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STUDIES ON THE ACUTE TOXICITY OF TWO-CYCLE OUTBOARD MOTOR EXHAUSTS TO SELECTED FISH SPECIES

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Henry B. S. Ghan

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Progress Report for Division of Water Pollution Control,
Massachusetts Water Resources Commission.
Contract Number 15-51451.

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PREFACE

This progress report is the second in a series of detailed progress reports prepared for the Division of Water Pollution Control, Massachusetts Water Resources Commission, Contract Number 15-51451, "Effect of outboard motor exhausts on water quality and associated biota of small lakes".

This report focuses on the acute toxicity effects of exhausts from a 7.5 horsepower outboard engine on two species of freshwater fish, fathead minnows and bluegills. It represents a portion of the research activities by the authors during the period from September, 1970 to February, 1972. The authors are, respectively, assistant professor, and graduate research assistant, Department of Civil Engineering and Professor, Department of Zoology, University of Massachusetts at Amherst.

This report will be brought to the attention of various agencies, organizations, companies, industries, and individuals interested in the preservation of our natural resources.

ABSTRACT

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Little is known on the effects of outboard-motor-subsurface-exhaust recipient-water (OMSE-recipient water) to fish. It was the purpose of this investigation to evaluate the acute lethal toxicity of OMSE-recipient water to fathead minnows (Pimephales promelas Rafinesque) and bluegills (Lepomis macrochirus Rafinesque). The short-term (96-hour) static bioassay technique was employed and the 24, 48, and 96 hour TL_{50} values were derived for each of the respective test species. A survey for histopathological effects on the dead and surviving fish in the bioassay chambers was also made according to standard histological techniques. The results of the experiments demonstrated the following: (1) the 24, 48, and 96-hour TL_{50} values for fathead minnows were 0.047, 0.038 and 0.032 percent concentration (or 2,150/l, 2,640/l and 3,130/l) of OMSE-recipient water, respectively; (2) the 24, 48, and 96-hour TL_{50} values for bluegills were all 0.044 percent concentration (or 2,260/l) of OMSE-recipient water; and (3) no overt significant damage to the gill structures of the test species exposed up to 0.079 percent concentration (or 1,270/l) of OMSE-recipient water could be found. A suggested safe concentration for fathead minnows and for bluegills is 0.0016 percent (or 62,500/l) and 0.0022 percent (or 45,500/l) of fuel consumed respectively.

In addition, it was found that aging the OMSE-recipient water for six days or more could result in an increase in toxicity of the toxic material.

The early spring collected species and the smaller test species were also found to be more sensitive to the OMSE-recipient water.

Finally, the two test species were found to be relatively equal in sensitivity to the test material.

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INTRODUCTION

INTRODUCTION

The increasing awareness concerning environmental quality has prompted several studies on the pollutional effects of outboard motor operations on inland waters. These studies (Lagler, et al., 1950; English, et al., 1963a, b; Kempf, et al., 1967; Environmental Engineering, Inc., 1969; Stillwell and Gladding, Inc., 1969; Schenck and Weber, 1970; and Shuster, 1971), generally sponsored by either Federal agencies or outboard motor manufacturers, have resolved only some of the controversies that have developed regarding the impact of outboard motors on water quality and aquatic life.

A report by the Boating Industry Association (1970) stated that approximately 7,215,000 outboard motors were in use in this country at that time and of these, 98 percent were of two-cycle design. The pollutional effects from two-cycle outboard motors have been the major concern of many environmentalists. Stewart and Howard (1968) reported that fish showed an attraction for "gas polluted water" and that oil in water attached to the surface of unicellular plankton and inhibited cultures of diatom algae. Oil slicks, scums and unpleasant odor have been observed in waters where outboard motors have been operating (Wilber, 1969). Anderson (1971) warned that many bodies of water do not contain enough bacteria "to consume the gush of oil and gas" from two-cycle outboard motors and that "the residue fouls the shorelines, kills fish, pollutes drinking water and greases the skins of swimmers." On the other hand, however, Taylor (1971), charged that the pollutional effects from outboard motors had been inflated and that there was no knowledge

of any incident where outboard motors were polluting the water.

Recent studies on the pollutional effects from outboard motors were focused on the root of the problem which was to determine whether two-cycle outboard motors discharged any pollutant into the water and if so, to what extent. A study by Stillwell and Gladding (1969) found that two-cycle outboard motors discharged unburned fuel into the water. The cause of the problem was considered to be due to the inefficient design of the engine. The amount of unburned fuel discharged into the water was found to vary depending on the horsepower of the engine (Ferren, 1970), the operating speed of the engine (Ferren, 1970; and Shuster, 1971) and the condition of the engine (Shuster, 1971). As much as 55 percent of the original fuel could be released unburned from an outboard motor (Ferren, 1970). The unburned fuel, together with the combustion products, when discharged into the water will hereinafter, be referred to as outboard-motor-subsurface-exhaust-recipient water or OMSE-recipient water.

The pollutional effects from the OMSE-recipient water on fish have been investigated to varying degrees by Lagler, et al., (1950); English, et al., (1963a), b); and Schenck and Weber (1970). Of these investigators, only English, et al., (1963a) conducted bioassays to determine the TL_{50}[1] values for test fish species exposed to OMSE-recipient water. It was felt in this investigation that further TL_{50} values needed to be derived using a recent model outboard motor wherein the prescribed fuel: oil ratio

[1] TL_{50} or tolerance limit for 50 percent survival is the concentration of the tested material in a suitable diluent at which just 50 percent of the test animals are able to survive for a specified period of exposure. The commonly used exposure periods are 24, 48, or 96 hours.

would be more meaningful with regard to the majority of outboard engines in use today.

English, et al., (1963a) also found that the toxicity component of OMSE-recipient water to fish was not highly persistent since no mortalities were recorded with tests using 4-day old OMSE-recipient water. It was felt in this study that this needed to be verified.

Several investigators (Mitrovic, et al., 1968; Brown, et al., 1968; and Fujiya, 1965) have demonstrated that fish mortality caused by phenol,,zinc, detergent and pulp mill wastes usually result from damage to the gills. However, no study has yet been done on the gills of fish exposed to OMSE-recipient water. Wood (1960) has recommended that identification of histopathological effects on gill tussue be included in fish toxicology studies.

Based on the above considerations, the objectives of this study, using fathead minnows (Pimephales promelas Rafinesque) and bluegills (Lepomis macrochirus Rafinesque) as test species, were as follows:

1. To determine the 24, 48, and 96-hour TL_{50} values for the two test species exposed to OMSE-recipient water.
2. To investigate the persistence of the toxic properties of OMSE-recipient water to the test species.
3. To search for, and possibly identify, any histopathological effects of OMSE-recipient water on gill tissue of the test species.

LITERATURE REVIEW

LITERATURE REVIEW

Scope

In order to understand the stress of OMSE-recipient water on fish, it is essential first to consider the emissions, factors influencing the amount of emission and the emission characteristics of a two-cycle engine. In addition, other effects of OMSE-recipient water on fish, specifically with regard to fish flesh tainting, chronic effects and its effect in relation to fish behavior, fish production and angling success, are equally important. For an in-depth discussion of these factors, the reader is referred to a review by Kuzminski and Jackivicz (1972).

Bioassay, as a general technique in measurement of toxicity to fish for a large variety of compounds, has recently been reviewed by Sprague (1969, 1970, 1971). It, therefore, will not be reviewed in close detail in this study. A review will, however, be made of studies in which bioassays were employed to determine the acute and chronic lethal effects of OMSE-recipient water to fish.

Effects of Two-Cycle Outboard Motor Emissions in Water on Fish

As mentioned above, the various aspects of the operation of two-cycle outboard motors to fish have been investigated to varying degrees by several investigators (Lagler, et al., 1950; English, et al., 1963a, b; Kempf, et al., 1967; Environmental Engineering, Inc., 1969; and Schenck and Weber, 1970). These investigators were generally concerned with one or more of the following: a) the lethal concentration or TL_{50} values of OMSE-recipient water to fish; b) the chronic effects of OMSE-recipient water as measured by fish reproductive capabilities; c) off-flavoring of

fish flesh exposed to OMSE-recipient water; and d) operation of outboard motors in relation to fish behavior, fish production and angling success. Each of these general goals of the investigations are reviewed.

Acute Lethality of OMSE-recipient Water to Fish

Short-term bioassays are often employed to determine the acute lethality of a material to fish. The results from these tests are usually expressed as the limit of tolerance for 50 percent of the test species or TL_{50} using survival and time periods as the criterion. A recommended safe concentration based on dilution of the substance within the recipient water may then be established by the use of a safety or application factor to the TL_{50} values derived in the laboratory.

A survey of the studies made on the lethality of OMSE-recipient water to fish showed that only English, et al., (1963a) derived any TL_{50} values and subsequently a recommended safe concentration based on the TL_{50} values derived. Kempf, et al., (1967) and Environmental Engineering, Inc. (1969) did not establish or determine any TL_{50} values. Schenck and Weber (1970) stated that their own future bioassay work on OMSE-recipient water would involve establishing acute toxicity values; however these have not as yet been reported in the literature.

A full-scale bioassay conducted by English, et al., (1963a) yielded 96-hour TL_{50} values of 1,700/l[2], 1,800/l and 1,900/l for fathead minnows (averaging 2 1/2-inch length; 1 1/2 g.) and 96-hour TL_{50} values of 1,600/l and 2,500/l for bluegills (averaging 2 1/4-inch length; 2 g.). The

[2]parts dilution water per parts fuel mixture consumed by outboard motor.

recommended safe concentration established to protect aquatic life was 19,000/l (based on 10 times a TL_{50} value). No indication was given why the TL_{50} value of 1,900/l was used to establish the safe concentration. A TL_{50} of 16,000/l was further reported to have a "significant toxic effect on fish life." The degree of effect at this concentration of OMSE-recipient water was not reported. All the TL_{50} values derived were based on using a prepared soft, diluent water with the following characteristics: D.O., 8 mg/l; pH, 7.4; alkalinity ($CaCO_3$), 18 mg/l; hardness ($CaCO_3$), 20 mg/l; and temperature, 25°C.

Kempf, et al., (1967) reported that through aquarium testing, the lethal dose of OMSE-recipient water for common domestic fish such as trout (Salmo trutta fario) and carp (Cyprinus carpio) was found to be quite variable. However, the range of lethal concentrations, test conditions and methodology was not reported.

No explanation was given by Environmental Engineering, Inc., (1969) why TL_{50} values were not derived in their study. The bioassays conducted were reported to conform to procedures as outlined in Standard Methods (12th Edition). Lake X water was used as the dilution water and although a single day characterization was made, it was not clear if this singular water sample was used in the bioassays as dilution water. The OMSE-recipient water was prepared by running a 4-horsepower motor at 1,000 rpm in a 50-gallon drum. The engine was operated for 1, 4 and 8 hours and the amount of gasoline-oil (50:1) mixture consumed during these times were 730 ml, 5 lbs and 8 lbs 10 ozs respectively. Bluegills (2-3 inches long) were used in all tests. An interpretation of data in terms of TL_{50} values was not reported.

To the authors' knowledge, no additional reports have been made in the literature other than those reviewed herein on acute toxicity effect of OMSE-recipient water to fish.

Chronic Effects of OMSE-recipient Water to Fish

Results of laboratory tests conducted by English, et al., (1963a) showed little chronic or accumulative effect of OMSE-recipient water on fish for exposure times of up to 15 days. The 24-hour, 48-hour, 96-hour, 5-day, 10-day and 15-day TL_{50} values derived from the long term bioassays were 1,900/l, 1,900/l, 2,200/l, 2,400/l, 2,400/l and 2,500/l respectively. Only a brief description was given of the bioassays technique from which these values were derived. These same investigators further reported that the 24-hour, 48-hour and 96-hour TL_{50} values derived from a static test were all 1,900/l. A comparison of the results obtained in a static bioassay with those from a continuous flow bioassay showed little differences in the TL_{50} values for exposure periods of up to 96 hours. This suggests that a continuous flow technique may not always be required for fish toxicity studies.

An experiment conducted by Schenck and Weber (1970) on fathead minnows exposed to a continuous flow of OMSE-recipient water showed that at some concentration higher than 10,000/l, a noticeable effect on reproduction could be found. No definite values regarding the chronic effects on reproduction (e.g., spawning numbers, spawnings per female, percent hatching success and percent fry survival) or on the growth of the fish were derived by these investigators as fish mortality, not related to the OMSE-recipient water, was observed during the course of the investigations. The cause of fish mortality was not resolved.

EXPERIMENTAL METHODOLOGY

EXPERIMENTAL METHODOLOGY

Fundamentals of Bioassays

Biological assay or bioassay may be defined as the determination of the quality of toxicant necessary to affect a test species based on a specified response criterion under stated laboratory conditions. The standard of measurement is expressed in tolerance limits for 50 percent test species survival (TL_{50}) for a specified exposure time (e.g., 96-hour TL_{50}).

The bioassay technique was originally developed by pharmacologists and statisticians as a standard method of analysis for testing drugs (Sprague, 1969). Hart, et al., (1945) first introduced it as a technique for the evaluation of the toxicity of industrial wastes, chemicals and other substances to fresh-water fishes and the technique was later refined and officially recommended by Doudoroff, et al., (1951) as a standard method for evaluating the lethality of industrial wastes to fish. This method of analysis has recently gained wider acceptance as a technique in pollution analysis and control. Agencies recommending bioassay as a standard technique to evaluate the lethality of polluted water to fish are the American Society for Testing and Materials (Manual on Industrial Water and Industrial Waste Water, 1966) and the Federal Water Pollution Control Federation, American Water Works Association, and American Public Health Association (Standard Methods, 1960).

The bioassay technique discussed in this work has been based on the quantal response, that is, an all-or-nothing reaction using death as the criterion. In other words, the fish has to be presumed dead. The number

of fish killed by the pollutant at a specified time is recorded and the results plotted on semi-logarithmic graph paper to obtain the respective TL_{50} value. Additional details concerning the treatment of data are presented in a subsequent section of the Methodology.

Routine Procedure for Static Bioassays

Test Species and Laboratory Acclimation

Fathead minnows (Pimephales promelas) and bluegills (Lepomis macrochirus) were selected as test species in this study for several reasons. They have been shown to be sensitive to OMSE-recipient water by previous investigators (English, et al., 1963a, b; and Schenck and Weber, 1970) and therefore facilitate comparison of results with these investigators. The fish are adaptable to laboratory conditions and sufficient quantities of each species were available from nearby ponds and hatcheries. In addition, both species have been suggested as standard fresh water species for use in bioassay and bluegills are a significant species to Massachusetts inland waters. Finally, their respective positions in the food chain may offer additional significance to this study.

The test fish were either purchased from private hatcheries or seined from local ponds. In either case, the fish were obtained from water sources where there was no known evidence of outboard motor usage. Fathead minnows (1 1/2" - 2" fork length) were obtained from a private hatchery in South Windsor, Connecticut. Bluegills (1" - 1 1/2" fork length) were purchased from a private hatchery in West Willington, Connecticut. Larger bluegills (2" - 2 1/2" fork length) were, however, seined from the University East Orchard pond located at the north-east

corner of the Amherst campus. All fish were transported in two, 20-gallon plastic containers (approximately 250 fish/container) from the source to the laboratory and approximately fish were collected every two weeks. Upon arrival at the laboratory, the containers were immediately aerated with an aquarium air pump. The fish, however, were left in their respective containers to slowly adjust to the laboratory temperature. When the temperature of the plastic containers and the stock tank differed by not more than ± 2°C, the fish were then transferred and allowed to acclimatize to laboratory feeding and diluent water in the stock tanks for a minimum period of 10 days. During this period, the fish were fed daily to satiation or as much as they could eat in five minutes with a variety of commercially prepared food such as dried daphnia and freeze dried turbifex worms (both manufactured by Longlife Fish Products) and tetramin (manufactured by Tetra Werke of Germany). Feeding was terminated two-days prior to each bioassay. In addition, no food was given during the bioassay exposure period. The fish were judged fit for a bioassay only if mortality during a period of 4-days immediately preceding a test was less than five percent and only if the fish showed no evident symptoms of disease or any abnormalities at the time of their transfer to test containers.

Dilution Water

Amherst tapwater, aged and aerated for a minimum period of ten days was used as an acclimatizing medium in the stock tanks and subsequently as a diluent in bioassays. The water was vigorously aerated with regular aquarium pumps and filtered with internal carbon filters during storage to remove any traces of free chlorine and chloramines.

A characterization of the water was made prior to each bioassay for the selected quality characteristics of total chlorine, copper, hardness, and alkalinity. Chlorine (starch-iodide method), hardness, and alkalinity were determined according to Standard Methods (1971). Copper was determined as specified in Analytical Methods for Atomic Absorption Spectrophotometry (March, 1971). The following instrumental parameters were used:

Instrument -- Perkin Elmer Model 303
Stomic Absorption Spectrophotometer

Light Source -- Copper-Hollow Cathode Lamp

Wavelength -- 3247A°

Current -- 15 Milliamperes

Oxidant -- Air

Fuel -- Acetylene

Sensitivity -- 0.10 ug/ml Copper for 1 percent absorption

Other Experimental Conditions

The bioassay temperature was maintained at 20°C ± 1°C during the acclimation and experimentation period. Twelve 10-gallon glass aquaria with stainless steel frames (19 1/2"L x 10"W x 11"D) were used as experimental tanks for each bioassay. Two bioassays were performed concurrently for each 4-day exposure period. Duplicate sets with ten fish per aquarium were used per concentration of OMSE-recipient water. Duplicate aquaria were also maintained for the control. These controls were maintained under similar experimental conditions as the test aquaria. In bioassays where mortality in the control exceeded 10 percent during the 4-day exposure time, the test was discontinued. Only in two instances throughout the entire investigaiton did this actually happen. After each

bioassay, the aquaria were detergent cleaned and rinsed repeatedly with both tap and distilled water. They were then allowed to dry before being used in subsequent experiments.

Experimental Procedure for Static Bioassays

Flow Diagram

The flow diagram of the experimental procedure used in this investigation to evaluate the lethality of OMSE-recipient water to fish is given in Figure 1. Note that the source of fish, the acclimation of fish to laboratory conditions and the characterization of the dilution water were discussed in earlier sections and therefore they would not be included in the subsequent discussion.

The concentrations of OMSE-recipient water in this study are expressed as percent by volume of fuel consumed or as a dilution factor of parts dilution water per parts of fuel consumed. If expressed as percent by volume, a 10 percent dilution equals one part of fuel consumed in nine parts of dilution water.

Preparation of Test Aquaria and Control

Twenty-four hours prior to each bioassay, each of the test and control aquaria was filled with the aged dilution water at varying conditions depending on the concentration of OMSE-recipient water desired. In each bioassay performed, a batch of sixty fish were removed from the stock tank, placed in a 5-gallon plastic container and then randomly distributed with one fish to each of the six-aquaria (consisting of five concentrations of OMSE-recipient water and a control) until a total of ten fish were placed in each of the aquaria. This process was repeated for

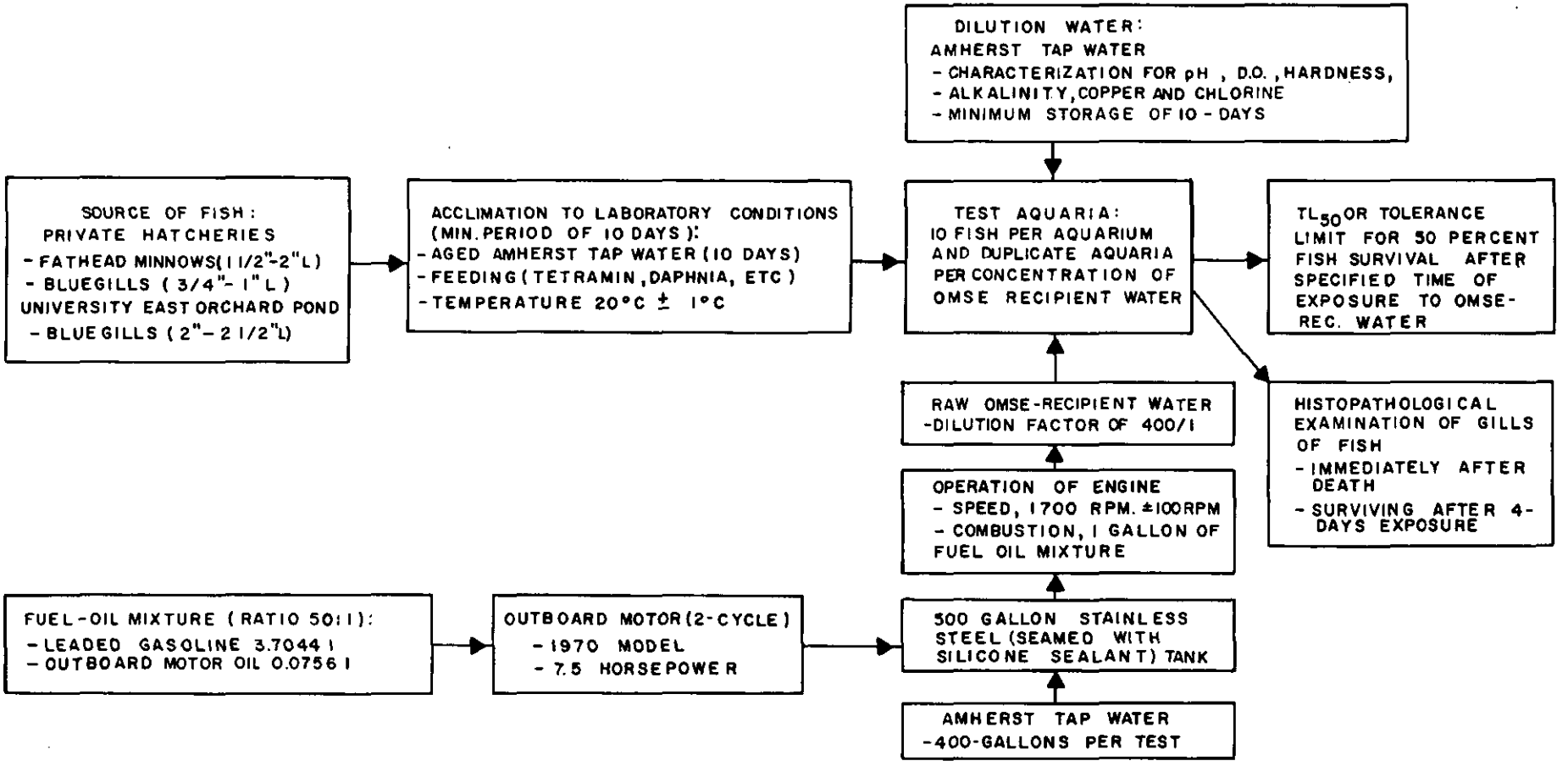

FIGURE 1 — FLOW DIAGRAM OF EXPERIMENTAL PROCEDURES FOR a) DERIVING TL_{50} VALUES AND b) HISTOPATHOLOGICAL EXAMINATION OF GILLS

each set of aquaria constituting a bioassay test.

The aeration to each test chamber was then adjusted by means of a brass control valve until a rate of 30 to 50 bubbles of air per minute was obtained through the 3/32" diameter glass tubing. This low rate of aeration is necessary to prevent possible volatization of organic compounds and it is also recommended by Standard Methods (1971) as a satisfactory rate to maintain a minimum dissolved oxygen of 5 ppm in the test solution for most bioassays. Measurements of dissolved oxygen using a calibrated D.O. meter in all the bioassays conducted in this study showed this to be a sufficient rate to maintain a minimum dissolved oxygen of 5 ppm. Environmental monitoring data throughout the 96-hour bioassay exposure are presented with the fish survival data in the Results and Discussion Section of this report.

Preparation of OMSE-recipient Water

A 7.5 horsepower, 1970 model Kiekhaefer Mercury Outboard Motor equipped with a standard engine propeller was used to prepare the OMSE-recipient water in this investigation. The outboard motor was operated in a 500-gallon stainless steel tank (69.5"L x 47"W x 47"D). The stainless steel tank (or motor tank) was seamed internally with silicone sealant to avoid contamination of the OMSE-recipient water by solder used in seaming construction.

Prior to each bioassay, the motor tank was first filled with 400 gallons of Amherst tap water. One gallon of a mixture of 50 parts regular Gulf-leaded (automobile) gasoline and 1 part oil[3] was then measured and

[3]0.1 New Formula 50 Quicksilver Super Outboard Motor Oil manufactured and recommended by Kiekhaefer Mercury. This 2-cycle engine oil is for Mercury Outboard Motors approved for the gasoline-oil ratio of 50:1 (1963 model and later).

added to the fuel tank connected to the outboard engine. The engine was then allowed to operate until the gallon of gasoline-oil mixture was exhausted into the water to produce 0.25 percent concentration (or 400/1) or OMSE-recipient water. Since a representative portion of the sample was preferred, the OMSE-recipient water tank was therefore stirred manually with a paddle before it was pumped into the (two) twenty-gallon plastic containers. The twenty-gallon plastic containers were used to transport the OMSE-recipient water from the motor tank to the bioassay laboratory.

Exploratory Bioassays

Exploratory bioassays were performed whenever applicable to determine the general range of toxicity of the OMSE-recipient water to the test fish. A range of concentrations of the OMSE-recipient water was first assumed from published data (English, et al., 1963a) and a bioassay then conducted to determine the sensitivity of the fish to the assumed range of concentrations. The bioassays were conducted according to the procedure outlined in Standard Methods (1971) and the results from these will be discussed in the subsequent section on Full Scale Bioassays. By trial and error, a general range of toxicity of the OMSE-recipient water to the test fish was determined.

Full-Scale Bioassays

To conduct the bioassay, a measured volume of the OMSE-recipient water (from the motor tank) was diluted further into each of the test aquaria to obtain the desired concentrations. These concentrations were derived by applying the decilog intervals as recommended by Doudurоff, et

al., (1950) to the range of concentrations derived in the exploratory tests.

The time interval which elapsed between the stoppage of outboard motor operation and the dilution of the OMSE-recipient water in the test aquaria was never more than two hours. In addition, 30 liters of test solution mixture for a single bioassay or approximately 1.5 liter of test solution per gram of fish was maintained in all test aquaria.

The number of mortalities of the fish in each test aquaria was observed and recorded at 24, 48, and 96 hours after their introduction. A fish was considered "dead" at the time of observation when respiration and movements had appeared to stop and only when the fish did not respond to prodding with a glass rod. Dead fish were removed as soon as they were observed. In addition, the behavior of the fish that were still alive was also noted and recorded.

Derivation of 24, 48 and 96-hour TL_{50}

The 24, 48, and 96 hour TL_{50} values were derived by straight-line graphical interpolation method as recommended in Standard Methods (1971). Briefly, this procedure involved:

a) The plotting of experimental data on semi-logarithmic paper with concentration of OMSE-recipient water on the logarithmic scale and fish survival percentage on the arithmetic scale.

b) The generation of a straight line between two points which represented survival percentages at two successive concentrations of OMSE-recipient water that were lethal to more than 50 percent and less than 50 percent of the test fish.

c) The extension of a vertical line from the 50 percent fish survival point on the abscissa to intercept the survivor curve. The TL_{50} value is that concentration of OMSE-recipient water on the ordinate corresponding to the point of intercept on the survivor curve.

Static Bioassay Using Aged OMSE-recipient Water

The bioassay procedures for the toxicity of aged OMSE-recipient water on fathead minnows were conducted similarly to the procedures described in earlier sections (Routine and Experimental Procedures for Static Bioassays). An exception was that raw OMSE-recipient water, in contrast, was aged under quiescent conditions for 4, 6, 10, and 15 days in the 500-gallon stainless steel tank (immediately after combustion of one gallon of fuel-oil mixture) before it was used in the full-scale bioassays.

A Beckman Model 915 Total Organic Carbon Analyzer was used to determine the total carbon and the total inorganic carbon in the 4, 6, 10, and 15-day old OMSE-recipient water which was withdrawn directly from the 500-gallon tank. In these carbon analyses, a 20 µl sample was injected into either the total carbon or the total inorganic carbon sampling port and the results were recorded on a strip chart recorder. The peak height were then measured from these charts and by reference to the calibration curves (Figure 2 and 3) a direct reading in terms of mg/l of carbon was noted for the total carbon and the total inorganic carbon. The total organic carbon for the samples was determined by difference from the values obtained from the total carbon and total

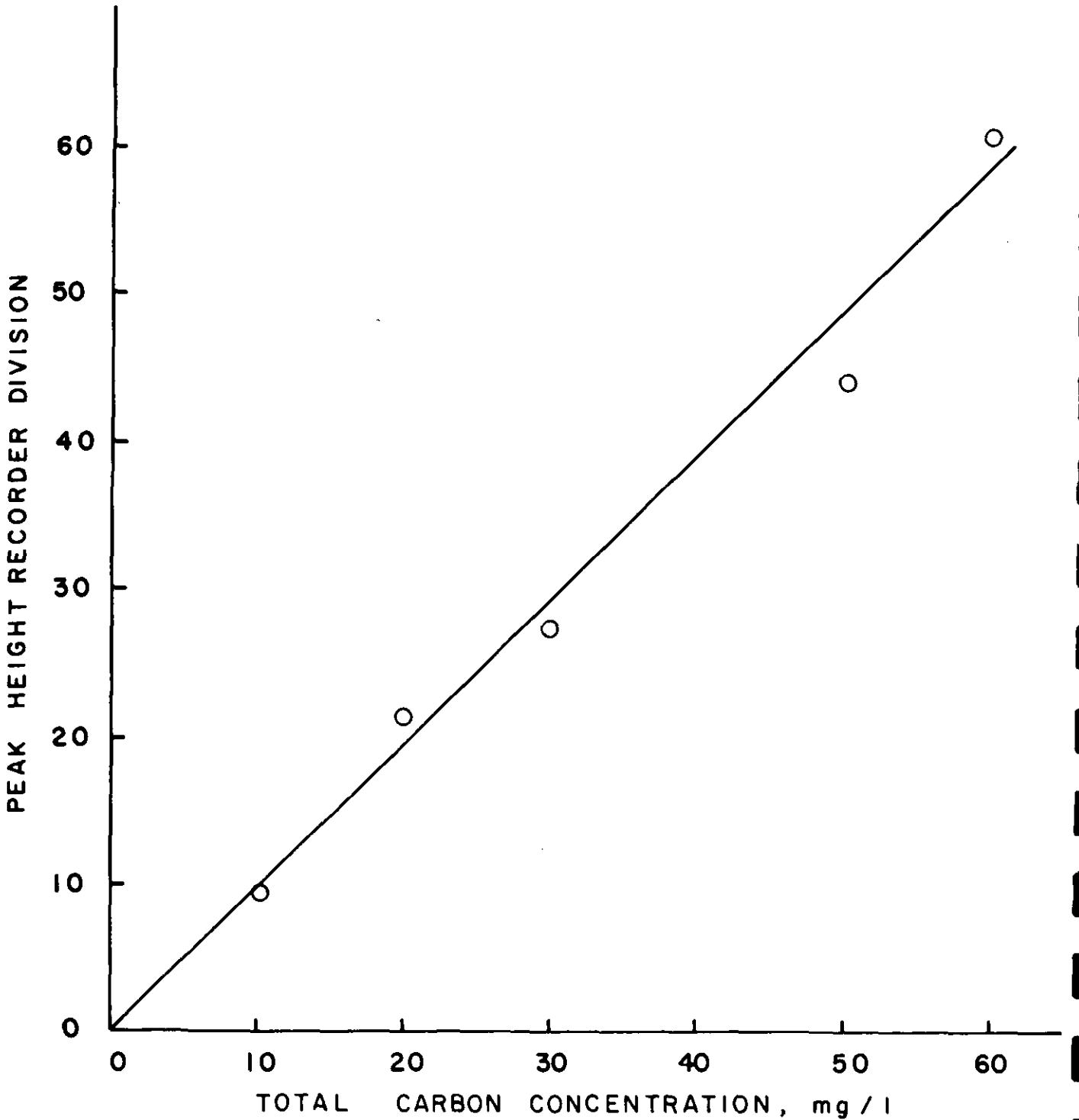

FIGURE 2 - CALIBRATION CURVE FOR TOTAL CARBON (SAMPLE 20 μ g/l)

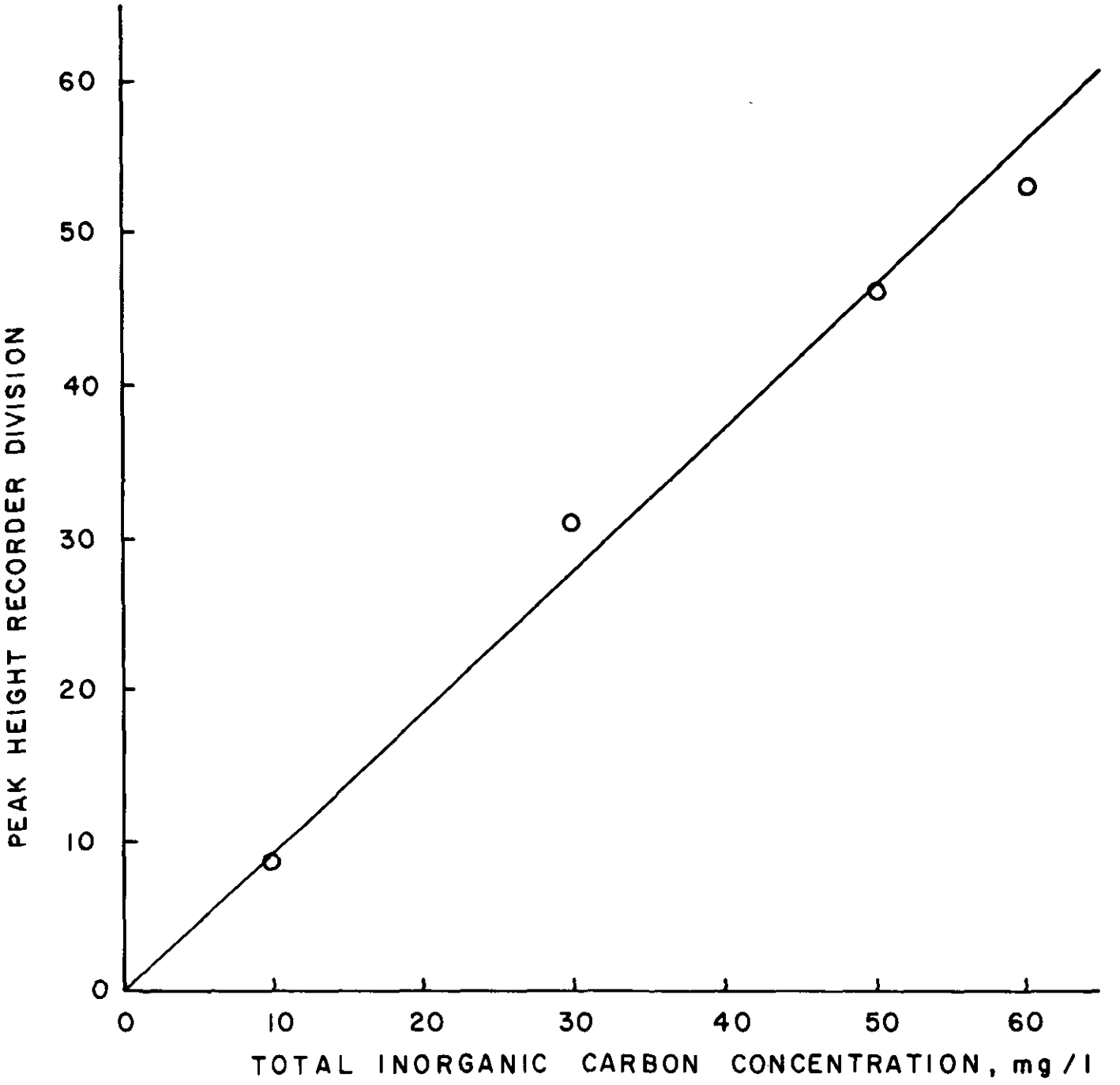

FIGURE 3 - CALIBRATION CURVE FOR TOTAL INORGANIC CARBON
(SAMPLE 20 μ g/l

inorganic carbon analyses.

Histopathological Effects on the Gills

Fathead minnows and bluegills in the bioassay tanks were investigated for histopathological effects on the gills immediately after death and at the end of the 4-day exposure period. At least two fish were removed from each concentration of OMSE-recipient water and the control chamber. The gills of the fish removed were immediately excised and fixed in Bouin's solution for a minimum period of 24 hours. After the fixation, the gills were run through three hourly changes of 70 percent alcohol and then only once after three hours. They were then placed in small metal receptacles in an automatic tissue processor or "Tissue-maton" and the timing device set for the intervals desired.
After this process, the gills were then embedded in paraffin wax with a "Tissuemat" and the paraffin was allowed to solidify around and within the gills. The embedded paraffin blocks were then trimmed and sectioned into specimens 9μ thick with a "Microtome." This sectioned specimens were then mounted on slides, dried, and stained using the Delafield haematoxylineosin. All the slides were microscopically examined under a Wild M-20 light microscope at 150 X magnification for possible damage to the gill epithelium. At least two photographs were taken from the slide for each concentration of OMSE-recipient water and control with an Olympus PM-7 camera using Panatonic-X Kodak film.

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

Dilution Water Characteristics

A summary of the aged dilution water characteristics with respect to total chlorine residuals, copper, hardness, and alkalinity analysed prior to each of the bioassays in this investigation are summarized in Table 1. As shown in this table, total chlorine residuals, as determined by the starch-iodide titration technique, were immeasurable in all dilution waters used for bioassays. Similarly, copper was undetectable with the technique used in this study except for one instance where 0.065 mg/l of copper was measured. Therefore, any toxic effects to test species due to chlorine and copper were considered to be negligible.

From Table 1, hardness of the dilution water characteristics throughout this investigation were found to vary from 14.5 to 30 mg/l as $CaCO_3$. Likewise, alkalinity for the same water were found to vary from 4.5 to 50 mg/l as $CaCO_3$. The analyses suggest that a soft water was used as the medium to prepare the OMSE-recipient water.

Acute Toxicity of OMSE-recipient Water to Fathead Minnows

Exploratory Bioassays

Exploratory bioassays conducted in April, 1971, revealed that the general toxic range of concentrations of OMSE-recipient water to fathead minnows was likely to be between 0.005 to 0.20 percent concentration (or a dilution factor of 20,000/1 to 5,000/1). A previous test based on an assumed range of concentrations of 0.010 to 0.100 percent concentration (or 10,000/1 to 1,000/1) was found to be inadequate as less than 50 percent fish survival was observed almost immediately after introducing the first

Table 1. SUMMARY OF DILUTION WATER CHARACTERISTICS FOR ACUTE LETHAL TOXICITY OF OMSE-RECIPIENT WATER TO FATHEAD MINNOWS AND BLUEGILLS

Bioassay Test No.	OMSE-Recipient Water	Fish		Dilution Water Characteristics			
		Species	Size (ins.)	Total Residual Chlorine (mg/l)	Copper (mg/l)	Hardness (mg/l as $CaCO_3$)	Alkalinity (mg/l as $CaCO_3$)
1	fresh	Fathead Minnows	1 1/2-2	0	0	14.5	4.5
2	"	"	"	"	"	"	"
3	"	"	"	0	0	28	48
4	"	"	"	"	"	"	"
5	"	"	"	0	0	20	38
6	"	"	"	"	"	"	"
7	4-days	"	"	0	0	30	50
8	6-days	"	"	"	"	"	"
9	10-days	"	"	0	0	20	16
10	15-days	"	"	"	"	"	"
11	fresh	Bluegills	3/4 - 1	0	.065	18.5	7
12	"	"	"	"	"	"	"
13	"	"	2 - 2 1/2	0	0	17	5
14	"	"	"	"	"	"	"

concentration of 0.010 percent concentration of OMSE-recipient water. A suitable range of concentrations should not provide for less than 50 percent fish survival at the lowest level nor more than 50 percent fish survival at the higher level. This is necessary in order to derive the TL_{50} values as discussed in Experimental Methodology.

A second exploratory test was performed in August, 1971, as the fish survival at the highest concentration of 0.020 percent was more than 50 percent. The range of concentrations established in this second exploratory bioassay test was 0.010 to 0.100 percent concentration (or 10,000/l to 1,000/l), and accordingly, this concentration range was felt suitable for full-scale bioassay.

Full-Scale Bioassays

The results from the full-scale bioassays conducted for fathead minnows are listed in Appendix A, Tables A-1 to A-6inclusive. Date in these tables not only indicate the number of fish surviving at 24, 48, and 96-hours but also the initial and final measurements of pH and dissolved oxygen for each of the test aquaria, including controls. Data in Tables A-1 and A-2 were obtained using fish which were obtained in the spring season of the year while that in Tables A-3 to A-6 inclusive were generated using fish obtained in the late summer of the year. Mention is made of this fact since a difference in response to OMSE-recipient water was observed between the two groups of fish. Further discussion to this difference is given in a subsequent section.

Note from each of the Tables that less than 50 percent fish survival was recorded at the highest concentration of OMSE-recipient water and

more than 50 percent fish survival was recorded at the lowest concentration or OMSE-recipient water. In addition, the pH recorded was near neutrality and the dissolved oxygen measured was greater than 5 mg/l in all cases. This indicated that any toxic effects exerted on the fish were not due to unfavorable pH or lack of D.O., but was more likely due to the toxicant added to the test aquaria. Furthermore, fish in the control aquaria appeared healthy and vigorous throughout the bioassay period as did the fish in the stock aquaria.

Survivor curves based on the fish survival data in Tables A-1 to A-6 inclusive are presented in Appendix A, Figures A-1 to A-6, respectively. From each of these figures, the 24, 48, and 96-hour TL_{50} values were derived from their respective survival curves as discussed previously.

The 24, 48, and 96-hour TL_{50} values derived for the spring-season collected fathead minnows as shown in Figure A-1 are 0.0180, 0.0180 and 0.0135 percent concentration (5,550/l, 5,550/l, and 7,400/l), respectively. The 24, 48, and 96-hour TL_{50} values derived for this same group of fish as shown in Figure A-2 are 0.0155, 0.0155 and 0.0146 percent concentration (6,440/l, 6,440/l and 6,850/l), respectively. Average 24, 48, and 96-hour TL_{50} values of the two figures would therefore be 0.017, 0.017 and 0.014 percent concentration (6,000/l, 6,000/l and 7,150/l), respectively. All of the above described TL_{50} values obtained for this group of fathead minnows are summarized in Table 2.

Figures A-3 to A-6 inclusive, show the survival curves for fathead minnows collected in the latter part of the summer. The 24-hour TL_{50} values generated from these figures ranged from 0.042 to 0.052 percent concentration (or 2,380/l to 1,920/l). Similarly from the same figures,

Table 2. SUMMARY OF TL_{50} VALUES FOR ACUTE LETHAL TOXICITY OF OMSE-RECIPIENT WATER TO FATHEAD MINNOWS

Test No.	Season	TL_{50} Values					
		% by vol. fuel consumed			parts dil. water/parts of fuel consumed		
		24-hr	48-hr	96-hr	24-hr	48-hr	96-hr
1	spring collected	0.018	0.018	0.013	5,550/1	5,550/1	7,400/1
2	" "	0.015	0.015	0.014	6,440/1	6,440/1	6,850/1
average	spring collected	0.017	0.017	0.014	6,000/1	6,000/1	7,150/1
3	summer collected	0.052	0.048	0.040	1,920/1	2,080/1	2,500/1
4	" "	0.045	0.040	0.040	2,220/1	2,500/1	2,500/1
5	" "	0.052	0.028	0.026	2,380/1	3,580/1	3,840/1
6	" "	0.047	0.036	0.022	2,120/1	2,780/1	4,550/1
average	summer collected	0.047	0.038	0.032	2,150/1	2,640/1	3,130/1

the 48-hour TL_{50} values estimated ranged from 0.028 to 0.048 percent concentration (or 3,580/l to 2,680/l) and the 96-hour TL_{50} values derived ranged from 0.022 to 0.040 percent concentration (or 4,550/l to 2,500/l). The average TL_{50} values and the individual 24, 48, and 96-hour TL_{50} values obtained in each bioassay for this group of fish are summarized in Table 2.

A plot of the average TL_{50} values for all full-scale bioassays done on the two groupings of fathead minnows as a function of exposure time is presented in Figure 4. The curve for fish obtained in the late summer shows a typical bioassay response plot with the gradient of the curve leveling off with time. As shown in this figure, the TL_{50} values gradually decreased from 0.047 to 0.032 percent concentration (or 2,150/l to 3,130/l) at 24 and 96 hours respectively. By comparison, this curve obtained from fishccollected in the spring shows a constant TL_{50} value of 0.017 percent concentration (6,000/l) at 24 and 48 hours and a decrease in TL_{50} value from 0.017 to 0.014 percent concentration (or 6,000/l to 7,150/l) at 48 and 96 hours respectively. The significance of this difference (0.017 percent versus 0.014 percent) is questionable but each valuewas obtained from duplicate bioassays wherein 10 fish were exposed to every toxicant concentration used in each bioassay. In order to obtain a more complete typical bioassay response, observation times shorter than 24 hours should in the future be used with fish collected at a similar stage of their life cycle. Both survivor curves show that the lethal concentration for 50 percent fish survival (or the concentration at infinite time derived by passing a straight line parallel to the time axis and asymptotic to the survival curve) is achieved or at least

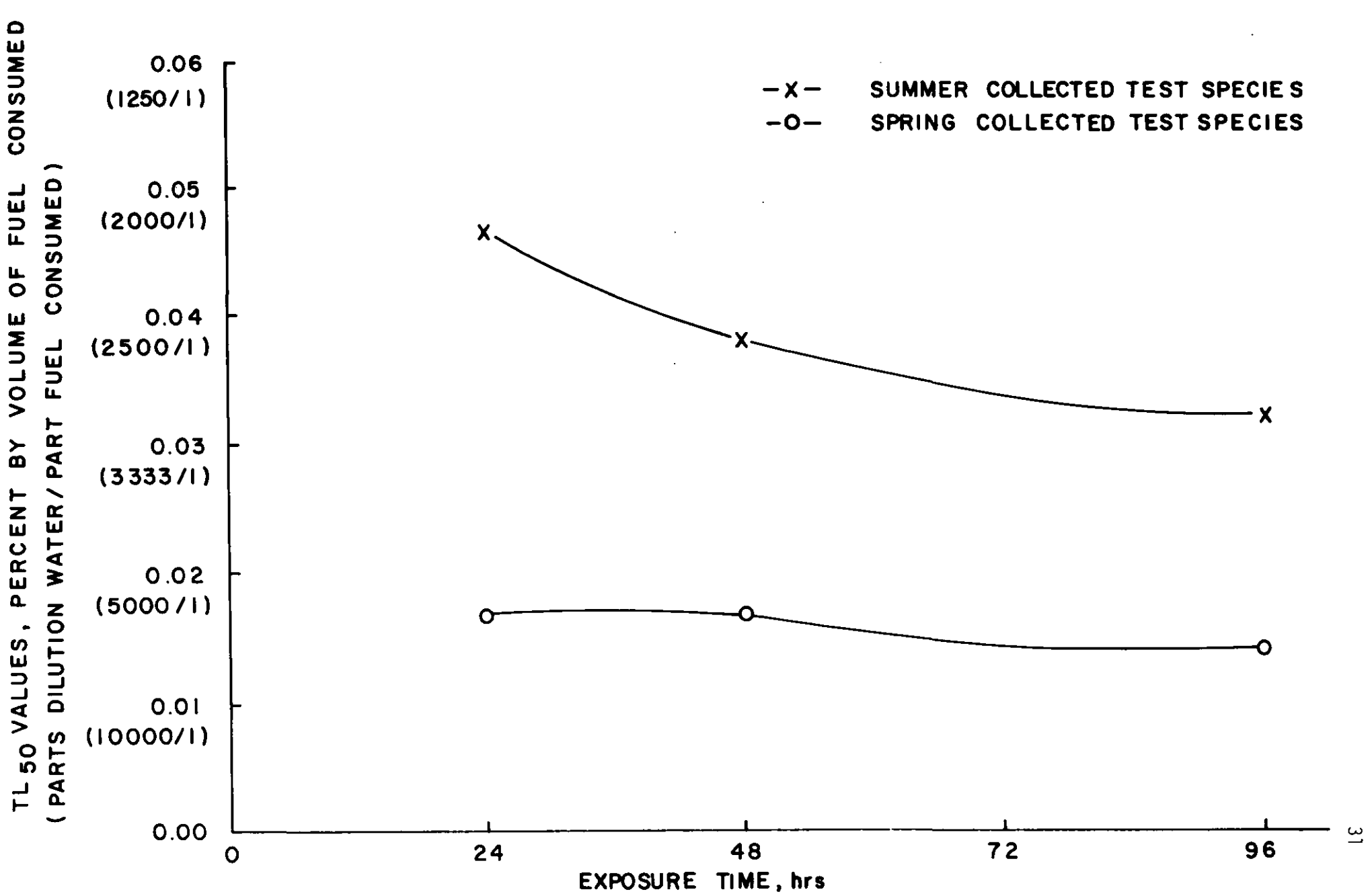

FIGURE 4 - PLOT OF SURVIVOR CURVES BASED ON AVERAGE TL_{50} VALUES FOR THE SPRING AND SUMMER COLLECTED FATHEAD MINNOWS

nearly achieved by the 96th hour. This suggests that the 96-hour exposure period as adopted in this study is probably a sufficient time limit for occurrence of acute lethal toxicity of OMSE-recipient water.

Acute Toxicity of OMSE-recipient Water to Bluegills

Exploratory Bioassays

No exploratory bioassays were conducted for bluegills. It was assumed that fathead minnows and bluegills were approximately equal in sensitivity to OMSE-recipient water (English, et al., 1963a). The range of concentration established in the exploratory tests for fathead minnows was 0.01 to 0.10 percent by volume of fuel consumed. Hence, a similar range of concentrations was assumed to be adequate for full-scale bioassays on bluegills.

Full-Scale Bioassays

The results from the full-scale bioassays conducted for bluegills are listed in Appendix A, Tables A-7 to A-10 inclusive. Data in Tables A-7 and A-8 were obtained for bluegills which ranged in length from 3/4 to 1 inch while that in Tables A-9 and A-10 were obtained using bluegills with a 2 to 2-1/2 inch length range. Both size groupings of bluegills were obtained in the early autumn. Differences in TL_{50} values for these two groups of bluegills will be discussed later.

The number of fish surviving at 24, 48 and 96 hours and the initial and final measurements of pH and dissolved oxygen for each of the test aquaria and controls are included in these tables. Note from the tables that fish survival at the lowest and highest concentrations were more and less than 50 percent respectively and that pH and D.O. measured in all cases were near neutrality and more than 5 mg/l, respectively. As with

the fathead minnow bioassays, neither pH nor lack of D.O. was felt to exert a deleterious effect on the experimentation fish. A 100 percent fish survival was observed in the control chamber in all bioassays and fish in these chambers appeared healthy and vigorous throughout the bioassay period. Hence, fish mortality in the experimentation chambers was attributed to the OMSE-recipient water.

Survivor curves for the bioassays on bluegills are plotted in Appendix A, Figures A-7 to A-10 inclusive. The plots in these figures are based on the experimental data shown in Tables A-7 to A-10, respectively.

The 24, 48, and 96 hour TL_{50} values for bluegills (3/4" - 1") determined from Figure A-7 are 0.037, 0.035 and 0.034 percent concentration (2,080/l, 2,820/l and 2,940/l), respectively. Similarly, the 24, 48 and 96 hour TL_{50} values for the same group of fish from Figure A-8 are 0.026, 0.024 and 0.023 percent concentration (or 3,810/l, 4,150/l and 4,270/l), respectively. The average 24, 48 and 96-hour TL_{50} values of these two figures would be 0.032, 0.030, and 0.029 percent concentration (or 3.130/l, 3,340 and 3,450/l), respectively. These average TL_{50} values together with the TL_{50} values from the two figures are summarized in Table 3.

A plot of average TL_{50} values for all full-scale bioassays done on the two sizes of bluegills as a function of exposure time is presented in Figure 5. The survivor curve for bluegills (3/4" to 1") shows that the lethal concentrations of 3,450/l is achieved after 60 hours of exposure time. By comparison, the survivor curve for bluegills (2" - 2-1/2") shows that the lethal concentration of 2,280/l is achieved by the

Table 3. SUMMARY OF TL_{50} VALUES FOR ACUTE LETHAL TOXICITY OF OMSE-RECIPIENT WATER TO BLUEGILLS

Test No.	Fork Length inches	TL_{50} Values					
		% by vol. fuel consumed			parts dil. water/parts of fuel consumed		
		24-hr	48-hr	96-hr	24-hr	48-hr	96-hr
11	3/4" - 1"	0.037	0.035	0.034	2,680/1	2,820/1	2,940/1
12	"	0.026	0.024	0.023	3,810/1	4,150/1	4,270/1
average	3/4" - 1"	0.032	0.030	0.029	3,130/1	3,340/1	3,450/1
13	2" - 2-1/2"	0.0460	0.046	0.046	2,180/1	2,180/1	2,180/1
14	"	0.042	0.042	0.042	2,360/1	2,360/1	2,360/1
average	2" - 2-1/2"	0.044	0.044	0.044	2,280/1	2,280/1	2,280/1

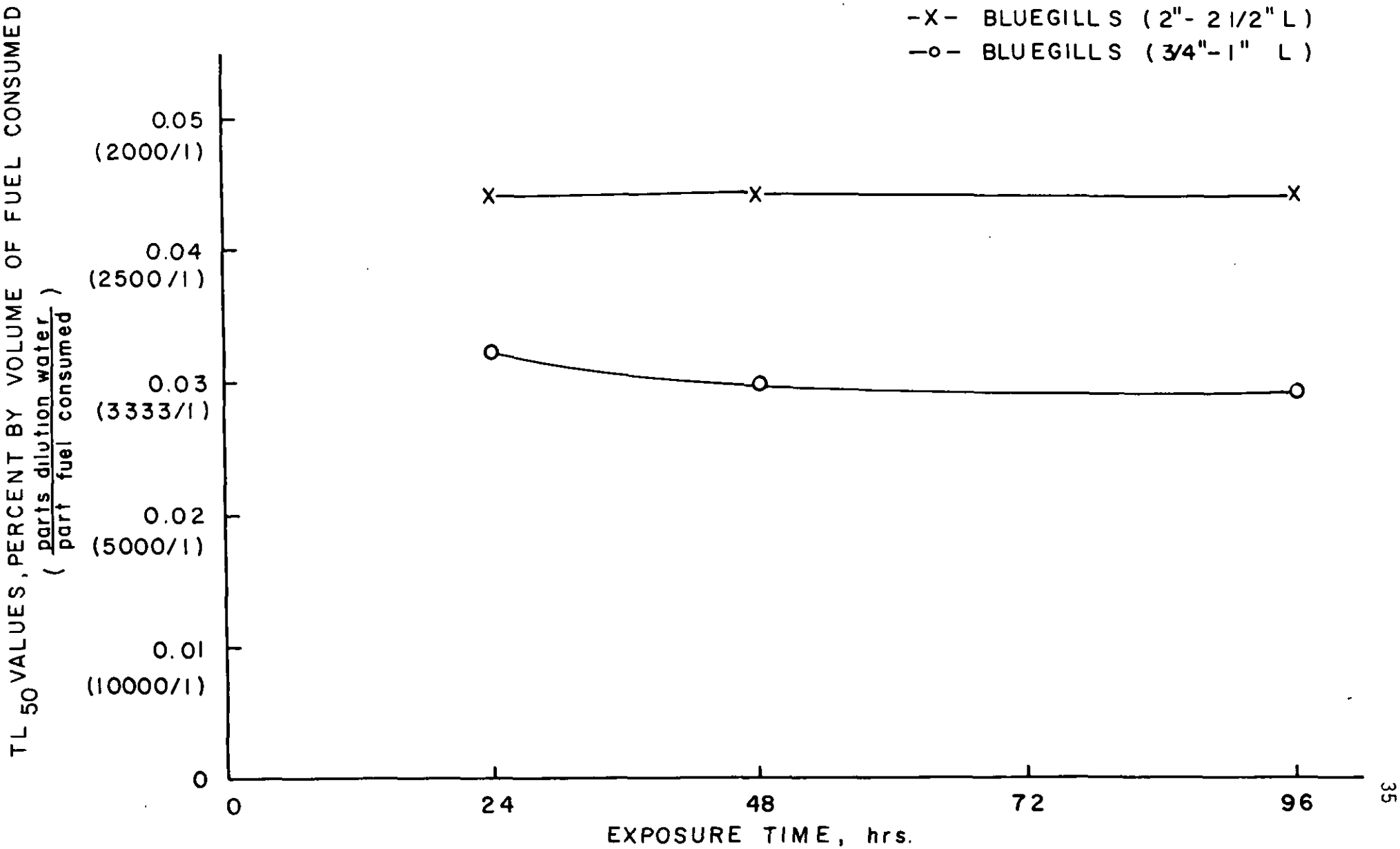

FIGURE 5.- PLOT OF SURVIVOR CURVES BASED ON AVERAGE TL_{50} VALUES FOR BLUEGILLS (3/4" - 1" L AND 2"- 2 1/2"L)

24th hour, thus indicating that observation times shorter than 24-hours should in the future be used for this group of fish with similar experimental conditions. Both survivor curves, however, suggest that the 96-hour exposure period used in this test was an adequate time limit for the occurrence of acute lethality of OMSE-recipient water to this group of fish.

Effects of Aging of OMSE-recipient Water on Acute Toxicity to Fathead Minnows

Full-scale bioassays were performed to determine the 24, 48 and 96-hour TL_{50} values for fathead minnows on exposure to OMSE-recipient water which had previously been aged for 4, 6, 10 and 15 days after combustion of one gallon of fuel-oil mixture. The results of these tests showing the extent of fish survival after 24, 48 and 96-hours and the pertinent pH and D.O. measurements are given in Appendix A, Tables A-11 to A-14 inclusive to correspond to bioassays run on OMSE-recipient water which had been aged for 4, 6, 10 and 15 days respectively. As may be observed from these tables, the fish survival at the lowest and highest concentrations of OMSE-recipient water were more than 50 percent and less than 50 percent respectively. The initial and final pH and dissolved oxygen were close to 7.0 and more than 5 mg/l respectively in all the test chambers in each of the bioassays performed. Similar to the previous described bioassays, neither pH nor D.O. insufficiency, were felt to be deleterious to the test fish.

Survivor curves from the fish survival data given in Tables A-11 to A-14 inclusive are plotted in Appendix A, Figures A-11 to A-14 respectively.

The 24, 48, and 96-hour TL_{50} values interpolated from each of these figures are summarized in Table 4.

With reference to these figures, the 24-hour TL_{50} values tabulated for the 4, 6, 10 and 15-day old OMSE-recipient water was 0.0415, 0.0640, 0.0610, and 0.0500 percent concentration (or 2,400/l, 1,560/l, 1,640/l, and 2,000/l), respectively. The 24-hour TL_{50} values showed an increase in percent concentration when the OMSE-recipient water used was 4 and 6 days old but a decrease in percent concentration was observed with the 10 and 15-day old OMSE-recipient water. A similar trend was obtained with the 48-hour TL_{50} values where the concentrations reported were 0.0386, 0.0430, 0.0385 and 0.0270 percent by volume of fuel consumed (or 2,590/l, 2,320/l, 2,600/l and 3,850/l) for the 4, 6, 10 and 15-day old OMSE-recipient water respectively. The 96-hour TL_{50} values, on the contrary, exhibit a definite decrease from 0.0358 to 0.0225 percent concentration (or 2,790/l to 4,450/l) when the OMSE-recipient water used in the bioassays was aged from 4 to 15 days.

Table 4 also lists the results of the total carbon, total inorganic carbon and total organic carbon analyses performed on the OMSE-recipient water. The total carbon reported ranged from 36.00 to 22.50 mg/l with the 4 and 15-day old OMSE-recipient water respectively. Similarly, with the same aged OMSE-recipient water, the total organic carbon reported ranged from 32.50 to 20.75 mg/l respectively. Both the total carbon and the total organic carbon data exhibited an almost linear decrease in concentration with OMSE-recipient water aging time as shown in Figure 6. Total inorganic carbon, on the other hand, decreases in concentration from 3.50 to 1.75 mg/l with the 4 and 6-day old OMSE-recipient water

Table 4. A SUMMARY OF TL_{50} VALUES AND TOTAL ORGANIC CARBON FOR ACUTE LETHAL TOXICITY OF 4, 6, 10 and 15 DAY OLD OMSE-RECIPIENT WATER TO FATHEAD MINNOWS

Test No.	Date	Age of OMSE Water (days)	TL_{50} Values						Carbon (mg/l)	Inorg. Carbon (mg/l)	Org. Carbon (mg/l)
			percent by vol.		fuel cons.	parts dil.	H_2O/parts	fuel cons.			
			24-hr	48-hr	96-hr	24-hr	48-hr	96-hr			
7	10/15/71	4	.0415	.0386	.0358	2,400/1	2,590/1	2,790/1	36.00	3.50	32.50
8	10/17/71	6	.0640	.0430	.0335	1,560/1	2,320/1	2,980/1	31.00	1.75	29.25
9	10/21/71	10	.0610	.0385	.0239	1,640/1	2,600/1	4,180/1	26.00	1.75	24.25
10	10/26/71	15	.0500	.0270	.0225	2,000/1	3,850/1	4,450/1	22.50	1.75	20.75

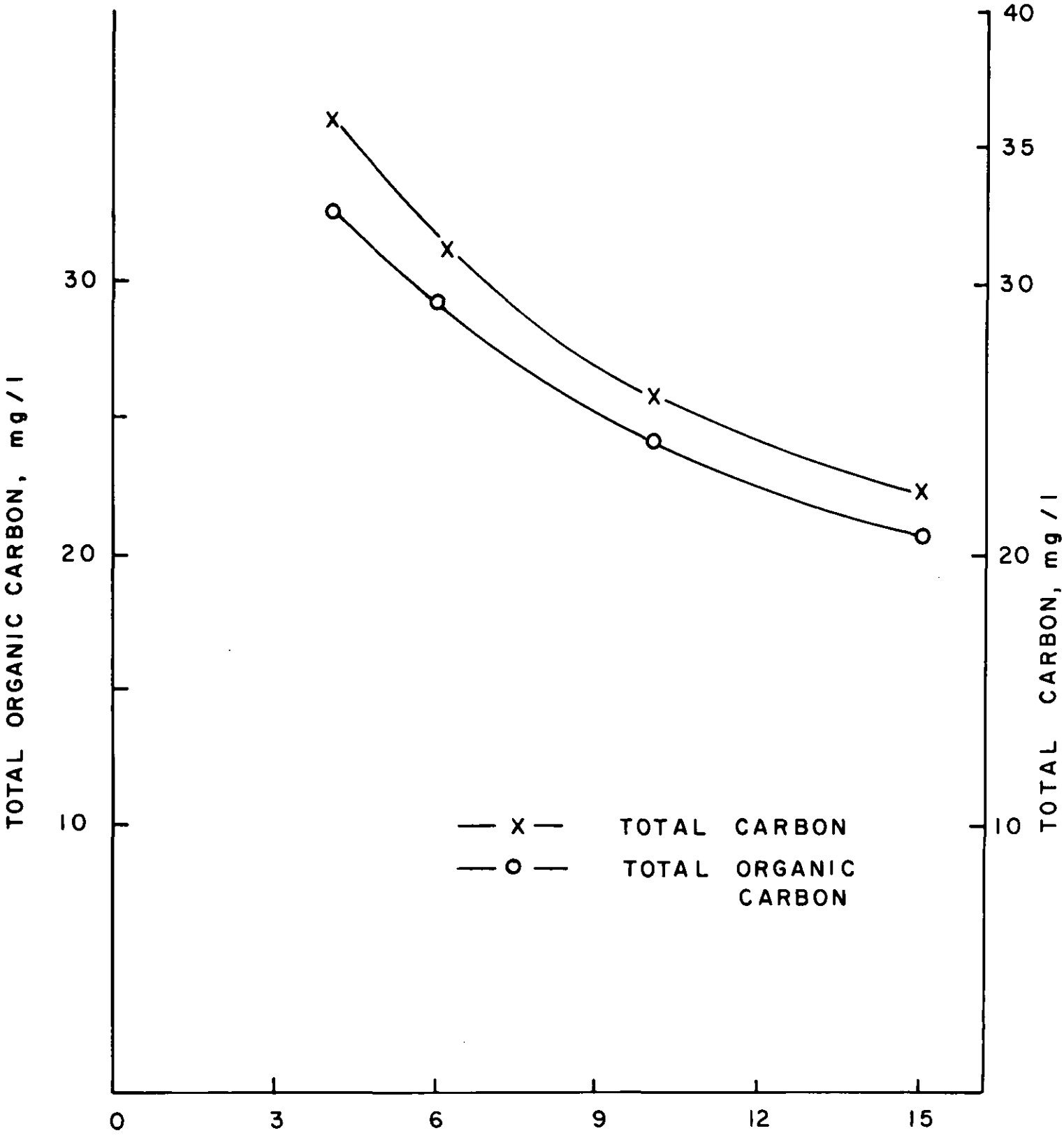

FIGURE 6.– PRESENCE OF TOTAL CARBON AND TOTAL ORGANIC CARBON IN AGED OMSE-RECIPIENT WATER

respectively but remains constant at 1.75 mg/l with the 10 and 15-day old OMSE-recipient water.

SURVEY OF HISTOPATHOLOGICAL EFFECTS ON THE GILLS OF FISH EXPOSED TO OMSE-RECIPIENT WATER

The structure of a normal gill is described in Appendix B. Figures B-1a , b, c, d, e and Figures B-2a, b, c, d, e, f are the gill structures of fathead minnows and bluegills respectively.

Gills of Fathead Minnows

Figure B-1a shows the normal gill structure of fathead minnows in the central chamber. Figures B-1b, c, d and e show the gills of fathead minnows exposed to 0.012, 0.020, 0.032, and 0.050 percent concentration (or 8,350/l, 5,000/l, 3.120/l and 2,000/l) of OMSE-recipient water, respectively. Gills of fathead minnows in Figures B-1d and e were exposed to OMSE-recipient water approximately 2-4 hours. The gills were excised from the fathead minnows immediately after death. The gills in Figures B-1a, b and c, on the other hand, were excised from fathead minnows which were still alive after 4 days of exposure to the toxicant. A comparison was made between Figures B-1b, c, d and e which show the gills of fathead minnows exposed to varying concentrations of OMSE-recipient water and Figure B-1a which shows the gill of fathead minnows in the control chamber. Comparisons were made particularly on the secondary lamellae, epithelial cells, cell mass, gill epithelium of primary and secondary filament, pillar cells and the pharyngeal wall. No abnormalities on these gill structures could be observed (Roberts, J., Personal Communication, 1971).

Gills of Bluegills

The normal gill structure of bluegills is shown in Figure B-2a. Figure B-2b, c, d, e and f show the gill structures of bluegills exposed to 0.020, 0.032, 01050 and 0.079 percent concentration (or 5,000/l 3,120/l, 2,000/l and 1,270/l) of OMSE-recipient water, respectively. Figures and show the gills of bluegills excised from the fish immediately after death. Death occurred in all cases between 2 to 4 hours of exposure time to the toxicant. Figures B-2a, b, c and d, on the other hand, show gills of fish which were still alive after the four-day exposure period.

A comparison of Figure B-2a showing the gill structure of bluegills in the control chamber was made with Figures B-2b, c, d, e, and f, all showing the gill structures of bluegills exposed to varying concentrations of OMSE-recipient water. Comparisons were also made here especially on the secondary lamellae, epithelial cells, cell mass, gill epithelium of primary and secondary filament, pillar cells and the pharyngael wall. No overt significant damage to these parts of the gills could be observed (Roberts, J., Personal Communication, 1971).

GENERAL DISCUSSION

GENERAL DISCUSSION

Acute Toxicity of OMSE-recipient Water to Fathead Minnows and Bluegills

The effect of bioassay exposure time on the TL_{50} values of OMSE-recipient water to the two groups of fathead minnows (spring collected versus summer collected) and bluegills (large versus small) is shown in Figure 7. The curves in this Figure are based on average 24, 48 and 96-hour TL_{50} values for the respective groups of fish.

With regard to the tolerance limit exposure time curves for fathead minnows, in general, it may be said that the fish collected in the summer were more tolerant to the OMSE-recipient water than the fish collected in the spring. The 96-hour TL_{50} values or the lethal concentration for 50 percent fish survival for the summer and spring collected fish were 0.032 and 0.014 percent concentration (or 3,130/l and 7,150/l), respectively. The spring collected fish were purchased in April for use in bioassays performed in April/May while the summer collected fish were purchased in August for use in bioassays conducted in September. No definite explanation can be given for the difference in tolerance limit of the two groups of fish to OMSE-recipient water. It was felt that perhaps the fish collected in the spring were affected by the spawning season and were therefore less resistant or more susceptible to the toxicant. McCarraher (1968) stated that fathead minnows have been known to spawn in the third week of April in a natural environment.

A difference in tolerance limits to the OMSE-recipient water for the larger and smaller bluegills was also observed in Figure 7. The lethal concentrations for 50 percent fish survival for bluegills with length

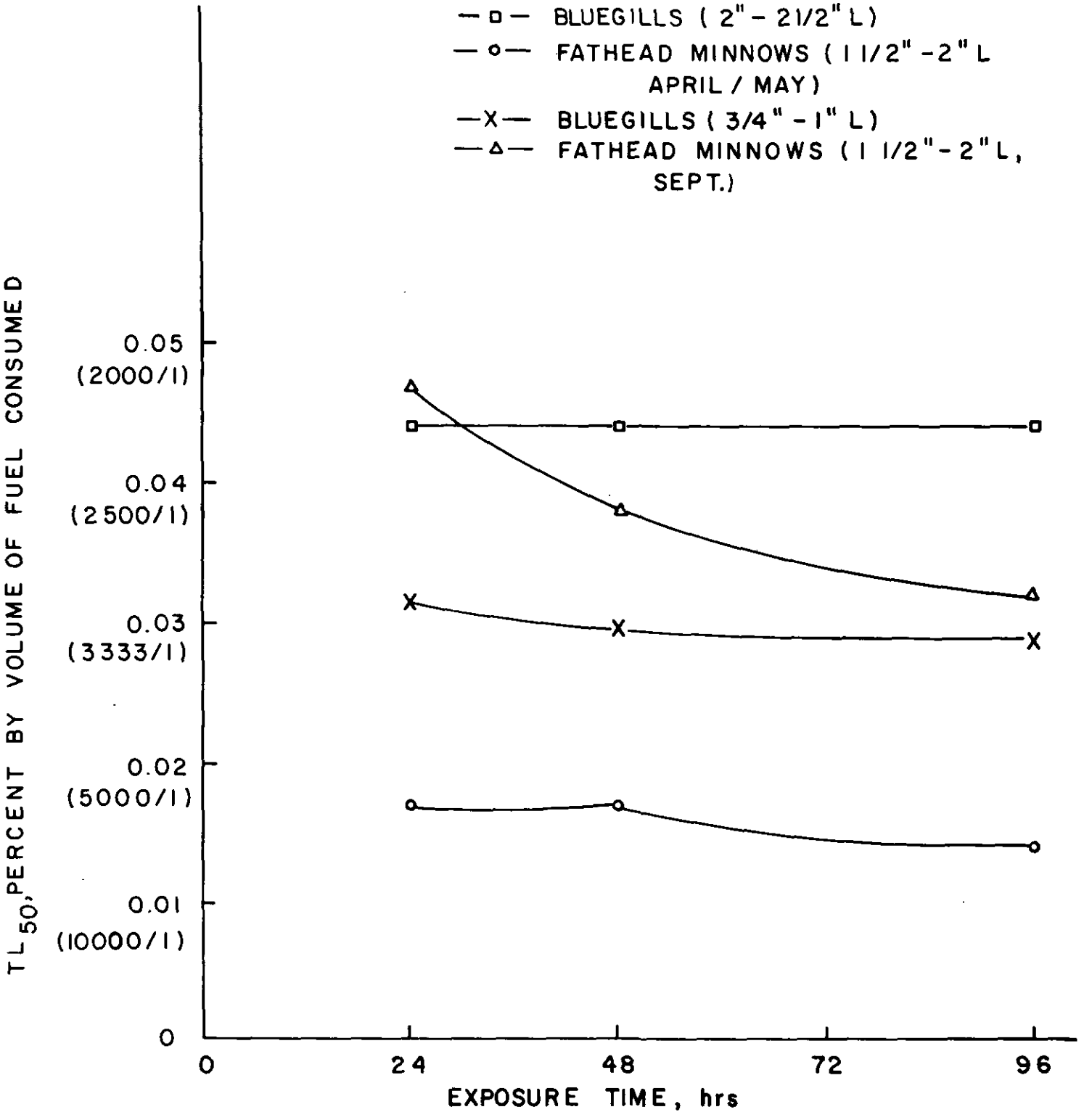

FIGURE 7. - PLOT OF SURVIVOR CURVES BASED ON TL_{50} VALUES FOR FATHEAD MINNOWS AND BLUEGILLS

ranging from 2" - 2-1/2" and those with length ranging from 3/4" - 1" were 0.044 and 0.029 percent concentration (or 2,280/l and 3,450/l), respectively. It was presumed that the smaller bluegills may be more susceptible to OMSE-recipient water than the larger fish of the same species.

Furthermore, it was also observed that lethality for the larger bluegills occurred in most cases during the first 6 hours of the bioassay period. In contrast, mortality for the smaller bluegills was observed throughout the 96-hour exposure period of the bioassay. This suggests that perhaps it may be possible to obtain significant toxicity data for the larger bluegills after the first 24-hour exposure period while for the smaller bluegills, the full 96-hour exposure period may be necessary.

A comparison of the TL_{50} values in Figure 7 for the two test species showed little differences in sensitivity to OMSE-recipient water. With reference to this figure, the TL_{50} values for fathead minnows (late summer) ranged from 0.032 to 0.046 percent concentration (or 3,130/l to 2,180/l) and the TL_{50} values for bluegills collected in the same period ranged from 0.029 to 0.045 percent concentration (or 3,450/l and 2,220/l). Other workers have also found equal sensitivity of test species when exposed to a material in bioassays. English, et al., (1963a) found a nearly equal sensitivity of fathead minnows and bluegills to OMSE-recipient water. Sprague (1970) reported that many investigators found an almost similar resistance with different species (not identified) of fish in bioassays for petrochemicals and heavy metals.

The TL_{50} values derived in this study are in general, slightly lower than those derived by English, et al., (1963a). Table 5 lists a summary

Table 5. COMPARISON OF RESULTS DERIVED BY ENGLISH, et al (1963a) AND THIS STUDY

			Fathead Minnows		Bluegills	
	Test Fish					
	Investigator		This study	English, et al (1963a)	This study	English, et al (1963a)
TL_{50}	24-hr	parts dil. water	2,150/1	1,760/1	2,260/1	1,430/1
	48-hr	parts fuel consumed	2,640/1	1,800/1	2,260/1	1,950/1
	96-hr		3,130/1	1,800/1	2,260/1	1,950/1
	24-hr	percent by volume of fuel consumed	.0465	.0569	.0443	.0700
	48-hr		.0379	.0556	.0443	.0513
	96-hr		.0320	.0556	.0443	.0513
Diluent Water Characteristics	D.O.	mg/l	6.8	8.0	7.2	8.0
	pH	--	7.3	7.4	6.6	7.4
	Alkalinity	mg/l as $CaCO_3$	43	18	7	18
	Hardness	mg/l as $CaCO_3$	24	20	18.5	20
	Water Temperature	°C	20	25	20	25

Note: The 24, 48, and 96-hr TL_{50} values indicated for both studies are average values

of the results obtained from both studies. Note that the 24, 48 and 96-hour TL_{50} values shown in Table 5 for this study is based on average values of all bioassays conducted for fathead minnows (spring and summer) and bluegills (larger and smaller), respectively, in order to facilitate comparison with previous investigations. The dilution water characteristics in both studies are similar. The 24, 48, and 96 hour TL_{50} values established by English, et al., (1963a) for fathead minnows were 0.057, 0.056, and 0.056 percent concentration (or 1,750/l, 1,800/l and 1,800/l), respectively. In this study, the 24, 48 and 96-hour TL_{50} values derived for fathead minnows were 0.047, 0.038 and 0.032 percent concentration (or 2,150/l, 2,640/l and 3,130/l), respectively. Similarly, the 24, 48, and 96-hour TL_{50} values derived by English, et al., (1963a) for bluegills were 0.070, 0.051 and 0.051 percent concentration (or, 1,430/l, 1,950/l and 1,950/l), respectively. By comparison this study established a constant TL_{50} value of 0.044 percent concentration (or 2,260/l) for the 24, 48 and 96-hour TL_{50} values for bluegills.

The TL_{50} values presented in Table 5 for the two separate investigations have been used to generate the tolerance limit exposure time curves in Figure 8. From these curves it may be generalized that the 96-hour test for acute toxicity of OMSE-recipient water to fish is adequate since the lethal threshold in most cases is attained by a maximum of 80 hours. On the contrary, however, it may also be noted that observation times shorter than 24 hours may be necessary to establish test species tolerances in those cases where the TL_{50} values did not differ or differed only slightly, at 24, 48 and 96 hours of exposure.

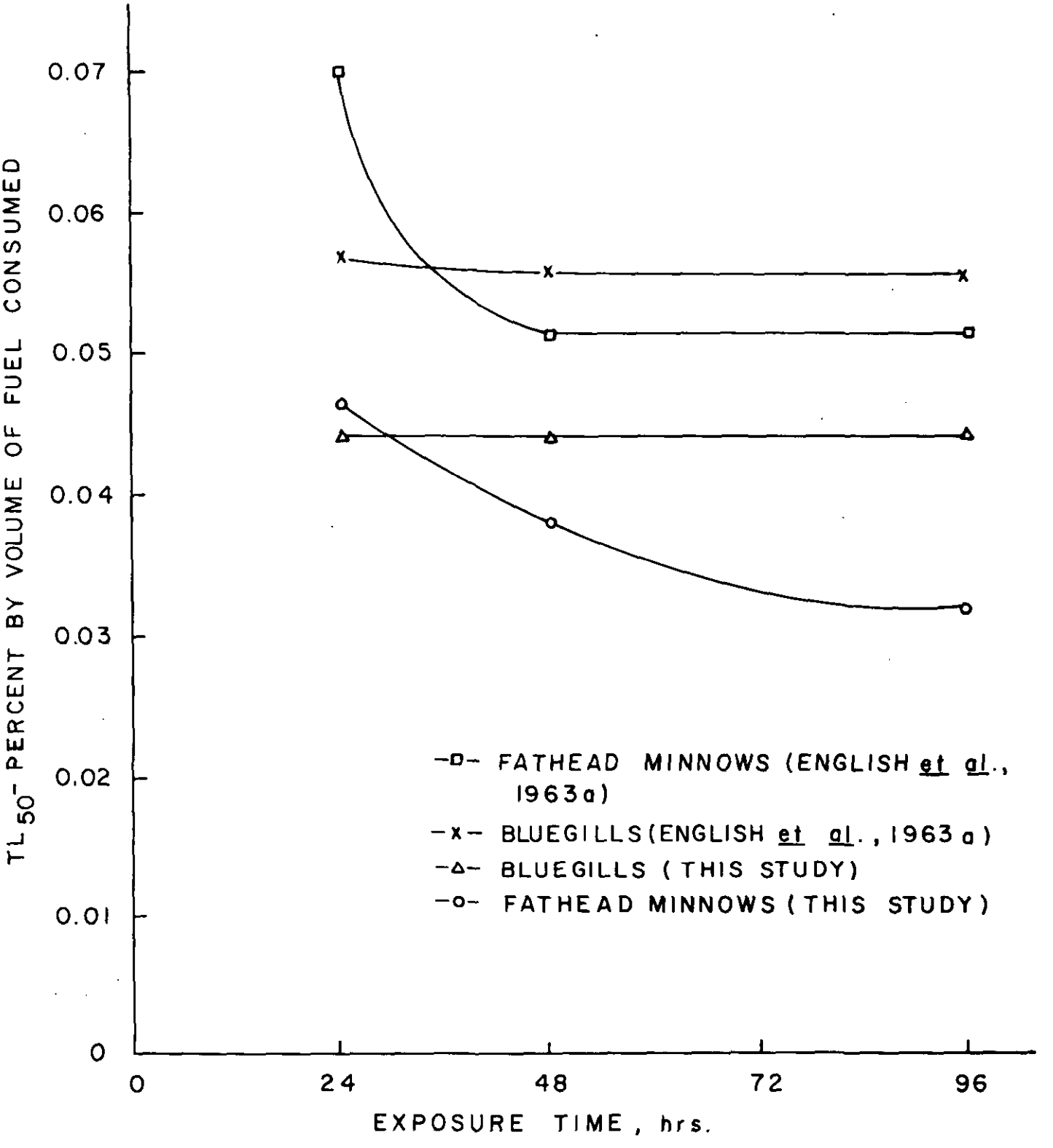

FIGURE 8. - GRAPHICAL COMPARISON OF SURVIVAL CURVE FOR FATHEAD MINNOWS AND BLUEGILLS DERIVED BY ENGLISH et al., (1963a) AND THIS STUDY

There has been some debate on methods of establishing "safe concentrations" or "water quality criteria" of pollutants for aquatic life based on the results obtained from short-term bioassays (Tarzwell, 1962). Many investigators have recommended using an application or safety factor based on a fraction of the 96-hour TL_{50} obtained from the short term bioassays and the concentration obtained either from a long-term exposure test or a field test. A review by Bender (1969) on application factors derived by other workers showed that the value recommended was never less than 0.04 of the 96-hour TL_{50}. English, et al., (1963a) adopted an application factor of 0.1 times the 96-hour TL_{50} from fish toxicity studies. The Ohio River Valley Water Sanitation Commission (1970) has recommended that the calculated concentration of a toxic material in a river does not exceed one-twentieth of the 96-hour TL_{50} for aquatic life. Hence, using the most recent recommendation, this study will use an application factor of 0.05 times the 96-hour TL_{50} value. The average 96-hour TL_{50} values derived for fathead minnows and bluegills in this investigation are 0.032 and 0.044 percent concentration (or 3.130/l and 2,260/l) respectively. Thus, a possible safe concentration derived in this study for fathead minnows and bluegills are 0.0016 percent concentration (or 62,500/l) and 0.0022 percent concentration (or 45,500/l) respectively. Note that these 'safe' concentrations are only hypothetical values and that definite values cannot be established unless an application factor for OMSE-recipient water is derived.

Effects of Aging OMSE-recipient Water on Acute Toxicity to Fathead Minnows

The toxicity behavior of OMSE-recipient water with aging may be

observed in Figure 9. The 24-hour survivor curve shows a definite decrease in toxicity from 0.041 to 0.064 percent concentration (or 2,440/1 to 1,560 1,560/1) when a four and six day old OMSE-recipient water was used as the experimental toxicant and an increase in toxicity from 0.061 to 0.050 percent concentration (or 1,640/1 to 2,000/1) when a 10 and 15 day old toxicant was used.

A similar trend in toxicity of the OMSE-recipient water was also observed with the 48-hour survivor curve. The 48-hour TL_{50} values reported for the 4, 6, 10 and 15 day old OMSE-recipient water were 0.038, 0.043, 0.038 and 0.027 percent concentration (or 2,630/1, 2,320/1, 2,630/1 and 3,700/1), respectively. The 96-hour TL_{50} survivor curve, on the contrary, exhibited gradual increase in toxicity with aging. A maximum 96-hour TL_{50} values of 0.022 percent concentration (or 4,550/1) was achieved after 12 days aging time.

It was at first thought that the toxicity of aged OMSE-recipient water could be associated with the concentration of total carbon or total organic carbon in the same water. A general decrease in the total carbon as primarily reflected by a decrease in total organic carbon was observed with an increase in toxicity of OMSE-recipient water. No definite correlation, however, could be made.

A graphical comparison of tolerance limit exposure time curves for aged OMSE-recipient water on fathead minnows between different investigations is presented in Figure 10. A difference in 96-hour TL_{50} (or increase in toxicity of OMSE-recipient water) of about 0.01 percent concentration may be observed between the 6 and 10 day old OMSE survivor curves. A possible explanation from this observation is that components that are

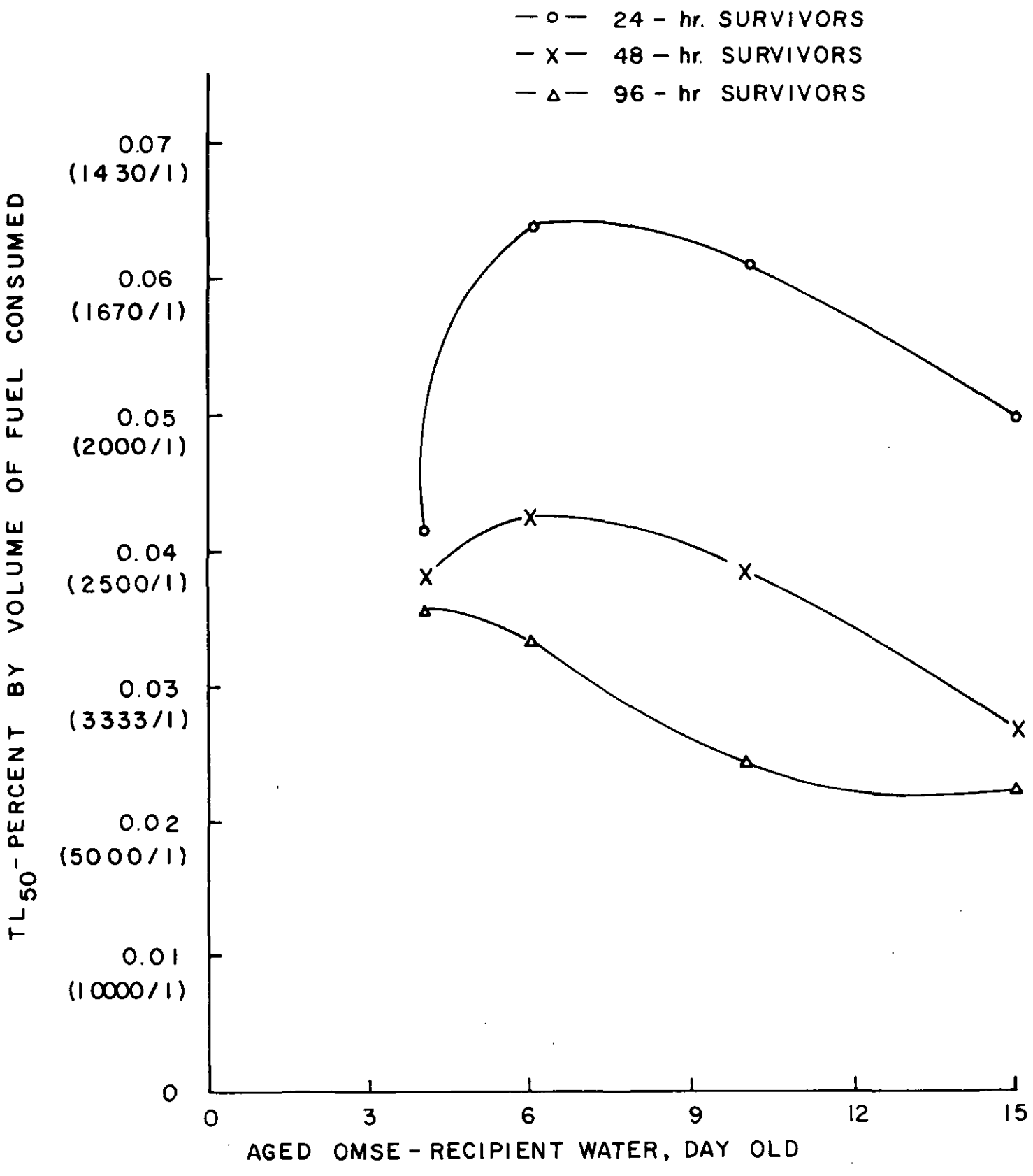

FIGURE 9. - THE TOXICITY BEHAVIOR OF OMSE -RECIPIENT WATER UPON AGING TO FATHEAD MINNOWS

more lethal to fish may be produced sometime between the 6th and 10th day due to the biological degradation of the OMSE-recipient water or the synergistic or antagonistic effect between the carbonaceous and non-carbonaceous materials in the OMSE-water.

Studies by English, et al., (1963a), on the contrary, showed a definite decrease in toxicity of OMSE-recipient water with aging. The 96-hour TL_{50} values reported by the investigator with fresh, 1 and 2 day old OMSE-water are 0.056, 0.063, and 0.083 percent concentration, respectively. No mortality was observed with the four-day old OMSE-water. Mortality was observed in this study when the 4, 6, 10 and 15 day old OMSE-water was introduced to the fish.

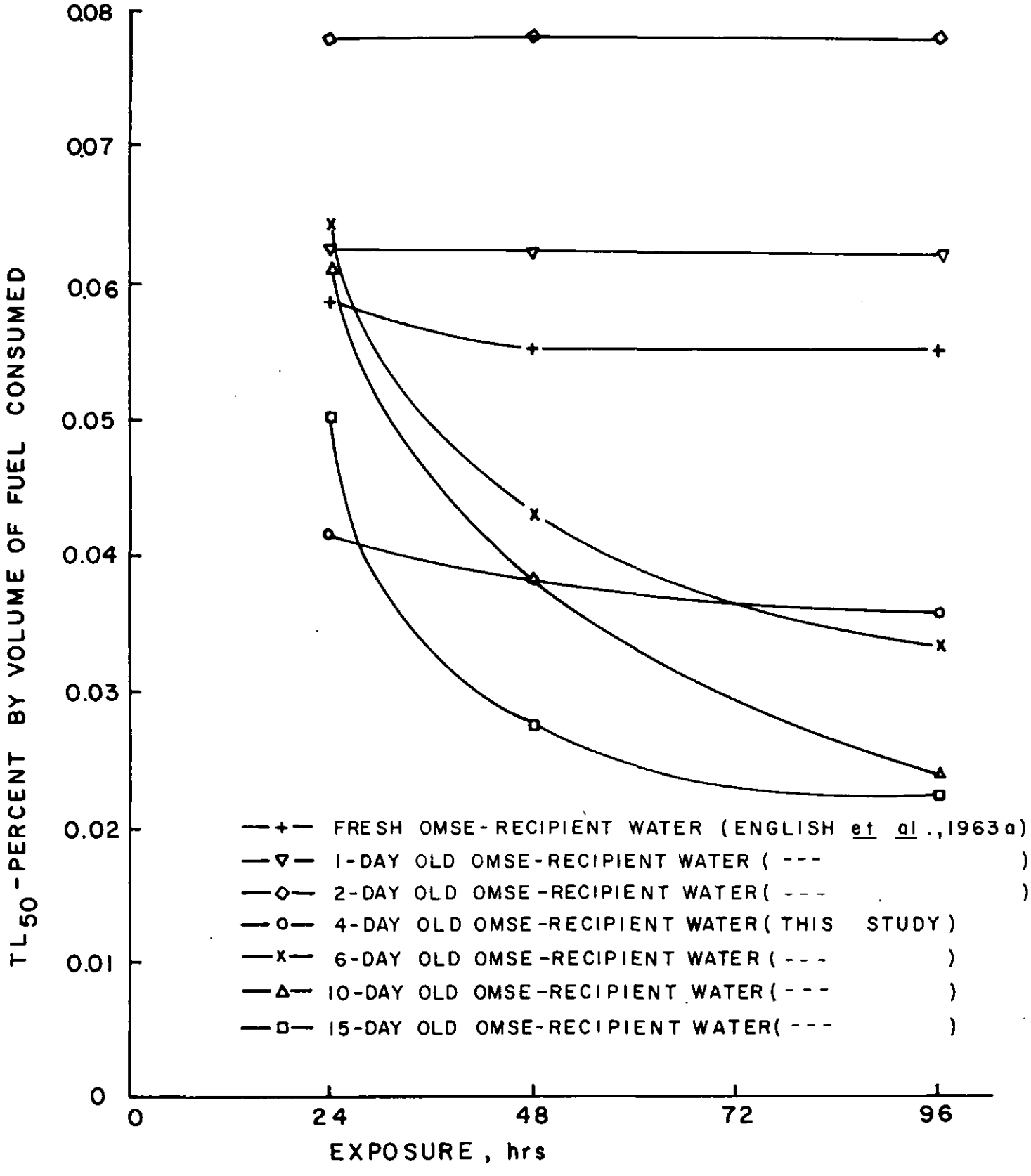

FIGURE 10.- GRAPHICAL COMPARISON OF THE SURVIVAL CURVE FOR AGED-OMSE RECIPIENT WATER ON FATHEAD MINNOWS

HISTOPATHOLOGICAL EFFECTS ON THE GILLS OF FISH EXPOSED TO OMSE-RECIPIENT WATER

The studies made during this investigation showed that no overt effects on the gill epithelium of fish exposed up to a concentration of 0.079 percent of OMSE-recipient water (or 1,270/1) could be observed based on the four day exposure test. A more refined technique to assay for specific enzymatic activity and cell organelles effect was, however, suggested as a possible future study. One possible area of study is the central nervous system of the fish.

The central nervous system of the fish has been suspected as a possible susceptible locus to OMSE-recipient water since a number of the fish during the initial period (24-hours) of the bioassays exhibited an inability to swim in a normal fashion. The initial reaction of the fat-head minnows was to separate from their school, which was then followed within minutes by either swimming in a gyrating fashion and hanging vertically from the surface of the water or laying motionless on one side at the bottom of the tank and respiring rapidly. The initial reaction of the bluegills, on the other hand, was to swim randomly in the tank accompanied within minutes by also swimming in a vertical and gyrating manner or skittering on one side at the bottom of the tank. A few bluegills also attempted to "jump out" from the test aquaria. Fish exhibiting this behavior usually did not survive beyond a 24-hour exposure period. However, no definite conclusions of the effects to the central nervour system of the fish can be made at this time.

It should be realized that this portion of the investigation was only

a preliminary attempt to identify possible gross histological effects on the gill structures from OMSE-recipient water. A limited number of gill samples were used for histological examination using a simple standard technique and staining. Furthermore, the fish were exposed to the toxicant only up to four days. To more accurately identify damage to the gill structures from OMSE-recipient water, a more sophisticated technique might be adopted. A larger number of gill samples should be used and larger areas of the gills examined. In addition, it would probably be more useful to conduct long-term exposure studies, in which chronic effects upon spawning, eggs per spawning, hatchability of eggs and survival of fry, could be evaluated.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

1. The average 24, 48 and 96-hour TL_{50} values based on static bioassays conducted for the spring and summer collected fathead minnows were 0.017, 0.017 and 0.014 percent concentration (or 6,000/l, 6,000/l and 7,150/l) and 0.047, 0.038 and 0.032 percent concentration (or 2,150/l, 2,640/l and 3,130/l) of OMSE-recipient water respectively.
2. The average 24, 48 and 96-hour TL_{50} values based on static bioassays conducted for bluegills with length ranging from 3/4" - 1" and length ranging from 2" - 2-1/2" were all 0.044 percent concentration (or 2,260/l) of OMSE-recipient water.
3. Based on an application factor of 0.05 and the average 96-hour TL_{50} values, a possible safe concentration for fathead minnows is 0.0016 percent (or 62,500/l) of fuel consumed. Similarly, a possible safe concentration for bluegills is 0.0022 percent (or 45,500/l) of fuel consumed.
4. In addition, it was found that a) the spring collected fathead minnows were more sensitive to OMSE-recipient water than the summer collected fathead minnows; b) the smaller bluegills (3/4" - 1" L) were slightly more sensitive to OMSE-recipient water than the larger bluegills (2" - 2-1/2" L); c) acute lethality for the larger bluegills (2" - 2-1/2" L) occurred in most cases during the first six-hours of exposure while acute lethality for the smaller bluegills (3/4" - 1" L) occurred during the full 96-hour exposure period to OMSE-recipient water; and d) fathead minnows and bluegills were relatively equal in sensitivity to OMSE-recipient water.

5. The 24 and 48-hour TL_{50} values showed a definite decrease in toxicity when a 4 and 6-day old OMSE-recipient water was used and an increase in toxicity when a 10 and 15-day old OMSE-recipient water was used as the experimental toxicant. On the contrary, the 96-hour TL_{50} values exhibited a gradual increase in toxicity with aging time of OMSE-recipient water.
6. No definite correlation could be made between toxicity of OMSE-recipient water and the total carbon or total inorganic carbon.
7. No overt significant damage to the gill structures of dead and surviving fathead minnows and bluegills exposed up to a concentration of 0.079 percent (or 1,270/1) of fuel consumed based on the 4-day exposure test.

RECOMMENDATIONS

1. Further tests be conducted on other fish species to determine its relative susceptibilities to OMSE-recipient water at different periods of the year (e.g., spring and summer) and different stages in life cycles.
2. Modifying factors for various abiotic conditions such as temperature and mineral content of the water be derived. A review of the techniques to derive these factors is given by Sprague (1970).
3. Long term exposure studies using the continuous flow system be performed to investigate for sublethal response such as reproduction, growth, respiration and avoidance behavior.
4. Field studies be conducted to verify the TL_{50} values and safe concentrations derived in this study.
5. A study be conducted to identify the components of OMSE-recipient water that is lethal to the fish and those that increase in toxicity with time.
6. Using a more refined technique such as the chemical autopsy method to identify for physiological effects in the fish from OMSE-recipient water. A possible area of investigation is for pathogenic effects on the central nervous system of the fish. Another possible method of investigation is to use histochemical procedures for microscopic examination with an electron microscope on the epithelial cell membranes and structure of pillar cells.

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LITERATURE CITED

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APPENDIX A

Survivor Data and Estimation of
24, 48 and 96-Hour TL_{50} Values
for Fathead Minnows and Bluegills

Table A-1. SURVIVOR DATA FOR FATHEAD MINNOWS (1 1/2" - 2"L) EXPOSED TO OMSE-RECIPIENT WATER, TEST No. 1

Tank	Concentration of OMSE-Water		No. of Test Fish	No. of Fish Surviving			pH		D.O. (mg/l)	
	percent*	dil. fac.		After 24-hr	After 48-hr	After 96-hr	initial	final	initial	final
1A	Control	Control	10	10	10	10	6.8	6.8	7.3	5.5
1B	"	"	"	10	10	10	6.9	6.8	7.3	6.5
2A	.0056	17880/1	10	10	10	7	6.7	6.9	7.3	6.2
2B	"	"	"	10	10	9	6.4	6.8	7.3	6.2
3A	.0075	13320/1	10	10	10	8	6.6	6.6	7.2	5.5
3B	"	"	"	10	10	8	6.8	6.8	7.3	5.4
4A	.0100	10000/1	10	10	10	5	6.8	6.7	7.3	5.2
4B	"	"	"	10	10	7	6.8	6.6	7.6	5.7
5A	.0135	7420/1	10	9	9	6	6.9	6.8	7.6	6.5
5B	"	"	"	7	7	4	6.8	6.7	7.6	5.2
6A	.0180	5560/1	10	6	6	3	6.9	6.8	7.5	5.1
6B	"	"	"	4	4	3	6.7	6.9	7.5	5.2

*percent by volume of fuel consumed

Table A-2. SURVIVOR DATA FOR FATHEAD MINNOWS (1 1/2" - 2"L) EXPOSED TO OMSE-RECIPIENT WATER, TEST No. 2

Tank	Concentration of OMSE-Water		No. of Test Fish	No. of Fish Surviving			pH		D.O. (mg/l)	
	percent	dil fac.		After 24-hr	After 48-hr	After 96-hr	initial	final	initial	final
1A	Control	Control	10	10	10	10	6.9	6.8	7.3	6.0
1B	"	"	"	10	10	10	6.8	7.0	7.2	6.2
2A	.0056	17850/1	10	10	10	10	6.6	6.6	7.3	6.3
2B	"	"	"	10	10	10	6.8	6.8	7.1	5.7
3A	.0075	13350/1	10	10	10	10	6.9	6.8	7.2	6.1
3B	"	"	"	10	10	10	6.8	6.8	6.9	6.8
4A	.0100	10000/1	10	10	9	9	6.7	6.8	6.7	6.6
4B	"	"	"	10	10	10	6.8	6.6	7.2	5.5
5A	.0135	7410/1	10	7	7	7	7.0	6.9	7.5	5.1
5B	"	"	"	6	6	5	6.6	6.5	7.3	5.2
6A	.0210	4760/1	10	2	2	1	6.8	6.8	7.1	5.8
6B	"	"	"	2	2	1	6.9	6.8	7.2	5.8

Table A-3. SURVIVOR DATA FOR FATHEAD MINNOWS (1-1/2" - 2"L) EXPOSED TO OMSE-RECIPIENT WATER, TEST NO. 3

Tank	Concentration of OMSE-Water		No. of Test Fish	No. of Fish Surviving			pH		D.O. (mg/l)	
	percent	dil.fac.		After 24-hr	After 48-hr	After 96-hr	initial	final	initial	final
1A	Control	Control	10	10	10	10	7.4	7.4	8.0	7.5
1B	"	"	"	10	10	10	7.4	7.5	7.8	7.6
2A	.016	6250/1	10	10	10	10	7.4	7.4	8.0	7.6
2B	"	"	"	10	10	10	7.3	7.4	8.0	7.7
3A	.025	4000/1	10	10	10	10	7.6	7.5	7.8	7.6
3B	"	"	"	10	10	10	7.4	7.5	8.0	7.6
4A	.040	2500/1	10	9	9	8	7.6	7.5	7.7	7.6
4B	"	"	"	5	4	2	7.4	7.4	8.0	7.5
5A	.063	1590/1	10	1	0	0	7.0	7.4	8.0	7.8
5B	"	"	"	6	6	3	7.2	7.0	8.1	7.6
6A	.100	1000/1	10	0	0	0	7.4	7.3	8.0	7.6
6B	"	"	"	0	0	0	7.2	7.4	8.0	7.5

Table A-4. SURVIVOR DATA FOR FATHEAD MINNOWS (1-1/2" - 2"L) EXPOSED TO OMSE-RECIPIENT WATER, TEST NO. 4

Tank	Concentration of OMSE-Water		No. of Test Fish	No. of Fish Surviving			pH		D.O. (mg/l)	
	percent	dil.fac.		After 24-hr	After 48-hr	After 96-hr	initial	final	initial	final
1A	Control	Control	10	10	10	10	7.4	7.3	7.8	7.6
1B	"	"	"	10	9	9	7.4	7.2	7.7	7.5
2A	.0125	8000/1	10	10	10	10	7.4	7.4	7.5	7.5
2B	"	"	"	10	10	10	7.6	7.4	7.5	7.3
3A	.0200	5000/1	10	10	10	10	7.4	7.5	7.8	7.7
3B	"	"	"	10	8	8	7.4	7.4	7.8	7.4
4A	.0315	3180/1	10	10	8	8	7.5	7.5	7.7	7.3
4B	"	"	"	10	8	8	7.4	7.4	7.8	7.3
5A	.0500	2000/1	10	4	4	4	7.5	7.6	7.8	7.5
5B	"	"	"	3	1	1	7.6	7.5	7.6	7.5
6A	.0790	1270/1	10	0	0	0	7.3	7.4	7.5	7.3
6B	"	"	"	0	0	0	7.4	7.4	7.9	7.0

Table A-5. SURVIVOR DATA FOR FATHEAD MINNOWS (1-1/2" - 2"L) EXPOSED TO OMSE-RECIPIENT WATER. TEST NO. 5

Tank	Concentration of OMSE-Water		No. of Test Fish	No. of Fish Surviving			pH[1]		D.O.[2] (mg/l)	
	percent	dil.fac.		After 24-hr	After 48-hr	After 96-hr	initial	final	initial	final
1A	Control	Control	10	10	10	10	7.2	7.1	7.7	6.0
1B	"	"	"	10	10	10				
2A	.016	6250/1	10	9	9	7	7.0	6.8	7.8	5.9
2B	"	"	"	9	7	6				
3A	.025	4000/1	10	9	8	6	7.2	7.2	8.2	7.5
3B	"	"	"	10	5	5				
4A	.040	2500/1	10	6	1	1	7.3	7.2	7.8	6.0
4B	"	"	"	5	1	1				
5A	.063	1590/1	10	0	0	0	7.4	7.2	7.7	7.0
5B	"	"	"	1	0	0				
6A	.100	1000/1	10	0	0	0	7.3	7.2	5.4	5.2
6B	"	"	"	0	0	0				

[1]Based on average values of replicate aquaria for a specified toxicant concentration.

[2]Same as No. 1

Table A-6. SURVIVOR DATA FOR FATHEAD MINNOWS (1-1/2" - 2"L) EXPOSED TO OMSE-RECIPIENT WATER, TEST NO. 6

Tank	Concentration of OMSE-Water		No. of Test Fish	No. of Fish Surviving			pH[1]		D.O.[2] (mg/l)	
	percent	dil.fac.		After 24-hr	After 48-hr	After 96-hr	initial	final	initial	final
1A	Control	Control	10	10	10	9	7.2	7.2	8.0	7.4
1B	"	"	"	10	10	9				
2A	.0125	8000/1	10	10	9	6	7.2	7.2	8.2	7.8
2B	"	"	"	10	8	6				
3A	.020	5000/1	10	10	9	7	7.3	7.1	7.8	7.4
3B	"	"	"	10	8	4				
4A	.0315	3180/1	10	9	7	4	7.2	7.1	8.0	6.8
4B	"	"	"	9	6	2				
5A	.050	2000/1	10	4	2	0	7.1	7.2	8.2	7.8
5B	"	"	"	5	0	0				
6A	.079	1270/1	10	0	0	0	7.2	7.2	8.0	6.2
6B	"	"	"	1	0	0				

[1]Refer to footnote No. 1 on Table 6

[2]Same as No. 1

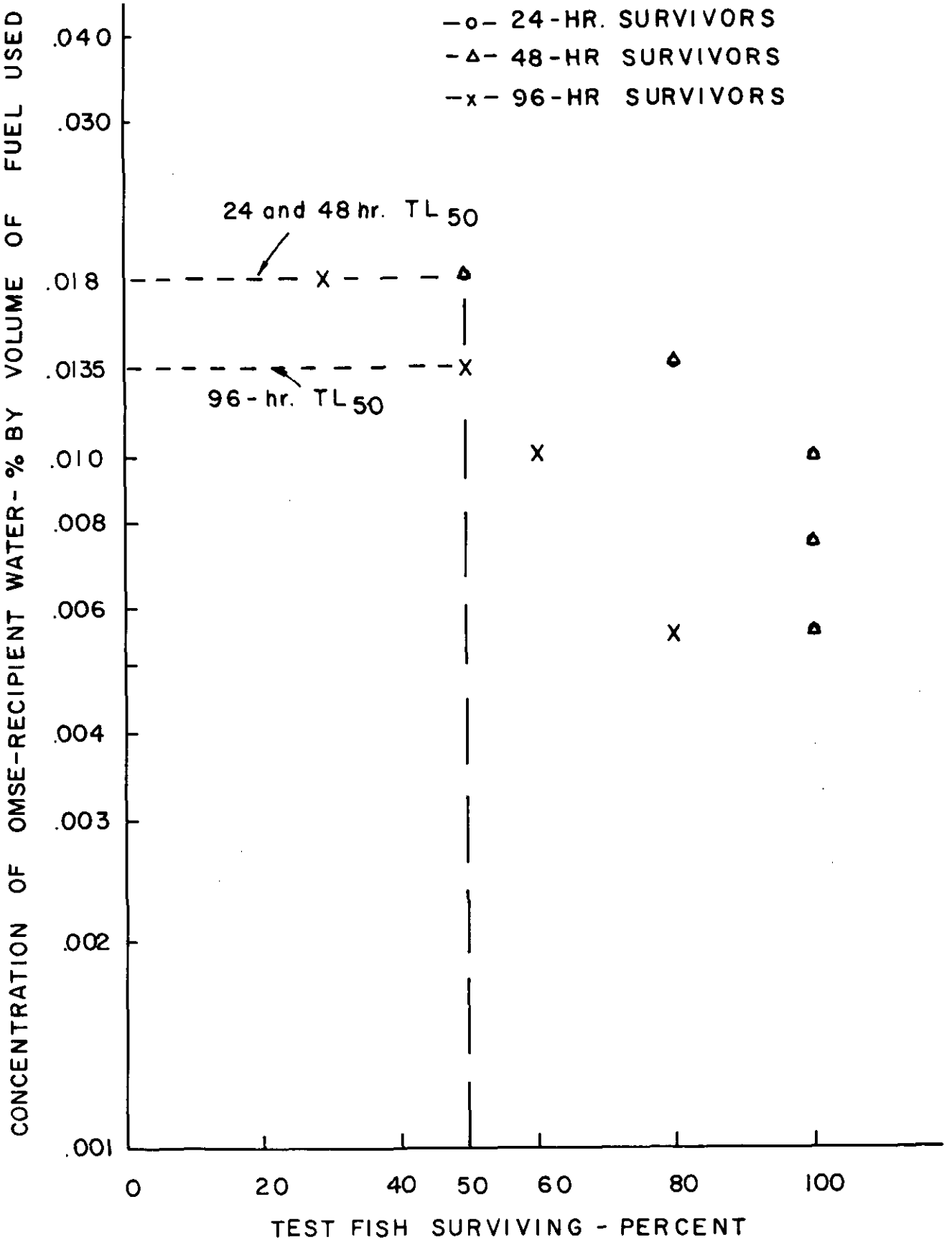

FIGURE A-1. ESTIMATION OF 24,48 AND 96-HR. TL_{50} VALUES FOR FATHEAD MINNOWS BY STRAIGHT-LINE GRAPHICAL INTERPOLATION METHOD, TEST NO. 1

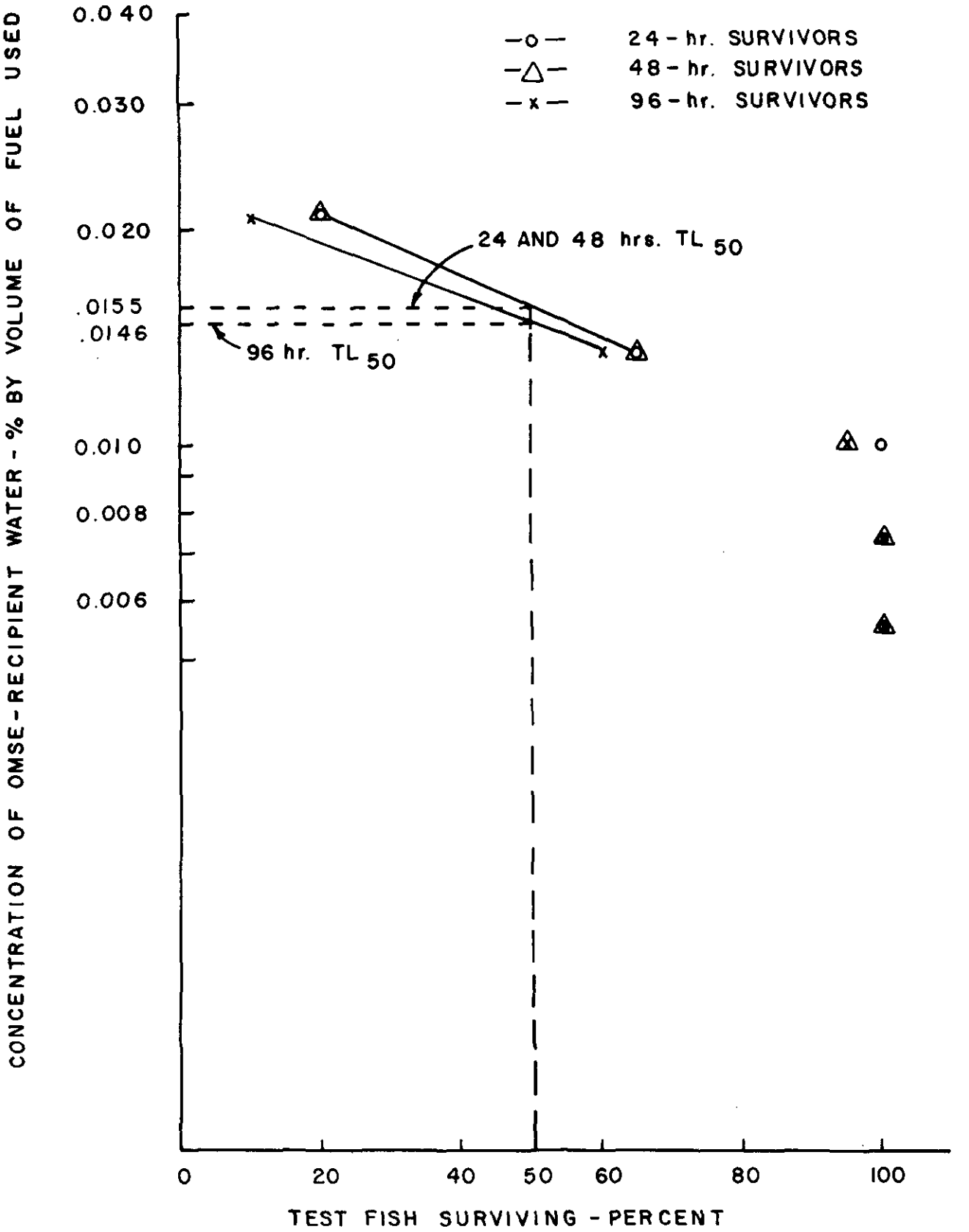

FIGURE A-2 ESTIMATION OF 24, 48 AND 96-HR. TL_{50} VALUES FOR FATHEAD MINNOWS BY STRAIGHT-LINE GRAPHICAL INTERPOLATION METHOD, TEST NO. 2

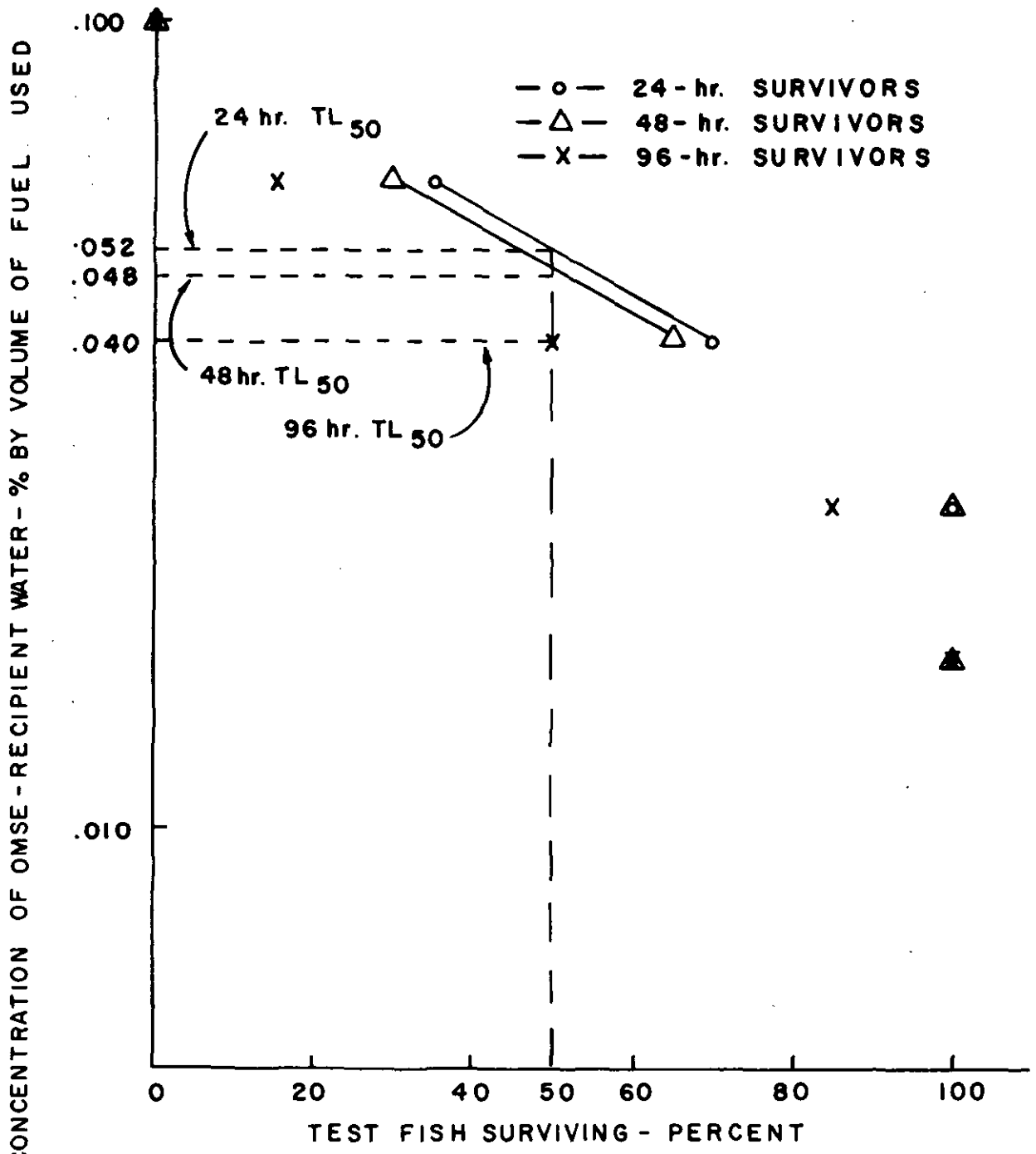

FIGURE A-3 ESTIMATION OF 24,48 AND 96 HR. TL_{50} VALUES FOR FATHEAD MINNOWS BY STRAIGHT LINE GRAPHICAL INTERPOLATION METHOD, TEST NO 3

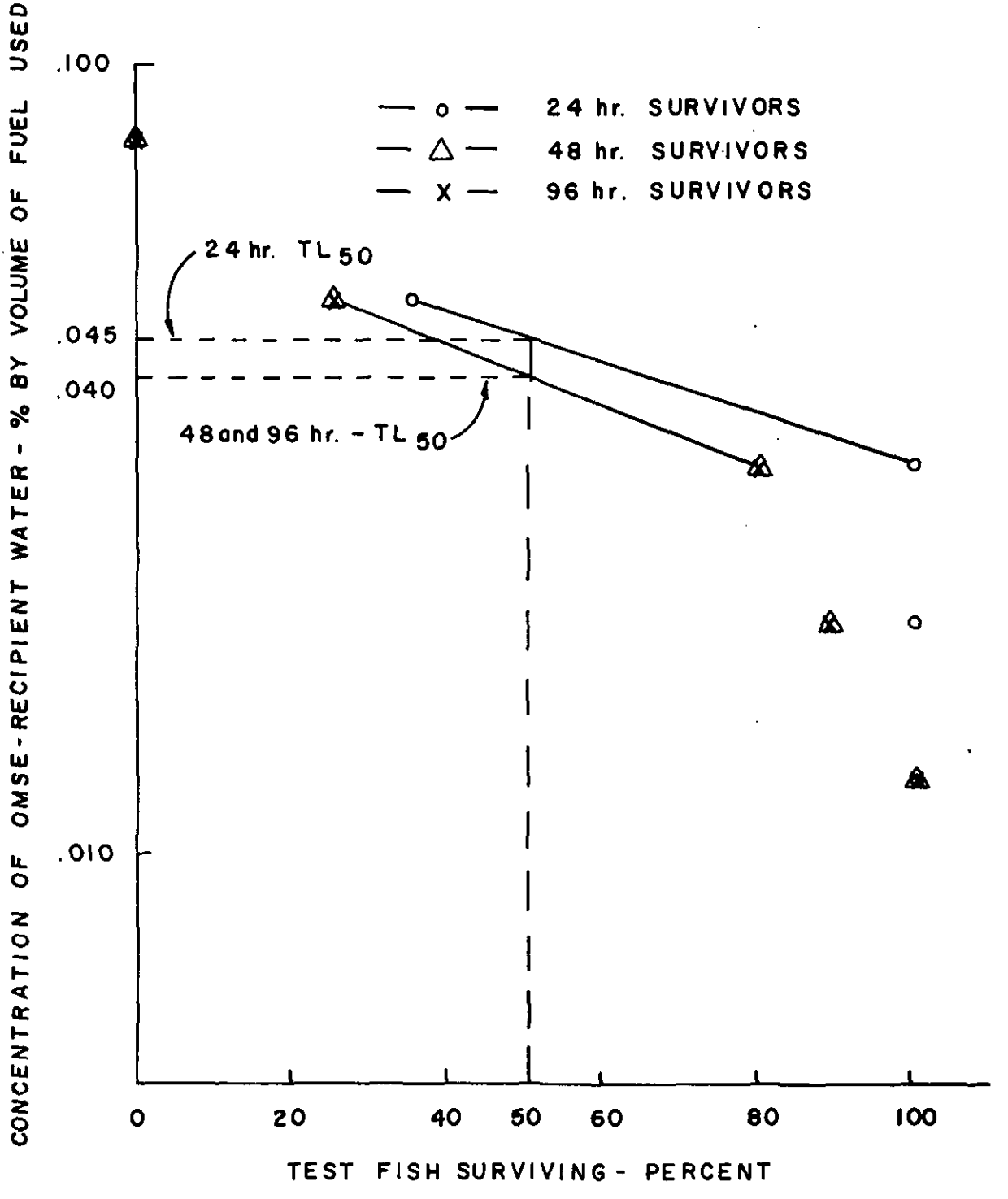

FIGURE A-4 ESTIMATION OF 24, 48 AND 96-HR TL_{50} VALUES FOR FATHEAD MINNOWS BY STRAIGHT LINE GRAPHICAL INTERPOLATION METHOD, TEST NO. 4

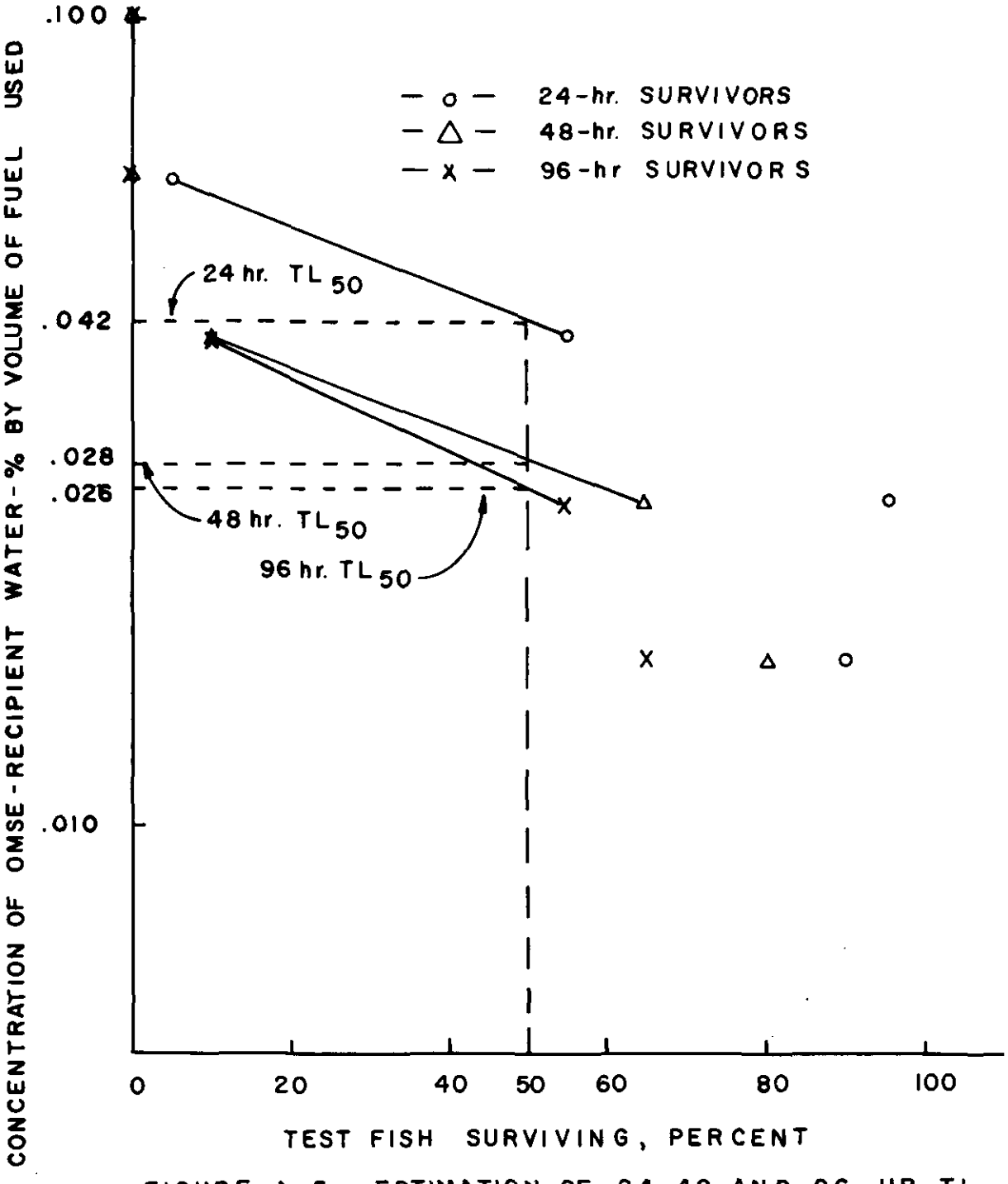

FIGURE A-5 ESTIMATION OF 24, 48 AND 96-HR TL_{50} VALUES FOR FATHEAD MINNOWS BY STRAIGHT LINE GRAPHICAL INTERPOLATION METHOD, TEST NO. 5

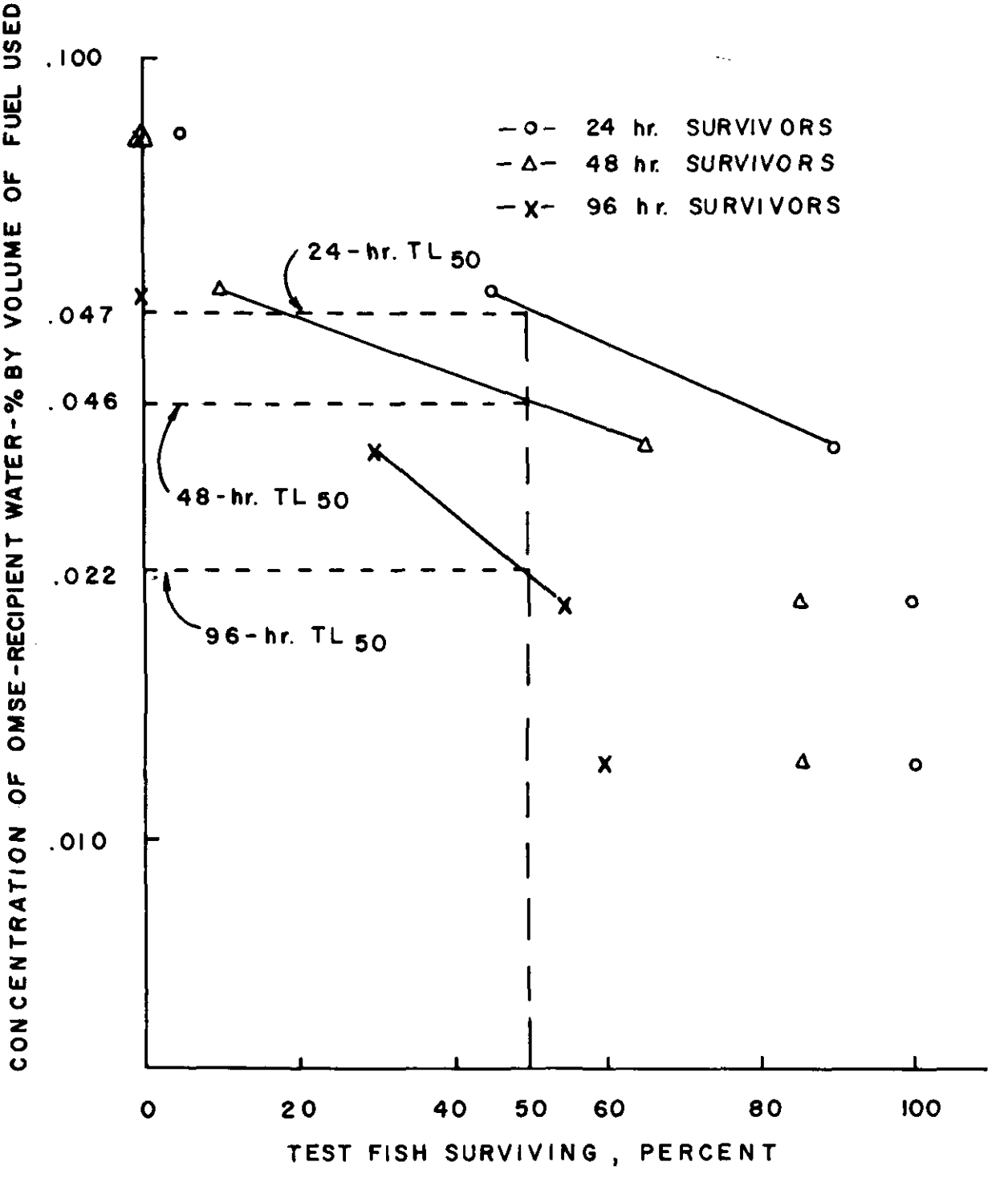

FIGURE A-6 ESTIMATION OF 24, 48 AND 96 HR. TL_{50} VALVE FOR FATHEAD MINNOWS BY STRAIGHT LINE GRAPHICAL INTERPOLATION METHOD TEST NO. 6

Table A-7. SURVIVOR DATA FOR BLUEGILLS (3/4" - 1"L) EXPOSED TO OMSE_RECIPIENT WATER, TEST NO. 11

Tank	Concentration of OMSE-Water		No. of Test Fish	No. of Fish Surviving			pH[1]		D.O. (mg/l)	
	percent	dil.fac.		After 24-hr	After 48-hr	After 96-hr	initial	final	initial	final
1A	Control	Control	10	10	10	10	6.9	6.5	9.0	8.1
1B	"	"	"	10	10	10				
2A	.0125	8000/1	10	10	10	10	6.6	6.6	7.5	7.2
2B	"	"	"	10	10	10			8.9	7.2
3A	.0200	5000/1	10	10	9	9	6.6	6.6	8.1	6.8
3B	"	"	"	10	6	6			9.0	8.1
4A	.0315	3180/1	10	8	8	7	6.5	6.5	7.5	6.1
4B	"	"	"	8	6	5			8.2	7.6
5A	.0500	2000/1	10	0	0	0	6.4	7.3	7.9	7.3
5B	"	"	"	0	0	0			8.3	7.3
6A	.0790	1270/1	10	0	0	0	6.3	6.3	7.9	6.5
6B	"	"	"	0	0	0			8.3	6.2

[1]Refer to footnote No. 1 in Table 6

Table A-8. SURVIVOR DATA FOR BLUEGILLS (3/4" - 1"L) EXPOSED TO OMSE-RECIPIENT WATER, TEST NO. 12

Tank	Concentration of OMSE-Water		No. of Test Fish	No. of Fish Surviving			pH[1]		D.O. (mg/l)	
	percent	dil.fac.		After 24-hr	After 48-hr	After 96-hr	initial	final	initial	final
1A	Control	Control	10	10	10	10	6.6	6.5	7.9	7.0
1B	"	"	"	10	10	10			8.7	6.3
2A	.0125	8000/1	10	10	10	10	6.6	6.2	8.0	7.3
2B	"	"	"	10	10	10			8.3	7.7
3A	.0200	5000/1	10	7	5	5	6.8	6.6	8.0	7.0
3B	"	"	"	9	8	8			8.2	7.5
4A	.0315	3180/1	10	3	3	3	6.7	6.5	8.1	6.6
4B	"	"	"	3	3	3			8.5	7.6
5A	.0500	2000/1	10	0	0	0	6.6	66.6	8.1	6.7
5B	"	"	"	0	0	0			8.2	7.1
6A	.0790	1270/1	10	0	0	0	6.6	6.2	7.7	6.5
6B	"	"	"	0	0	0			7.9	5.5

[1]Refer to footnote No. 1 in Table 6

Table A-9. SURVIVOR DATA FOR BLUEGILLS (2" x 2-1/2"L) EXPOSED TO OMSE-RECIPIENT WATER, TEST NO. 13

Tank	Concentration of OMSE-Water		No. of Test Fish	No. of Fish Surviving			pH[1]		D.O.[2] (mg/l)	
	percent	dil.fac.		After 24-hr	After 48-hr	After 96-hr	initial	final	initial	final
1A	Control	Control	10	10	10	10	6.3	6.3	6.8	6.4
1B	"	"	"	10	10	10				
2A	.0125	8000/1	10	10	10	10	6.1	6.1	6.5	6.2
2B	"	"	"	10	10	10				
3A	.0200	5000/1	10	10	10	10	6.1	6.1	6.9	6.8
3B	"	"	"	10	10	10				
4A	.0315	3180/1	10	10	10	10	6.0	6.0	6.6	6.6
4B	"	"	"	9	9	9				
5A	.0500	2000/1	10	5	5	5	6.1	6.0	7.8	7.1
5B	"	"	"	3	3	3				
6A	.0790	1270/1	10	0	0	0	6.2	6.0	7.8	7.7
6B	"	"	"	0	0	0				

[1]Refer to footnote No. 1 in Table 6

[2]Same as No. 1

Table A-10. SURVIVOR DATA FOR BLUEGILLS (2" - 2-1/2"L) EXPOSED TO OMSE-RECIPIENT WATER, TEST NO. 14

Tank	Concentration of OMSE-Water		No. of Test Fish	No. of Fish Surviving			pH[1]		D.O.[2] (mg/l)	
	percent	dil.fac.		After 24-hr	After 48-hr	After 96-hr	initial	final	initial	final
1A	Control	Control	10	10	10	10	6.3	6.2	7.4	7.2
1B	"	"	"	10	10	10				
2A	.0125	8000/1	10	10	10	10	6.2	6.0	6.7	6.5
2B	"	"	"	10	10	10				
3A	.0200	5000/1	10	10	10	10	6.3	6.1	6.8	6.1
3B	"	"	"	10	10	10				
4A	.0315	3180/1	10	10	10	10	6.2	6.0	6.5	5.8
4B	"	"	"	9	9	9				
5A	.0500	2000/1	10	0	0	0	6.1	6.1	7.4	7.4
5B	"	"	"	5	5	5				
6A	.0790	1270/1	10	0	0	0	6.3	6.0	7.8	7.4
6B	"	"	"	0	0	0				

[1]Refer to footnote No. 1 in Table 6

[2]Same as No. 1

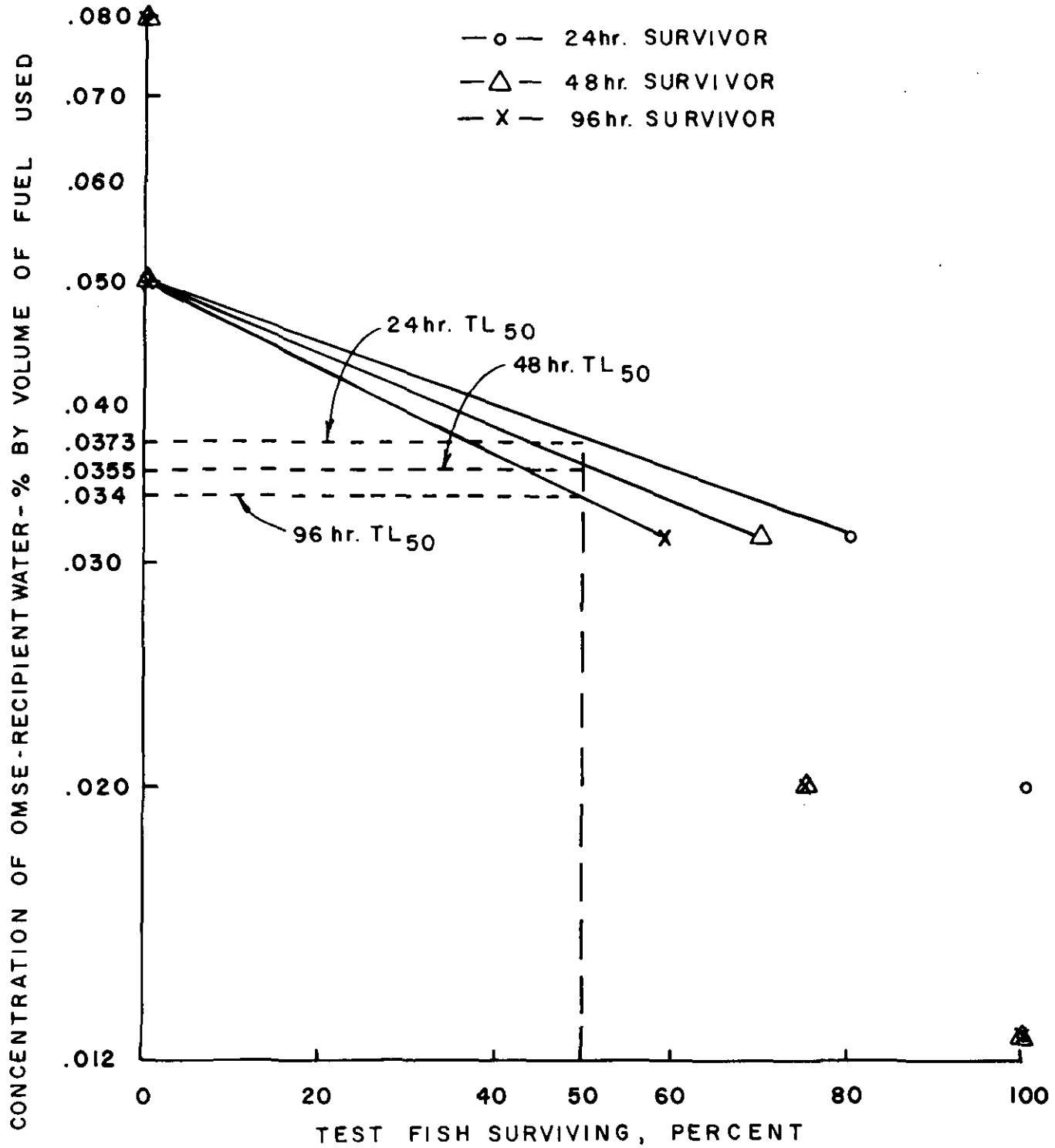

FIGURE A-7 ESTIMATION OF 24, 48 AND 96 HR. TL_{50} VALUES FOR BLUEGILLS BY STRIGHT-LINE GRAPHICAL INTERPOLATION METHOD, TEST NO. 5

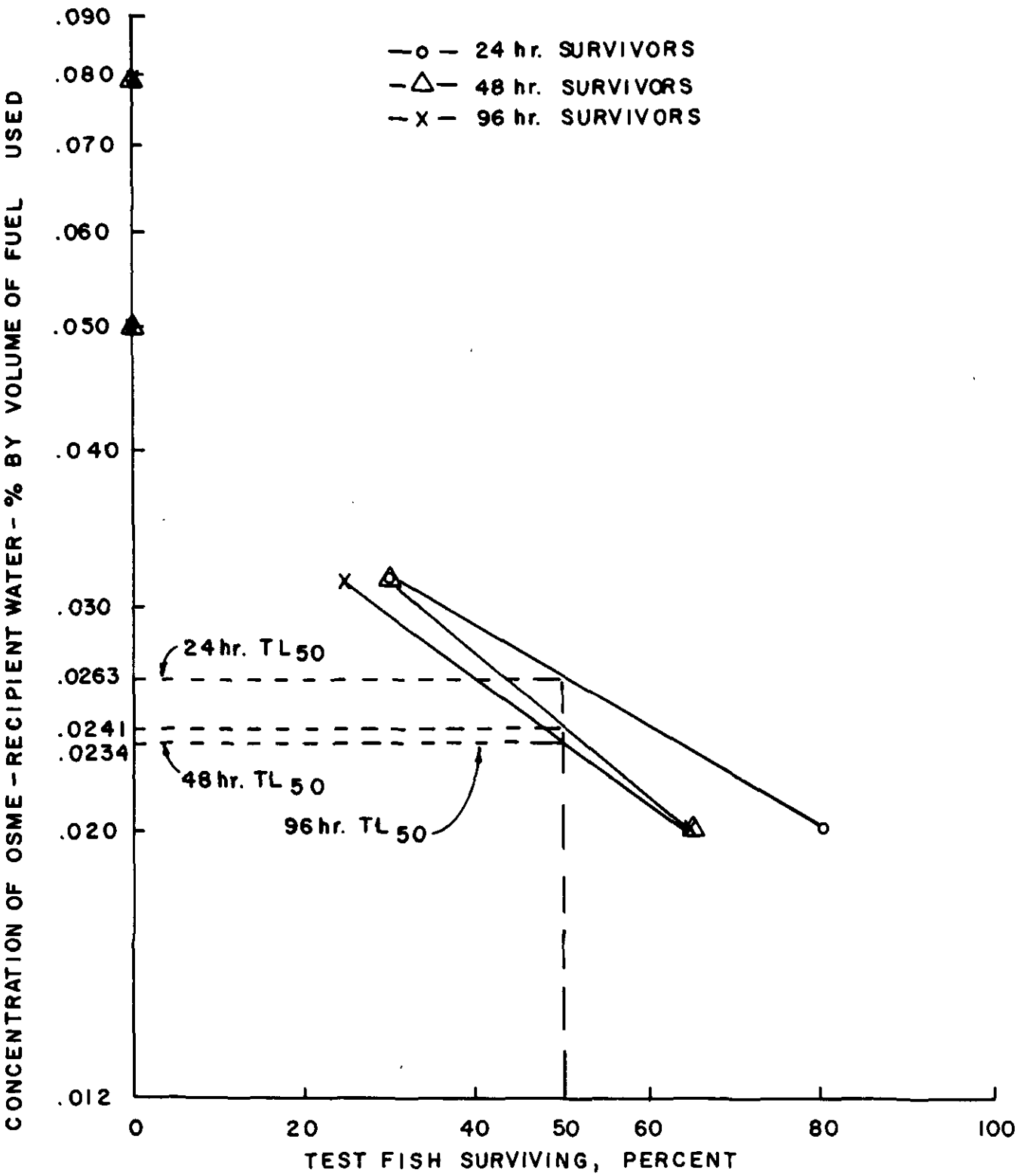

FIGURE A-8 ESTIMATION OF 24,48 AND 96 HR. TL_{50} VALVES FOR BLUEGILLS BY STRAIGHT-LINE GRAPHICAL INTERPOLATION METHOD, TEST NO. 12

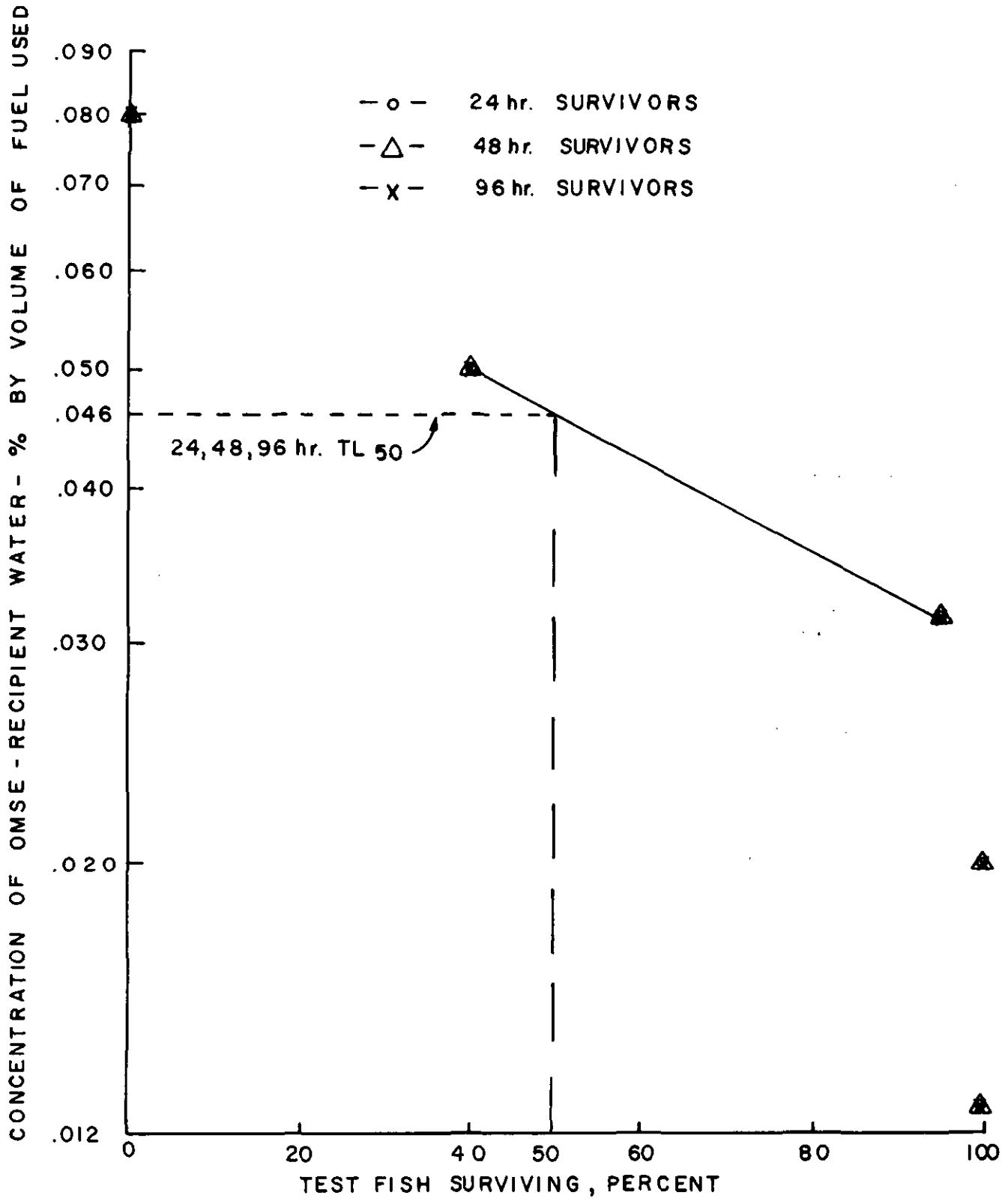

FIGURE A-9 ESTIMATION OF 24, 48 AND 96 TL_{50} VALUES FOR BLUEGILLS BY STRAIGHT-LINE GRAPHICAL INTERPOLATION METHOD, TEST NO. 13

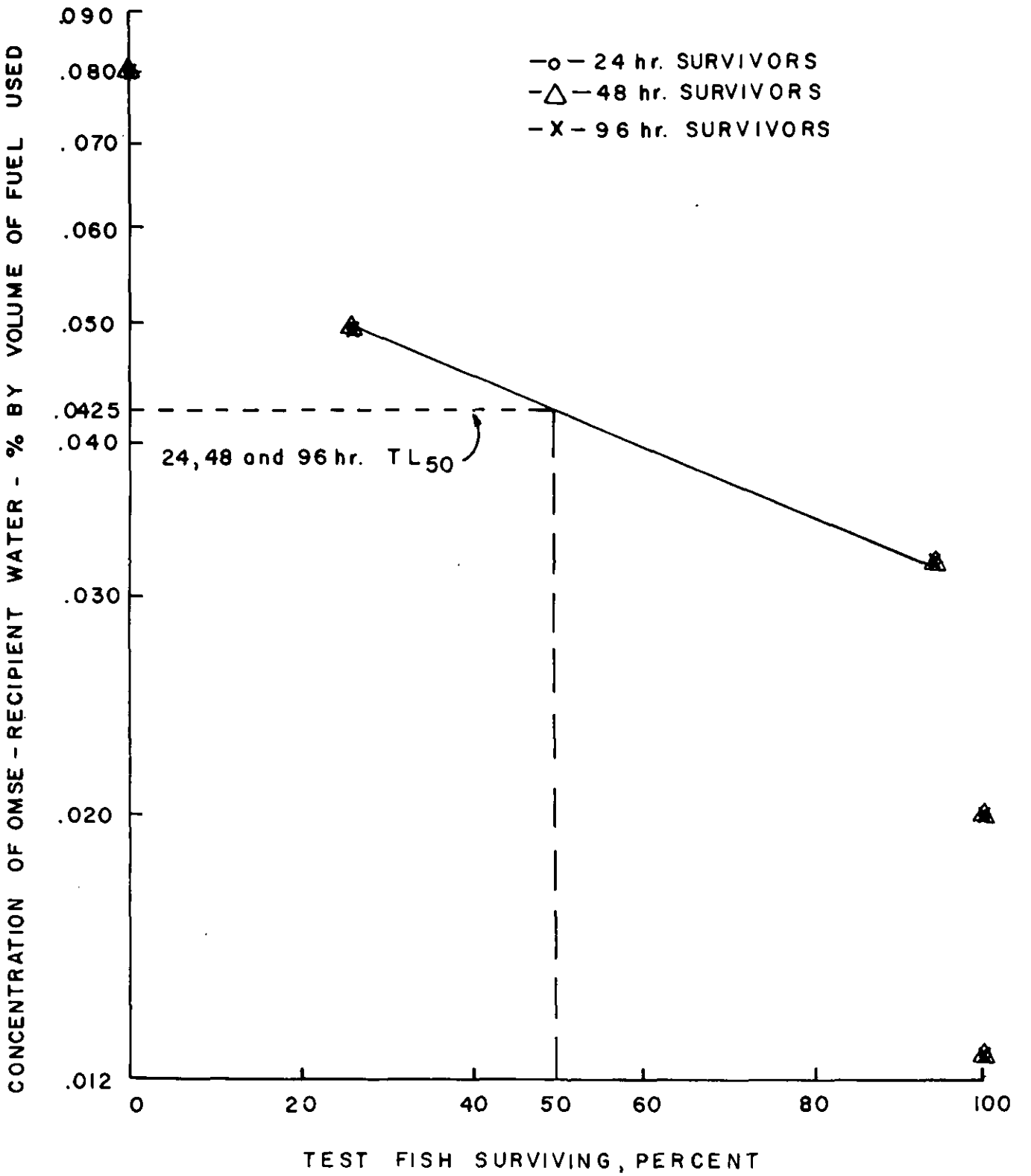

FIGURE A-10 ESTIMATION OF 24,48 AND 96-HR TL_{50} VALUES FOR BLUEGILLS BY STRAIGHT-LINE GRAPHICAL INTERPOLATION METHOD, TEST NO. 14

Table A-11. SURVIVOR DATA FOR FATHEAD MINNOWS (1-1/2" - 2"L) EXPOSED TO 4-DAY OLD OMSE-RECIPIENT WATER, TEST NO. 7

Tank	Concentration of OMSE-Water		No. of Test Fish	No. of Fish Surviving			pH		D.O. (mg/l)	
	percent	dil.fac.		After 24-hr	After 48-hr	After 96-hr	initial	final	initial	final
1A	Control	Control	10	10	10	10	7.2	7.1	6.8	5.1
1B	"	"	"	10	10	10	7.2	7.1	6.8	6.0
2A	.0125	8000/1	10	10	10	10	7.4	7.1	7.0	5.2
2B	"	"	"	10	10	10	7.1	7.1	7.2	6.6
3A	.0200	5000/1	10	10	10	10	7.3	7.1	6.8	5.4
3B	"	"	"	10	10	8	7.4	7.4	6.8	6.6
4A	.0315	3180/1	10	10	9	7	7.4	7.3	6.8	5.4
4B	"	"	"	10	9	7	7.2	7.1	7.0	5.8
5A	.0500	2000/1	10	0	0	0	7.2	7.2	6.9	6.0
5B	"	"	"	3	0	0	7.2	7.2	7.2	5.4
6A	.0790	1270/1	10	0	0	0	7.3	7.1	6.8	6.0
6B	"	"	"	0	0	0	7.2	7.2	6.6	5.2

Table A-12. SURVIVOR DATA FOR FATHEAD MINNOWS (1-1/2" - 2"L) EXPOSED TO 6-DAY OLD OMSE-RECIPIENT WATER, TEST NO. 8

Tank	Concentration of OMSE-Water		No. of Test Fish	No. of Fish Surviving			pH		D.O. (mg/l)	
	percent	dil.fac.		After 24-hr	After 48-hr	After 96-hr	initial	final	initial	final
1A	Control	Control	10	10	10	10	7.4	7.0	6.6	6.0
1B	"	"	"	10	10	10	7.2	7.2	7.0	6.0
2A	.0125	8000/1	10	10	10	10	7.2	7.2	6.7	5.4
2B	"	"	"	10	10	10	7.3	7.2	6.1	5.2
3A	.0200	5000/1	10	10	10	7	7.2	7.0	6.6	5.4
3B	"	"	"	10	10	8	7.2	7.2	6.9	5.3
4A	.0315	3180/1	10	10	8	5	7.3	7.2	7.4	6.4
4B	"	"	"	9	8	6	7.0	7.1	6.8	5.2
5A	.0500	2000/1	10	8	2	1	7.2	7.3	7.0	6.0
5B	"	"	"	9	5	3	7.2	7.2	6.9	5.7
6A	.0790	1270/1	10	3	0	0	7.4	7.0	6.9	5.5
6B	"	"	"	3	0	0	7.1	7.0	6.8	5.2

Table A-13. SURVIVOR DATA FOR FATHEAD MINNOWS (1-1/2" - 2"L) EXPOSED TO 10-DAY OLD OMSE-RECIPIENT WATER, TEST NO. 9

Tank	Concentration of OMSE-Water		No. of Test Fish	No. of Fish Surviving			pH		D.O. (mg/l)	
	percent	dil.fac.		After 24-hr	After 48-hr	After 96-hr	initial	final	initial	final
1A	Control	Control	10	10	10	10	7.0	6.8	7.2	6.6
1B	"	"	"	10	10	10	7.0	6.9	7.4	6.6
2A	.0125	8000/1	10	10	10	10	6.9	6.9	5.6	5.2
2B	"	"	"	10	10	10	6.9	7.0	5.8	5.4
3A	.0200	5000/1	10	10	8	6	6.8	7.0	6.4	6.0
3B	"	"	"	10	10	8	7.0	7.1	5.9	5.2
4A	.0315	3180/1	10	10	7	2	7.2	7.2	6.2	5.2
4B	"	"	"	10	7	2	7.4	7.3	7.5	6.2
5A	.0500	2000/1	10	8	1	0	7.2	7.0	7.6	6.1
5B	"	"	"	8	4	0	7.2	7.1	7.6	6.2
6A	.0790	1270/1	10	2	0	0	7.3	7.4	7.1	5.4
6B	"	"	"	1	0	0	7.2	7.1	6.0	5.1

Table A-14. SURVIVOR DATA FOR FATHEAD MINNOWS (1-1/2" - 2"L) EXPOSED TO 15-DAY OLD OMSE-RECIPIENT WATER, TEST NO. 10

Tank	Concentration of OMSE-Water		No. of Test Fish	No. of Fish Surviving			pH[1]		D.O.[2] (mg/l)	
	percent	dil.fac.		After 24-hr	After 48-hr	After 96-hr	initial	final	initial	final
1A	Control	Control	10	10	10	10	7.2	7.0	6.5	5.5
1B	"	"	"	10	10	10				
2A	.0125	8000/1	10	10	10	10	7.2	7.0	6.8	5.4
2B	"	"	"	10	10	10				
3A	.0200	5000/1	10	10	6	6	7.4	7.2	6.6	5.2
3B	"	"	"	10	8	6				
4A	.0315	3180/1	10	8	4	3	7.1	6.9	6.8	5.6
4B	"	"	"	8	4	1				
5A	.0500	2000/1	10	5	3	2	7.2	7.0	6.6	5.5
5B	"	"	"	5	2	0				
6A	.0790	1270/1	10	1	0	0				
6B	"	"	"	0	0	0				

[1]Refer to footnote No. 1 in Table 6

[2]Same as No. 1

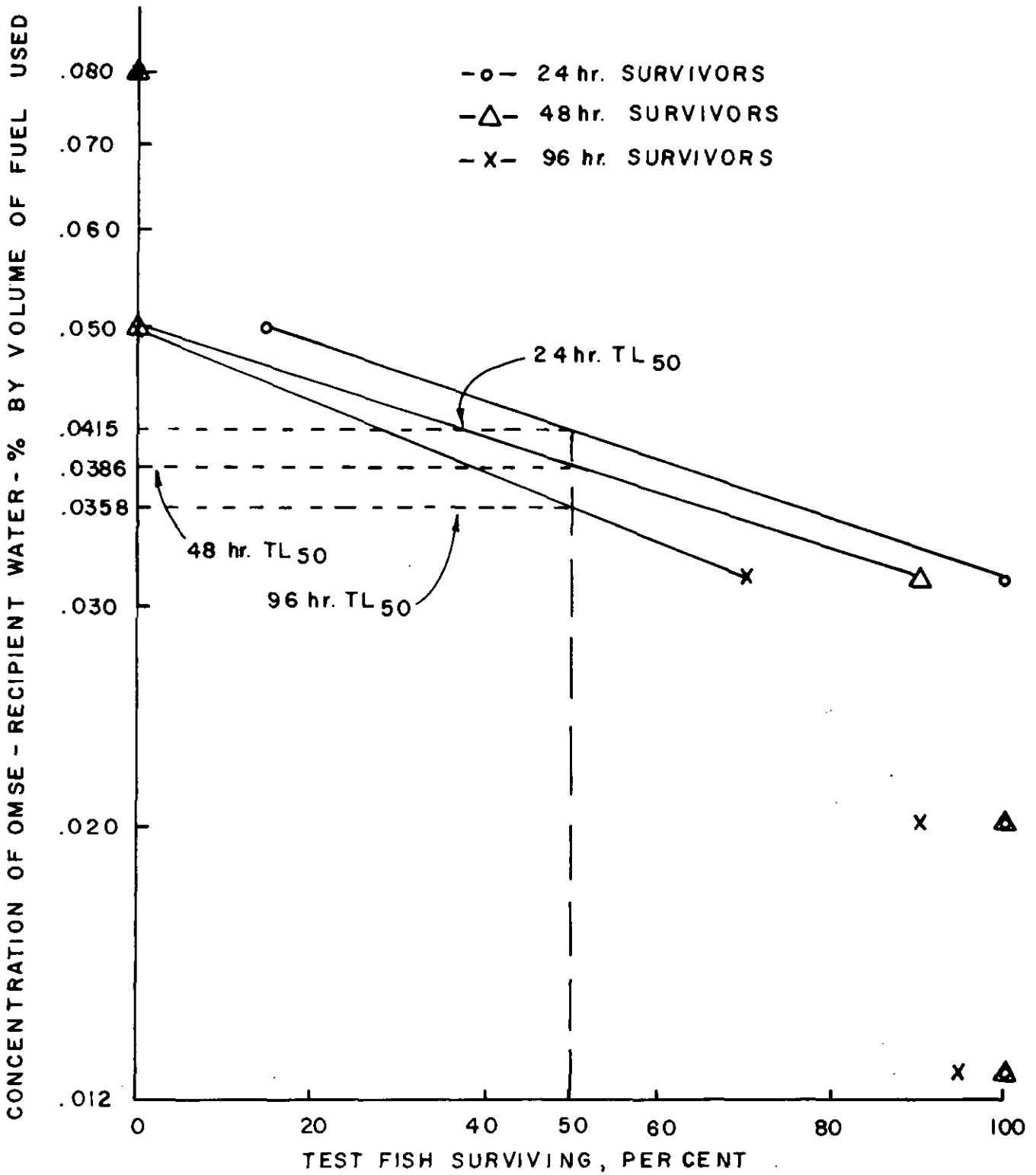

FIGURE A-II ESTIMATION OF 24, 48 AND 96 HR. TL_{50} VALUES FOR FATHEAD MINNOWS BY STRAIGHT LINE GRAPHICAL INTERPOLUTION METHOD, TEST NO. 7

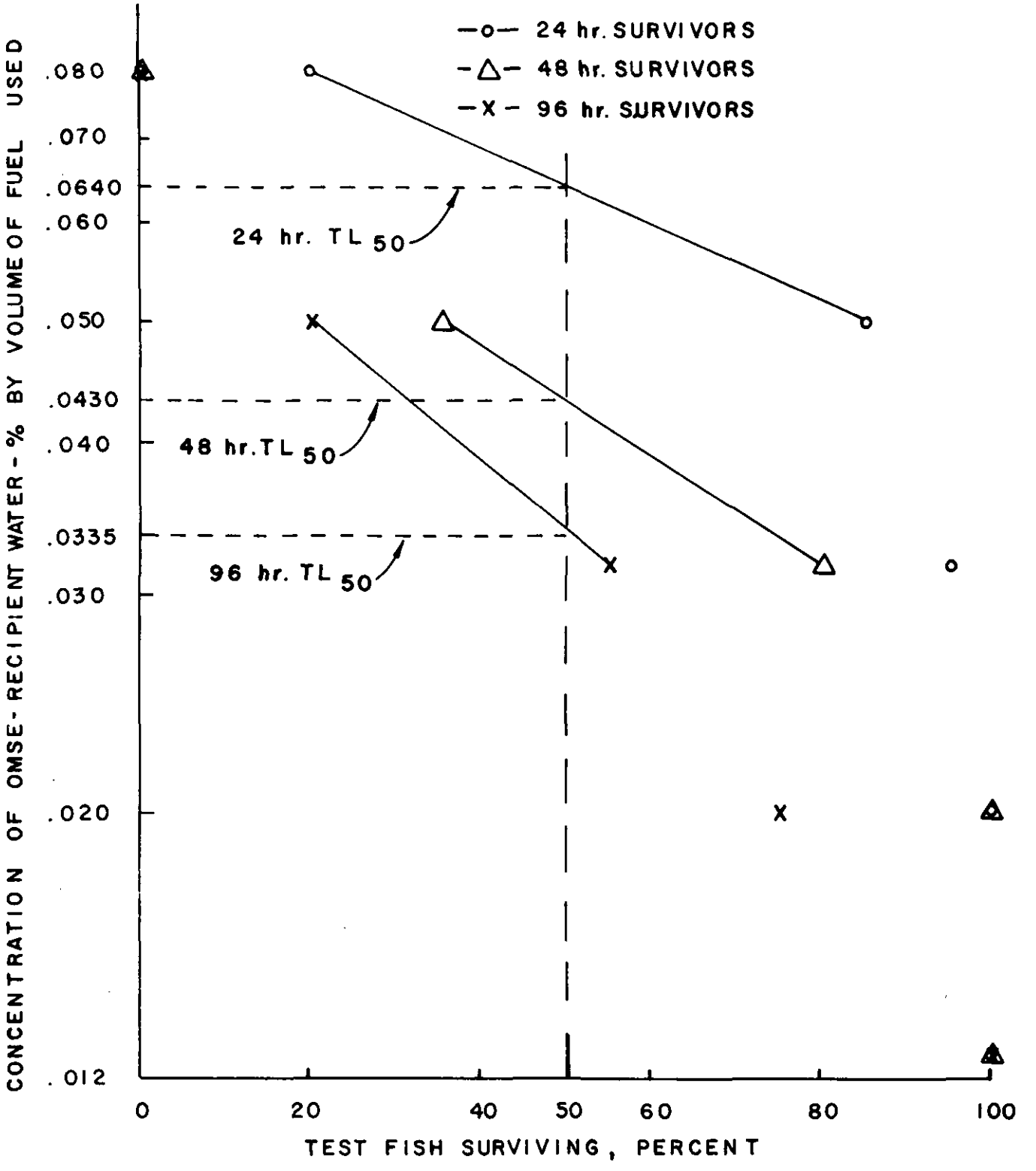

FIGURE A-12 ESTIMATION OF 24, 48 AND 96 HR. TL_{50} VALUES FOR FATHEAD MINNOWS BY STRAIGHT-LINE GRAPHICAL INTERPOLATION METHOD TEST NO. 2

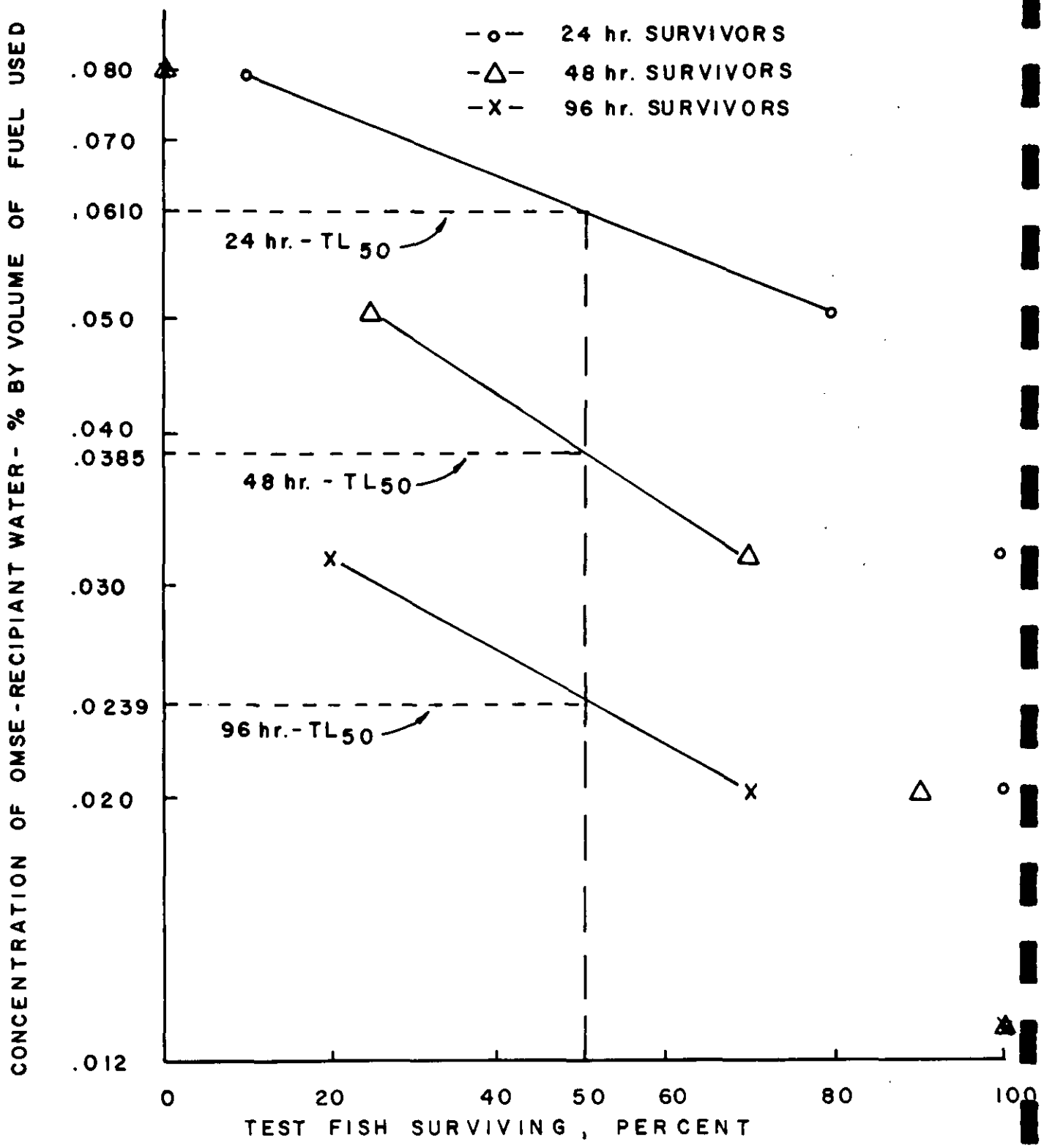

FIGURE A-13 ESTIMATION OF 24, 48 AND 96-HR. TL 50 VALUES FOR FATHEAD MINNOWS BY STRAIGHT LINE GRAPHICAL INTERPOLATION METHOD

TEST NO. 9

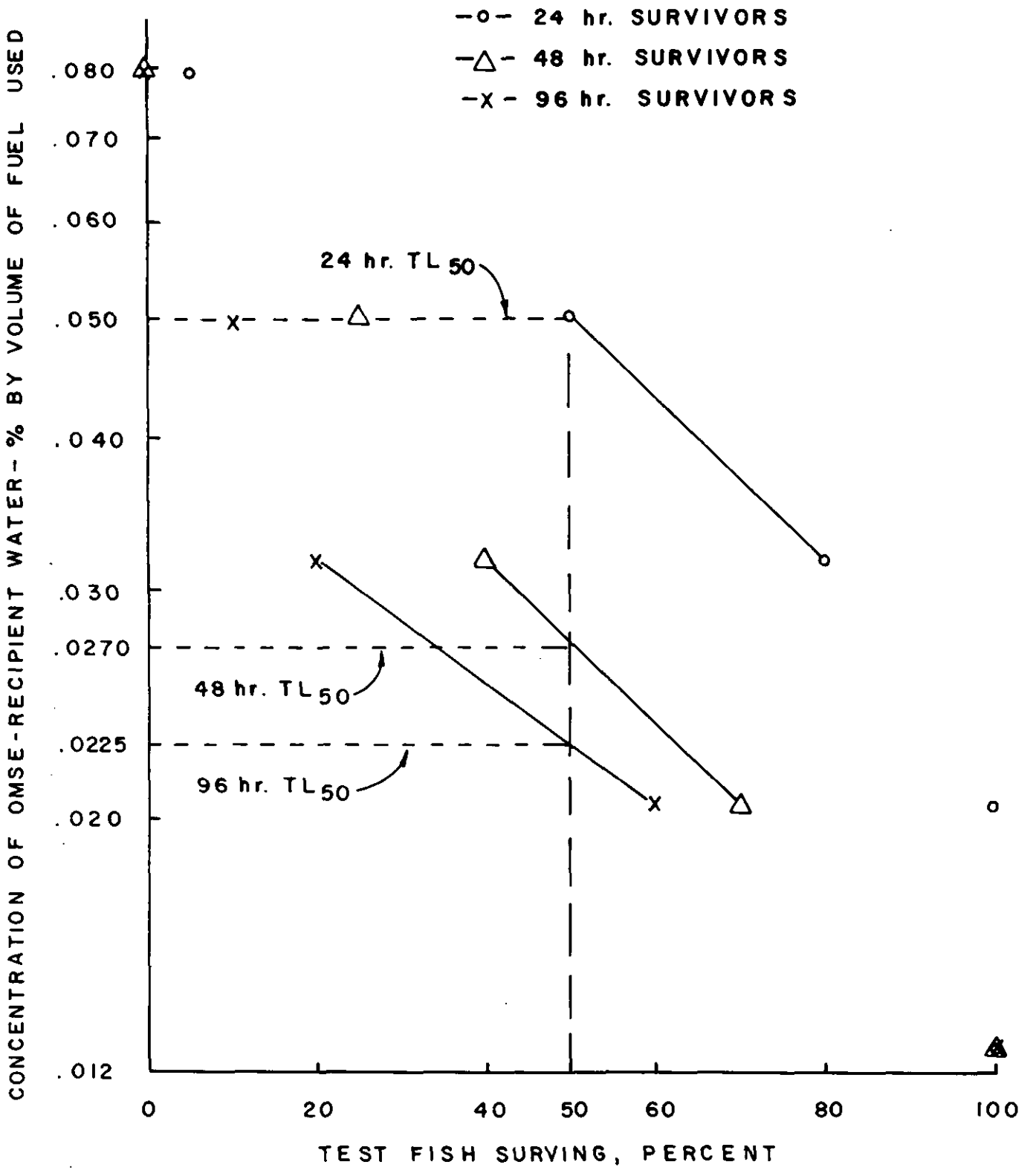

FIGURE A-14 ESTIMATION OF 24,48, AND 96 HR TL_{50} VALUE FOR FATHEAD MINNOWS BY STAIGHT-LINE GRAPHICAL INTERPOLATION METHOD
TEST NO. 10

APPENDIX B

Structure of a Normal Gill and Gills of Fathead Minnows and Bluegills Exposed to OMSE-Recipient Water

Figure B-1a. Gills of Fathead Minnow (Control, alive)

Figure B-1b. Gills of Fathead Minnow Exposed to 0.0125 percent concentration of OMSE-recipient Water (4 days exposure; alive).

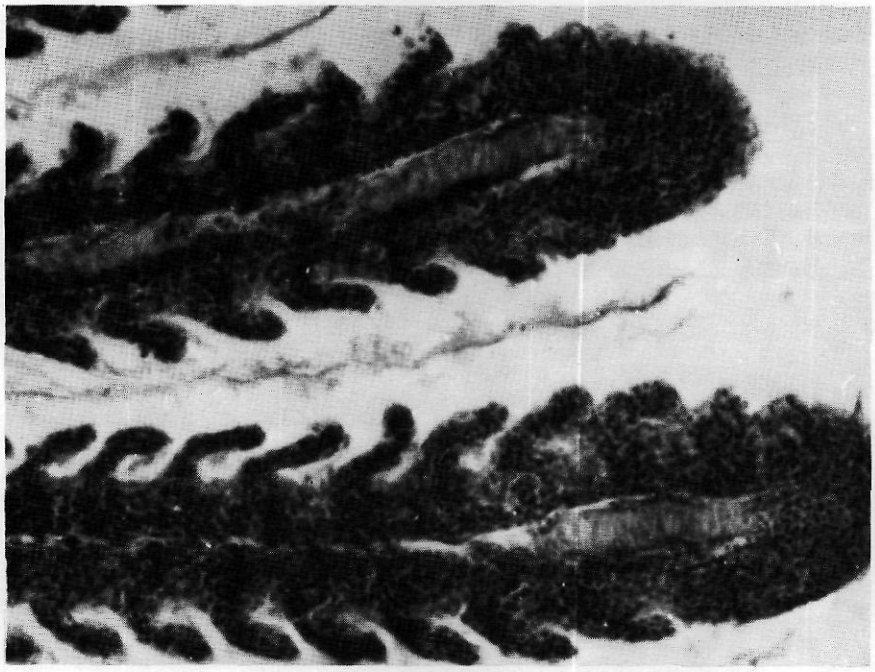

Figure B-1a. Gills of Fathead Minnow (Control, alive)

Figure B-1b. Gills of Fathead Minnow Exposed to 0.0125 percent concentration of OMSE-recipient Water (4 days exposure; alive).

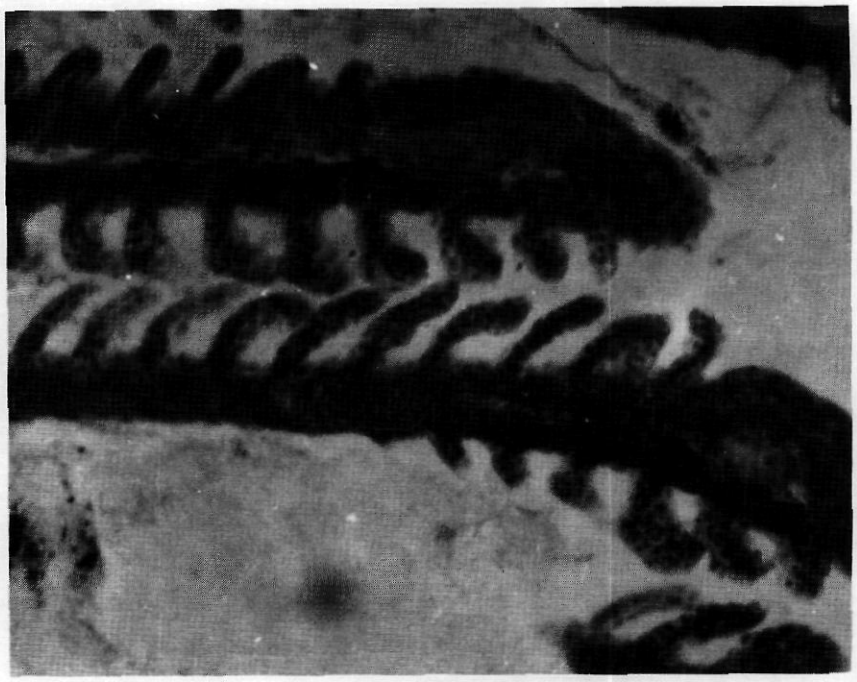

Figure B-1c. Gills of Fathead Minnow Exposed to 0.020 percent concentration of OMSE-recipient Water (4 days exposure; alive)

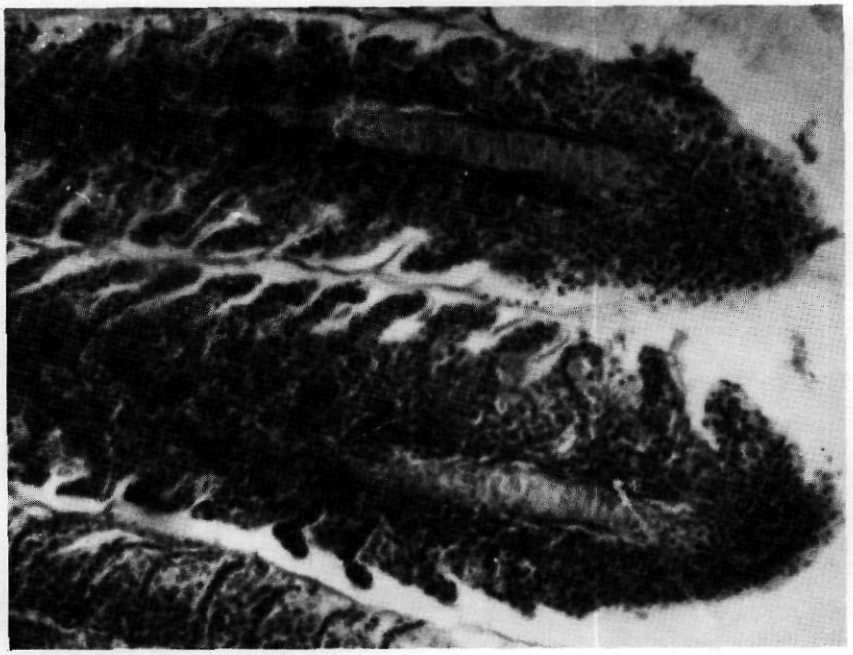

Figure B-1d. Gills of Fathead Minnow Exposed to 0.0315 percent concentration of OMSE-recipient Water (2-4 hrs. exposure; dead).

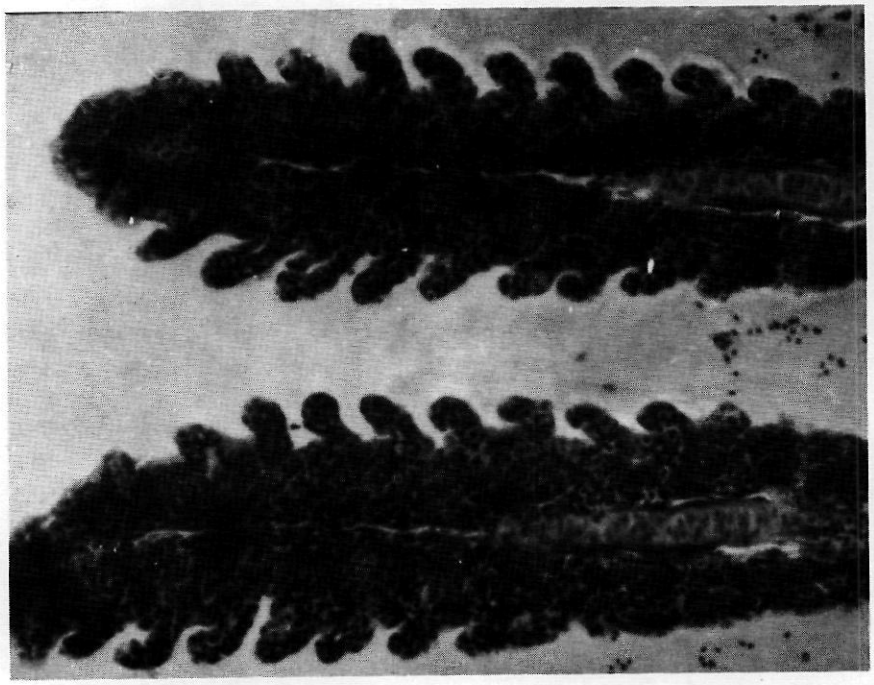

Figure B-1e. Gills of Fathead Minnow Exposed to .050 percent concentration of OMSE-recipient Water (2-4 hrs. exposure; dead).

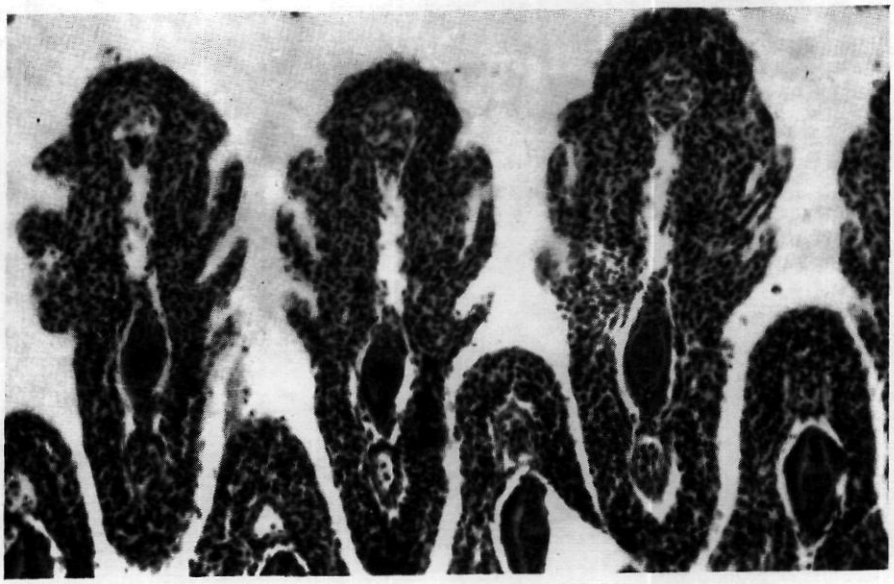

Figure B-2a. Gills of Bluegill (Control; alive).

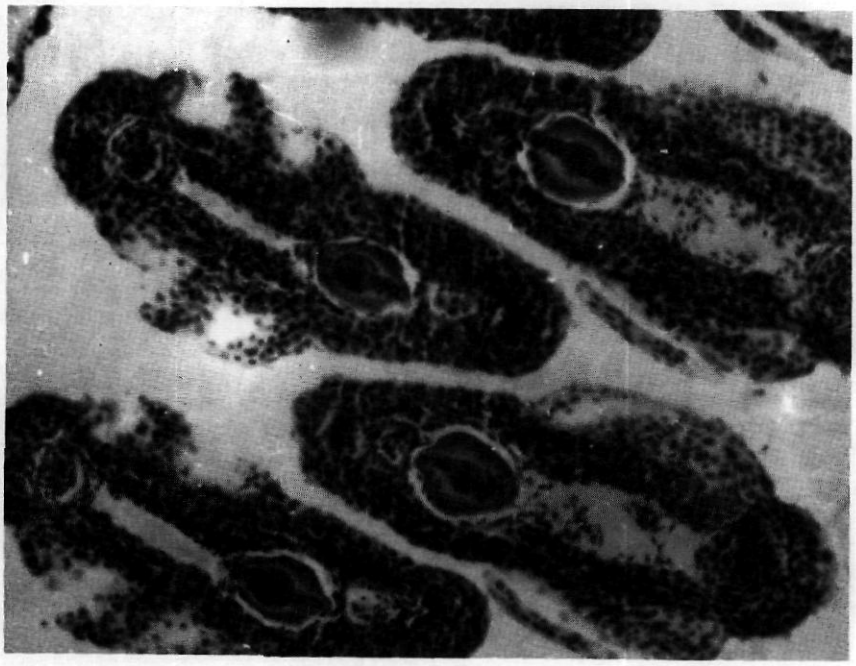

Figure B-2b. Gills of Bluegill Exposed to 0.020 percent concentration of OMSE-recipient Water (4-days exposure; alive).

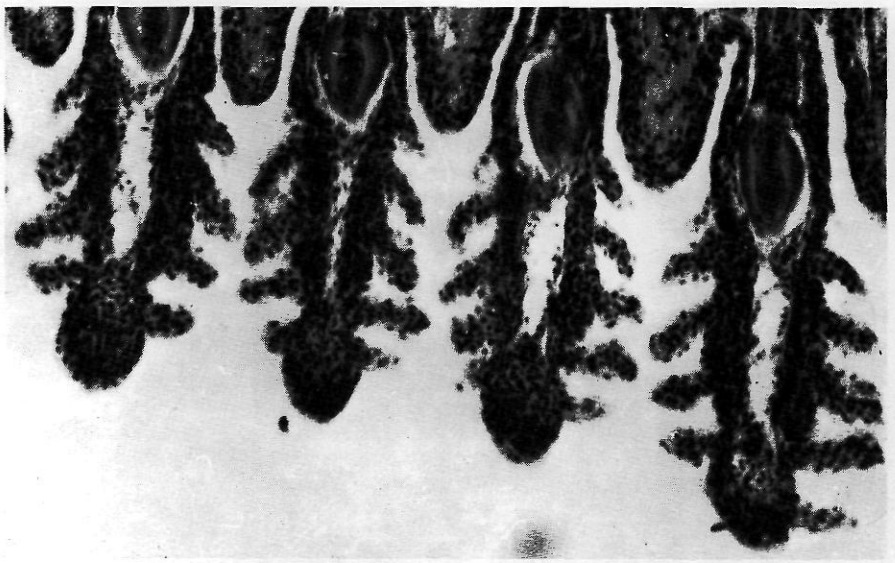

Figure B-2c. Gills of Bluegill Exposed to 0.0315 percent concentration of OMSE-recipient Water (4-days exposure; alive)

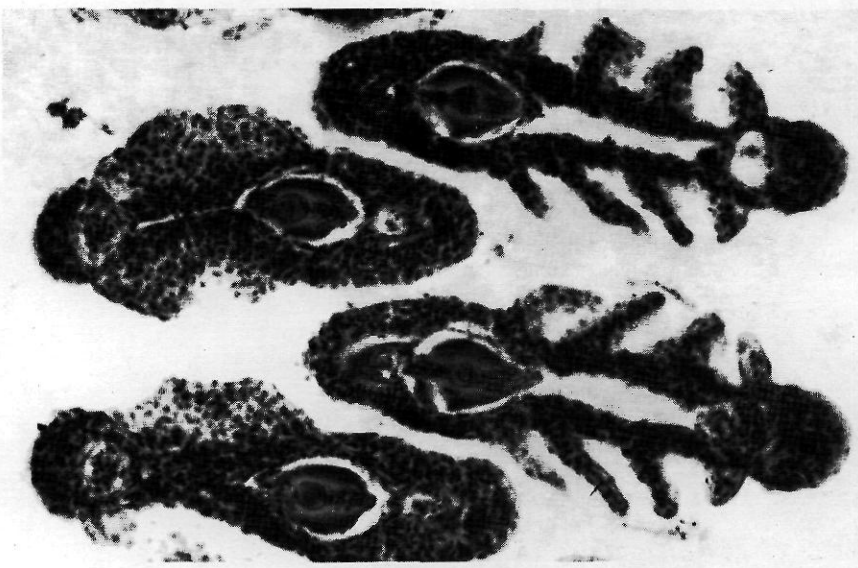

Figure B-2d. Gills of Bluegill Exposed to 0.050 percent concentration of OMSE-recipient water (4-days Exposure; alive).

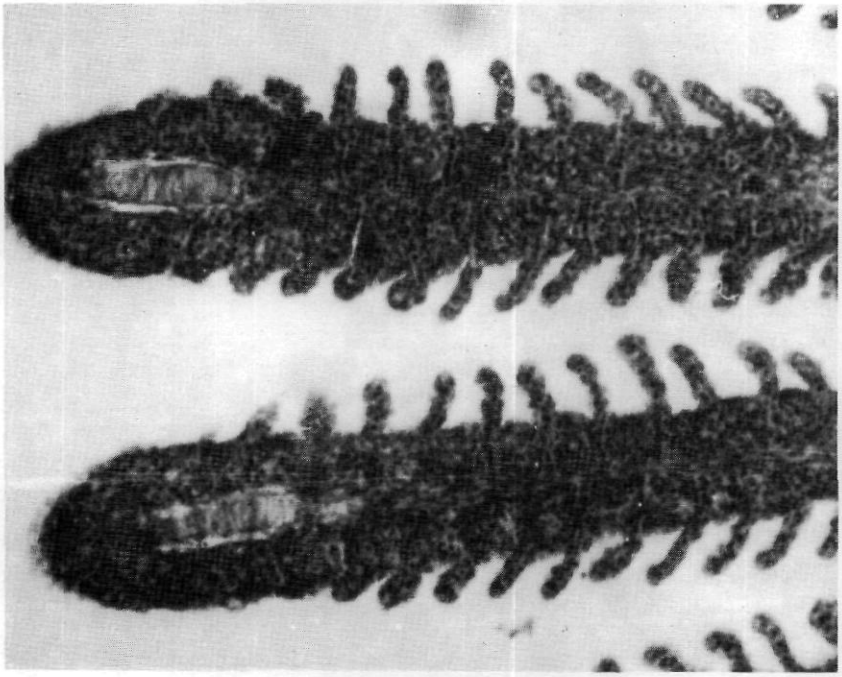

Figure B-2e. Gills of Bluegill Exposed to 0.050 percent concentration of OMSE-recipient Water (2-4 hrs. exposure; dead).

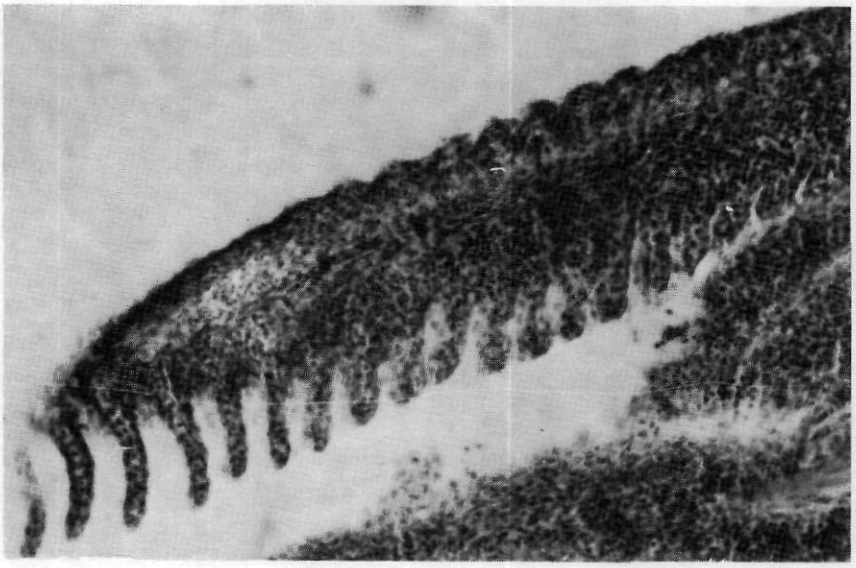

Figure B-2f. Gills of Bluegill Exposed to 0.079 percent concentration of OMSE-recipient Water (2-4 hrs. exposure; dead).