permanent location.

THIS PAGE IS INTENTIONALLY BLANK

THIS PAGE IS INTENTIONALLY BLANK

THIS PAGE IS INTENTIONALLY BLANK

THIS PAGE IS INTENTIONALLY BLANK