

WATER POLLUTION CONTROL WITH TOXICANT AVOIDANCE TESTS

by

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This thesis is dedicated to
the eternal memory of my beloved father
ARISTIDES
and to my friends
of the Sign of the Theotokos Orthodox Church in Montreal

.....

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ABSTRACT

An extensive review of all the avoidance apparatus which have been used to date in chemical avoidance tests and also an analysis of the trends and needs for new improved designs led to the design of a new avoidance apparatus. The new apparatus is a valuable tool for a three-dimensional analysis of fish behavioral responses to potentially toxic effluents. The method is quick, sensitive and relatively easy to use if sufficient fish stock is available.

The variable parameters of the avoidance reaction were three: a) the type of the pollutant, b) the injection system, and c) the concentration of the pollutant.

Statistical analysis for the experiments included preliminary calculations, time series analysis, analysis of variance and Duncan's tests.

Preliminary tests evaluated the general disposition of fish in the channel, the influence of a water acclimation jet and also the effects of noise, light and temperature. Experiments were performed to define the optimal number of fish per experiment, the influence of video analysis and also of variability, adaptability and continuity.

The types of pollutants used were D.S.S. (dodecyl sodium sulfate) - a well-known reference pollutant, polymers, monomers, a plating industrial effluent and its components (Cr, Ni, Fe).

For all of these, horizontal and vertical distributions were produced together with avoidance curves and avoidance reaction representations.



The role of the avoidance reaction in relation to lethal and sublethal levels has been defined; with evaluations of synergistic and antagonistic response considerations, adjustments for safe toxicant levels are proposed.

R E S U M E

Tous les appareils d'évitement qui ont été utilisés jusqu'à ce jour ont été évalués. Une analyse des tendances et des besoins de nouvelles conceptions améliorées a conduit à l'élaboration d'un nouvel appareil et d'une procédure nouvelle de l'étude de l'évitement. L'appareil conçu est un instrument utile pour analyser le comportement tri-dimensionnel de poissons en réponse à des effluents potentiellement toxiques. La méthode est rapide, sensible et relativement facile à utiliser si suffisamment de poissons sont disponible.

Trois éléments de la réaction d'évitement ont été variés au cours de nos expériences:

- a) le type de polluant
- b) le système d'injection
- et c) la concentration du polluant.

Les calculs préliminaires, une analyse time-series, une analyse de la variance et des essais Duncan's ont formé une analyse statistique.

Les essais préliminaires ont servi à évaluer le comportement générale des poissons dans le canal, l'influence de l'ensemble de l'acclimatement à l'eau, et aussi l'effet du bruit, de la lumière et de la température.

Des essais ont été fait pour déterminer le nombre optimal de poissons par essais, l'influence de enregistrement sur video, et aussi des essais sur la variabilité, l'adaptabilité, et la continuité.

() Les types de polluants utilisés étaient le D.S.S. (dodecyl sulfate de sodium) un polluant de référence bien connu, des polymères, des monomères, un effluent d'industrie de placage et ses composantes (Cr, Ni, Fe).

Des distribution horizontales et verticales ainsi que des courbes d'évitement et des représentations de la réaction d'évitement ont été produites pour tout ceci.

Le rôle de la réaction d'évitement par rapport aux concentrations létales ou sub-létales a été déterminé; avec l'évaluation du synergisme et d'antagonisme on a établi des ajustements importants pour le développement de concentrations sécuritaires.

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LIST OF SYMBOLS

LC50	=	Lethal concentration at which 50% of the test organisms are killed on exposure to the test material
EC50	=	Effective concentration at which 50% of the test organisms show a particular sublethal effect
T	=	Temperature
t	=	time
%	=	percentage
C	=	concentration
ANOVA	=	Analysis of variance
°C	=	degrees Celcius
TL _m	=	Median tolerance limit
S.C.	=	safe concentration
MATC	=	maximum allowable toxicant concentration
Q	=	flow
std.dev	=	standard deviation
R	=	range
A.F.	=	application factor
G.C.	=	growth curve
L	=	distance in minutes
σ_e	=	fluctuation in % around the mean avoidance line
gpm	=	gallons per minute
cc/min	=	cubic centimeters per minute
ml/min	=	milliliters per minute

LIST OF SYMBOLS (continued)

mg/l	=	milligrams per liter
ppm	=	parts per million
exp	=	experiment
A.R.R.	=	avoidance reaction representation
D.O	=	dissolved oxygen
SS	=	suspended solids
R^2	=	variation of the dependent variable
F	=	F statistic
C.V.	=	coefficient of variation
PR > F	=	the significance probability for the F value
MS	=	error mean square

PART A

CHAPTER I

"INTRODUCTION"

INTRODUCTION

1.1 General

Until recently, aquatic biologists engaged in research in government and academic institutions focused on ecological studies of lakes and rivers, or an evaluation of LC50 for various species and isolated chemicals. Conversely, environmental engineers were primarily concerned with stereotyped designs of wastewater treatment plants for the removal of BOD and suspended solids. If the two disciplines met, it was usually unrelated to professional interests or activities.

In the past few years, however, the picture has undergone substantial change. An increasing number of industries and regulatory agencies are attempting to relate pollution or discharges to the overall effect on the aquatic environment rather than to restricted specific effects. These facts have lead to a professional linkage between the engineer responsible for removing or modifying pollutants and the biologist responsible for defining its effect on ecological systems.

There has recently been a trend towards a multi-disciplinary approach in solving water pollution problems (Buikema 1979). Computer technology has also been increasingly applied in this direction (Cairns 1980).

Natural scientists and engineers tend to do research on the same subjects, using each other's tools but approaching them from different perspectives and with different objectives. Collaboration in seeking common solutions will hopefully become more frequent.

Virtually every industry, with or without treatment of its liquid effluents, discharges them into sewers, rivers, lakes or the sea. Most of these industrial effluents include toxic compounds. The toxicity of effluents may have acute or long-term effects on aquatic life. Motile aquatic organisms often exhibit sensitive behavioral changes to the toxic pollutants (i.e., avoidance). The existing "industrial pollution control" recommends the use of acute lethal bioassays in order to fulfill their particular water quality objectives (EPS - Environment Canada, 1980).

The research to be presented in this thesis deals with the acute avoidance behavior of motile ecosystem members to sublethal concentrations of toxicants in water. An interdisciplinary approach has been adopted utilizing bioassay techniques. The need for such a study arises from the fact that acute lethal bioassays ignore the possible sublethal and chronic effects brought about by the industrial discharge of toxic effluents into bodies of natural water.

Since the early 1960's when general public awareness of and concern for the biological environment led to increased funding of research related to pollution, a vast amount of information has been accumulated on the acutely morbid and lethal effects of pollutants on aquatic organisms. Particular emphasis was placed on the relationships between these effects and the exposure time and the concentration of the pollutants studied.

It is evident that a toxicant may affect fish populations in ways other than acute mortality, but until recently this concept has not been extensively applied. Now, substantial attention is being given to sublethal effects which can affect living organisms. Field studies of fish have shown that toxic products may render large portions of an environment undesirable or uninhabitable. The behavior of fish that enter these areas is often modified; any such behavior patterns that allow fish to escape or avoid deleterious conditions and move to more favourable locations within the environment contribute to their survival and well being (Cherry, 1982).

Important decisions must soon be made to limit the further degradation of water resources. Unfortunately, zero discharge is an unrealistic goal (Cairns 1978). Criteria must therefore be formulated to help establish standards that can adequately protect our water resources.

Avoidance curves and thresholds provide valuable insight regarding the survival of fish in toxic solutions that cannot be obtained from static or chronic bioassays. Fish may successfully survive brief exposures to toxic concentrations greater than their 96-hour lethal concentrations by avoidance behavior and retreat to more favorable locations in their environment. Therefore studies of these phenomena may provide extremely useful information from which criteria for protection of water resources can be established.

Analytical methods used for water pollution control studies are numerous and varied. Table 1.1 depicts schematically the various

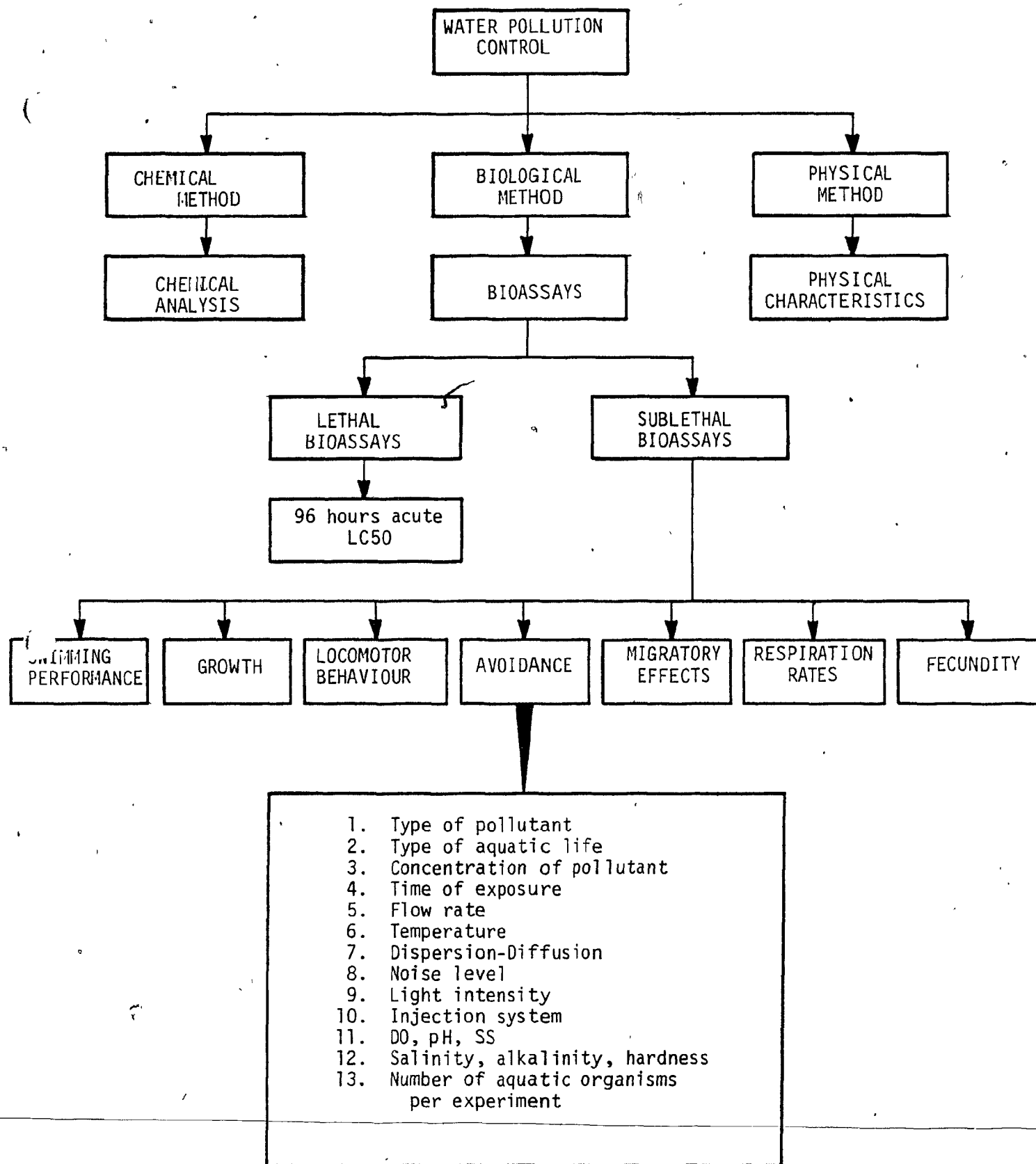


TABLE 1.1 ANALYTICAL METHODS OF
WATER POLLUTION CONTROL

techniques which may be employed. Within this schema, the relative role of avoidance studies can be seen, and various parameters which this present study will examine, are indicated.

1.2 Objectives and Scope of this Study

The first specific objective of this study was to analyze the advantages and disadvantages of existing avoidance apparatus in order to develop a tool which would more accurately reproduce the conditions prevailing in bodies of natural water, and which would permit the application of advanced analytical techniques and the introduction of various engineering parameters.

A second specific objective was to evaluate the avoidance responses of sublethal toxic effluent discharges on organisms utilizing the newly developed tool. These results were then compared with dynamic and static acute toxicity measurements to determine more realistic predictors of "safe level" concentrations.

A more general objective of the study was to provide data which would substantiate the inadequacy of existing toxicity guidelines by measuring the sublethal responses of aquatic organisms living in the vicinity of effluent discharges.

A detailed outline of the scope of this study is given in the following section.

1.3 Thesis Outline

The Thesis is divided into three parts.

Part A includes: general introduction concepts, a review of previous investigations, the experimental set-up, the selection of the parameters to be studied, and a description of the statistical

analyses employed (Chapters I, II, III and IV).

Part B comprises experimental data and statistical analyses from experimental work: preliminary testing, tests for continuity variability, adaptability, optimal number of fish, DSS, polymers, monomers, actual industrial effluent and its components (Chapters V-X).

Part C contains the implementation of the results, guidelines, suggestions and recommendations for future experiments, and the summary and conclusions of the overall study. References and appendices are also included in Part C.

CHAPTER II

"REVIEW OF PREVIOUS INVESTIGATIONS"

2.1 Toxicity Tests

Toxicity tests have been developed and employed in response to a growing awareness of water pollution and its effects on the environment.

To be useful in pollution control, toxicity tests must provide information in three main areas:

1. PREDICTION of environmental effects of a waste disposal.
2. COMPARISON between different toxicants, test organisms and test conditions.
3. REGULATION of discharges (how much, where, how, when, ... etc.).

Toxicology or the study of the effects of materials or substances on organisms came about as a result of the development of organic chemistry in the 19th and 20th centuries. The capacity of a chemical to have both beneficial and adverse effects was recognized very early (Hueppe, 1896), but it was only in the 1940's that studies began on the effects of chemicals and wastes on non-human organisms. Biologists had already recognized the enormous differences between groups of organisms that lived in polluted water and those that did not. As awareness of these effects grew, studies were made of the nature and extent of damage due to the toxicity of chemicals on fish. In these studies fish were exposed to industrial wastes in order that the potential damage of a chemical might be predicted. Toxicity tests have been used to answer the following types of questions:

(Buikema, Medelehner, Cairns, 1982)

- (1) Is the material lethal to the test organism and at what concentration?
- (2) What are the effects on organisms exposed to sublethal concentrations of a toxicant for part or all of a life cycle?

- (3) Which waste, or waste component, is most toxic?
- (4) Which organism is most sensitive?
- (5) Under which conditions are wastes most toxic?
- (6) Does toxicity change when the material enters the environment?
- (7) Does the waste or pure compound pass or fail some regulatory standard?
- (8) How much of the receiving system is affected?
- (9) What are the short term effects of episodic spills?

Work has been done in the development of toxicity testing techniques, but there is still much room for improvement. With increasing governmental regulations, major emphasis must be placed upon the development of meaningful toxicity tests to specific management goals.

Table 2.1 illustrates the goals and the subdivisions of toxicity tests.

2.2 Preference-avoidance Tests

Among the seven toxicity tests depicted in Table 2.1, preference-avoidance reactions may be considered particularly important and practical. They can detect adverse effects to pollutants at sublethal concentrations, the tests themselves are of relatively short duration (normally a matter of hours), and their outcome is measured by a perceptible alteration in subject behavior, rather than by more irreversible effects such as death. As may be noted, the sensitivity of the test is only limited by the acuity of the sensory mechanisms of the organisms tested.

In practice, toxicity tests of most types are meant to yield concentrations above which species may be endangered. In contrast, avoidance tests are meant to exploit the refined ability of an organism to withdraw from an environment that may impair its survival.

The interest in avoidance studies arose from practical situations in which lethal or known sublethal tests proved to be inadequate for assessing waste discharge management. Questions, such as what organisms are attracted by lethal concentrations, or which

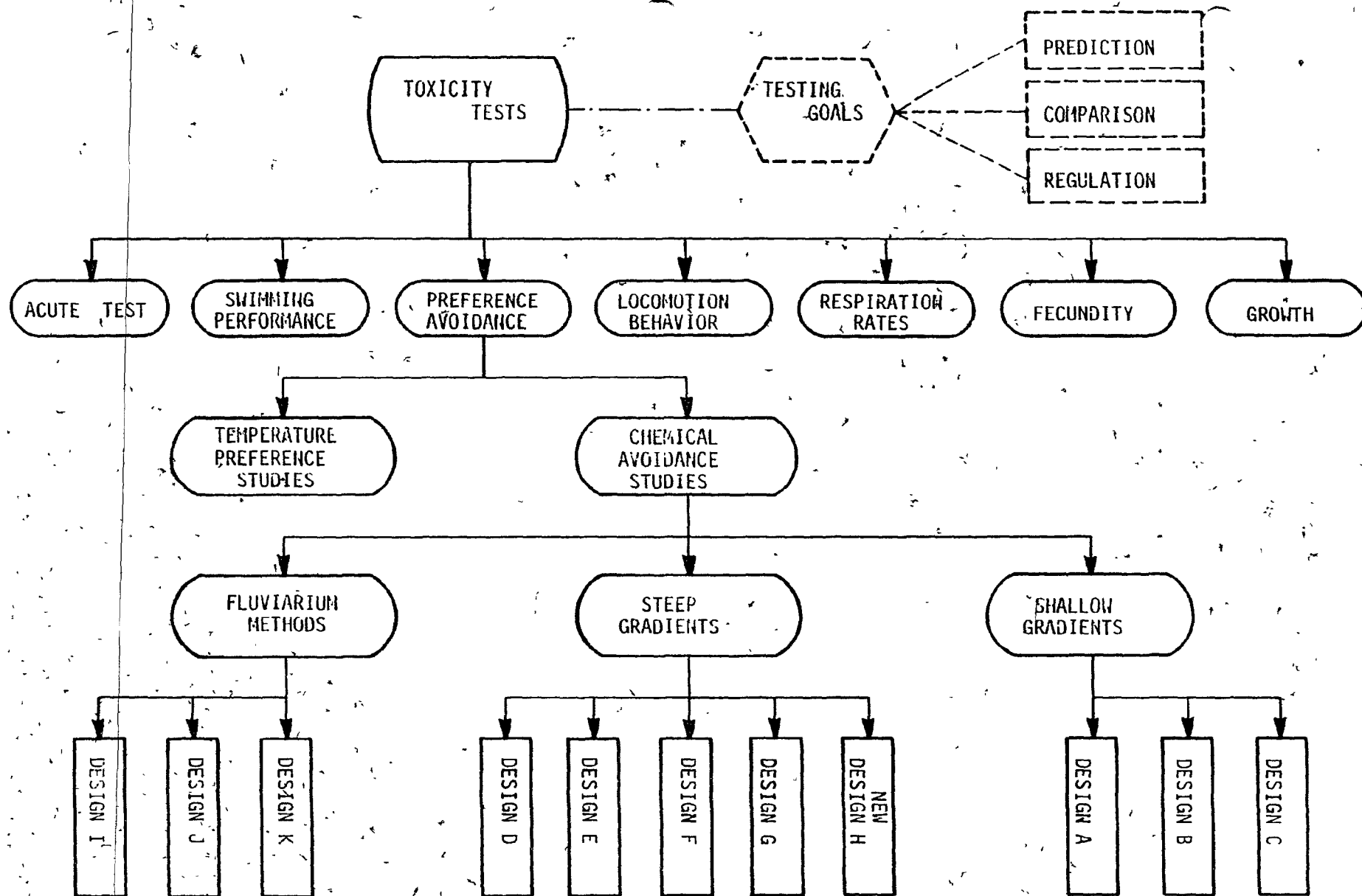


TABLE 2.1 GOALS AND SUBDIVISIONS OF THE TOXICITY

organisms lack the ability to detect lethal concentrations, made the preference-avoidance research essential for a better understanding of the actual environmental hazards.

Fish and other motile organisms by their movements have relative control over the environmental conditions to which they wish not to be exposed. Avoidance-preference tests are based on the fact that aquatic organisms often have the ability to detect harmful factors within their environments and to respond to toxicants by moving into preferable surroundings when given the choice. Testing the direct response to toxicants can determine:

- (a) whether the species can detect the toxicant, and, if so,
- (b) whether preference for the toxicant will prevail (regardless of outcome) or avoidance will provide a chance for escape.

Preference-avoidance tests may be divided into two groups:

- 1) temperature preference-avoidance studies, and
- 2) chemical avoidance studies.

Because temperature is a major ecological factor, many studies have been carried out to determine its effect on aquatic life. More than ten different apparatus have been designed for temperature avoidance studies; McCauley (1977) made a complete inventory of them.

In this study the emphasis is on chemical avoidance tests which can be much more complicated. An analytical description of the various chemical avoidance tests follows.

2.3 Chemical Avoidance Tests

Once the presence of a toxicant at a given concentration has been detected, fish may respond in several ways. A detailed analysis of these responses (kinesis, orthokinesis, klinokinesis, taxes, tropotaxis, etc.) and their probable causes can be found in research literature of pure biology. In general it is not known if, in some instances, fish have specific toxicant receptors or not. In the case of chlorine it may simply act as an irritant over portions of the

body, thereby providing the stimulus for toxic behavior (Cherry 1977).

In attempting to simulate as closely as possible the effects of a toxicant in a natural body of water, the most important decision to be made is the selection of an apparatus appropriate for the particular test situation.

From 1913 until the present, many designs for chemical avoidance apparatus have been introduced. These can be grouped into three basic categories: 1) the shallow gradient apparatus, 2) the steep gradient apparatus, and 3) the fluvial systems.

Each one of these three categories contains a number of different designs. The advantages and disadvantages of these designs and an analytical description of all of them appears in Sections 2.3.1 to 2.3.3 inclusive.

Table 2.2 (page 35) shows the simplified designs of chemical avoidance apparatus. Table 2.3 (page 37) summarizes the experimental designs of chemical avoidance studies in chronological order and the type of design that each investigator used. Table 2.4 (page 39) reviews concisely the experimental designs of chemical avoidance studies reported in the literature, which employed assorted fish species exposed to a variety of chemical toxicants.

2.3.1 Shallow Gradients

2.3.1.1 Design A: "Three region horizontal gradient"

In 1913 Shelford and Allee introduced for the first time the use of shallow concentration gradients.

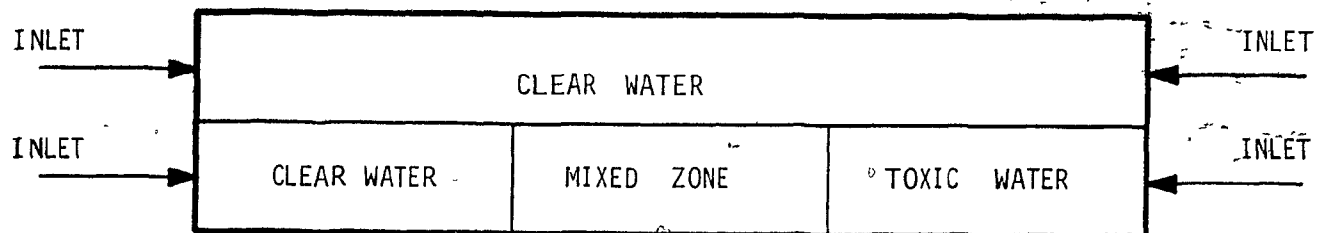


FIGURE 2.1 DESIGN A
Three region horizontal gradient
(Shelford and Allee 1913) - Plan view

Their apparatus (Fig. 2.1) consisted of two parallel boxes, each 120 cm. long, enclosed by curtains to minimize external disturbances. Toxic water entered both ends of the reference box. In the experimental box, toxic and clear water entered at opposite ends creating three relatively distinct regions: a region at each end filled with the water introduced on that side, and a central mixing zone. An experimental trial consisted of placing one fish in each trough and recording the relative position of the fish in the trough. Unequal residence time or the number of entrances into the treated water indicated avoidance of the toxicant.

Wells in 1915 modified the previous method. Additional outlets at the central drain prevented the formation of vertical gradients and a series of stopcocks was placed along the bottom of the trough to facilitate the removal of water samples.

A major disadvantage of Design A is that it allows only one fish to be tested at a time.

2.3.1.2 Design B: "Fully developed horizontal gradient"

Ishio (1964) introduced a new shallow gradient tank which only allowed the toxic gradients to form horizontally (Fig. 2.2).

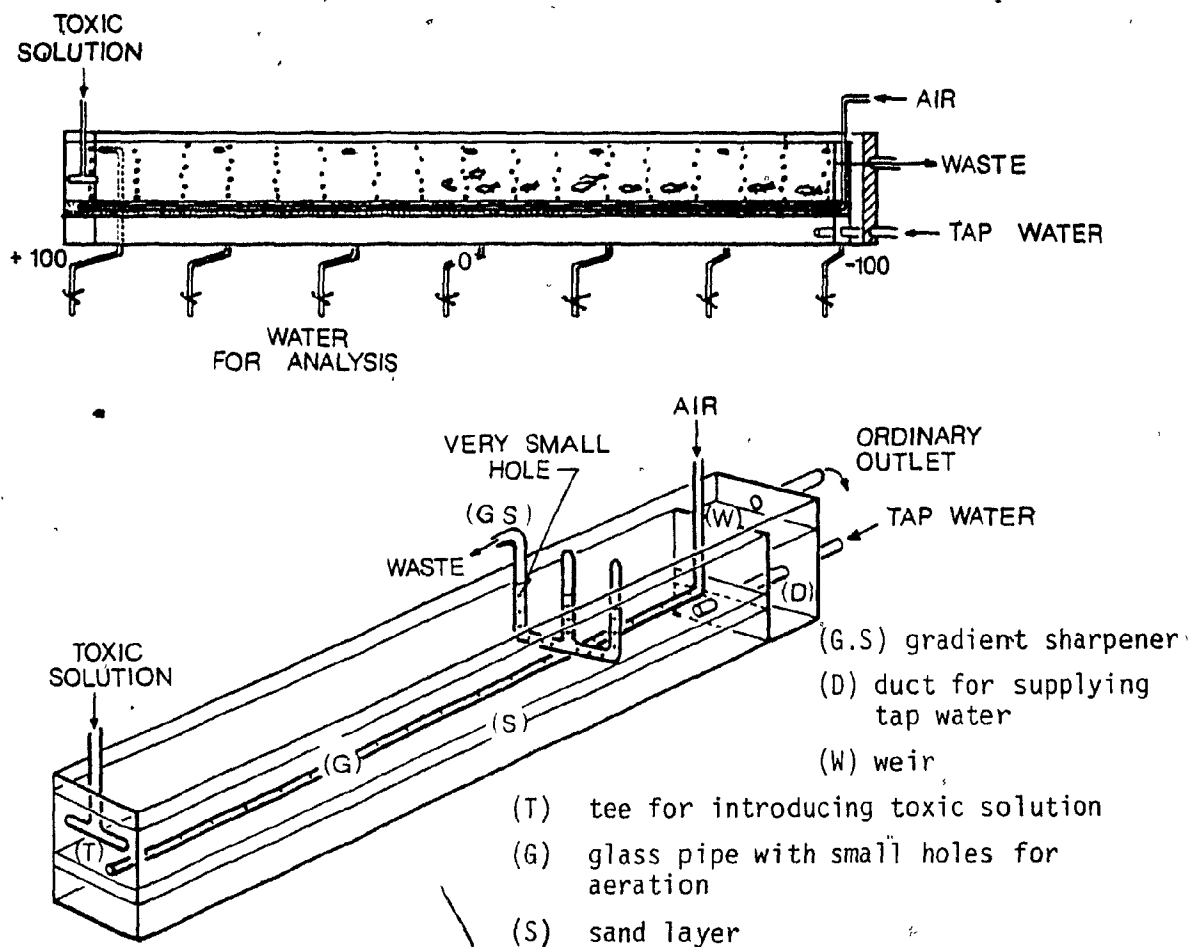


FIGURE 2.2 DESIGN B. FULLY DEVELOPED HORIZONTAL GRADIENT (ISHIO 1964)

The interior of the tank was divided into both upper and lower paths by a sand layer (S). Tap water was supplied into the lower path through a duct (D) at the right-hand end of the tank. The water in the lower path then ascended through the sand layer comparatively quickly, emerging into the upper path. It moved slowly towards a weir (W) increasing its speed and then went out of the tank. For interrupting certain surface currents, a transverse bar was inserted into the test stream at a depth of 1 cm, 1 cm from the weir. When a particular toxic solution was introduced through a tee (T) into the upper path at the opposite end of the tank, the toxic concentration gradually decreased with distance towards the weir by mixing with newly supplied tap water through the sand layer. There was also some assistance for establishing a complete horizontal gradient by the use of continuous gentle aeration through a series of small holes in the glass pipe (G) buried in the sand layer.

2.3.1.3 Design C: "Field vertical gradient"

Birtwell (1977) developed an experimental field technique to study preference and avoidance in polluted and unpolluted shallow stratified waters (Fig. 2.3).

The purpose of the apparatus was to create a vertical gradient in water columns using two components: the preference chambers and a flotation unit. The vertical preference chamber was intersected at intervals by horizontal aluminum screens whose central

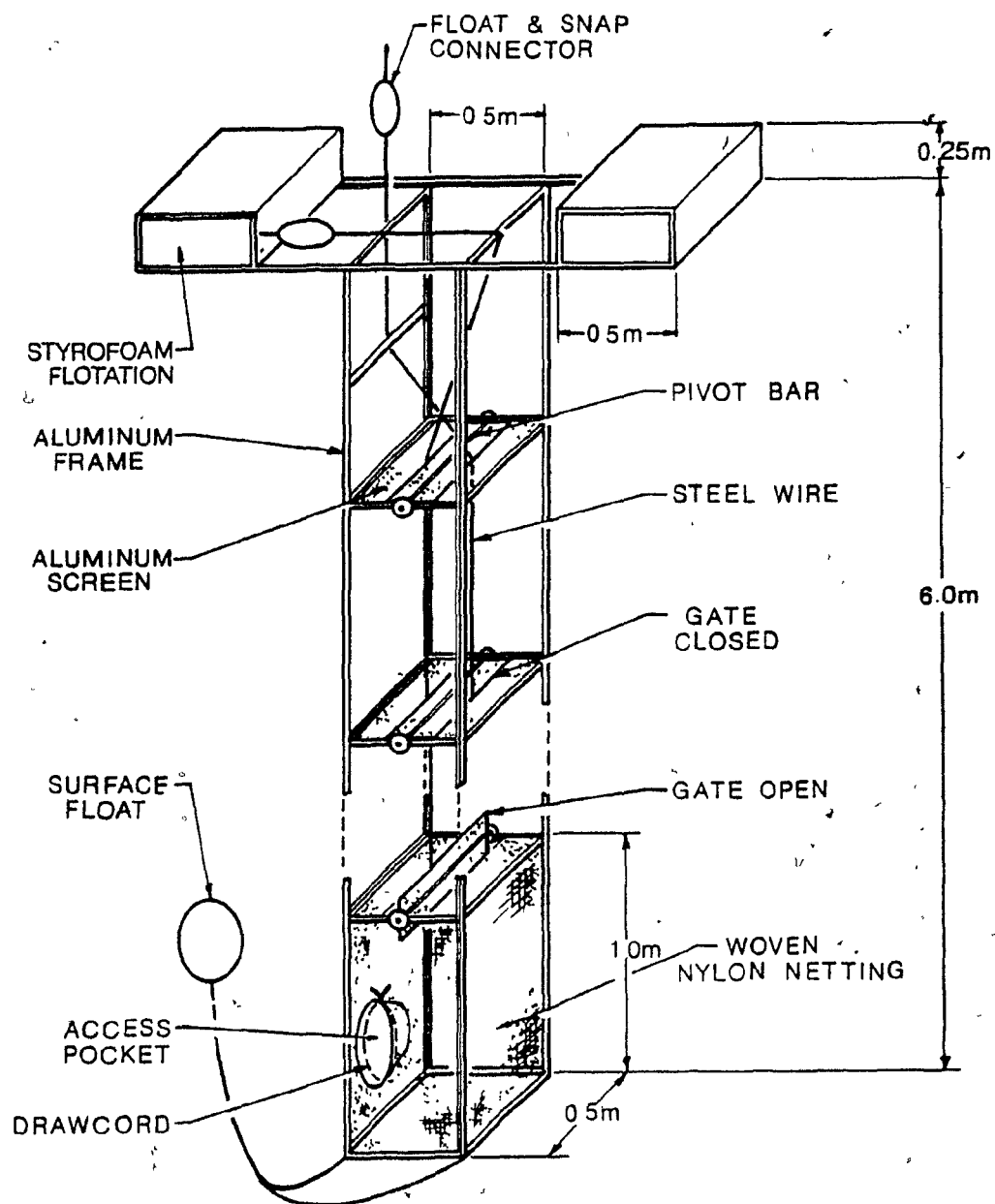


FIGURE 2.3

DESIGN C. BIRTWELL (1977)
FIELD VERTICAL GRADIENT

portions consisted of special gate mechanisms riveted onto pivot tubes passing through two sides of the screen supports. The gates were connected to each other by wires connected midway along the longer edges of each gate. Each wire terminated in a float and snap connector which allowed connection to the flotation unit and prevented movement of the gates.

The preference apparatus was moored initially to a fixed object via the flotation section and the gates between the partitions were closed. The apparatus was then drawn to the surface using a rope which was attached to its base. Thus the chambers pivoted around the flotation section so that the access pockets were uppermost. The access pockets were opened and ten fish were gently but quickly transferred into each chamber of the apparatus. The apertures on the access pockets were sealed after entry of the fish and the apparatus was gently lowered into the vertical position in the water column. The gates were opened and the opening time noted. The fish then had access to the total water column. After the test period, the gates were closed and the entire preference apparatus was raised to the water surface allowing for removal of the fish in each test chamber for analysis.

A serious limitation of this apparatus (Design C) is that it can be used only in situ for field studies.

2.3.1.4 Advantages and disadvantages of shallow gradients

A. ADVANTAGES:

1. They are relatively inexpensive.
2. Their theoretical rationale is basically simple.
3. They can be used to simulate the majority of toxic environments- a distinct advantage.

B. DISADVANTAGES

1. None of the systems are computerized.
2. It is impossible to reproduce identical gradients for repetition of experiments.
3. The threshold avoidance concentration is difficult to ascertain in a continuous gradient unless it lies directly at the sampling point.
4. Fish placed in a gradual concentration gradient may choose to remain within their initial favorable location, rather than migrating there to avoid a particular toxic concentration.
5. Most importantly, shallow gradients cannot provide distinct directional clues for fish orientation.

General comments: For all these reasons the use of shallow gradients has recently decreased.

2.3.2 Steep Gradients

2.3.2.1 Design D: "Simple and double Y maze"

Hansen in 1969 and 1972-73 and Folmar in 1976 used the single and double Y maze in which a toxicant was introduced into one Y-shaped arm and water into the other (Figures 2.4 and 2.5).

The two media then drained into a circular holding area.

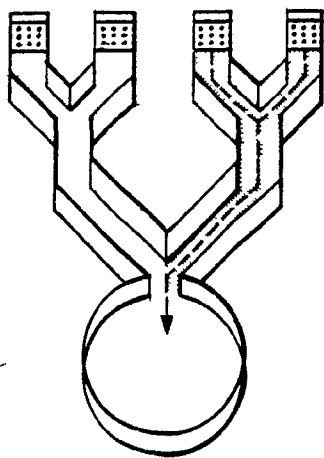


FIGURE 2.4 DESIGN D
Y MAZE
(Hansen et al 1972)

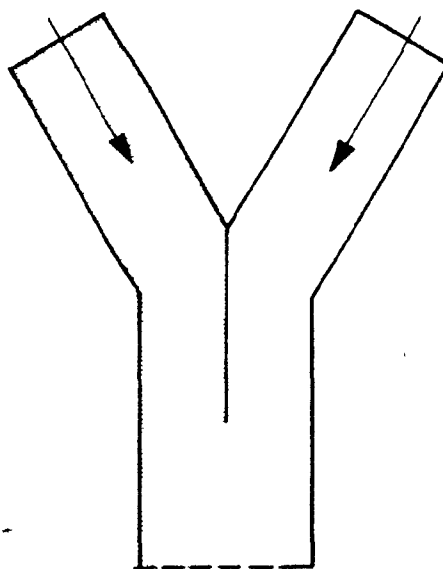


FIGURE 2.5 SIMPLIFIED DESIGN D
- Plan view

The disadvantages of Design D are:

1. Aquatic organisms may be affected by eddies and areas of no current in the holding area.
2. Toxicant concentration in the circular holding area may not be homogeneous because of turbulence.
3. Use of the apparatus has been limited to testing herbicides and pesticides.

2.3.2.2 Design E: "Counter-current tube, tank or system"

Jones in 1947 proposed an apparatus which consisted of a 58 cm plexiglass tube with inlets at both ends and an outlet through central drains (Fig. 2.6).

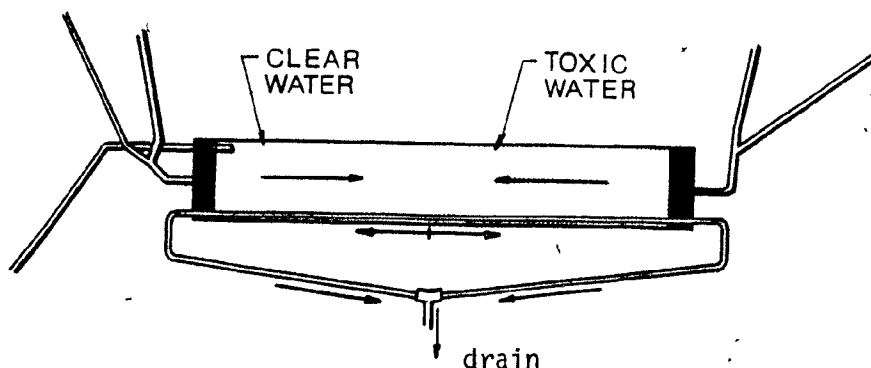


FIGURE 2.6 DESIGN E - SIDE VIEW
Counter-current tube (Jones 1947)

Untreated water was initially released into the tube at both ends and exited through a central drain. A fish was introduced at the open end of the apparatus and then monitored in the trough. A test solution was released at one end of the trough and the movement of the fish was recorded on graph paper. The above technique was used by Jones (1948, 1951, 1952) and Bishoi (1962a,b).

Sprague (1964) modified Jones' idea for studies with metal pollutants. He increased the length of the tube and added a new drainage system consisting of four central hoses which improved the separation of the two bodies of water. This system was employed by Sprague (1965, 1968), Hill (1968), Sprague and Drury (1969) and Renwoldt and Bide^{ll} (1970).

Scherer and Nowak in 1973 used a rectangular counter-current system with an automated recorder (Fig. 2.7).

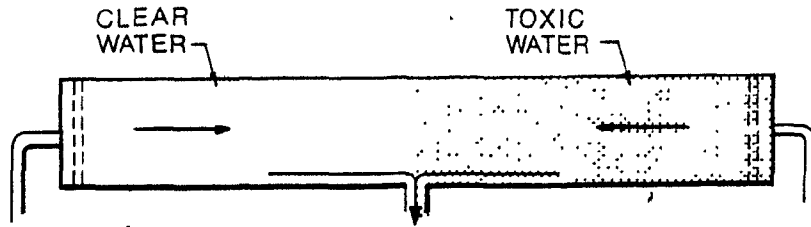


FIGURE 2.7 DESIGN E
Rectangular current stream - side view
(Scherer and Novak 1973)

Design E is characterized by the introduction of a toxicant into one end of the tube and water into the other end. The two media meet at the centre of the draining tube and create a toxicant water interface. A disadvantage of Design E is that the change in current direction at the drain may effect the rheotropic behavior of aquatic organisms by, for example, reducing the frequency of interface crossings.

A simplified Design E appears in Figure 2.8.

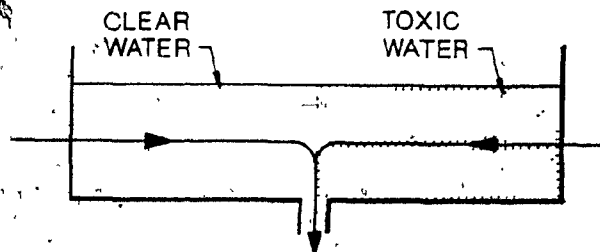


FIGURE 2.8 SIMPLIFIED DESIGN E
- side view

2.3.2.3 Design F: "Open field system"

Westlake and Lubinski (1976) designed the open field system (Fig. 2.9). The same system was used in 1977 by Sprague and Fogels with minor modifications.

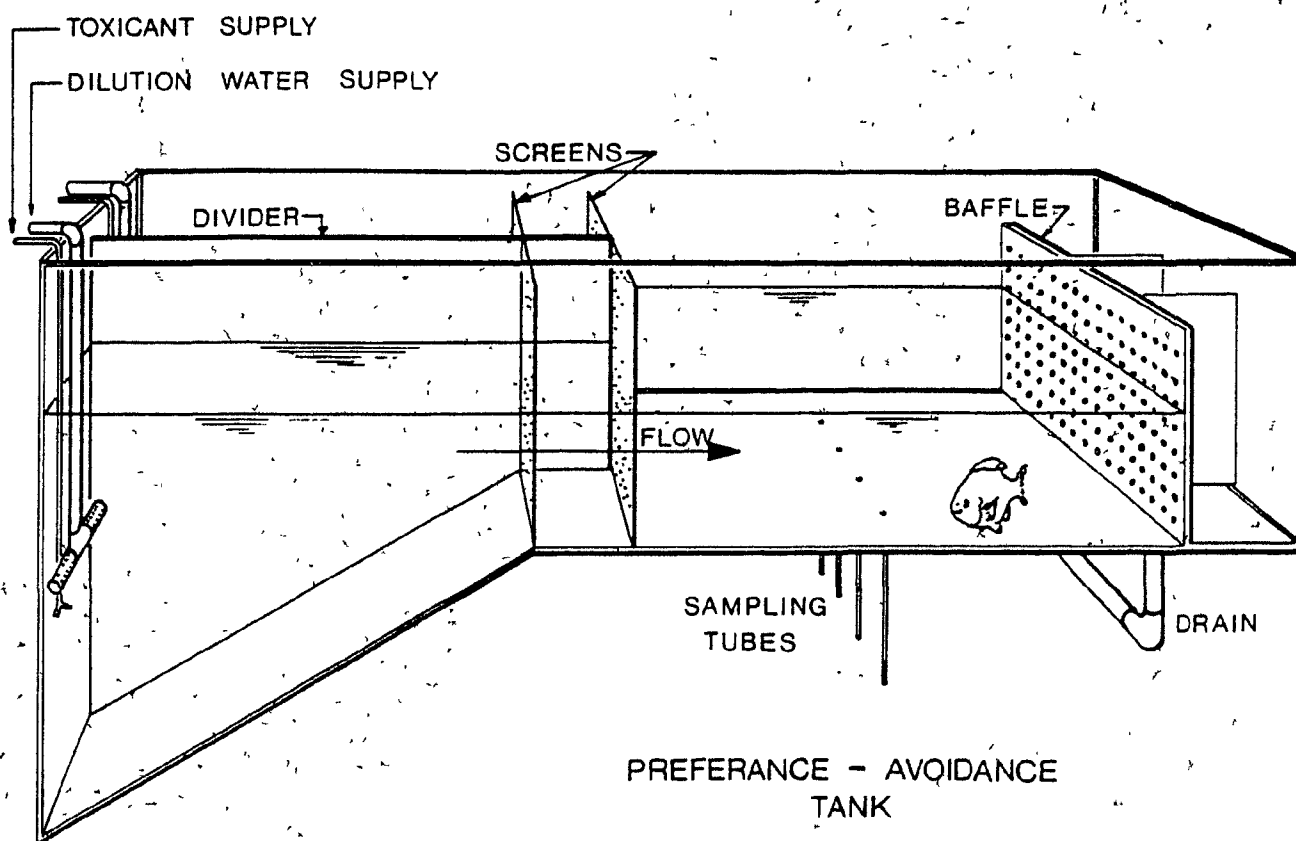


FIGURE 2.9

DESIGN F — OPEN FIELD SYSTEM (WESTLAKE AND LUBINSKI, 1976)

In the open field system the toxicant and the water flowed side-by-side in the same direction across a 50 x 50 cm observation area.

The position of the fish was recorded with respect to 16 square regions on the tank bottom. The deep end of the tank was separated into 2 equal sides by a divider. Water on either side of the divider mixed and moved towards the shallow end, increasing in velocity due to a decrease in the cross-sectional area. The entire monitoring system consisted of the preference avoidance tank, an external housing, a toxicant delivery system, and an overhead television monitor and receiver.

Recently Lubinski (1978-1980) improved the system using a television camera, video monitor, video-microcomputer interface and a teletypewriter. Fish position and activity were monitored overhead from a variable voltage video signal produced by the camera as a result of the dark image of the fish on a light background. Signals from fish activity were interfaced into the microcomputer with commands issued from the observer at the teletypewriter.

Kleerekoper (1967 and 1972) constructed a unidirectional water flow system with a large (5 x 5 x 0.5 m) open area fed by treated water. Avoidance was monitored by a series of 1,936 photocells embedded in the floor of the system on which a fish's position was measured by an interfaced colorimeter. Experiments were carried out using three consecutive 8-hour recordings of the locomotion of a single fish.

Initially, the entire system was filled with untreated water, followed by the introduction of treated water into one-half of the test system. During the third 8-hour period, treated water was added to the remaining half of the test area.

Disadvantages of Design F include the following:

- 1) It is difficult to maintain the toxicant-water separation, and
- 2) There is a problem in comparing the results of a sharp gradient in small containers with the potential responses to gradual gradients which are found in nature.

A simplified version of Design F appears in Figure 2.10.

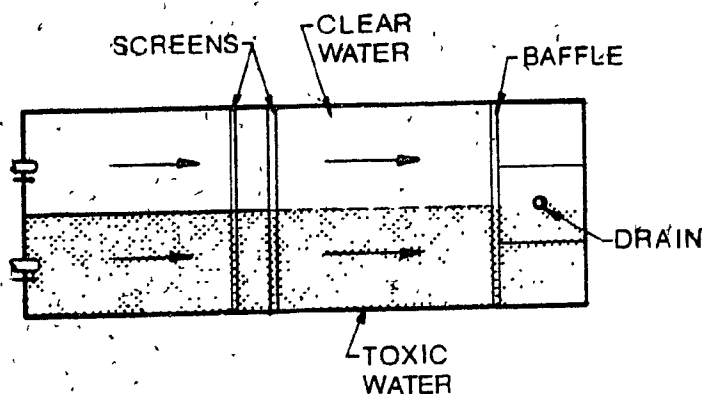


FIGURE 2.10
SIMPLIFIED DESIGN F - Plan view

2.3.2.4 Design G: "Open field small system with three regions"

Unlike the two compartments, steep gradient apparatus of Sprague (1964) and Scherer and Novak (1973) (in which fish avoiding the test water automatically select the clean reference water), Greer and Kosakoski in 1978 made the first attempt at creating a small open field system with 3 areas (Fig. 2.11). This system potentially allowed for more variability in the avoidance behavior to be measured. It was basically used to measure salinity responses.

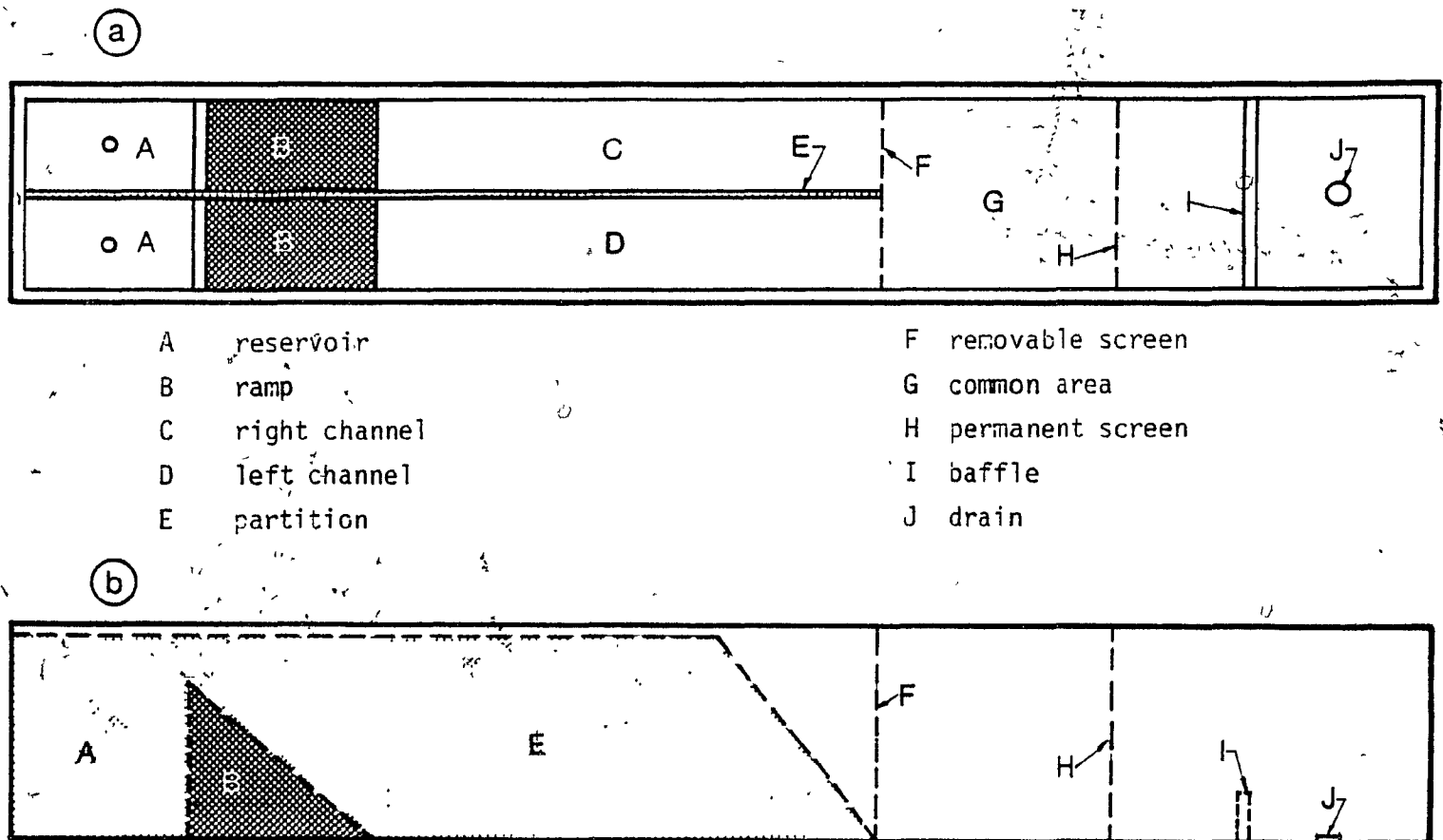


FIGURE 2.11

DESIGN G - OPEN FIELD SMALL SYSTEM WITH THREE REGIONS (GREER ET AL 1978)

(a) Plan view and (b) side view

The apparatus consisted of two reservoirs at the inlet end which led by way of two ramps into an experimental chamber. A longitudinal partition divided the chamber into two parallel channels of equal size around an undivided or common area downstream. Water from the

reservoir flowed down the ramps in two parallel streams and then through the common area to waste.

Disadvantages of Design G are:

1. It has a very small avoidance tank with a very shallow constant depth (3 cm).
2. The apparatus to date has not made provision for sophisticated analysis of the results (i.e., it is not computerized).

A simplified Design G is shown in Figure 2.12.

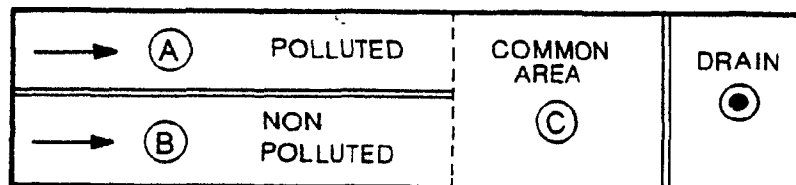


FIGURE 2.12

SIMPLIFIED DESIGN G - Plan view

2.3.2.5 Design H (new): "Flume hydraulic avoidance channel"

A detailed description of a Design H (new), employed in this study, appears in Chapter III, (page 59).

2.3.2.6 Advantages and disadvantages of steep gradients

A. ADVANTAGES:

1. The greatest advantage is that they provide the necessary clues for successful directed movements using a single toxic concentration.
2. Steep gradients represent the natural phenomenon which occurs at the confluence of two bodies of water or in the immediate vicinity of an industry's outfall.
3. Most of the steep gradient designs are partially or fully computerized.
4. Steep gradients have produced the most unbiased avoidance curves to date.
5. They can be used to study different species, chemicals and time exposures, and can make a replica of results accurately.
6. They are very easily applied in field conditions.
7. "Kineses" are apt to be more successfully executed in very steep gradients due to the proximity of favorable environmental conditions.
8. Steep gradients are the most commonly used in recent research

B. DISADVANTAGES:

1. Steep gradient conditions do not occur commonly in nature.
2. Steep gradients are more time-consuming than either shallow gradients or fluvial systems.
3. They are comparatively more expensive than the other two systems.

2.3.3 Fluvium Systems

2.3.3.1 Design I: "Basic fluvium"

Hoglund in 1951-53 attempted to integrate both the shallow and the steep gradient methods into a "Fluvium", now often referred to as the "Basic fluvium" (Fig. 2.13).

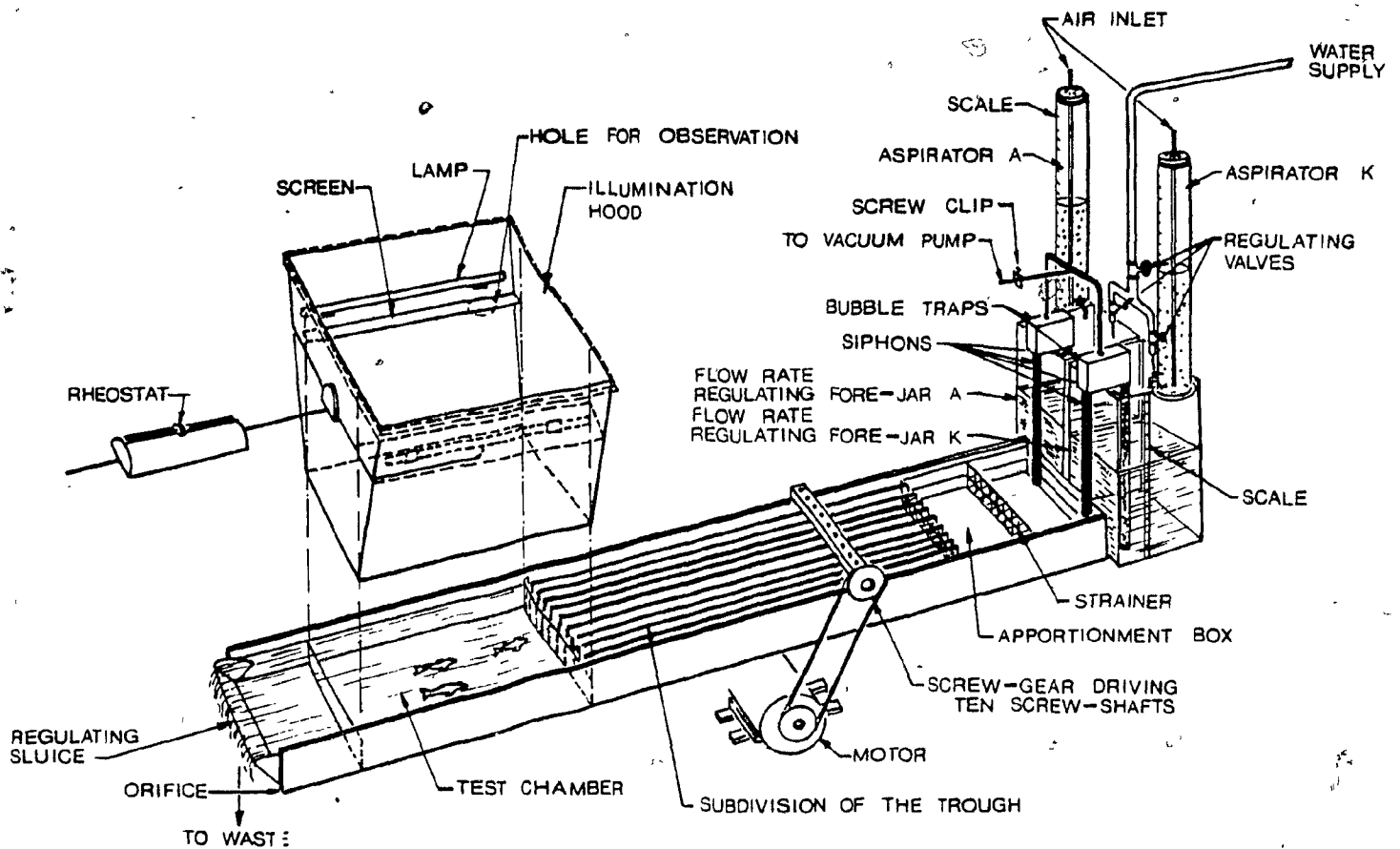


FIGURE 2.13
DESIGN I - GENERAL SCHEME OF THE FLUVIARIUM

The basic principle of the fluvium system was to permit fish to select the most favourable conditions. The apparatus was composed of a supply system, a tap water supply, two removable aspirators containing the test solution, two flow-rate regulating fore-jars and a trough. Into the trough was placed an apportionment box, a central section of nine vertical glass plates creating ten longitudinal sections and a test chamber to house the fish. An illumination hood above the test chamber functioned as a sheltering cover, prevented disturbances and housed an overhead recording camera.

With different diaphragm plates a series of gradient curves was produced after the calculation of the standard gradient curve for the plate. A motor driven system varies the angle of the plates. The subdivided trough preceding the test chamber, employs the steep gradient principle with the actual unpartitioned test chamber (typical of shallow gradient apparatus). The steep gradient conditions are largely maintained because of the relatively short length of the test section of the apparatus.

The total number of appearances in each concentration was presented by means of histograms, showing which sections were least frequented or avoided.

A simplified Design I appears in Figure 2.14.

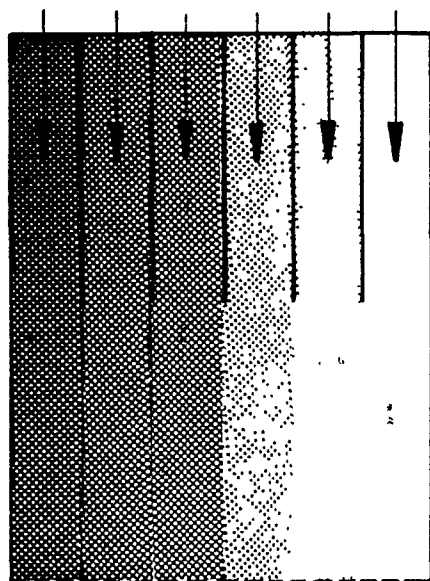
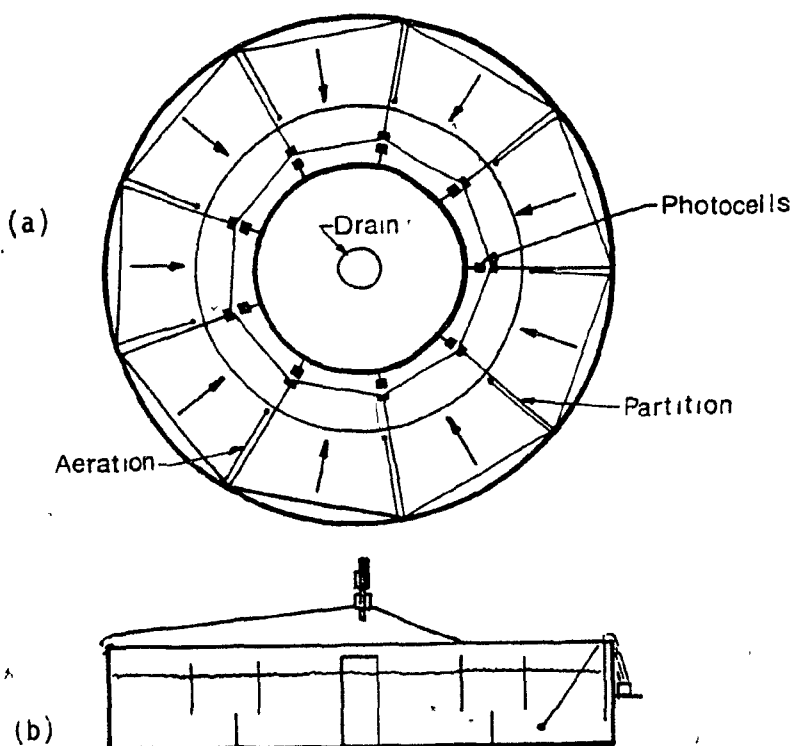


FIGURE 2.14 SIMPLIFIED DESIGN I
Plan View

2.3.3.2 Design J: "Rosette" gradient

Kleerekoper and Mogensen in 1963 introduced a circular fluvium system with the drainage at the centre. In this design a central introduction chamber was surrounded by a number of peripheral compartments. Small photocells were installed at the entrance to each compartment. Eight (8) different concentrations of a specific pollutant were introduced and the time spent by the fish in each compartment was recorded.

The same design, with slight modifications and described as a radial or "rosette" device, was used by McLean and Fry, University of Toronto, for temperature preference avoidance studies (Fig. 2.15).



SCALE 1:20

FIGURE 2.15 DESIGN J

A schematic diagram of a "rosette" gradient device (McLean & F.E.J.Fry, University of Toronto - unpublished paper)

(a) plan view (b) side view

A simplified Design J is shown in Figure 2.16.

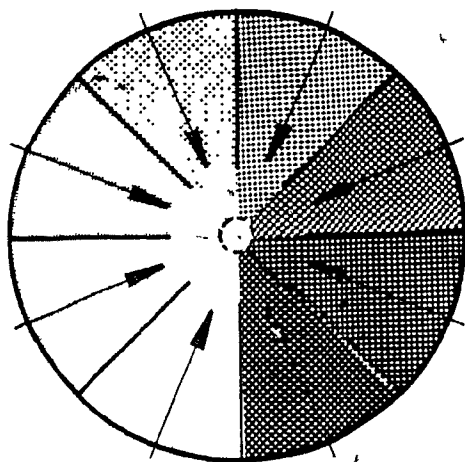


FIGURE 2.16 SIMPLIFIED DESIGN J
Plan View

2.3.3.3 Design K: "Two channel fluvium"

A two-channel fluvium system for avoidance studies for several heavy metals and organic compounds was introduced by Black and Birge (1980) (Figure 2.17).

A carbon-filtered water source was fed through a pressure regulator into a constant head-flow regulator and released as control water into one side of the fluvium (control channel). From a toxicant reservoir, a peristaltic pump delivered the toxicant into a mixing chamber which sent a selected concentration into the test chamber of the fluvium. At the base of each channel in the decision area, fish residence was monitored relative to location in treated and untreated water.

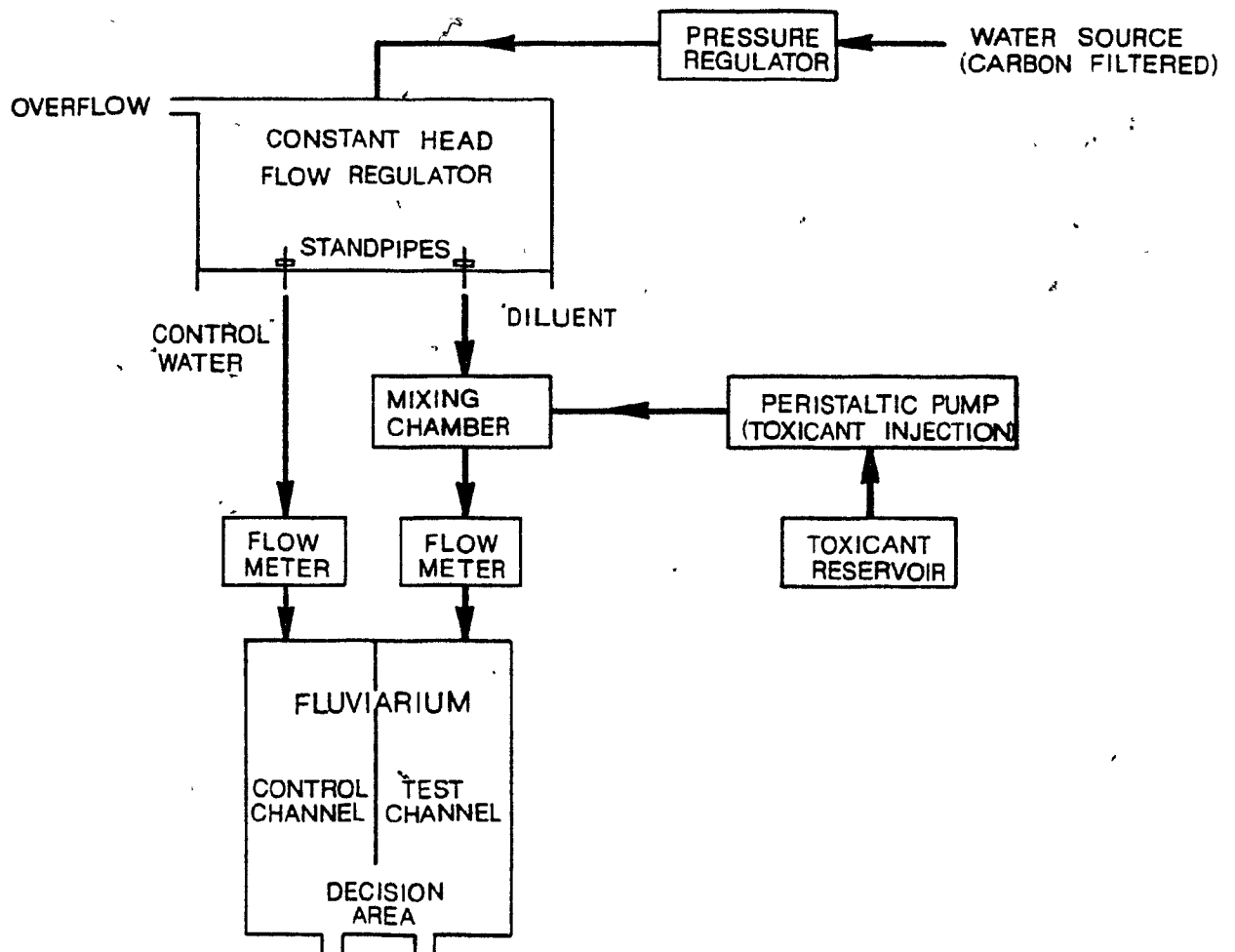


FIGURE 2.17

DESIGN K - "TWO CHANNEL FLUVIARIUM" (Black and Birge 1980)
(Schematic representation)

- A simplified Design K appears in the Figure 2.18.

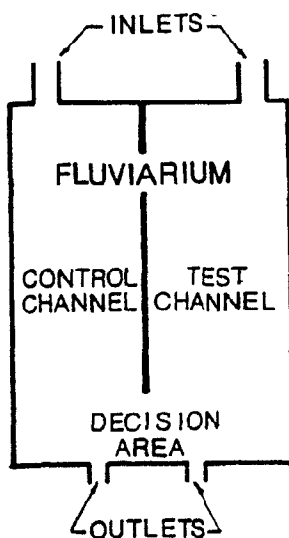


FIGURE 2.18
SIMPLIFIED DESIGN K - Plan view

2.3.3.4 Advantages and disadvantages of fluvium systems

A. ADVANTAGES:

1. They combine shallow and steep gradients in an effort to give a more integrated approach to natural phenomena.
2. They are not time consuming.
3. They are useful for studying specific effluents.

B. DISADVANTAGES:

1. The exposure of a fish to a gradient of several concentrations may alter its behavioral response to low effluent concentrations because of the possible

deleterious effects of initial exposure to higher toxic levels during the same test.

2. Fluvium systems do not provide a distinct and constant separation between the water and the effluent.
3. It is difficult to duplicate previous results with accuracy.

2.4. Trends and Needs for New Designs

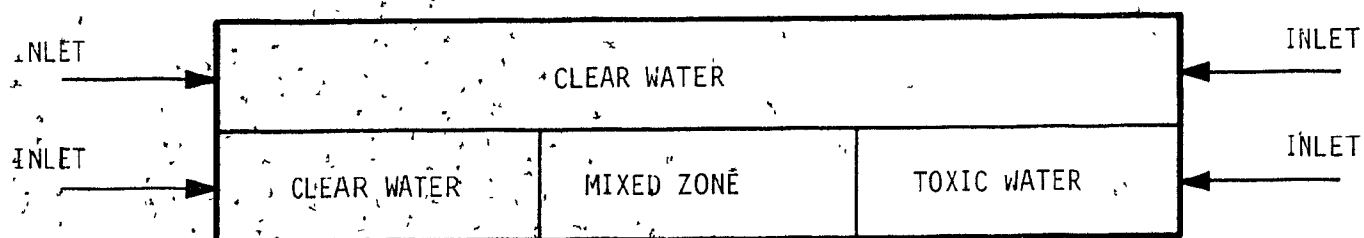
2.4.1 Trends

1. To increase the number of test organisms (i.e., from 1 or 5 to 50 or 100).
2. To use actual effluent in the testing rather than purified chemical components.
3. To store the results for re-examination.
4. To define the pattern of the toxicant flow using dyes and to relate the toxic concentrations found by analysis to the position of test organisms.
5. To separate the toxicant from the unpolluted water.
6. To use steep gradient designs rather than shallow or fluvium systems.

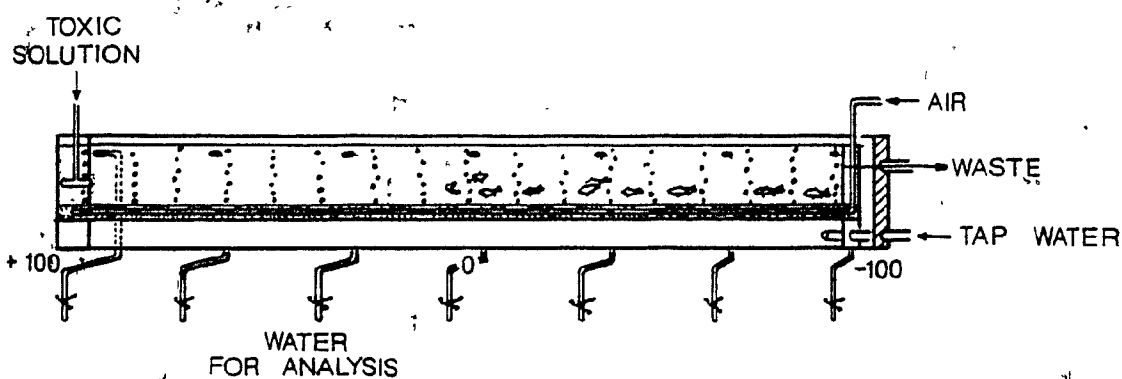
2.4.2 Needs

1. To have vertical, horizontal and lateral analysis of the organisms' position.
2. To use advanced techniques for analysis of data.
3. To introduce additional physical and engineering parameters (e.g., injection systems, hydraulics, etc.).
4. To use a larger tank to eliminate end effects and to simulate better the natural system.

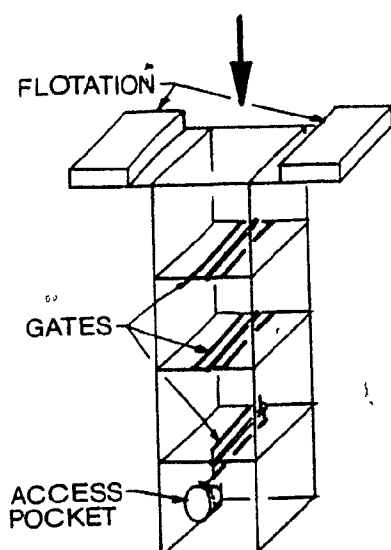
TABLE 2.2 Simplified designs of chemical avoidance studies



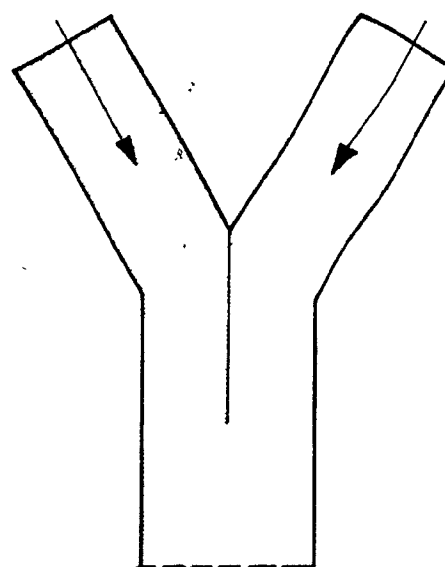
SIMPLIFIED DESIGN A - Plan view



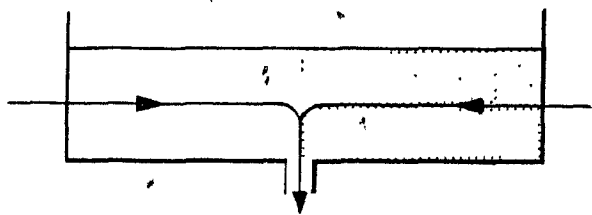
SIMPLIFIED DESIGN B - Side view



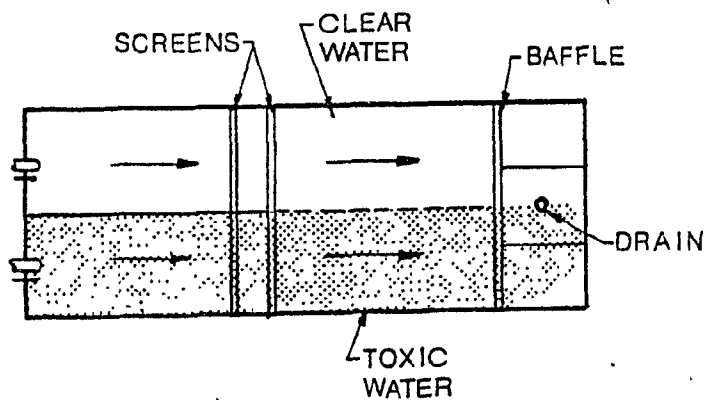
SIMPLIFIED DESIGN C - Side view



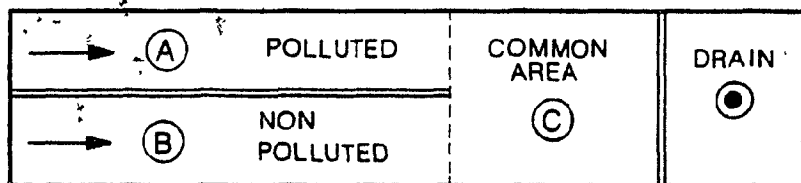
SIMPLIFIED DESIGN D - plan view



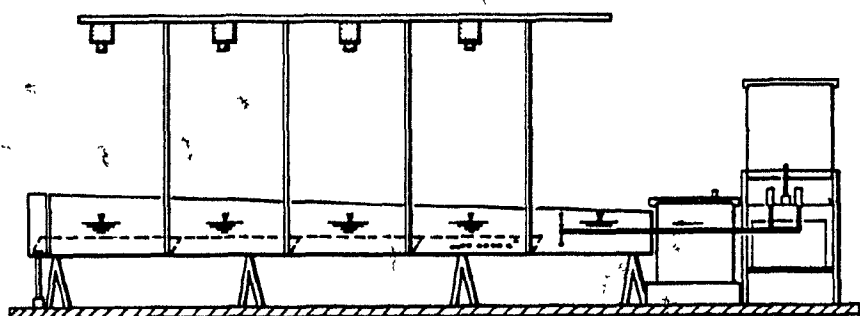
SIMPLIFIED DESIGN E - Side view



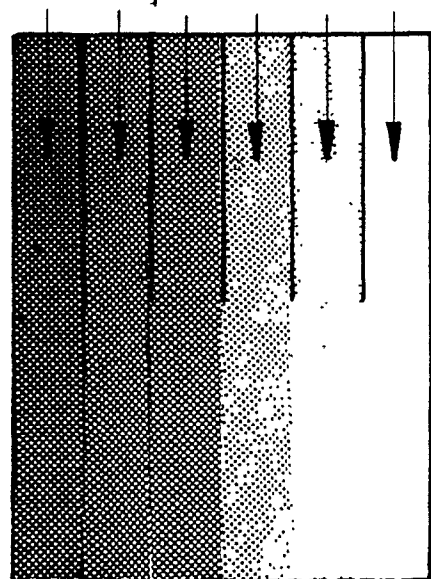
SIMPLIFIED DESIGN F - Plan view



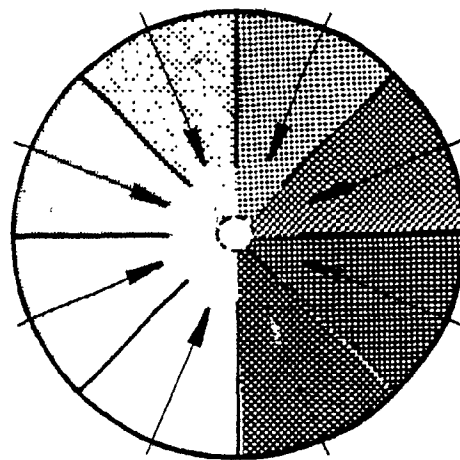
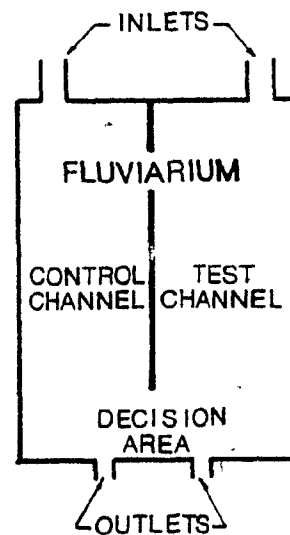
SIMPLIFIED DESIGN G - Plan view



SIMPLIFIED DESIGN H - Side view



SIMPLIFIED DESIGN I - Plan view

SIMPLIFIED DESIGN J
- Plan viewSIMPLIFIED DESIGN K
- Plan view

	<u>Author</u>	<u>Year</u>	<u>Type of Design</u>
1.	Shelford and Allee	1913-14	1-DES A
2.	Shelford and Powers	1915	1-DES A
3.	Wells	1915	1-DES A
4.	Jones	1947	2-DES E
5.	Jones	1948	2-DES E
6.	Jones	1951-52	2-DES E
7.	Hoglund	1951-53	3-DES I
8.	Lindahl and Marcstrom	1958	-
9.	Whitmore et al	1960	2-DES E
10.	Hoglund	1961	3-DES I
11.	Bishai	1962	2-DES E
12.	Behread and Betherman	1963	-
13.	Kleerekoper and Mogensen	1963	3-DES J
14.	Ishio	1964	1-DES B
15.	Sprague	1964	2-DES E
16.	Sprague et al	1965	2-DES E
17.	Warner et al	1966	-
18.	Costa	1966	2-DES D
19.	Summerfelt and Lewis	1967	1-DES B
20.	Hill	1968	2-DES E
21.	Sprague	1968	2-DES E
22.	Hansen	1969	2-DES D
23.	Sprague and Drury	1969	2-DES E
24.	Hoglund and Hardig	1969	3-DES J
25.	Anderson and Prins	1970	-
26.	Rehwoldt and Bida	1970	2-DES E
27.	Kleerekoper et al	1970	2-DES F
28.	Weir and Hine	1970	-

	<u>Author</u>	<u>Year</u>	<u>Type of Design</u>
29.	Hansen	1972	2-DES D
30.	Harfield and Johansen	1972	-
31.	Kleerekoper	1972	2-DES F
32.	Hansen et al	1973	2-DES D
33.	Scherer and Novak	1973	2-DES E
34.	Wilson	1973	3-DES K
35.	Kynard	1974	2-DES E
36.	Scherer	1975	2-DES E
37.	Westlake and Lubinski	1976	2-DES F
38.	Muller and Fry	1976	2-DES J
39.	Fava and Isai	1976	-
40.	Folmar	1976	2-DES D
41.	Maciorowski and Benfield	1976	-
42.	Lewis and Livingston	1977	2-DES
43.	Scherer	1977	2-DES E
44.	Birtwell	1977	1-DES C
45.	Reynolds, W.	1977	-
46.	Greer and Kosakoski	1978	2-DES G
47.	Black and Birge	1980	3-DES K
48.	Lubinski	1980	2-DES F
49.	Hadjinicolaou and Spraggs	1981	2-DES H
50.	Spraggs, Gehr and Hadjinicolaou	1982	2-DES H
51.	Hadjinicolaou and Spraggs	1982	2-DES H

Key words: SH.GR = Shallow gradient = 1
 ST.GR = Steep gradient = 2
 FL.SY. = Fluvium system = 3
 DES = Design

TABLE 2.3. Experimental designs of chemical avoidance studies in chronological order

CHEMICALFISH SPECIESREFERENCEShallow Gradients

CO₂, O₂, N₂, acetic acid, NH₄

Abramis crysoleucus, Ambloplites rupestris,
Ameiurus melas, Catostomus commersoni,
Etheostoma coeruleum, Etheostoma zonale, Hybopsis
kentuckiensis, Lepomis cyanellus, Micropterus
dolomieu, Notropis atherinoides, Notropis
cronutus, Umbra limi

Shelford & Allee (1913, 1914)

H₂S, salinity, alkalinity,
acidity

Clupea pallasii, Lepidapsetta bilineata,
Oligocottus maculosus

Shelford & Powers (1915)

H₂CO₃, H₂SO₄, NH₄OH, Na₂CO₃
H₂CO₃, CuCl₂, NH₄OH, OH⁻

Ameiurus melas, Lepomis pallidus, Pomoxis annularis
Achellognathus limbata, Carassius auratus,
Cyprinus carpio, Gnathopagan gracilis, Lebistes
reticulatus, Lepomis macrochirus, Moroco
steinduchneri, Pimephales promelas, Pungtungia
herzi, Sarcocheilichthys variegatus, Tribolodon
hakonensis, Zucco platypus
Lepomis cyanellus

Wells (1915)

Ishio (1964)

ACP, chlordane, 1-4 dichloro-2-
Nitrobenzene, isobornyl
thiocyanoacetate

Bleached kraft
with effluent

Oncorhynchus keta
Oncorhynchus tshawytscha
Clupea harengus pallasii

Summerfelt & Lewis (1967)

Birtwell (1977)

CHEMICALFISH SPECIESREFERENCESteep Gradients

Alcohol, chloroform, formalin, mercuric-chloride, copper-sulphate, zinc-sulphate	<u>Pygosteus pungitius</u>	Jones (1947)
Calcium nitrate, sodium-sulphide, lead-nitrate zinc sulphate	<u>Gasterosteus aculeatus</u> , <u>Phoxinus phoxinus</u>	Jones (1948)
Phenol, para-cresol, ortho-cresol, O ₂	<u>P. phoxinus</u> , <u>G. aculeatus</u> , <u>Salmo trutta</u>	Jones (1951, 1952)
Sulfate waste, sulfite waste O ₂ , Copper sulfate, zinc sulfate ABS, BKME, phenol, chlorine	<u>Oncorhynchus kisutch</u> , <u>O. tshawytscha</u> , <u>Salmo gairdneri</u> <u>S. salar</u> , <u>S. trutta</u> <u>S. gairdneri</u> , <u>S. salar</u>	Jones et al (1956) Bishai (1962a,b), Sprague (1964, 1968); Sprague et al (1965) Sprague & Drury (1969) Hill (1968) Hansen (1969, 1972)
O ₂ DDT, endrin, dursban malathion, sevin, 2.4-D	<u>Chologaster agassizii</u> <u>Cyprinodon variegatus</u> , <u>Gambusia affinis</u>	
CuCl ₂	<u>Carassius auratus</u>	Kleerekoper et al (1973)
Copper ions	<u>Carassius auratus</u>	Kleerekoper and Westlake (1970)
Cr, Zn, Cd O ₂	<u>Roccus lineatus</u> <u>Lepomis macrochirus</u> , <u>Micropterus salmoides</u> , <u>O. kisutch</u> , <u>O. tshawytscha</u>	Rehboldt & Bida (1970) Whitmore et al (1960)
HgCl ₂ , fenitrothion	<u>C. auratus</u>	Scherer & Novak (1973); Scherer (1975)
DDT, toxaphene, endrin, parathion	<u>G. affinis</u>	Kynard (1974)
TRC, FRC, CRC	<u>Rhinichthys atratulus</u>	Fava & Tsai (1976)

CHEMICAL

Ammonia
nitrogen
BKME
Salinity
Bleached kraft mill
effluent

Ammonia
chloride
DSS
Monomers-Polymers
Plating industry
Ni, Cr, Fe

O₂, nickel nitrate, ferric
nitrate
2.4.6-trinitrophenol
Sulphite waste liquor, HCl.
O₂, NaOH, NaCl, pH, CO₂

Oil dispersants

FISH SPECIES

Lepomis
macrochirus
Lagodon rhomboides, Fundulus grandis
Oncorhynchus
gorbuscha

Lepomis
macrochirus
Salmo
gairdneri
Salmo
gairdneri

Fluvarium Methods

Leuciscus rutilus
L. rutilus
Coregonus nasus, Esox lucius, Gasterosteus aculeatus
Leuciscus idvarus, L. rutilus, Perca fluviatilis
Salmo alpinus, S. solar, S. trutta, Salvelinus fontinalis,
Tinca tinca
Herring and plaice larvae

REFERENCE

Westlake and
Lubinski (1976)
Lewis & Livingston (1977)
Greer and
Kosakowski (1978)

Lubinski (1980)

Hadjinicolaou,
Spraggs, Gehr (1981)

Hadjinicolaou,
Spraggs (1982)

Höglund (1951)

Lindahl & Marcstrom (1958)

Höglund (1951, 1961)

Höglund & Hardig (1969)

Wilson (1973)

<u>CHEMICAL</u>	<u>FISH SPECIES</u>	<u>REFERENCE</u>
Cadmium, copper, mercury, zinc, chloroform, dioctyl phthalate, trisodium nitrilotriacetic acid, phenol	<u>L. macrochirus</u> , <u>S. gairdneri</u> , <u>M. salmoides</u>	Black & Birge (1980)
	<u>Time (Response-Shock) Interval</u>	
As, Hg, Ph, Sc, DDT, methoxychlor, sumithion abate	<u>C. auratus</u>	Anderson & Prins (1970); Hatfield & Johansen (1972); Weir & Hine (1970); Warner et al (1966); Behread & Bitterman (1963)
2,4-dichlorophenoxyacetic acid (2,4-D amine)	<u>C. auratus</u>	Rand & Barthalmus (1980)

(modified after D. Cherry and J. Cairns, Jr. 1982 - Biological monitoring Part V)

TABLE 2.4 Review of the experimental designs of chemical avoidance studies
of fish exposed to various chemicals

2.5 Review of results of previous toxicity tests

2.5.1 Introduction

Bioassays used for testing water contaminated by effluent discharges may be classified according to:

- (1) Outcome:
 - a) lethal
 - b) sublethal
- (2) Duration of exposure:
 - a) acute
 - b) subacute
 - c) subchronic
 - d) chronic
 - e) long-term
- (3) Type of flow system used:
 - a) static
 - b) recirculation
 - c) flow-through or dynamic

Commonly, lethal tests are static bioassays whereas sublethal tests may be either static or dynamic. However, regardless of the type of assay used, some form of comparative evaluation scale is necessary in order to derive safe level concentrations for application to a particular chemical or effluent. Such a scale would hopefully permit existing and future data from different types of assays to be combined and ranked according to a "toxicity classification" such as the following:

<u>Toxicity Classes</u>	<u>(mg/l)</u> <u>lowest lethal toxicity</u>
A. extremely toxic	< 0.01
B. very highly toxic	0.011-0.1
C. highly toxic	0.11-1.0
D. moderately highly toxic	1.1-10
E. moderately toxic	10.1-100
F. fairly toxic	101-1000
G. slightly toxic	1000-10000
H. practically nontoxic	10000-50000
I. relatively harmless	> 50000

With the recent development of new tests and techniques, the amount of information available has increased, especially concerning sublethal effects, thereby contributing to a better understanding of the toxic effects of chemicals and effluents on aquatic life.

Although a systematic procedure for evaluating the toxicity of chemicals (especially new compounds) at both lethal and sublethal concentrations is lacking, the present study provides certain indications of the direction towards which future research must be directed.

2.5.2 Lethal Levels

2.5.2.1 Lethal Threshold Concentration

Threshold concentration is the concentration at which a particular lethal or sublethal effect is first noted. Lethal threshold is the concentration at which the first lethal effect appears. The threshold concentration alone is a biased measurement, especially in static tests, because the individual test organism's resistance will influence the results. For example, in a static test which uses 10 fish, the one which is least resistant or "healthy" is likely to die first. The lethal threshold concentration is usually noticeable in the data. The lethal threshold concentration, if it is demonstrated, should be reported to permit comparison. Absence of any apparent threshold is equally noteworthy.

2.5.2.2 Acute Static LC50

A relatively short-term lethal effect (acute) is usually defined as one occurring within four days for fish, and within a shorter time

for smaller organisms. The lethal concentration (L.C.) is the concentration which will produce death in the test organisms. This is usually defined as the median (50%) lethal concentration which is the concentration which kills 50% of exposed organisms at a specific time of observation; for example, 24 hr. LC50, 48 hr. LC50, 96 hr. LC50, etc. It has been described in the literature as a numerical value of toxicity (Cairns, J. Jr., 1982) and it is in fact the only legally recognized value used in managing Canadian environmental schemes. The LC50 is not a valid measure of toxicity for a given product or effluent. Instead, it is a range of concentrations where a toxicant may kill under a given set of artificial test conditions. Comparing LC50's for various products will yield information on the relative toxicity of products to a variety of test species, the higher the LC50, the lower the toxicity (Sprague 1970).

2.5.2.3 Median Tolerance Limit - Asymptotic LC50

The median tolerance limit (TL_m) is the test material concentration at which 50% of test organisms survive for a specific exposure time. This term has been superseded by the LC50.

(ex: $TL_m 90 = LC10$, $TL_m 10 = LC90$, $TL_m 50 = LC50$).

The asymptotic LC50 is the toxicant concentration at which LC50 becomes a constant for a prolonged exposure time, i.e., the concentration at which acute toxicity ceases, and 50% of the population can live for an indefinite period of time (more accurately referred to as the Incipient Lethal Level).

2.5. Avoidance Reactions

2.5.3.1 Effective Concentration (E.C.)

Effective concentration (E.C.) is the concentration which produces a specific response (e.g., altered respiration rate, loss of equilibrium,

avoidance reaction) in a given time, i.e., the concentration at which effects other than lethality are noted. As for lethal tests, the effective concentration usually is defined as the median concentration (EC50) at which 50% of the exposed organisms are affected by the toxicant. The most common exposure time is 96 hr. (96 hr EC50). The EC50 values and the tests from which they are obtained are not legally recognized. When testing to determine the toxicity of a product the EC50 can be used to support the results of an acute toxicity test.

2.5.3.2 Avoidance Reaction Evaluation

The avoidance/preference tests are dynamic sublethal tests. The evaluation of the avoidance reaction can be done by knowing at any time the position of the fish - horizontally, vertically and laterally in relation to the position of the pollutant. The horizontal distribution, the vertical distribution and the avoidance curve, illustrate the results horizontally, vertically and laterally, respectively. The avoidance reaction representation contributes additional information related to the stability of the responses with time and to their variation around the mean value. Also from the avoidance curve the avoidance threshold can be derived, defined as the intersection of the avoidance curve with the 50% line (% of time spent in clear water).

2.5.4 Evaluation and Prediction of Safe Toxicant Levels

The 48 and 96 hr. LC50 values are useful measures of relative acute lethal toxicity to organisms under specified conditions. These values do not represent safe concentrations in natural habitats. Long-term exposure

to much lower concentration may be lethal to fish and other organisms and may cause sublethal impairment of their function. The possibility of synergism and antagonism and other multiple effects of multiple toxicants must be considered. To evaluate and predict safe toxic levels for particular chemicals or effluents, information is needed both at lethal and sublethal levels from all exposure and flow classes. Substantial data on long term effects and safe levels are available for only a few toxicants. Information is being gathered on reproduction, growth, performance, avoidance reaction and social behavior of fish. Also important is the sensitivity of organisms at various life stages. Many organisms are more sensitive in the larval, nymphal, molting or fry stages; some are most sensitive in the egg and sperm stage.

Four different parameters have been introduced so far for the evaluation of safe toxicant levels. These are:

- (1) survival time
- (2) maximum allowable toxicant concentration
- (3) safe concentration, and
- (4) application factor.

2.5.4.1 Survival Time (ST)

Survival time is the time that a particular species can live after being exposed to a certain pollutant concentration. Survival time varies with concentration, and this makes the applicability of the parameter impractical most often. However, survival time can be a very useful indicator in particular cases, especially when a certain chemical or effluent is discharged during a fixed time period, at a constant rate.

2.5.4.2 Maximum Allowable Toxicant Concentration (MATC)

MATC is the concentration of toxic waste which may be present in the receiving water without causing significant harm to productivity as well as all water uses.

MATC is determined by long-term tests of either a partial life cycle at sensitive life stages or a full life cycle of test organism. Acceptable concentrations of toxicants to which organisms are exposed continually must be lower than the higher concentrations that may be reached occasionally but briefly, without causing damage.

2.5.4.3 Safe Concentration (SC)

Safe concentration is the maximum concentration of a toxicant that has no observable harmful effects after long-term exposure over one or more generations. It is the best indicator for toxicological safety and is the most strict criterion for toxicity management (Sprague 1971).

Normally, safe concentration is determined taking into account every small sublethal effect which has been reported in the literature. Recently the advanced techniques used in sublethal testing have produced "safer" safe concentrations and more strict effluent limit discharges. The distinction between S.C and MATC exists because what is allowed is not always safe. The MATC is based on short-time concentrations and the safe concentration belongs to a more restricted range for continuous exposure. Both parameters are useful.

2.5.4.4. Application Factor

This is a fractional factor applied to acute toxicity tests to estimate the toxicant concentration that may be safe for chronic or lifetime exposure of test organisms. An application factor is unitless

and is calculated as the ratio of MATC to the 96 hour (or incipient) LC50. Sometimes safe concentration (S.C.) is used instead of MATC,

$$A.F. = \frac{MATC}{\text{inc. or 96 hr. LC50}} \text{ or } A.F. = \frac{SC}{\text{inc. or 96 hr. LC50}}$$

For each toxicant there is obviously a numerical value for the ratio of the safe concentration to the acutely lethal concentration. This "safe-to-lethal ratio" is often an expedient suggesting that a level of protection will be offered without necessary experimental work.

For most toxicants, safe levels have not been determined and must be arrived at by some empirical and often unreliable mathematical methods.

In the past, the assumption that application factors are similar for related groups of chemicals led to disappointing results. Ideally, an application factor has to be determined for each waste material in question. To do this, it is necessary first to determine the lethal concentration of the toxicant, and then to define the safe concentration of the same toxicant for the same species at various physiological states and under the same environmental conditions. This necessitates extensive research into physiological, biochemical and behavioral effects, and the study of growth, reproduction, avoidance and other sublethal effects in both the laboratory and the field.

Table 2.5 shows the application factors for a number of potential aquatic pollutants determined experimentally by Environmental Protection Agency in 1973, and given in the well known "Blue Book" of Water Quality Criteria. Because determination of the safe levels of pollutants is an extremely involved process, interim procedures for estimating tolerable concentrations of various wastes have been used.

Material	Species of Animal	Safe-to-lethal Ratio
LAS (linear alkylate sulfate)	Fathead minnow (<u>Pimephales promelas</u>)	Between 0.14 and 0.28 (= about 0.21)
Chlorine	Fathead minnow	0.16
	<u>Gammarus</u>	0.16
Sulfides	Fathead minnow and white sucker (<u>Catostomus commersoni</u>)	± 0.1
	Walleye pike (<u>Stizostedion vitreum v.</u>)	± 0.22
Copper	Several species of fish	close to 0.1
Trivalent Chromium	Fathead minnow	0.037
Hexavalent Chromium	Fathead minnow	0.03
	Brook trout (<u>Salvelinus fontinalis</u>)	0.012
	Rainbow trout (<u>Salmo gairdneri</u>)	0.04
Malathion	Fathead minnow and bluegill (<u>Lepomis macrochirus</u>)	0.03
Carbaryl	Fish species	0.02
Nickel	Fathead minnow	0.02
Lead	Rainbow and Brook trout	< 0.02
Zinc	Fathead minnow	0.005

TABLE 2.5 Ratios between the safe concentration and the lethal concentration which have been determined experimentally for potential aquatic pollutants (EPA 1973).

To meet this situation, in 1973, three universal application factors were selected on the basis of knowledge, experience and judgment at that time:

- (a) 0.1 of 96 hr. LC50 for nonpersistent and non-cumulative toxic materials;
- (b) 0.05 24 hr. LC50 for nonpersistent and non-cumulative toxic materials, or
96 hr. LC50 for persistent and non-cumulative toxic materials;
- (c) 0.01 96 hr. LC50 for persistent and cumulative toxic materials.

For mixtures of two or more toxicants basically the premise is used that their lethal actions are additive if the level is above 0.1 of the lethal concentration or 0.01 of the sublethal; for lower levels, potential additive effects are ignored. Despite all the apparent sophistication of the foregoing, a recent review (Mount 1977) questions the whole concept of application factors and considers unacceptable the spread in their numerical value. According to Mount, factors such as chemical effects of the water on the pollutant, experimental error and biological variability must contribute to this spread thereby making a determination of their real validity difficult. Mount who was first an enthusiast of application factors now believes that MATC is a better prediction for safe toxicant levels. Other biologists continue to believe that MATC is at present another "shifting" baseline from which to base an application factor (LaRoche, 1983).

2.5.5 Contribution of Avoidance Reaction to the Estimation of Safe Toxicant Levels

Avoidance-preference reactions, being one of the sublethal tests, play an important role in the evaluation of the MATC, the safe concentrations and the derivation of the application factors.

Avoidance reaction results can be derived for:

- (a) different species
- (b) new chemicals
- (c) mixtures of toxicants
- (d) actual municipal effluents
- (e) actual industrial effluents

This type of study possesses several advantages when compared with other sublethal tests:

- (1) A good avoidance apparatus design minimizes the differences between laboratory and field experiments.
- (2) Avoidance results can be easily reproduced.
- (3) The experiments and the data analysis can be fully computerized.
- (4) Avoidance reactions are amongst the most unbiased, sensitive and integrated behavioral tests available, provided that sensory acuity of test organisms is not diminished. In other words avoidance/preference responses are as sensitive as the sensory mechanisms of test species. In the case of most fish species those responses are remarkably sensitive for most products tested. Experimental work in avoidance, it has been shown that some of the presumed safe concentrations based on LC50 and application factors (especially for new or untested chemicals) were not strict enough and produced harmful effects on the aquatic life.

More information about sublethal effects is needed for thousands of chemical compounds to prevent environmental hazards. It is this particular consideration which renders avoidance tests extremely important and even necessary.

2.6 Physiological mechanisms of behavioral responses of fish

2.6.1 Behavioral toxicology

In behavioral toxicology the primary problem is not to devise a behavioral characterization of a transitory pollutant effect but rather to detect the effect of an agent that may accumulate very slowly through time and produce discernible behavioral effects only after it reaches a critical concentration (Mello, 1975).

Behavioral changes may serve as the earliest indicators that some, as yet covert, toxic action is occurring, perhaps at a time when the process can still be reversed. It is suggested that nervous tissue, especially the brain, is more sensitive to many foreign chemical substances than has previously been suspected, and that toxic effects may be manifested as subtle disturbances of behavior long before any classical symptoms of poisoning become apparent (Spyker, 1975).

More specifically, in experimental behavioral toxicology, the task is not only to detect and measure the behavioral effects of known pollutants, but also to devise sensitive techniques to evaluate the possible consequences of exposure to new and untested chemicals. The emergence of behavioral toxicology is a relatively recent development and has received little formal recognition prior to 1969 (Weiss, 1969). It is now increasingly acknowledged that the behavioral analysis of toxicological effects is critical for providing a sound empirical basis for legislative regulations concerning the maximally acceptable concentrations of pollutants in the environment and industry (Mello, 1975).

2.6.2 Physiological mechanisms of behavioral responses

In humans as well as in other mammals, exposure to toxic chemicals may adversely effect a number of organ systems. The hepatic, cardiac, pulmonary and central nervous systems may be severely compromised. In some instances, the concomitant behavioral change may be undetectable, as in the case of asbestos which may eventually result in the development of rapid pathological sequences after a 25 to 30 years induction period following exposure (Sherill, 1973). In contrast, other toxic agents such as lead and methylmercury may produce early and direct behavioral effects which range from interference with sensory and motor integration to profound intellectual deficits and emotional disturbances (Evans, 1975).

Some behavioral and neurological consequences of representative pollutants are shown in Table 2.6.

	Carbon disulfide	Asbestos	Mercury	Lead	Carbon monoxide	Noise
Tremor			X			
Muscle weakness	X		X		*	
Headache			X		*	
Dizziness			X			
Seizures				X		
Sensory loss				X	*	X
Mental retardation				X	X	
Memory defects	X		X			
Severe insomnia	X					X
Fatigue			X		*	X
Drowsiness			X		*	
Irritability	X		X	X		X
Uncontrolled anger	X		X	X		
Homicide attempts	X					
Sexual impotence	X					
Crying spells			X			
Depression	X					
Delusions	X					
Hallucinations	X					
Confusion	X				X	
Disorientation					X	
Autism					X	
Suicide	X					
Death - direct medical consequence		X			X	

* Effects of acute intoxication x Chronic effects

TABLE 2.6 BEHAVIORAL AND NEUROLOGICAL CONSEQUENCES OF REPRESENTATIVE POLLUTANTS IN HUMANS (MELLO 1975)

In fish, the avoidance-preference reaction appears to be related to the sensory perception of fish, and more specifically to the effect which pollutants have on chemoreceptors (Bardach et al, 1965). These researchers showed that as little as 0.5 mg/l of a detergent concentration could damage chemoreceptors in time (4 hours), so that behavior was impaired. Also in 1969 Sprague, inspired by the results of Hasler (1950), suggested that fish had their sensory perception dulled by phenol and p-chlorophenol. In this instance avoidance responses would be mitigated by this dulling of the sensory perception.

It is expected that defects in a developmental process may not become evident for years. A morphological and biochemical lesion can be dormant and not manifest itself until later in life as disorders, including behavioral, or overt functional impairments (Nair 1968). There may be compensatory mechanisms that initially mask the defect, but usually these are not adequate, as aging, repeated exposure and cell death occur. Delayed effects can be uncovered only by long-term evaluations (Spyker, 1975).

In recent years a review of the literature by Stebbing (1982) has shown that a broad phenomenon is being linked to effects of low pollutant concentrations. The term used to describe this phenomenon is "hormesis".

Specifically, hormesis is the name given to the stimulatory effects caused by low levels of potentially toxic agents. In this case, the suggestion that low doses produce harmful effects in proportion to dosage is invalid. Hormesis is a general phenomenon in which exposure of organisms to traces or low levels of hazardous substances or agents actually stimulate physiological mechanisms in a manner that appears to benefit health and possible survival (Luckey, 1980). Luckey, reviewing in 1975, the types of

effects caused by pollutants and toxic agents, identified four types of concentration-response curves (Figure 2.19).

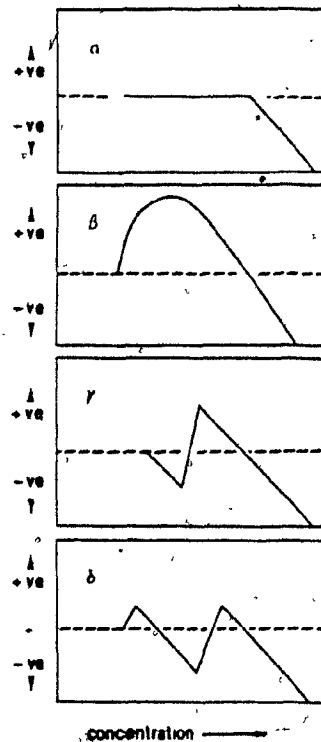


Fig. 2.19 Various types of concentration-response curves identified by Luckey (1975).

The α -curve is the familiar pattern commonly observed for the effect of a toxic substance, showing no departure of the process or state from normal at low concentrations, followed by a progressive inhibition above a threshold concentration. Curve β shows hormesis and is the response most frequently observed when tests with low concentrations are analysed. This has a single stimulatory peak at concentrations immediately below those that are inhibitory. Curves γ and δ are specific types of dose-

response relationships and must remain questionable until more data is available.

Stebbing, trying to define the causes of hormesis in his review paper in 1982, stated that: Hormesis is a consequence of a response common to different organisms to some stimulus shared by different toxicants, alternatively that hormesis is due to a response to low levels of inhibitory challenge.

However, it is expected that, further evidence will confirm this biological response that transcends the animal world. It is also inevitable that molecular biology will offer plausible mechanisms.

In summary, it is well documented that hormesis is a response that occurs in living organisms in the form of a stimulation in biological activities at the cellular level (Stebbing, 1982). To date, hormesis has not been associated with behavioral responses. However, since behavior may be described as resulting from a sum of physiological responses it appears logical to advance that some of the behavioral findings in this thesis are expressions of hormesis.

CHAPTER III

"EXPERIMENTAL SET-UP OF THE NEW DESIGN H"

CHAPTER III

3.1 Introduction

In an attempt to overcome some of the deficiencies of existing avoidance apparatus, and considering the trends and needs enumerated at the end of Chapter II, a new design H, the "flume hydraulic avoidance channel", was developed for use in this present study. It is of the steep gradient type, in view of the above-described advantages of this class of apparatus. It aims to produce a more realistic and unbiased approach to the study of avoidance reactions of fish, and to simulate more closely the actual conditions prevailing in rivers.

Figure 3.1 shows a simplified plan and side view of the channel of this new design H. The remainder of this chapter is devoted to a detailed consideration of its facilities, with a summary of its innovative characteristics in Section 3.3 below.

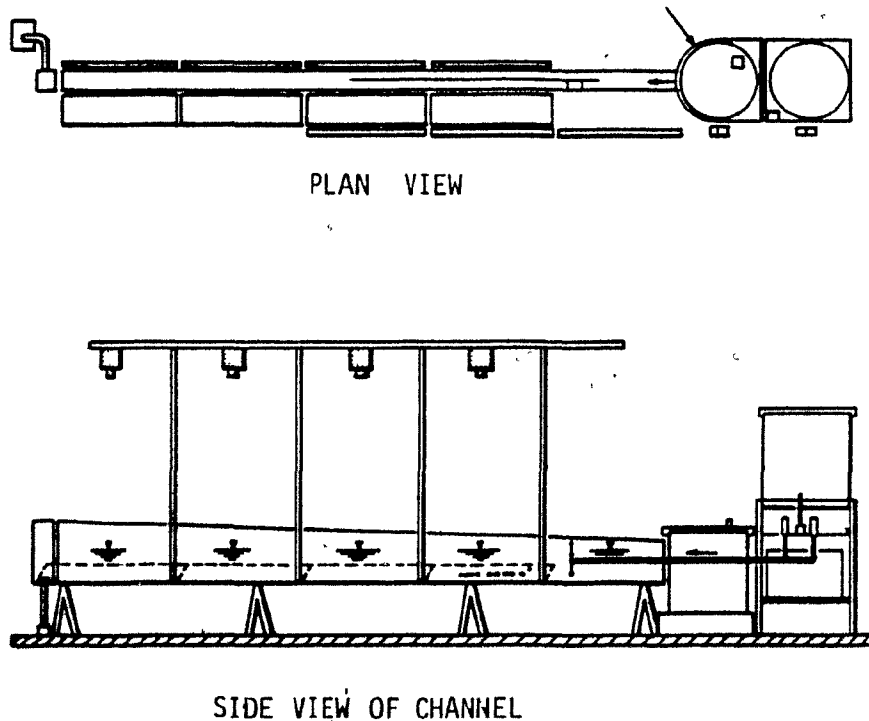


FIGURE 3.1 SIMPLIFIED DESIGN H

3.2 Design and set-up of facilities

A description of the continuous flow-through system used in this present study will now be presented. Figures 3.2, 3.3 and 3.4 depict the system schematically. A numbered key below each diagram indicates its constituent parts.

The entire design can be divided into five basic components:

1. The water treatment facilities.
2. The holding facilities.
3. The temperature control system.
4. The channel.
5. The data acquisition facilities.

An analytical presentation of each of these components is given in the following sections (3.2.1 to 3.2.5, inclusive).

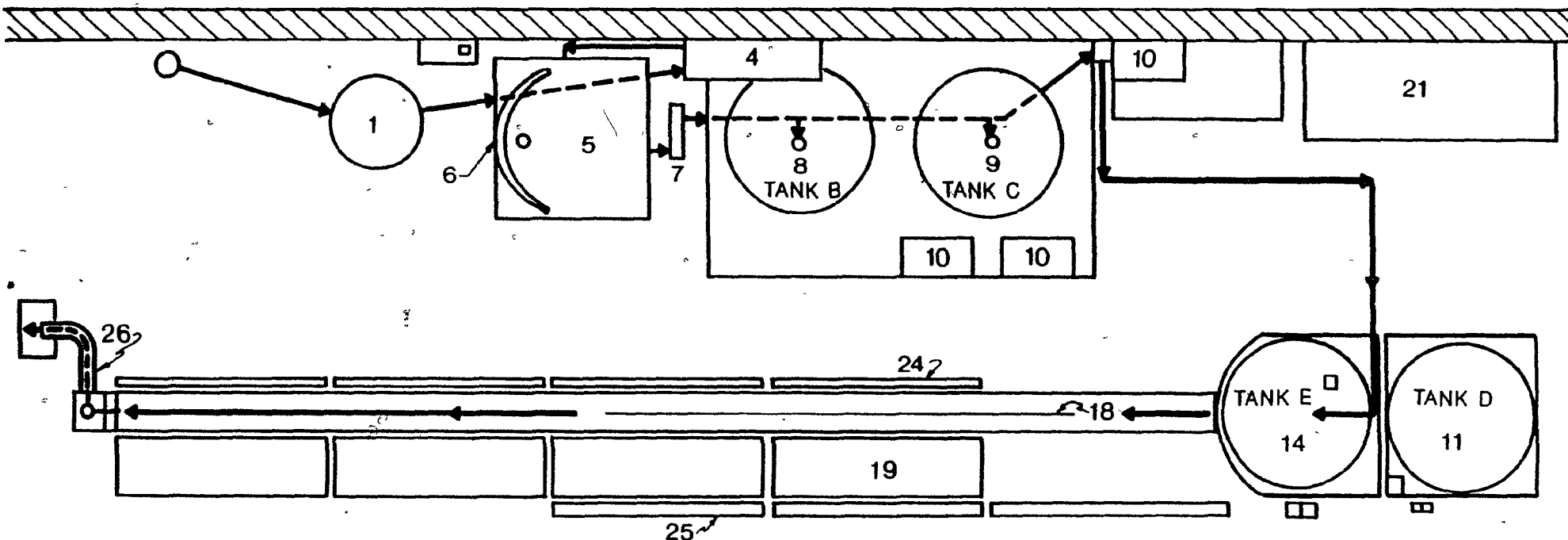
3.2.1 Water treatment

Water for the system is taken from the domestic supply of the City of Montreal. Prior to actual use, it is treated to render it suitable for fish survival. Trout used in the study have continued to thrive and grow in the water with a growth rate of approximately 2 cm per month for fish that were originally 2-3,5 g in weight.

Water analysis tests have been done monthly from different sampling spots in the system to check the efficiency of the water treatment equipment used and also to evaluate the water quality before its entrance into the holding facilities. A report of this routine analysis is included in the Appendix A4 (page 302).

The water treatment facilities contain an activated carbon unit and ultraviolet lights to kill bacteria.





1	ACTIVATED CARBON UNIT	9	FISH HOLDING TANK C	19	MIRRORS
4	ULTRA VIOLET LIGHT	10	SMALL WATER COOLERS	21	VIDEO SYSTEM
5	STORAGE RESERVOIR	11	EFFLUENT TANK D	24	CHANNEL LIGHTING
6	HEATING COIL	14	ACCLIMATION TANK E	25	SIGHT AND SOUND BARRIERS
7	MAIN PUMP	18	CENTER SEPARATION UNIT	26	END GATE AND DRAINAGE
8	FISH HOLDING TANK B				

SCALE 1:40

FIGURE 3.2 FLOW DIAGRAM AND PLAN VIEW OF AVOIDANCE APPARATUS DESIGN H

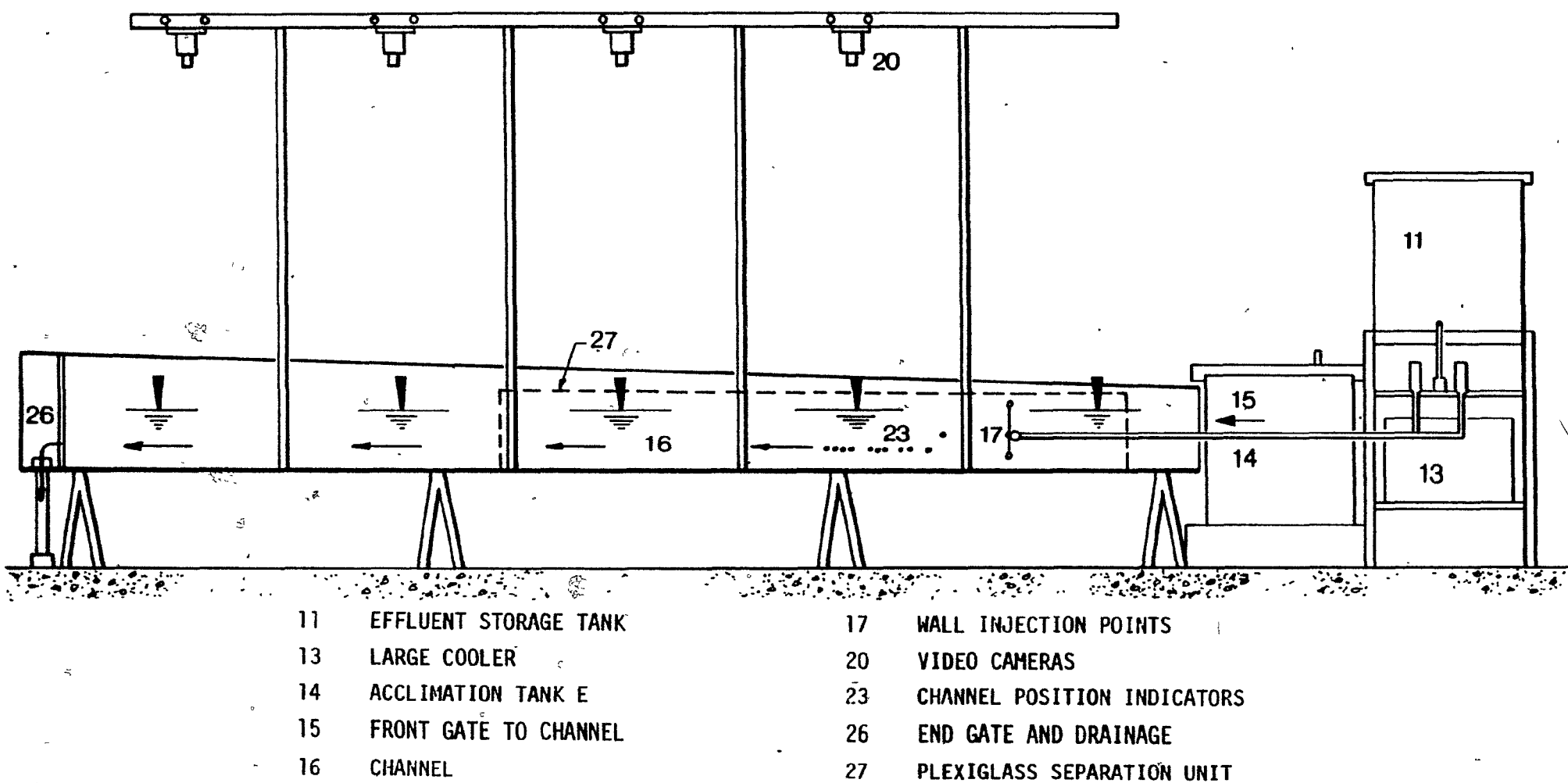
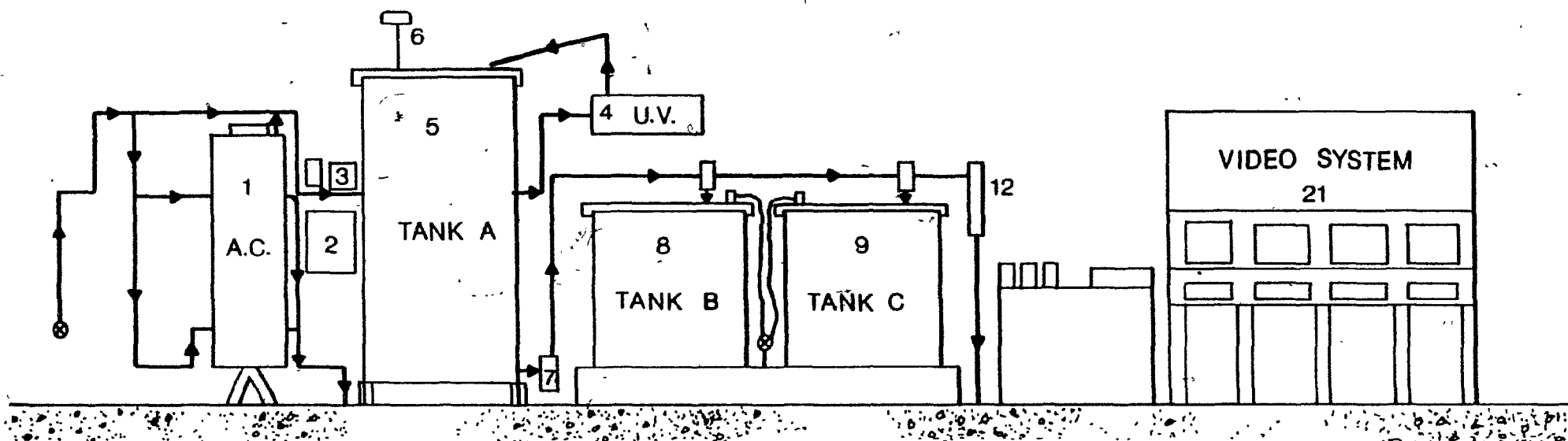


FIGURE 3.3 SIDE VIEW OF AVOIDANCE APPARATUS DESIGN H,
WITHOUT THE MIRRORS



1	ACTIVATED CARBON UNIT	7	MAIN PUMP
2	HEATING CONTROL UNIT	8	FISH HOLDING TANK B
3	AUTOMATIC TEMPERATURE CONTROL	9	FISH HOLDING TANK C
4	ULTRA VIOLET LIGHTS	12	FLOWMETER
5	STORAGE RESERVOIR	21	VIDEO SYSTEM
6	HEATING COIL		

FIGURE 3.4 SIDE VIEW OF FISH HANDLING APPARATUS, WATER TREATMENT UNITS AND VIDEO ACQUISITION DESK.

3.2.1.1 Activated carbon unit (A.C.)

Model: DÜRO AAC-24 single

The unit consists of a filter tank, operating under pressure, which contains the filter media. City water enters the system and passes into a single activated carbon filter with automatic multi-valve control. It contains 3 cu. ft. of activated carbon, 140 lb. of superfine gravel in a 24" x 60" galvanized tank (left-hand in Figure 3.5). When in operation, the water is directed to the top of the tank through the control valves and flows downward through the filter media. The dirt and turbidity are filtered out by the filter media. Clean, filtered water is supplied in the outlet line. As the filter cycle progresses, the filter bed becomes dirty and "packs", thus increasing the pressure drop across the unit. When the pressure drop reaches the maximum allowable, the filter must be backwashed.

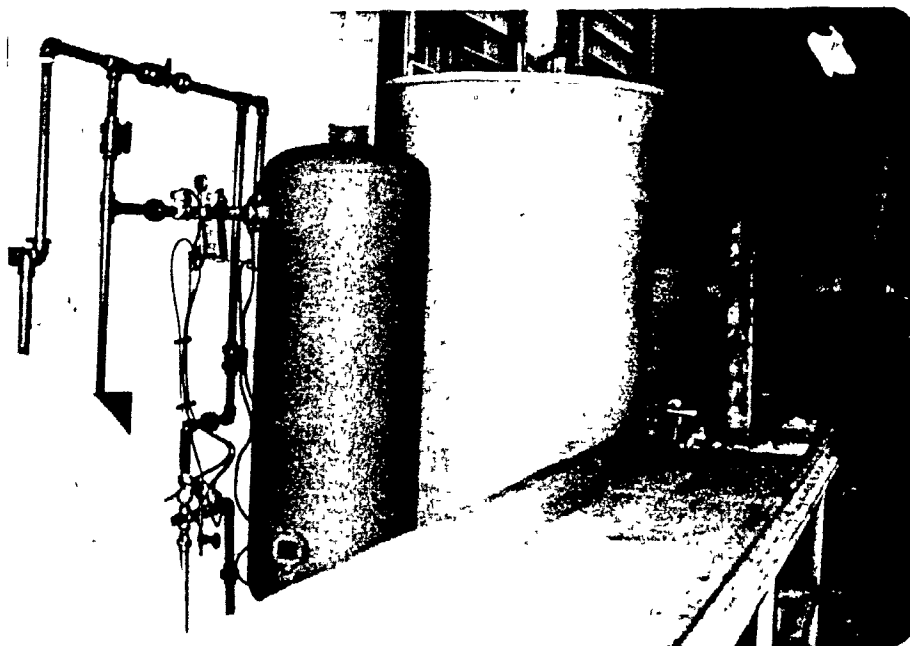


FIGURE 3.5

Activated carbon unit and Tank A

3.2.1.2 Ultra-violet lights

After being treated in the A.C. unit, the water passes through the aquafine water sterilizer (ultra-violet lights) to kill bacteria which might remain and represent a health hazard to the fish (Figure 3.6).

Model: UP-25L, H.G.Kalish Co. Ltd.

The aquafine Ultraviolet Sterilizer provides in excess of 99% reduction of all micro-organisms across the sterilizer. The basic sterilizer is comprised of an electrical housing containing ballast, and ultraviolet sterilizer manifold. The main housing has a front cover which is removeable and provides access to all interior components. Within the sterilizing manifold or chambers are a series of specially designed discs which cause the water to spin rapidly through the equipment. This is intended to prevent laminar flow and to optimize the performance of the sterilizer. The sterilizing manifold is comprised of two sterilizing chambers. Each chamber is intended for one U.V. lamp. These sterilizing chambers are designed to operate in series, as this has proven to be the most efficient design for such sterilizers.



FIGURE 3.6
Ultra violet lights

3.2.2 Holding facilities

3.2.2.1 Storage reservoir - Tank A

Model: 373-1 Polyethylene drum

Tank A, with a capacity of 500 gallons, is used as the storage reservoir for the cleaned water. The purpose of this reservoir is to provide a clean water source in the event of a failure in the incoming supply or during the cleaning of the activated carbon tower. In addition, the reservoir acts as a thermal buffer in that the retention time is sufficiently long to allow for a nearly constant temperature. A floater in the tank automatically controls the water level and the heating and cooling units control the temperature of the water being supplied to the fish. A polyethylene cover at the top of the tank protects the water from dust. A partial view of Tank A is illustrated in Figure 3.5.

3.2.2.2 Tank B - Flowmeter 1 and Airmeter 1

Model: G45-1 Polyethylene drum

Tank B, with a capacity of 300 gallons, is used to hold the larger fish (5-7 g). A flowmeter is attached with a 1" pipe to the tank and regulates a 2 gpm flow continuously (Model: 1358-V Brooks Instrument Division of Emerson Electric with valve and max. flow of 5 gpm). Also, an airmeter (Model 2700 V, 5-50 SCFH*, Brooks Instrument) regulates an air flow of 10 SCFH into the tank. A standard 2" pipe controls the overflow in the center of the tank.

* SCFH = standard cubic feet per hour

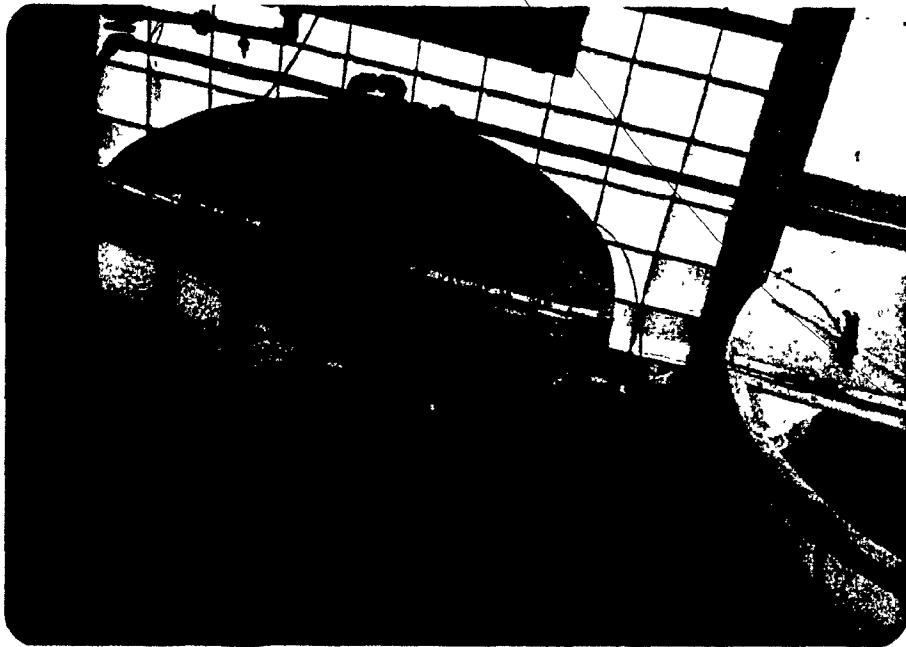


FIGURE 3.7
Tank B

3.2.2.3 Tank C - Flowmeter 2 and Airmeter 2

Tank C is identical to Tank B described above, except that the smaller fish are acclimated in this tank. When the fish reach the size specified for the experiments, they are moved to Tank B or the experimentation Tank E. Also Flowmeter and Airmeter 2 are identical to Flowmeter and Airmeter 1.

Tanks B and C are both placed on a steel support structure covered by 1/4" plywood, 9" from the ground, which allows the proper connection of the drainage piping system. A 6" air-stone produces continuous bubbles to keep the water nearly saturated with oxygen. The covers of Tanks B and C are half plexiglass and half polyethylene, with a series of hinges in the middle, which permit the opening of the first half of the cover for cleaning and feeding purposes.

3.2.2.4 Tank D - Flowmeters 3 and 4 and Pump 1

Model: G45-1 Polyethylene Drum
Dwyer Flowmeter 0.2-2.2 gpm
Brooks tube size R-6-25-B (0.01-0.8 gpm)
AC-2CP-MD plastic pump

A 300 gallon capacity polyethylene tank has been mounted on a 6 foot high steel support structure to act as a storage reservoir for industrial effluents. The effluent is pumped through one of two flowmeters to give a constant flow of effluent into the channel (Figure 3.8). An agitator prevents the suspended solids of the effluent from settling and keeps the effluent in a completely mixed state.

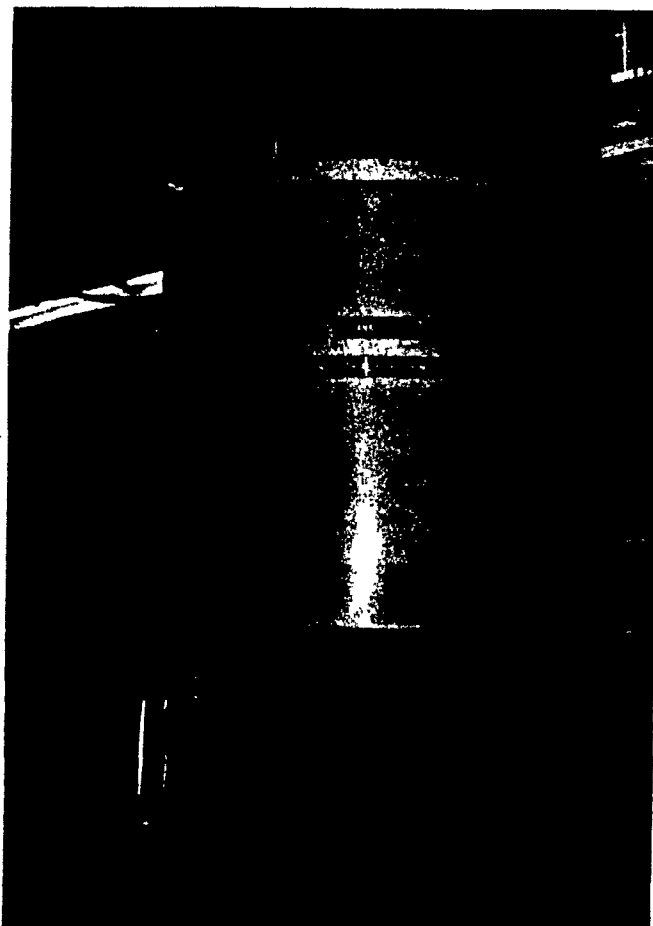


FIGURE 3.8
Tank D

3.2.2.5 Tank E - Airmeter 3

Model: 645-1 Polyethylene drum
2700V, 5-50 SCFH, Brooks

Tank E, with a capacity of 300 gallons receives 6-12 gpm from Flowmeter 5 and discharges into the channel (Figure 3.9). On one side of the tank a 16" x 12" hole has been made 10 inches from the top of the tank to allow the fish to move from the tank to the channel through the front gate. A 2" stand-pipe in the centre of the tank controls the overflow, and a split plexiglass polyethylene cover is placed over the tank for protection. An airmeter installed inside the tank allows 10 SCFH to pass through, and a 6" air-stone produces continuous bubbles to keep the water nearly saturated with oxygen (10 mg/l), in a working temperature of 15°C. Tank E holds the fish during the last stage of their acclimation period before the tests.

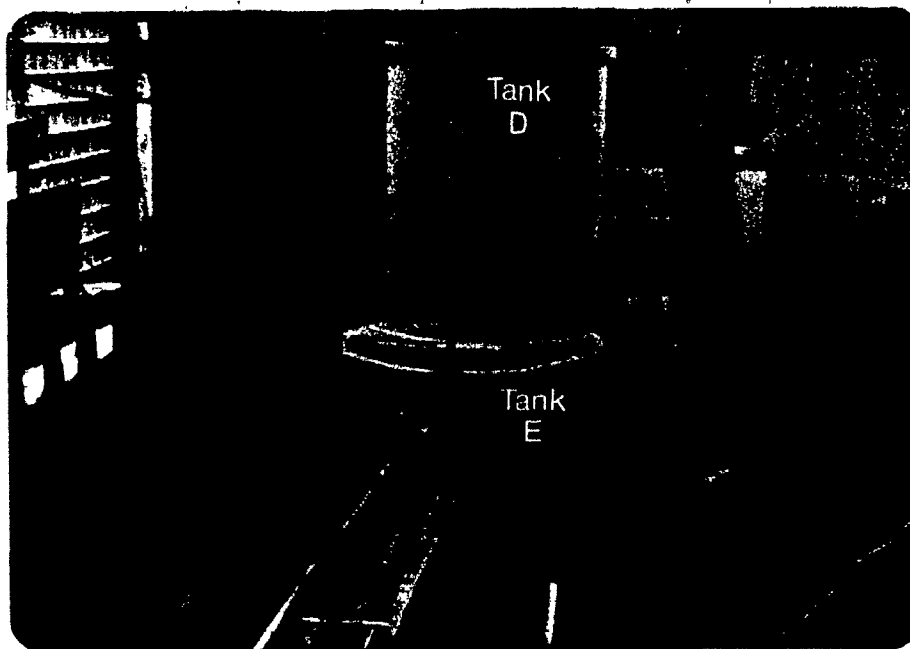


FIGURE 3.9
Tank E

3.2.2.6 Pump 2

Model: DP-GT-MD Magnetic drive pump

The water has to be raised from the bottom of Tank A (Figure 3.5) to a level of 5 feet in order to be distributed to the other tanks and the channel. For distribution a pump with a capacity of 35 gpm (32 ft.-head), has been installed in the line.

3.2.2.7 Flowmeter 5

Model: 1110 size 10 $\frac{1}{4}$ " NPT-Brooks

A flowmeter regulates the flow of water into the experimentation acclimation tank (Tank E). The water passes through Tank E and into the channel. Consequently, the fish are being tested in the same water that they were acclimated in. Flow through Tank E and the channel can be as high as 15 gpm but is generally maintained at 6 gpm to avoid turbulence.

3.2.3 Temperature Control

A submerged heating unit in Tank A and an automatic temperature control unit provide a continuous temperature of 15°C in the system. In addition, cooling units are used to cool the water to 15°C in the summer.

Ten waterproof temperature probes have been strategically located throughout the system (channel, holding tanks, etc.) and connected to a manually operated multi-channel thermometer for rapid recording of the temperature at any point in the system (YSI Model 44 Tele-Thermometer). Figure 3.10 shows the position of the temperature probes throughout the system.

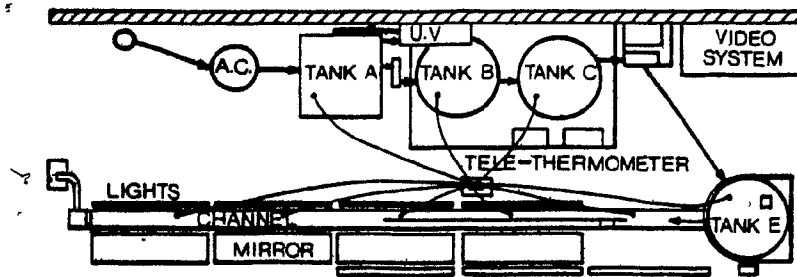


FIGURE 3.10

POSITION OF THE TEMPERATURE PROBES THROUGHOUT THE SYSTEM

3.2.3.1 Heater

Model: KTL - 318 - 1 WMMR

An immersion heater has been installed in Tank A to heat the treated water to the required temperature (continuous element 3 ft. high, 600 v., 60 amps.).

3.2.3.2 Heating Control Unit

Model: HCP-60-(Chromalox Canada)

Control of the submersed heating unit is done through a control panel with a magnetic contactor of 40 amps. and a main disconnect switch of 60 amps. A dedicated 575 volt line is connected to the control panel to supply power for the heater and peripheral control.

3.2.3.3 Automatic Temperature Control (2 units)

Model: 40-702010-402 (Chromalox Canada)

These two control units have been installed to provide a constant temperature of 15°C in the system.

3.2.3.4 Cooler for Tank E

Because the flow of water into Tank E is large (6-12 gpm), a large individual cooler has been installed to keep the temperature

of the tank and channel at 15°C during the warm summer period (Figure 3.11). This cooling system is independent of the other coolers and it is therefore possible to maintain the fish stock in the event of a failure in either system.

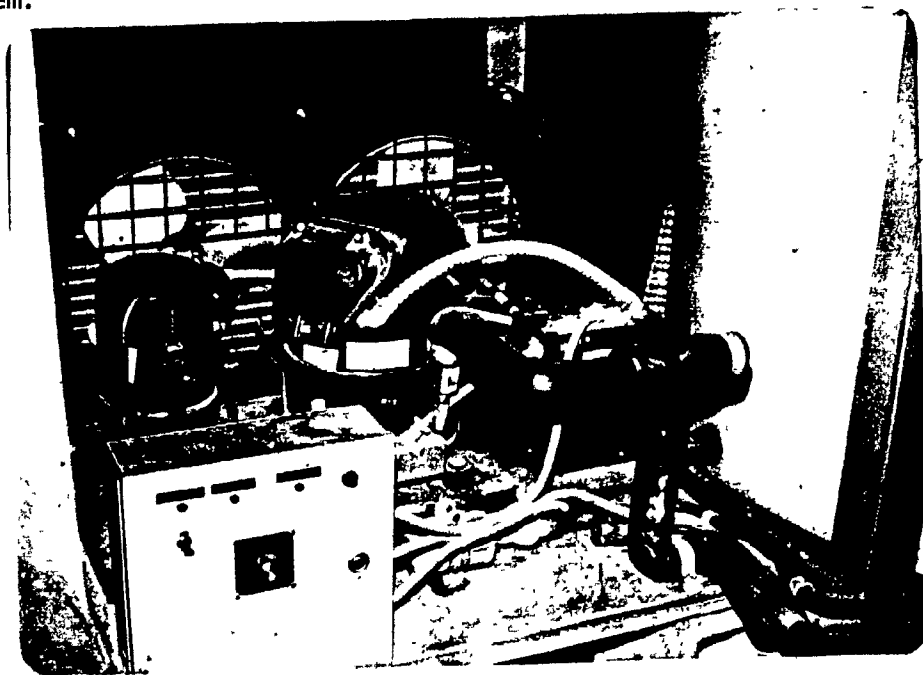


FIGURE 3.11
Cooler for Tank E

3.2.3.5 Small Coolers

During the summer months and the beginning of fall, five small coolers are used to keep the temperature in Tanks B, C and D, as close as possible to 15°C .

3.2.4 The Channel

The experimental channel is 30 ft. long, 1 ft. wide and has a continuous water elevation of 13" (max. up to 18"). It is divided into 5 sections of 6 ft. each. The first section is constructed of plexiglass

to allow for holes for jets and diffusers while the other four are made of glass. In the first section of the channel, 5 ft. from the front gate and 1 ft. from the second section, provision has been made for the injection of the effluents and dyes from the effluent tank. Three injection points have been located in the wall: (a) 4" from the bottom, (b) 9" from the bottom, and (c) 17" from the bottom.

A 1/4" plexiglass barrier with holding devices has been placed in the centre of the channel extending from 4 ft. upstream of the first section through to the third section (14 ft.). This separation unit has been introduced to give the fish a chance to choose either the polluted or the non-polluted side of the channel.

Because it is desirable to have a three-dimensional analysis of the disposition of the fish in the channel, mirrors have been placed along one side of the channel. The mirrors are placed at an angle of 45° and, when photographed from above, both the horizontal and the vertical projections are shown on the screen. Consequently, any avoidance in either the lateral, horizontal or vertical direction is recorded. Direct horizontal coverage is provided by four overlapping cameras along the length of the channel.

The bottom and one side of the entire channel are divided into 4" x 4" squares with black stripes. Each of these squares has been colour-coded horizontally and vertically to aid in determining the position and behaviour of the fish in the channel.

Because the light reaching the iris of the cameras was not adequate and because the picture on the monitors from the mirrors must be clear, fluorescent lights were placed along the length of the channel. These lights have a 12-hour photo period.

Large portable modular wall panels have been located along one side of the channel to minimize both noise and visual disturbances while experiments are in progress.

A plexiglass box with a perforated end gate has been built at the downstream end of the channel. The perforated end gate is required to allow for flow which approximates parallel flow in a cross-section of a river while at the same time holding the fish within the channel. The diameter of the holes (1") and width of slots (3") in the end gate prevents small fish from passing through. The end gate is removeable to allow for rapid drainage and easy cleaning of the channel (Figures 3.12 and 3.13).

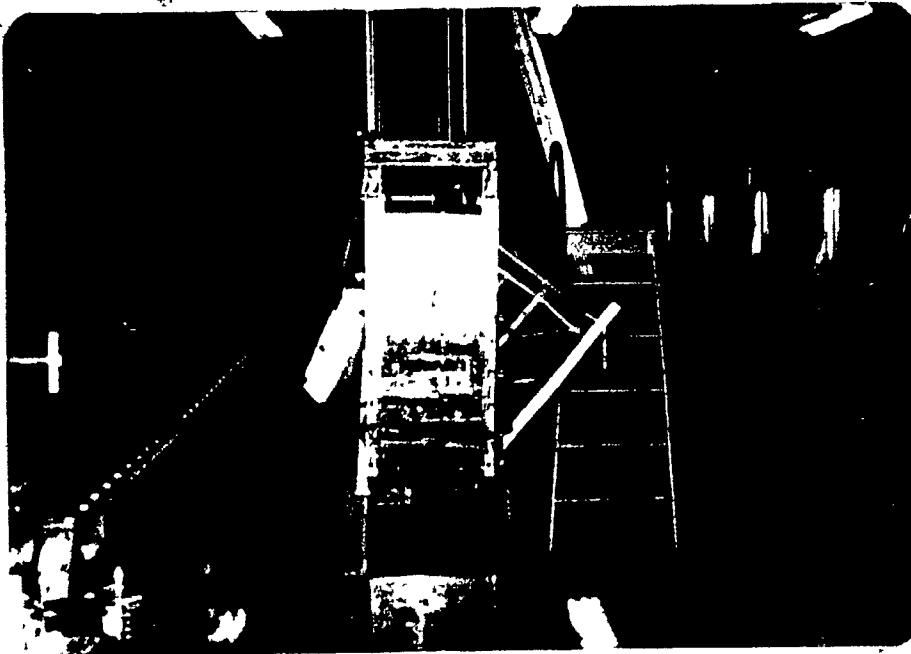


FIGURE 3.12
End Gate

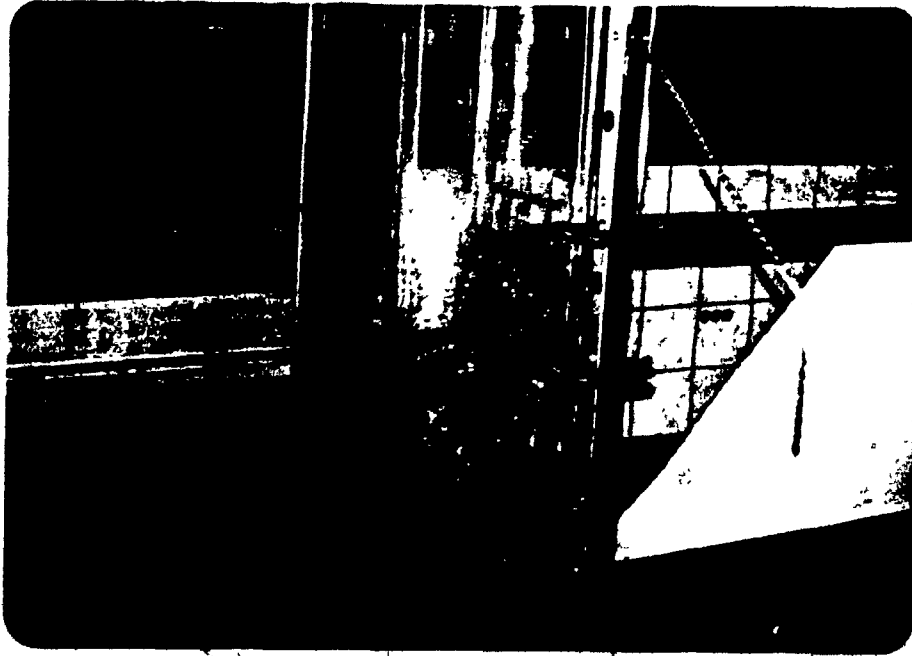


FIGURE 3.13
End Gate

The channel has been designed deliberately with different depths at either end, 18 inches at the entrance and 36 inches at the end gate. A pivot can drop one end of the channel, thereby converting the existing system, which simulates a river, into a simulated lake system (as shown in Figure 3.14).

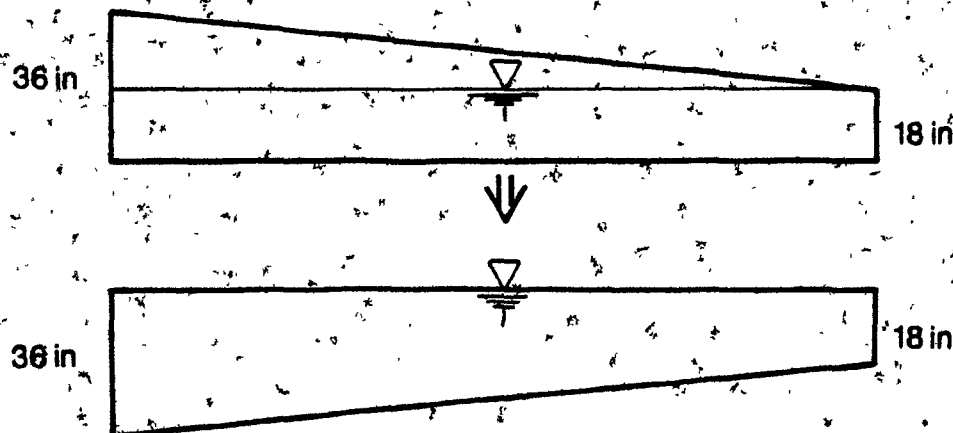


FIGURE 3.14

MODIFICATION OF THE CHANNEL FROM A RIVER SIMULATION
TO A LAKE SIMULATING SYSTEM

After extensive initial testing it was concluded that the channel modifications and extensions provide a facility that can be adapted to a number of practical applications. The design incorporates most of the needs and advantages discussed earlier in Chapter II

Figures 3.15 to 3.19 illustrate different views and sections of the channel.

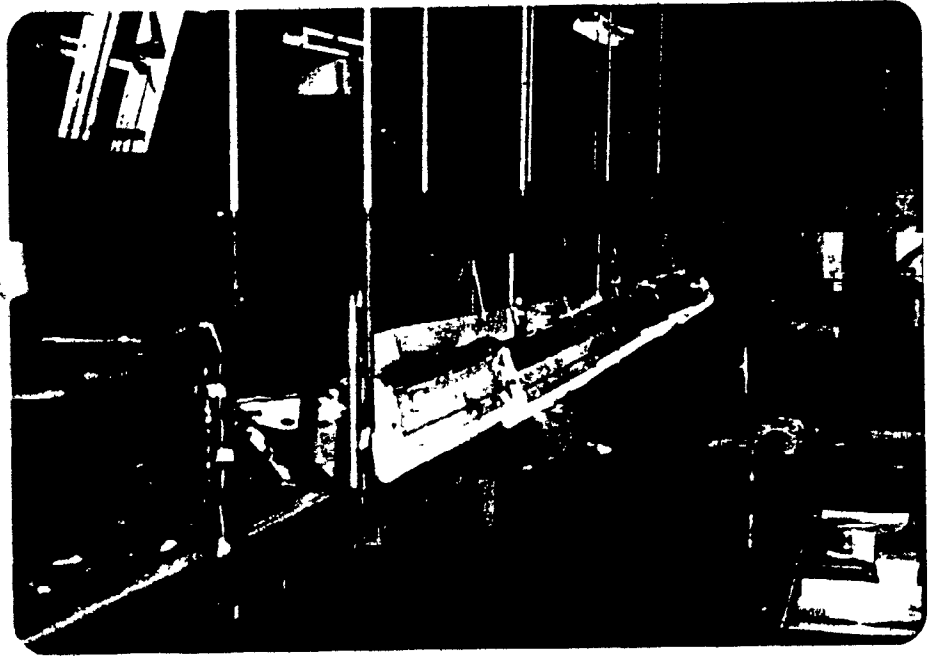


FIGURE 3.15

Channel: Overall view with Section 4 at the far end and Section 0 nearest the camera



FIGURE 3.16

Channel: Side view of Section 0

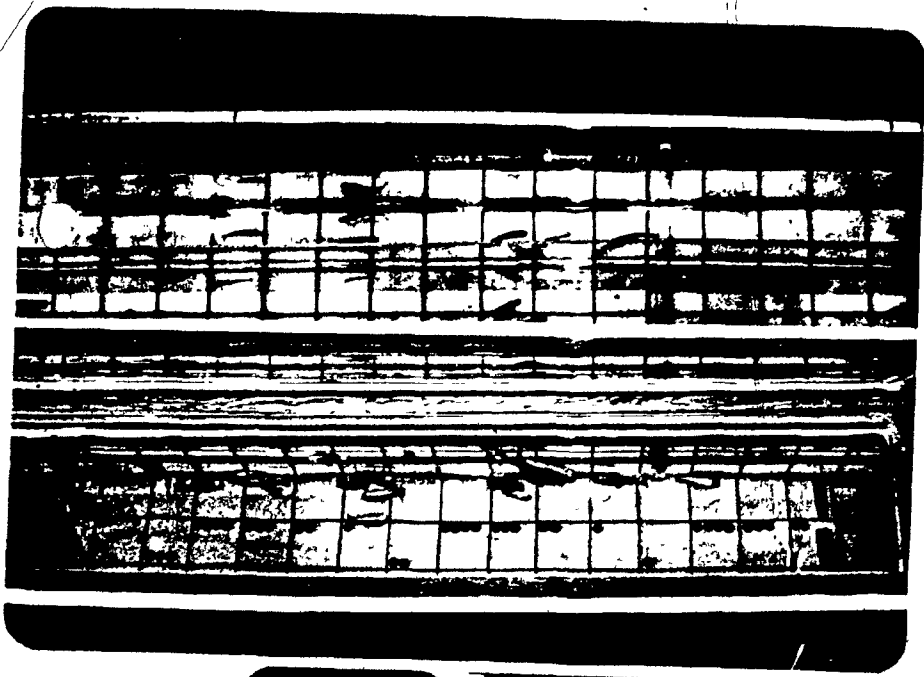


FIGURE 3.17
Channel: Overhead view of
Section 1

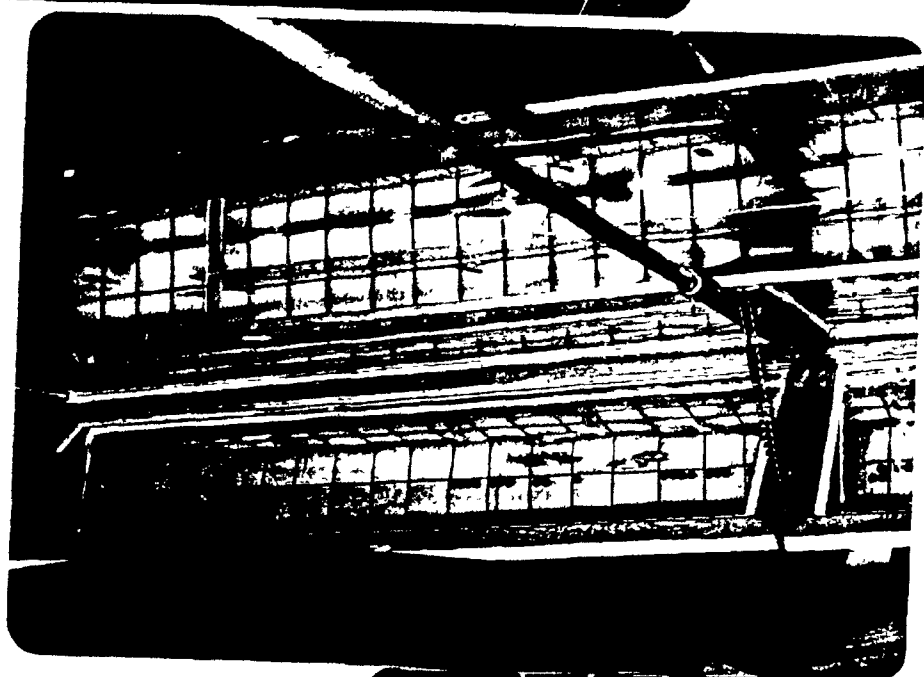


FIGURE 3.18
Channel: Overhead
view of Section 2

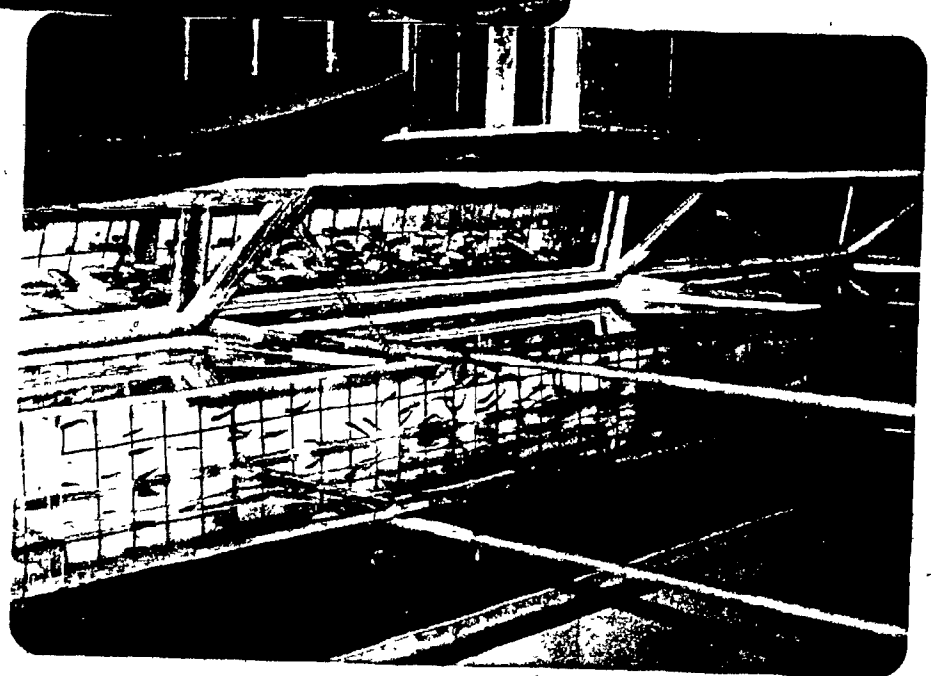


FIGURE 3.19
Channel: Overhead view of
Sections 3 and 4

3.2.5 Data Acquisition

3.2.5.1 Video Cameras

Model: Panasonic WV3320, WV3200

Four cameras have been mounted on moveable trolleys 9.5 ft. above the channel (Figure 3.20). Each camera can cover approximately 6 feet of the channel, resulting in total coverage of the area where the fish are able to swim. The cameras project on to the monitors:

- a) An overhead view which provides observation of the horizontal and lateral distribution of fish within the section and b) a side view through the 45° mirrors which provides information about the vertical distribution of fish within the section. Once the cameras have been positioned and focused they remain immovable for the total duration of the experiment in question. In the present study, the cameras have been fixed in position for the duration of the research project (Figure 3.20).

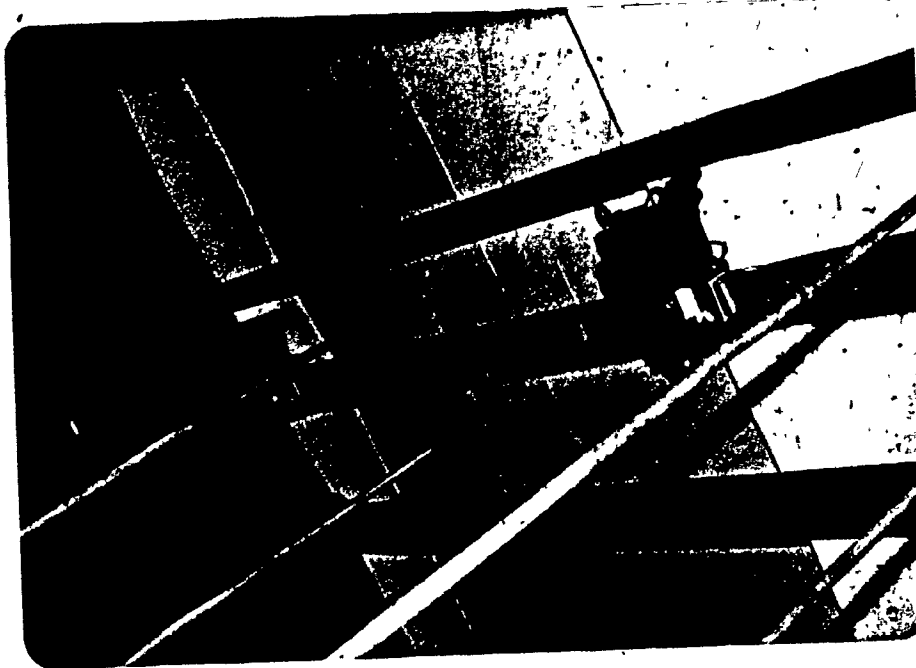


FIGURE 3.20

Video cameras seen from water surface
of channel

3.2.5.2 Video Monitors

Model: Panasonic CT1310MC, CT1309C

Pictures from the four cameras are displayed on a separate video monitor for each section of the channel.

The image in each monitor is comprised of two parts:

1. The upper part which corresponds to the vertical distribution (Depths A,B,C) of the particular section, and
2. The bottom part which illustrates the horizontal distribution (Sections 1, 2, 3 and 4) and the lateral distribution (Sides A and B) within the particular section.

The number of fish in each part of the image should be equal, thereby providing a check during the counting process.

Figure 3.21 shows a schematic view of the four TV monitors.

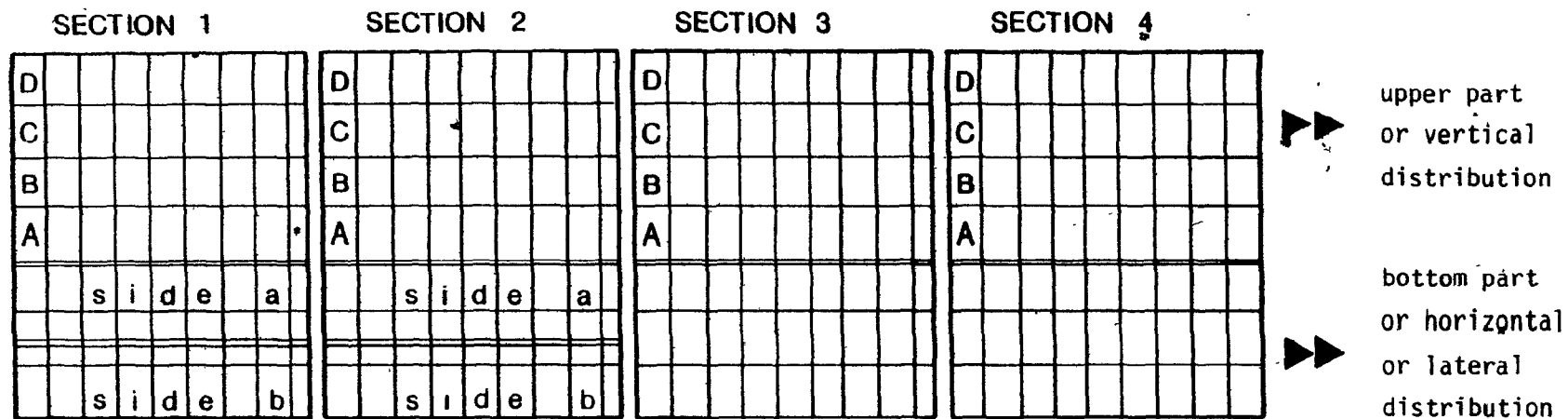
3.2.5.3 Video Recorders

Model: Panasonic NV-8200, NV-8410

Simultaneously with the pictures, long playing video recorders are used to record the video signals for later analysis. Recorder 1 is equipped with audio dubbing capabilities to allow for audio documentation of archive tapes.

The four video cassette recorders (Nos. 1, 2, 3, 4) store the events of every experiment, both vertically and horizontally. For experiments of two-hour duration or less recording is continuous, while selected intervals give representative coverage for experiments ranging from two to eight hours.

Following the experiment an analysis of the tapes is made at 5 minute intervals. At these intervals the tapes are stopped and the



Horizontally -----> top view - Sections 1, 2, 3, 4
 Vertically -----> through the mirrors - Depth A, B, C, D
 Laterally -----> top view - Sides a, b

FIGURE 3.21 VIEW OF THE T.V. MONITORS

distribution of the fish in the channel is quantitatively determined by a count of the number of fish resident in each 4 x 4 inch square.

The result of each count is recorded on counting sheets for subsequent statistical analysis (Figure 3.22). To complete the analysis all the important portions of the tapes are transcribed to storage tapes for future reference and comparison. Figure 3.23 show the recorders and the monitors.

The cost analysis of the avoidance apparatus - Design H (Appendix A8) shows that the installation cost in 1981 was \$37,000 and the cost per set of experiments (7-12 tests) is presently \$7,000 per month.

HORIZONTALLY

	NUMBER OF SQUARES			
	1-6	7-12	13-18	TOTAL
NUMBER OF FISH				

VERTICALLY

	DEPTH			
	A	B	C	TOTAL
NUMBER OF FISH				

LATERALLY

	SIDES		
	SIDE A	SIDE B	TOTAL
NUMBER OF FISH			

FIGURE 3.22
COUNTING SHEET

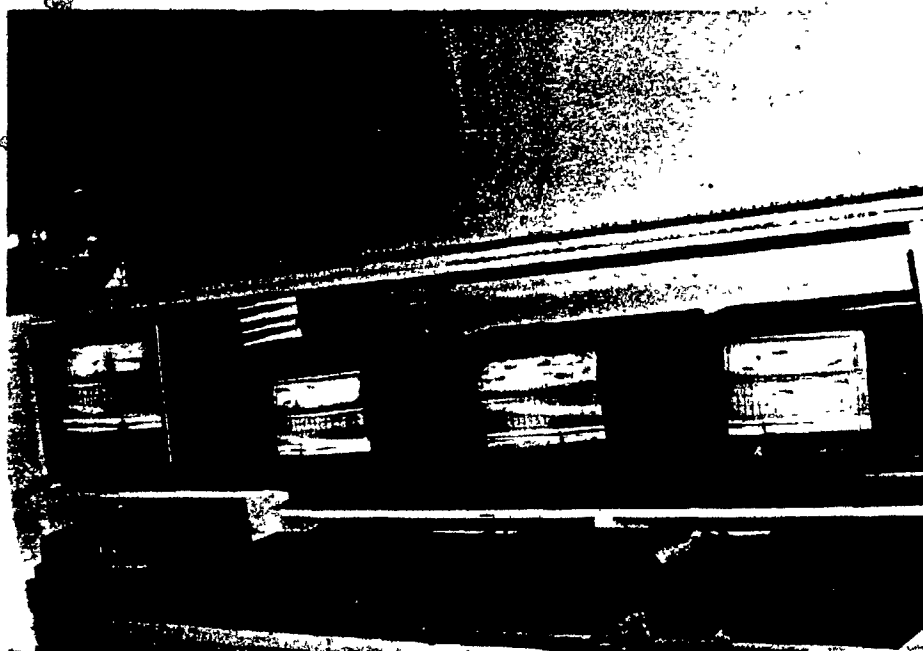


FIGURE 3.23
The entire data acquisition desk
with 4 monitors and 4 recorders

3.3 Innovative Characteristics of Design H

1. The use of a large number of test organisms (100 or more fish).
2. The use of both total separation and side-by-side toxicant and water separation simultaneously.
3. The capacity for vertical, horizontal and lateral analysis of the position of test organisms.
4. The use of a large tank (33 ft. long), which eliminates end effects.
5. The inclusion of the injection system, not previously used in avoidance studies.
6. The ability to store results for later re-examination.
7. The use of advanced video techniques for data acquisition.
8. The ability to test any kind of pollutant (actual industrial effluent or a single chemical component), without making modifications in the system.
9. The opportunity for fish to swim in: a) a non-polluted area; b) a polluted area with a certain toxicant concentration; and c) a polluted area with progressively less toxic concentrations (shown in Fig. 3.24)

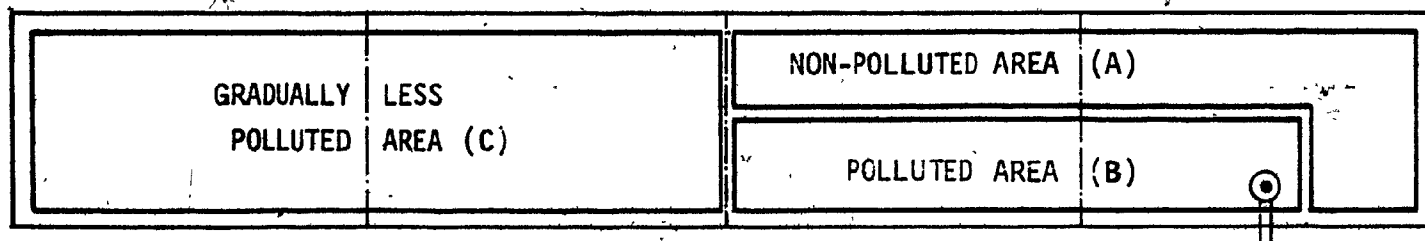


FIGURE 3.24 /
SIMPLIFIED PLAN VIEW SHOWING AREAS OF RELATIVE POLLUTANT EXPOSURE

10. The creation of a test environment which closely simulates natural conditions.

CHAPTER IV

"SELECTION OF PARAMETERS - DESCRIPTION OF STATISTICAL ANALYSIS"

CHAPTER IV

4.1 Selection of Parameters4.1.1 Parameters of the Problem

Avoidance reactions may be influenced by the following parameters:

1. Type of pollutant
2. Type of aquatic life
3. Concentration of pollutant
4. Time of exposure
5. Flow rate
6. Temperature
7. Dispersion-diffusion
8. Noise level
9. Light intensity
10. Injection system
11. Dissolved oxygen, suspended solids, pH
12. Salinity, alkalinity, hardness
13. Number of aquatic organisms per experiment.

These parameters can be grouped to give different categories, as shown in Table 4.1: independent, physical, chemical, hydraulic and water quality parameters.

Because the parameters are so numerous, the following rationale was accepted. Ten of these parameters were fixed, remained constant or had no effect on the results of the experiments. Parameters which might vary during the experiment were tested to determine their effect on the behaviour of the test organisms and then fixed at an appropriate value. Three of the parameters: type of pollutant, concentration of pollutant and injection system were selected as major parameters and were varied throughout the study to form the basis for the research.

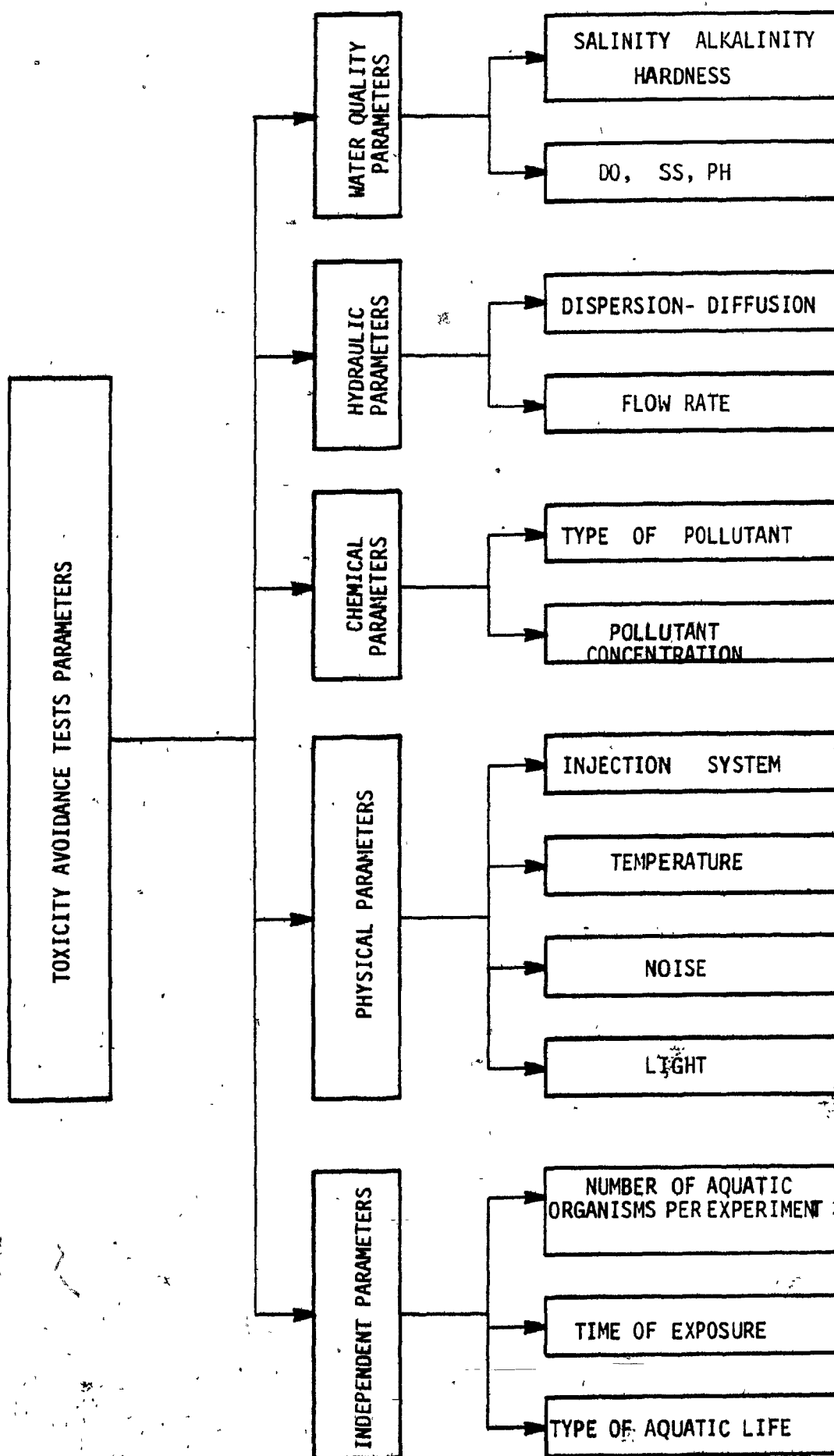


TABLE 4.1 GROUPING OF THE PARAMETERS OF THE PROBLEM

Table 4.2 illustrates the grouping into fixed and variable parameters for this particular research study. An analytical discussion of each one of the parameters follows.

4.1.2 Fixed Parameters

4.1.2.1 Selection of Aquatic Life

Among the factors affecting the results, the selection of a suitable test species is very important. The final selection of SALMO GAIRDNERI (rainbow trout) for test species was based on the following considerations:

1. Strong recommendation for the usage of rainbow trout was found in "Standard methods for the examination of water and wastewater", 15th ed. (APHA-AWWA-WPCF 1980).
2. The known sensitivity of trout to metal toxicity.
3. The availability of trout throughout the year from local hatcheries.
4. The fact that the rearing methods and holding requirements of trout are well known from a number of sources.
5. The recommendation of trout as standard species for temperate waters (Davis 1977).
6. The use of rainbow trout as test species in federal government laboratories.
7. The possibility for comparing results with those from lethal (static LC50) and sublethal tests done with trout.
8. The biological homogeneity of trout supplies.

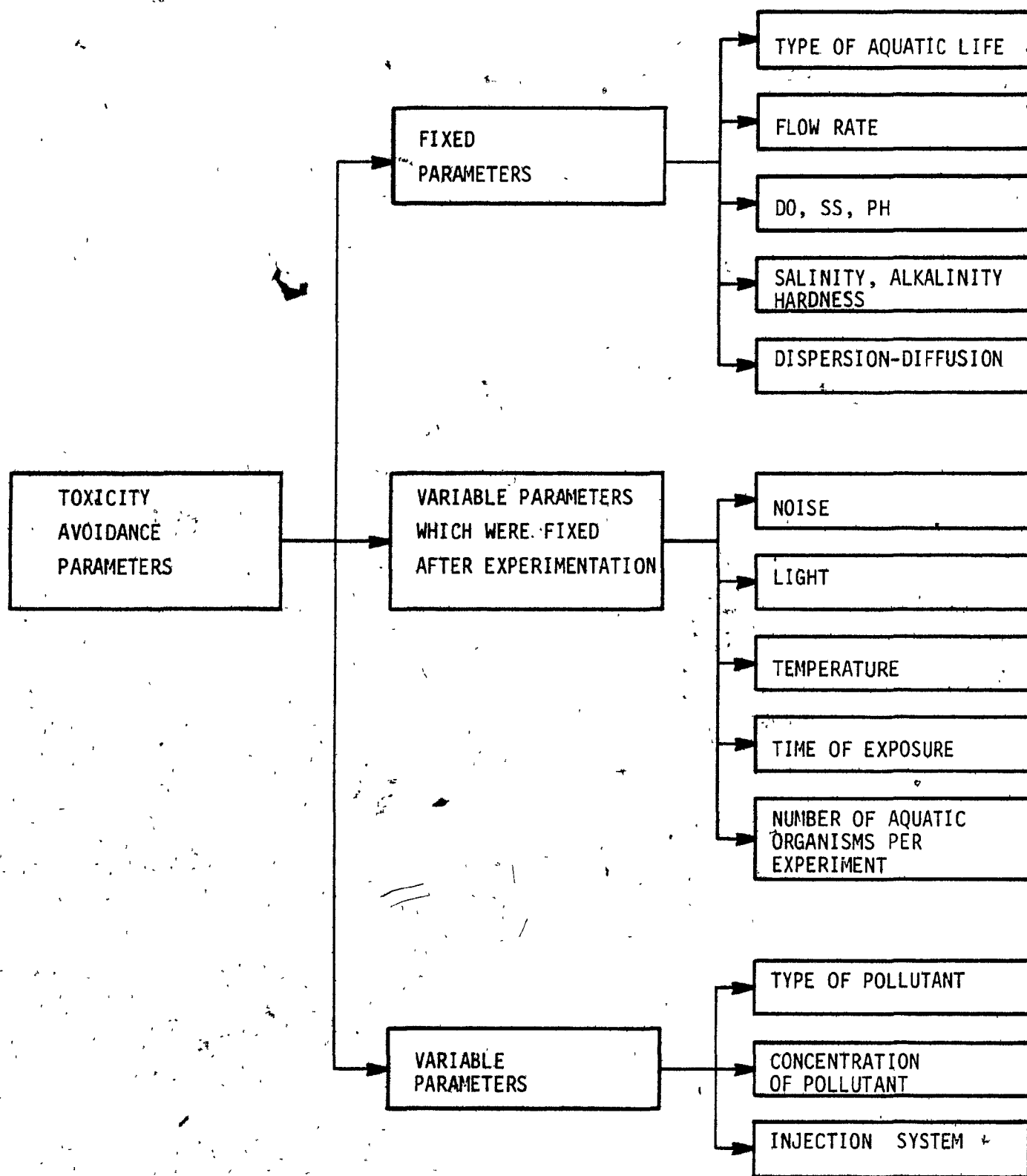


TABLE 4.2 FIXED AND VARIABLE PARAMETERS
OF THE PRESENT AVOIDANCE STUDY

Rainbow trout, from two Quebec hatcheries, were used throughout the entire research study, with the sole exception of a particular set of experiments which were done with algae (Selenastrum capricornutum) and bacteria in Chapter VIII.

Table 4.3 summarizes previous research studies which have used rainbow trout in toxicity avoidance preference tests.

TOXICITY PREFERENCE-AVOIDANCE TESTS
WITH RAINBOW TROUT

<u>AUTHORS</u>	<u>CHEMICALS USED</u>
1. Jones et al (1956)	Sulfate and sulfite waste
2. Sprague (1964, 1968)	Copper sulfate, zinc sulfate
3. Sprague et al (1965)	Copper, zinc
4. Sprague and Drury (1969)	ABS, BKME, phenol chlorine
5. Black and Birge (1980)	Cadmium, copper, mercury
6. Hadjinicolaou, Spraggs (1981)	Dodecyl sodium sulfate
7. Spraggs, Gehr, Hadjinicolaou (1982)	Monomers, polymers
8. Hadjinicolaou, Spraggs (1982)	Chromium, nickel, iron, plating industry waste.

TABLE 4.3 Chronological list of researchers who used rainbow trout in preference - avoidance studies.

4.1.2.2 Selection of Flow Rate

Flow rate is one of the hydraulic parameters which has an important impact under natural field conditions. It is well known that turbulent flow is characteristic of most of the natural settings whose problems were to be studied.

In order to apply turbulent flow in a laboratory setting, one needs first to have information concerning the laminar zone:

In every new design or approach it is considered wise to begin with laminar flow on account of difficulties in handling the large quantities of water required for turbulent flow (60 gpm, compared with the 20 gpm maximum used in this study). If the experimental conditions permit, it is then possible to integrate the laminar approach with the use of turbulent flow.

In a continuous flow-through system, because the amount of treated water needed is very large, there appears to be a choice between two options: either to use a very small apparatus with turbulent flow or to use a larger apparatus with laminar flow. The situation which corresponds more closely to the actual field situation is to have a large apparatus with turbulent flow, but for practical reasons, this is very difficult to accomplish in the laboratory (huge amount of treated water; very expensive; large installations, etc.).

For the present research a large apparatus was chosen (channel length of 33 feet) with laminar flow, because the maximum quantity of treated water available from water treatment facilities was 20 gpm.

A proportion of that amount of water went into the holding facilities for the trout with the remainder going into the channel. In the channel, flows of up to 15 gpm were obtained, which is still in the laminar range, yielding a maximum Reynolds number of 2000.

In designing the channel, the first objective was to create uniform flow by proper construction of the front and the end gates of the channel. A further objective was to maintain the depth constant at 13".

After testing with dyes, the first choice was the establishment of a flow rate of 6 gpm which gave a completely uniform flow at a velocity of 0.156 "/sec and a constant ~~depth~~ of 13" in the channel. This flow rate of 6 gpm remained constant throughout the experiments.

A suggestion for future experiments mentioned in Chapter XI would be to investigate the influence of laminar and turbulent flow on avoidance behavior.

4.1.2.3 DO, SS, PH

During the course of the experiments three major water quality parameters were measured periodically (First set).

The dissolved oxygen (D.O.) level, measured weekly, remained stable throughout the study, keeping the water oversaturated (between 10-12 mg/l). This was achieved by the constant aeration of the water in the holding tanks by means of airstones (5" and 10" long) connected to an oil-free air supply.

The pH was measured weekly and was maintained between 7 and 7.5. A number of studies appear in the literature, evaluating the effect of pH on the behavioral changes of aquatic organisms (Baird (1978), Kwain (1975), Miller (1979), McKim (1976), Sellers (1975), Warwood (1978)). In particular, avoidance-preference toxicity studies have been used to estimate the environmental damage caused by acid or alkaline waters.

The suspended solids (S.S) were measured once a month after the water treatment, as an additional control on the efficiency of the activated carbon and ultra-violet light treatment units. The suspended solids in the treated water were always negligible.

4.1.2.4 Dispersion-Diffusion

While it is obvious that the natural processes of dispersion and diffusion play an important role in the effluent concentration in the channel it is difficult to compute or determine what the concentration actually is at any given point. One possible method would be to apply a mathematical model to the system and compute the concentration at each location for any time. While this method gives exceptionally good results for a channel, the disturbance created by the fish totally changes the diffusion process and the mathematical model does not produce viable results. This latter point was verified by injecting dyes which the fish did not find objectionable.

As a result of the dye studies (see Appendix A6) and as a result of the diffusers used, it was determined that there is complete mixing of the effluent within the first section of the channel due to the locomotion activity of the fish. Consequently, a simple mass balance could be performed to determine quantitatively the concentration of the toxicant throughout the channel.

An interesting area for future research would be to study the modification of the diffusion-dispersion equations, taking into account the movements of the living organisms present in the system.

4.1.2.5 Salinity, Alkalinity and Hardness

Periodic analysis of the source water indicated that the quality remain relatively constant. While there was some seasonal changes in the parameters, they were insufficient to have any impact on the experiments being carried out. Salinity, alkalinity and hardness belong to the second set of water quality parameters commonly used.

Salinity levels influence the problem, mainly in estuarine and coastal situations and sometimes, in particular, industrial effluent discharges. The design J for a preference-avoidance apparatus introduced first by Shepard (1948) and modified and improved by Greer and Kosakoski (1978) has been used, especially for industrial discharges with different salinity levels.

In the present research study the salinity level remained close to zero.

There is little information in the literature describing the effects of alkalinity or hardness on avoidance reactions, but meanwhile, it is well known that hardness variation affects significantly the chemical speciation of metals and thus toxicity responses (Leland (1979), Miller (1979), Toveil (1974)). In the present study, a bi-monthly water analysis was made and the results indicated that the alkalinity and hardness of the water used in the system remained stable around 50 ppm and 100 ppm respectively.

4.1.3 Variable parameters which can be fixed after experimentation

The light intensity, the noise level, the temperature, the time of exposure to a certain pollutant and the number of aquatic organisms per experiment are variable parameters which were fixed after initial experimentation.

In this present research study Chapter V describes the preliminary testing carried out to set appropriate values for light, noise and temperature, and Chapter VI discusses the tests for exposure time and the number of fish to be used per experiment.

4.1.4 Variable Parameters

Three parameters - type of pollutant, pollutant concentration and the injection system - were chosen as major parameters which were varied throughout the study to form the basis of the research.

4.1.4.1 Type of Pollutant

Table 2.4 (Chapter II) shows the chemicals (pollutants) which have been used in previous preference-avoidance studies.

For most of the pollutants selected for the present study, there is no information available concerning either toxic levels, or the avoidance reactions of aquatic organisms to them.

These pollutants were:

- A. DSS (Dodecyl sodium sulfate) (Chapter VII)
- B. Polymers (Chapter VIII)
- C. Monomers (Chapter VIII)
- D. Plating-industry effluent (Chapter IX)
- E. Components of the plating industry effluent
(nickel, chromium, iron) (Chapter X)

During the initial planning of this research it was determined that an actual industrial effluent should be used to perform the main body of the avoidance research. One practical criterion in selecting the effluent was that it should be from an industry in close proximity to Montreal to reduce transportation problems. To determine which effluent would be appropriate, the following guidelines were prepared:

1. The effluent should be relatively toxic and homogeneous;
2. The total solid matter should be less than 500 ppm;
3. The suspended matter should be less than 100 ppm;
4. The oil and grease content should be below 50 ppm;
5. The volatile constituents (phenols and ammonium) should be as low as possible.

Using the above criteria, existing data about local industries were examined to determine a source of effluent.

Initially, three different types of industries were chosen as being representative of the general industrial activities in Montreal. However, it soon became apparent that one particular industry (the plating sector) produced an effluent which generally met the above criteria. (see App. A5) Selection of a metallurgical industry for the effluent provided an added advantage in that considerable work had already been done on the acute toxic effects of purified heavy metals. Consequently, this present research extends the knowledge about the effects of heavy metals and serves to corroborate and to add a realistic dimension to laboratory work done previously.

Following the initial selection of effluent, exhaustive tests were completed to determine both the persistence and the consistence

of the effluent. Persistence of toxicity was confirmed by performing a series of static fish, algal and bacterial toxicity tests from a single sample of effluent. Static tests were made at 1, 2, 4 and 14-day intervals and the results compared. Consistence of the effluent was confirmed by running a series of static toxicity tests on several samples of the effluent taken on different days but at the same hour.

Results of the testing indicate that the effluent is persistent and while toxicity varies throughout the day it is generally consistent at specific times of the day. The general characteristics of the effluent are as follows:

1. total solid matter content (540 ppm);
2. low volatiles (<2.5 ppm);
3. high Chromium (20 ppm), Iron (4.5 ppm) and Nickel (50 ppm);
4. slightly acidic ($\text{pH} = 6.8$);
5. no organic matter ($\text{TOC} = 8 \text{ ppm}$);
6. low grease and oils (<2.5 ppm).

Although the effluent does not adhere strictly to the ideal guidelines described above, it was judged to be suitable and was used for the avoidance research of this study (Chapters IX and X).

4.1.4.2 Concentration of the Pollutant

To produce a complete set of curves for an individual chemical pollutant, 7-10 tests are required at various concentrations. To find the most sensitive concentration range, a number of preliminary experiments (usually three) must be conducted.

The concentrations of toxicants may be expressed as functions of the LC50 of the particular chemical under study (i.e., 1:1000 LC50, 1:100 LC50, 1:1 LC50, 5:1 LC50 etc.). This makes it easier to establish the threshold concentration, and, in addition, gives a good visual understanding of the effect of a particular chemical on the aquatic life. The concentrations of chemicals may also be expressed in ppm (parts per million or mg/l) of pollutant, diluted in the water column.

In studying an actual effluent, the determination of its possible toxic components through chemical analysis, and the calculation of its acute lethal values (LC50) were under study initially. After that, known concentrations could be produced in the channel by variations of the flowmeter readings, which could then be related to LC50 dilution ratios (or ppm) and used to compare sublethal responses to the particular effluent.

4.1.4.3 Injection System

Jets can be distinguished according to position (surface, wall and submerged jets), to characteristics (momentum and buoyant jets) and to dimensions (one, two or three dimensional jets).

All the jets in the experiments of this study were three-dimensional momentum jets (without density differences). Four different types of jet-diffusers were used as shown in Table 4.4 and Figures 4.1, 4.2, 4.3, 4.4.

<u>TYPE</u>	<u>DEPTH</u>	<u>INJECTION SYSTEM</u>	<u>FIGURE</u>
1. wall jet	4" from the bottom	A	4.1
2. wall jet	9" from the bottom	B	4.2
3. vertical- centre multidiffuser	4"-10" from the bottom	C	4.3
4. horizontal multidiffuser	9" from the bottom	D	4.4

TABLE 4.4 Types of different injections systems

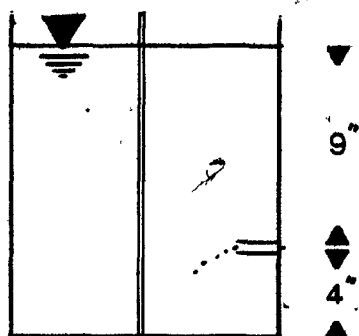


FIGURE 4.1
Injection System A

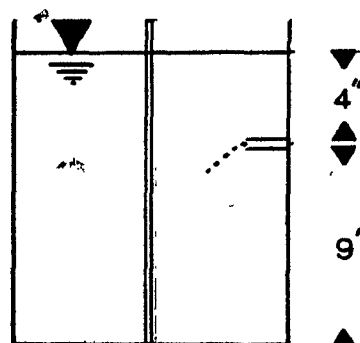


FIGURE 4.2
Injection System B

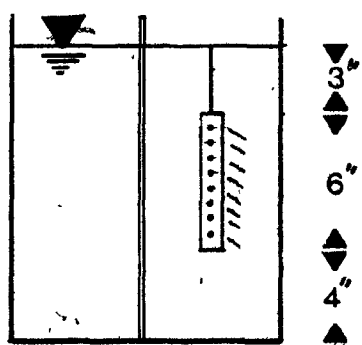


FIGURE 4.3
Injection System C

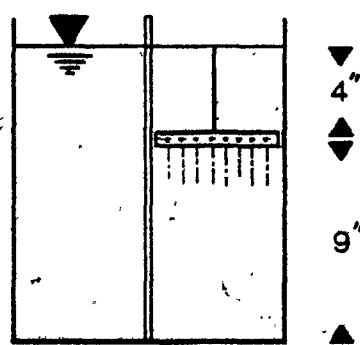


FIGURE 4.4
Injection System D

Two methods were used to inject the pollutant into the channel:

- a) With a proportional pump placed at the top of the channel (Injection Systems C and D), and
- b) With a pipe which delivered effluent from the effluent storage Tank D to the injection point of the channel. In the latter case, effluent was pumped through flow regulation valves to ensure a constant source of effluent in the channel

(Injection Systems A and B). The channel was modified so that

effluent could be injected from either side. All four injection systems were used in the experiments with the industrial effluent. In the remainder of the experiments, the most appropriate of the four for the particular case was chosen.

4.2 Description of Statistical Analysis

4.2.1 Description of the Procedure of a Typical Experiment

4.2.1.1. Before the Experiment

A. Acclimation

Acclimation of fish prior to testing is essential to ensure that they are not adversely conditioned before the experiment begins. The following guidelines were developed for the acquisition and acclimation of the trout stock as a result of preliminary testing.

Similar guidelines may be found in references APHA (1980), Buikema (1982).

1. Fish obtained from the hatchery were approximately 2-3 inches in length.
2. Fish were kept until 4-5 inches in length before being transferred to the channel acclimation unit.
3. Fish were acclimated for 14 days in the channel and acclimation Tank E.
4. Access to the channel was controlled to acclimate the fish to living either in the channel or in the Tank E.
5. Cleaning of the channel was performed daily and had to be completed eight hours before beginning an experiment.
6. Fish feeding was done at the same time each day at five specific locations in the channel.

7. No experiments began until three hours after feeding.
8. Fish had to be counted prior to the commencement of any experiment.
9. A complete record of the feeding rate in the channel and the holding tanks was kept.

B. Preparation for the experiment

1. An experimental protocol had to be designed and adhered to.
2. Recordings for noise, light and pH were taken according to a prescribed schedule.
3. The temperature of the effluent and the channel were checked every half hour.
4. The effluent was discharged for 15 minutes before actual recording began.
5. The experiment was continued either until a steady-state condition was achieved in the channel or until two hours had elapsed (whichever was shorter).
6. Before beginning an experiment, the laboratory superintendent was informed and all environmental laboratory conditions remained constant.

4.2.1.2 During the experiment

1. A switch box was connected to the four cameras. By turning the switch on, the experiment was simultaneously recorded on the four cassette recorders for two hours continuously.
2. Any changes in the ambient conditions had to be recorded and any resultant behaviour changes likewise noted.
3. The experiment was supervised during its entirety including changes in personnel.
4. After two hours, the experiment was terminated by turning the switch off.

4.2.1.3 After the experiment

Following any experiment, there was a series of procedures which were followed to ensure that subsequent experiments were not biased. These procedures were:

1. Cassettes were rewound and temporarily labelled.
2. Fish which had been exposed were removed and either destroyed or placed in appropriate containers for post analysis.

-----NO FISH WHICH HAD BEEN EXPOSED TO THE EFFLUENTS

WERE PLACED BACK IN THE EXPERIMENTAL TANK

-----NO FISH WHICH HAD BEEN MOVED TO THE EXPERIMENTAL

TANK WERE RETURNED TO THE STOCK TANKS.

3. The channel was drained and cleaned.
4. The channel was refilled and fish were allowed to move back into it.
5. Analysis of tapes was performed.
6. The next batch of fish was allowed to enter the channel for acclimation for the subsequent experiment.
7. The significant portions of the recordings were transferred to archive tapes for reference.

4.2.2 Procedure for Obtaining Results

4.2.2.1 Recording

Because 100 fish were used in each experiment their position in the channel had to be noted at selected time intervals.

This analysis required an initial visual count followed by an analysis of the position of the fish. To determine the relative avoidance of the fish, the individual squares were grouped for analysis.

The horizontal, vertical and lateral disposition of the fish were determined at each counting interval. The beginning of each experiment was indicated by the setting of the recorder counters to zero. After two hours of continuous recording the counter of the first recorder indicated 840 while the counters of the other three indicated 1848. In a two-hour tape, 24 still frames were obtained (one frame every five minutes) which corresponds to a counter interval of 35 on the first recorder and of 77 on the other three.

4.2.2.2 Counting

In each still-frame reading, eight (8) different numbers were obtained from the counting of each screen. These were:

1. Number of fish from square 1 up to square 6
2. Number of fish from square 7 up to square 12
3. Number of fish from square 13 up to square 18
4. Number of fish at depth A
5. Number of fish at depth B
6. Number of fish at depth C
7. Number of fish on side A
8. Number of fish on side B.

The number of readings in every tape was 24. A typical counter sheet appears in Appendix A1 (page 292).

Before beginning computations the initial readings were discarded if there were

- a) recording mistakes
- or b) disturbances in the experimental set-up.

4.2.3 Analysis of the Results

4.2.3.1 Preliminary Calculations

From the counting sheets the data were transferred into computer files and the preliminary calculations were done by computer using SAS (Program A in the Appendix B1). For every section of the channel, mean value, standard deviation, range and percentage were calculated horizontally, vertically and laterally (see Figure 4.5).

A typical output containing the preliminary calculations of the four sections of an experiment appears in Appendix C1.

4.2.3.2 Time Series Analysis

After the preliminary calculations, time series analysis was applied to the lateral distribution (Sides A and B) to evaluate the stability of the responses with time.

Program B in Appendix B2 plots the percentage of fish in clear water (the non-polluted side of the channel) versus the time in minutes (0-120 minutes), or in other words the avoidance response of the fish with time.

An additional contribution of Program B is the calculation of mean values, standard deviation, minimum and maximum values, standard errors, sums and variances of the readings of the lateral distribution. Also it calculates the previous parameters excluding the incomplete development of the fish distribution throughout the channel in the first twenty minutes of the experiment until stability was reached.

EXPERIMENT X

SECTION y^*

	LOCATION	READINGS																								PRELIMINARY CALCULATIONS			
		Q ₁	Q ₂	Q ₃	Q ₄	Q ₅	Q ₆	Q ₇	Q ₈	Q ₉	Q ₁₀	Q ₁₁	Q ₁₂	Q ₁₃	Q ₁₄	Q ₁₅	Q ₁₆	Q ₁₇	Q ₁₈	Q ₁₉	Q ₂₀	Q ₂₁	Q ₂₂	Q ₂₃	Q ₂₄	Mean Value	Standard Deviation	Range	% of fish present
HORIZONTALLY	1-6 squares	9	6	8	10	12	12	8	9	9	8	10	9	12	6	7	9	5	5	3	8	6	6	6	10	8.04	2.36	9	35.80
	7-12 squares	5	4	6	14	12	13	5	10	10	6	9	5	7	10	8	10	7	5	8	7	8	6	9	4	7.83	2.77	10	34.87
	13-18 squares	12	4	5	10	14	5	3	6	3	3	6	3	5	11	8	7	4	11	6	8	8	6	5	5	6.58	3.09	11	29.31
	TOTAL	26	14	19	39	38	30	16	25	22	17	25	17	24	27	23	26	16	21	17	23	22	18	20	19	22.45	5.86	24	100.00
VERTICALLY	Depth A	11	2	6	10	16	10	7	8	5	5	9	6	9	12	8	8	7	6	3	6	6	4	6	7	7.37	3.03	14	33.08
	Depth B	5	7	5	10	11	13	6	8	7	8	6	5	9	9	3	8	2	8	4	7	6	5	6	7	6.87	2.49	11	30.84
	Depth C	10	5	8	13	9	8	3	7	11	4	8	6	6	5	12	10	7	7	10	12	10	8	9	5	8.04	2.66	10	36.07
	TOTAL	26	14	19	33	36	31	16	23	23	17	23	17	24	26	23	26	16	21	17	25	22	17	21	19	22.29	5.58	22	100.00
LATERALLY	Side A	10	2	6	12	13	12	2	6	5	2	8	5	6	5	12	12	4	8	5	8	8	6	8	9	7.25	2.99	9	32.89
	Side B	15	12	12	20	23	19	14	18	17	15	16	12	18	21	11	14	12	13	12	15	13	12	12	9	14.79	3.13	12	67.11
	TOTAL	25	14	18	32	36	31	16	24	22	17	24	17	24	26	23	26	16	21	17	23	21	18	20	18	22.04	4.58	22	100.00

FIGURE 4.5

DIAGRAMMATIC SKETCH OF PRELIMINARY CALCULATIONS
OF A TYPICAL EXPERIMENT

* $y = 1, 2, 3$ or 4

An output of a time series analysis program for a typical experiment appears in Appendix C2 (page 320).

From the computer output of the time series analysis, the avoidance reaction representation was derived plotting T versus σ_e , where T = crossing time of the mean avoidance value line in minutes and σ_e the fluctuation over the mean avoidance value.

4.2.4 Production of Curves

4.2.4.1 Horizontal Distribution

For every section of the channel after the preliminary calculations, four numbers are produced. Three of them represent the mean value of the number of fish in the intervals between 1-6, 7-12 and 13-18 squares, respectively, and the fourth, the mean value of the total number of fish in the section (squares 1 to 18). Transforming the four total mean values (one for each section) into percentages and plotting them versus the fish concentration as %, the horizontal distribution curve of the particular experiment was obtained.

4.2.4.2 Vertical Distribution

For every section of the channel, after the preliminary calculations, four numbers are produced. Three of them represent the mean value of the number of fish in Depths A, B and C, and the fourth the mean value of the total number of fish in the section (Depths A + B + C). Having four means (one for each section) for every depth, the mean of the means for Depth A, B and C was calculated. These

three numbers transformed into percentages and presented in a graph form versus the fish concentrations in %, produce the vertical distribution curve of the experiment.

4.2.4.3 Lateral Distribution

For every section of the channel after the preliminary calculations and the time series analysis, two numbers are produced. The one represents the mean value of the number of fish on the non-polluted side and the other the mean value of the number of fish on the polluted side.

Having four (4) numbers (one for each section) for the non-polluted side, the main avoidance was obtained as the mean value of the two first sections in %, and the secondary avoidance as the mean value of the third and fourth sections also in %. In these calculations the first four readings are excluded because of the twenty minutes adaptation period. The main avoidance after the elimination of the bias factors, represents the avoidance value of the experiment and the secondary avoidance was used for additional information.

As part of an unrelated study, five experiments in this series were conducted with algae cages present in the channel. This caused a bias factor (max 5% avoidance on the side of the cages, see Appendix A 7). An additional bias factor was the influence of the water jet (max 5% preference to the injected side, see Section 5.2 and Appendix A 7). Plotting the final avoidance values after the elimination of bias factors in mg/l or ratios of LC50 versus the time spent in clear water as % of the total time, the avoidance curve for a set of experiments was obtained.

The horizontal distribution curve together with the vertical distribution curve and the avoidance curve give a three-dimensional understanding of the effects of a particular pollutant on the fish disposition in the channel.

4.2.5 Analysis of Curves

For every experimental series (normally seven tests for a complete set), a horizontal distribution containing seven curves, a vertical distribution containing also seven curves, an avoidance curve and an avoidance reaction representation were produced.

For the analysis of these curves, the following steps were necessary:

1. Basic Analysis

- (a) Trends
- (b) Obvious basic comparisons
- (c) Similarities and differences
- (d) Experimental errors
- (e) Necessary corrections

2. Analysis of Variance (using statistical analysis systems - SAS)

The analysis of variance tables (ANOVA), in this thesis are based on models which treat the variables as follows:

- a) For the horizontal distribution: Independent variables

Section of the channel and concentration of the pollutant

Dependent variable : Percent (%) of fish in clear water.

- b) For the vertical distribution

Independent variables: Depth of the channel and concentration of the pollutant

Dependent variable: Percent (%) of fish in clear water

- c) For the avoidance reaction

Independent variables : Pollutant and concentration of the pollutant

Dependent variable : Avoidance reaction

From this, values are produced for:

F = F statistic value for the model

(This value is the ratio produced by dividing the mean square for the model by the mean square for error, and tests how well the model as a whole accounts for the dependent variable's behavior.)

$PR > F$ = the significance probability for the F value

(If this value is < 0.05 , it indicates that the F is significant and therefore the null hypothesis is rejected at the 5% level).

R^2 = variation of the dependent variable

(R^2 measures how much variation in the dependent variable can be accounted for by the model, and is computed as the ratio of the sum of squares for the model divided by the sum of squares for the corrected total. The larger the R^2 value, the better the model fits the data.)

C.V. = coefficient of variation

(Is the standard deviation of the dependent variable divided by its mean times 100, and is often a preferred measure because it is dimensionless.)

STD.DEV. = standard deviation of the dependent variable

(Is equal to the square root of the mean square of the error term.)

The ANOVA tables also test the hypothesis of equal means for every independent variable of the models.

The analysis of variance is a supplementary statistical test for the evaluation of the significance between two factors or cases using F statistic and hypothesis tests.

In general, the goal of the analysis of variance is to compare two or more sample means and infer whether or not the corresponding population means are the same, or in other words, it is to provide a statistical procedure for determining whether or not the null hypothesis (equal population means) should be rejected based on the sample information.

The proper use of analysis of variance requires that certain assumptions regarding the nature of the data are satisfied. These assumptions are:

- a) The homogeneity of variances
- b) The normality of the populations
- c) The additivity of the model and
- d) The randomness of the error terms (independence assumption)

The homogeneity of variances requires that the population variances associated with each treatment in the experiment are equal.

The normality of the populations requires that each treatment population is normally distributed. Additivity requires that the effects in the model behave in an additive fashion. The independence assumption requires that the errors are statistically independent.

The ANOVA tables also test the hypothesis of equal means of all the independent variables of the model.

3. Duncan's tests

The goal of the Duncan's tests is to use multiple comparison techniques to analyse which means differ from the rest when it is found by ANOVA that the population means are likely to be different.

The observations are separated into groups based on the values of a classification variable and means are calculated for all the variables (For details see Ott (1977)). There are two restrictions that are placed on the Duncan's procedure. First it is required that all sample sizes be the same and secondly, the procedure should not be used when a priori one would expect certain of the population means to be very different from others.

The difference between the two Duncan's tests is that in the Waller-Duncan's k-ratio t-test, the k-ratio value describes the error seriousness ratio (level of significance), and in Duncan's multiple range test that is given by the alpha value. Reasonable values for k-ratio are 50, 100 and 500. The alpha value is 0.5 unless specified as 0.01 or 0.1.

When given a set of values, Waller-Duncan's k-ratio t-test and Duncan's multiple range test assign the same letter to the values within the group which are not significantly different.

To perform Duncan's tests the values from the models:

df = error degrees of freedom
MS = error mean square
F = F value for the model

are needed, and normally are derived from previously obtained ANOVA tables.

Analysis of variance and Duncan's tests were performed in this study for:

- (a) The avoidance of different injection systems (Chapter IX)
- (b) The avoidance of the industrial effluent and its components (Chapter IX&X)
- (c) The avoidance of different polymers and monomers (Chapter VIII)
- (d) The horizontal and vertical distribution analysis of temperature, noise and DSS tests (Chapter V and VII)
- (e) The horizontal and vertical distribution of the industrial effluent and its components (Chapters IX and X)

Program C in Appendix B3 illustrates the analysis of variance and the Duncan's tests.

4.2.6 Classification of Toxicity Avoidance Information

The classification of the avoidance information can be done in three different ways: a) by tabulation, b) by comparison of avoidance with the LC50 values, and c) by cluster analysis.

Tabulation may be used for the following purposes:

1. The different designs used in avoidance studies.
2. The chemicals-pollutants used.
3. The researchers and their major interests.
4. The different species used.
5. The pollutant concentrations used.
6. The different tests conducted.
7. The different parameters used.
8. The different laboratories equipped with avoidance apparatus.
9. The different criteria and standards used in avoidance studies.

The LC50 value distinguishes the lethal from the sublethal area for each pollutant. Knowing that value, the avoidance reaction can be placed accordingly and threshold concentrations, effective concentrations, safe concentrations, maximum allowable toxicant concentration, application factors and incipient lethal level concentrations can be estimated. Also comparison of LC50 values with avoidance reactions provides important information for the management of the environmental hazards produced.

Cluster analysis is a technique for the grouping of different curves into similar systems. By knowing to what group an avoidance curve for a specific pollutant belongs, possible effects on the environment can be predicted by studying the behavior of the group. Also by cluster analysis a clarification of the degree of toxic pollutants (high, medium and low) can be obtained, with respect to the avoidance reaction of the aquatic organisms. However, to be able to apply cluster analysis in a study it would be necessary to have information available about the avoidance curves of independent toxicants or effluents. Therefore cluster analysis is considered to be a future step in avoidance analysis.

In the present study, adjustments for safe toxicant concentrations have been proposed by combining data from two sources:

- a) comparison of LC50 values with the avoidance reactions; and
- b) information from the available literature.

PART B

CHAPTER V

"PRELIMINARY TESTING"

CHAPTER V

5.0 INTRODUCTION

The first objective of the preliminary testing was to establish fixed values for some of the variable parameters of the problem, and to evaluate the effect of the injected water jet. A second objective was to quantify the behavior of the fish and to acquire knowledge about the movement of fish in the channel.

Preliminary experiments were run with the following objectives:

1. To establish the general disposition of fish without any pollutant (7 tests);
2. To evaluate the effect of flow (clear water, dye or effluent: 5 tests);
3. To evaluate the effect of water temperature (7 tests);
4. To evaluate the effect of noise (4 tests);
5. To evaluate the effect of light intensity (3 tests).

5.1 General Disposition of Fish in the Channel5.1.1 General

The disposition of the fish in the channel had to be established to obtain reference behavior. These tests were conducted without the introduction of jets to establish baseline behavior and subsequently with a jet of acclimation water to determine the extent of hydraulic preference.

5.1.2 Test Results

Seven tests (Nos. 1,2,22,24,25,26,74) were conducted on different days and with different batches of fish to establish

a general pattern of fish behavior throughout the channel without any pollutant. The final results are given as baseline in Table 5.1.

As the results indicate, there was no significant preference for Side A or B. The fish generally had a preference for the bottom level (A), rather than for the upper levels (B and C). Also, they appeared to prefer the horizontal sections nearer the source of water input, i.e., with a decreasing preference for sections nearer the channel outfall (Figure 3.2). This horizontal preference can be explained because the fish were acclimated in the tank near Section 1 and because of their instinctive negative tropism (orientation movement of an organism in avoidance response to a stimulus). The results in Table 5.1 represent the mean values of these experiments.

5.2 Jet Effects

Five tests (Nos. 9,10,23,27,28) were conducted with a small clear water flow from one side (Side A) of the channel (0.00892 gpm; dilution factor 336) to estimate the effect of a small jet on the fish distribution. The results are given in Table 5.1 under the column heading "acclimation jet". The effect of the jet (injected with multiple diffuser at Depth B - Injection System C), increased the preference of the fish for the injected side (about 5%), and increased the percentage of the fish in the top vertical level (a movement from Depths A and B to C). It was concluded from these data that there was a slight preference of the fish to align themselves with a small hydraulic disturbance in the flow field. Consequently, this slight preference was incorporated in all subsequent results in which jets were used (see Appendix A7).

DISTRIBUTION IN PERCENT

		Baseline	Acclimation Jet
<u>Horizontal</u>			
Section 1		34.8	32.5
2		25.0	29.4
3		22.2	25.0
4		18.0	13.1
<u>Vertical</u>			
Depth	A	48.2	46.5
	B	22.8	20.7
	C	29.0	32.8
<u>Lateral</u>			
Side	A	49.0	54.0
	B	51.0	46.0

TABLE 5.1 General disposition of fish
in the channel and jet effect

Two additional tests using injection systems A and B showed that the 5% influence of the jet was the same for all injection systems tested.

5.3 Effect of Noise

5.3.1 General

Because the experimental channel was in a hydraulics laboratory, there might have been considerable change in the intensity of noise during an experiment. To test the effect of the noise variations several experiments were conducted at different noise levels and the results compared. The following levels of noise (in decibels), representing the range of variations in the laboratory, were tested:

- (a) 60 dBA (Experiment No. 5);
- (b) 64 dBA (normal environment) (Experiment No. 3);
- (c) 73 dBA (Experiment No. 6);
- (d) 80 dBA (highest level) (Experiment No. 4).

5.3.2 Apparatus

The apparatus used for the measurements of the noise level was a portable sound level meter type 2203 by Brüel and Kjaer. It complies with all existing standards for precision sound level meters. It is equipped with an individually calibrated condenser microphone, an internal reference voltage for calibration, weighting networks, AC output for connections to recorders, etc. External filters can be directly attached and a wide range of accessories is available.

5.3.3 Results

As Figure 5.1 shows, there was no significant change in the horizontal position of the fish with changes in the noise level.

In Figure 5.2 a slight preference for the deeper waters is indicated as the noise level increases. However, in the range between 64 dB and 73 dB, which was the normal noise level of the laboratory when the experiments were conducted, there was no significant change in the position of the fish.

In the analysis of variance for the horizontal distribution (ANOVA 18 in the Appendix C3) testing the hypothesis

H_0 : Section 1 = Section 2 = Section 3 = Section 4

H_A : Section 1 $\neq$ Section 2 $\neq$ Section 3 $\neq$ Section 4

F value = 1.63 was obtained with $PR > F = 0.25 > 0.05$

The hypothesis was accepted, which indicated that there was no significant difference between the four sections of the channel in the percentage of fish in the clean water, using four different noise experiments. Also testing the hypothesis

H_0 : 60 dBA = 64 dBA = 73 dBA = 80 dBA

H_A : 60 dBA $\neq$ 64 dBA $\neq$ 73 dBA $\neq$ 80 dBA

the F value was: 0.00 and $PR > F = 0.99 > 0.05$. The hypothesis was accepted, meaning that there was no significant difference between the four noise levels in the horizontal distribution. In the Duncan's tests grouped by section, all noise levels have found to belong to the same Group A.

In the vertical distribution (ANOVA 19 in the Appendix C3), testing the hypothesis:

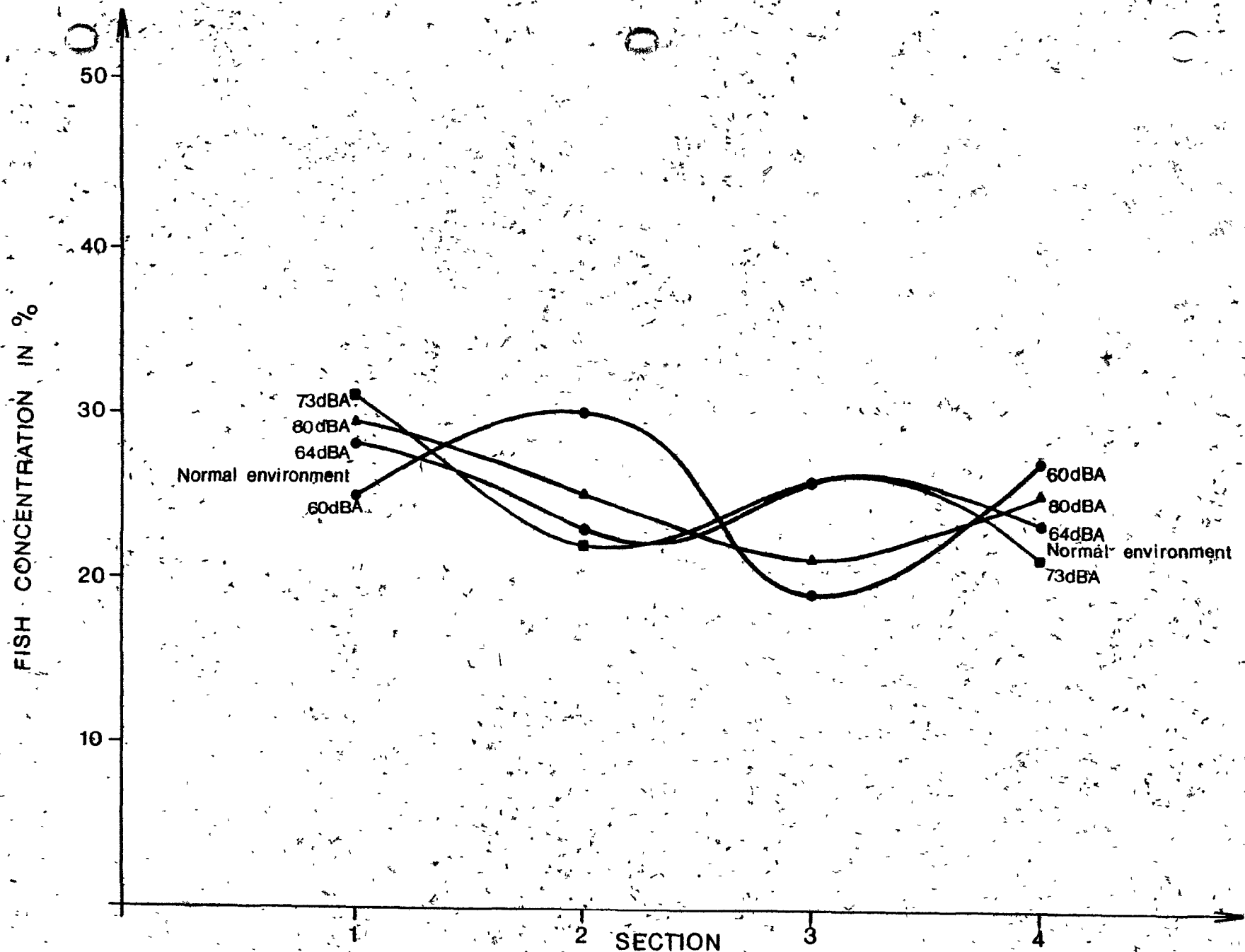


FIGURE 5.1
EFFECT OF NOISE - HORIZONTAL DISTRIBUTION

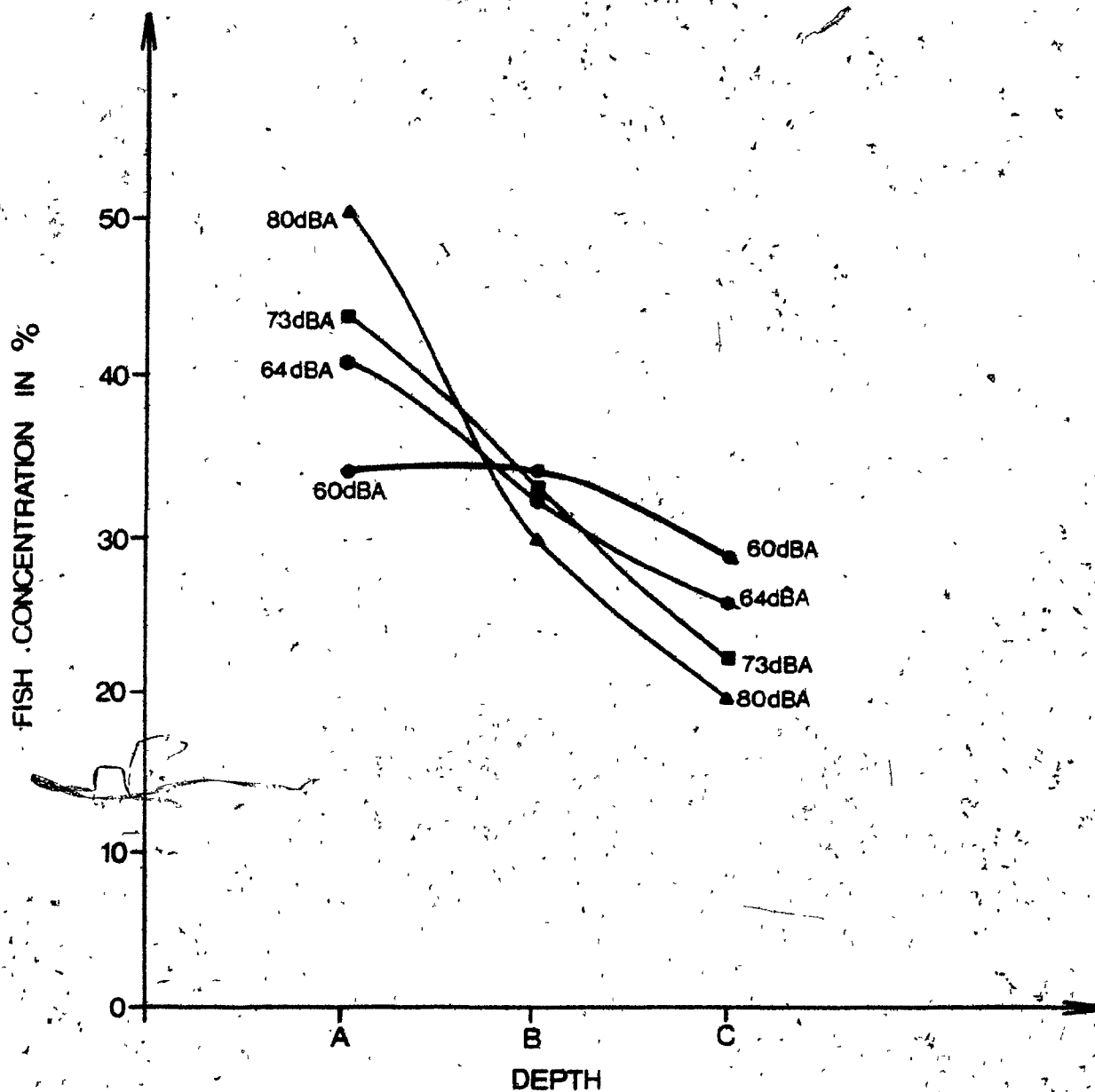


FIGURE 5.2
EFFECT OF NOISE - VERTICAL DISTRIBUTION

H₀: Depth A = Depth B = Depth C

H_A: Depth A ≠ Depth B ≠ Depth C

The F value was 10.83 and $PR > F = 0.01 < 0.05$.

The hypothesis was rejected, indicating that there was a significant difference between the three depths of the channel. However, all the values corresponding to different noise levels for each depth lay within the same group (Duncan's tests).

5.4 Effect of Light Intensity

5.4.1 General

Three experiments (Nos. 3, 7, 8) were conducted to determine the effect of light intensity on fish behavior:

- (a) with very low intensity in a dark environment (side lights) (Experiment No. 7)
- (b) with normal intensity (day lights and side lights) (Experiment No. 3)
- (c) in a high intensity (changing different light intensities in different sections) (Experiment No. 8).

The set-up of the lighting for these experiments throughout the different sections of the channel is illustrated in Figure 5.3.

5.4.2 Results

Because a constant medium level light source was required for the video recording system, a series of fluorescent lights was installed along the length of the channel. Opposite the lights, mirrors were installed and the light intensity in the channel was maintained at a constant level with a daily photoperiod of 12 hours (8 A.M. - 8 P.M.).

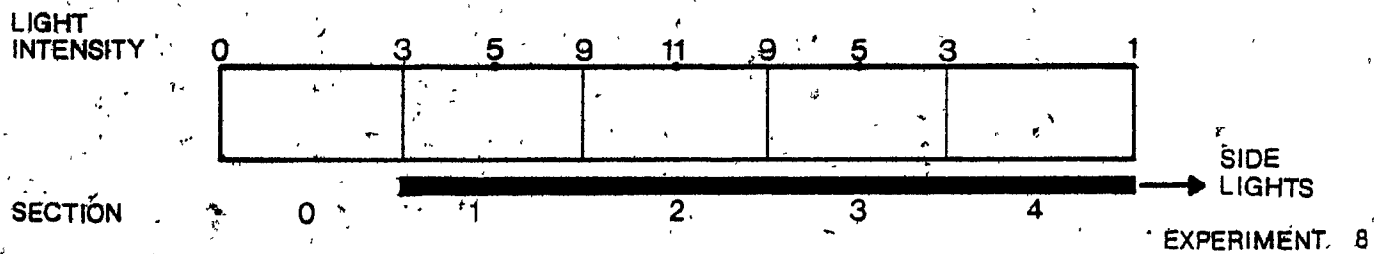
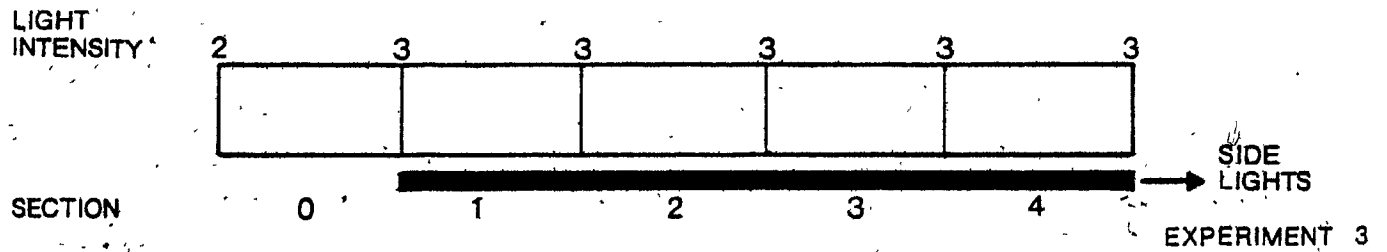
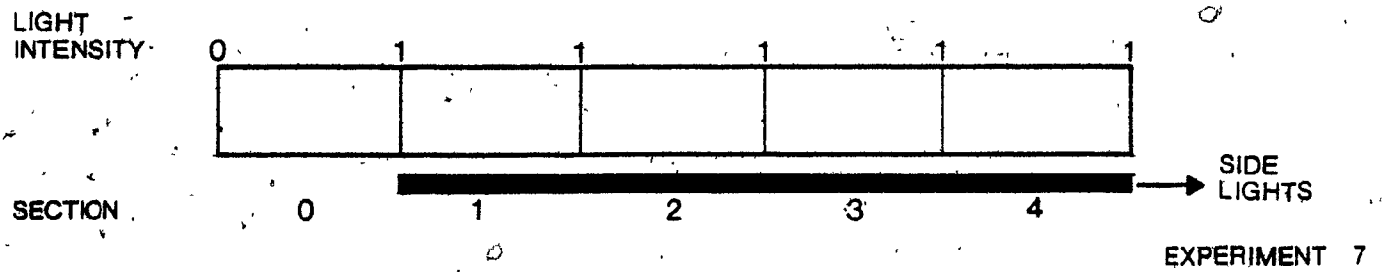


FIGURE 5.3

SET-UP OF THE LIGHTING FOR THE EVALUATION OF THE EFFECT OF LIGHT INTENSITY

The results (Figure 5.4 and 5.5) showed that low intensity of light did not affect the distribution of the fish in the channel. However, there appeared to be an increased preference for high intensity as there was an apparent correlation between the intensity of light and the change in distribution throughout the channel (Fig. 5.4). Very intensive permanent light attracted the fish whereas flashing light tended to frighten them away from the light source.

In the horizontal distribution of low lights (Figure 5.5) Section 0 of the channel was included. In the same figure an expected heavy concentration at depth A of the vertical distribution appears, showing the influence of the side lights (mainly depth A) in a low light intensity environment.

5.5 Effect of Temperature

5.5.1 General

Temperature is a major ecological factor affecting the distribution of fish. The effects of environmental factors, such as temperature, generally have been classified into five categories: lethal, controlling, limiting, masking and directive (Fry 1971). Many fish species prefer a temperature higher than the acclimation temperature during winter and spring conditions when tested in laboratory systems.

McCauley (1977) classified and reviewed ten different types of gradient devices used in the laboratory to determine temperature preferences of fish. The relationship of preference temperatures compared with the upper avoidance and lethal temperature limits on a seasonal basis needs to be better established. In any set of avoidance-preference studies, if preference avoidance data is to be

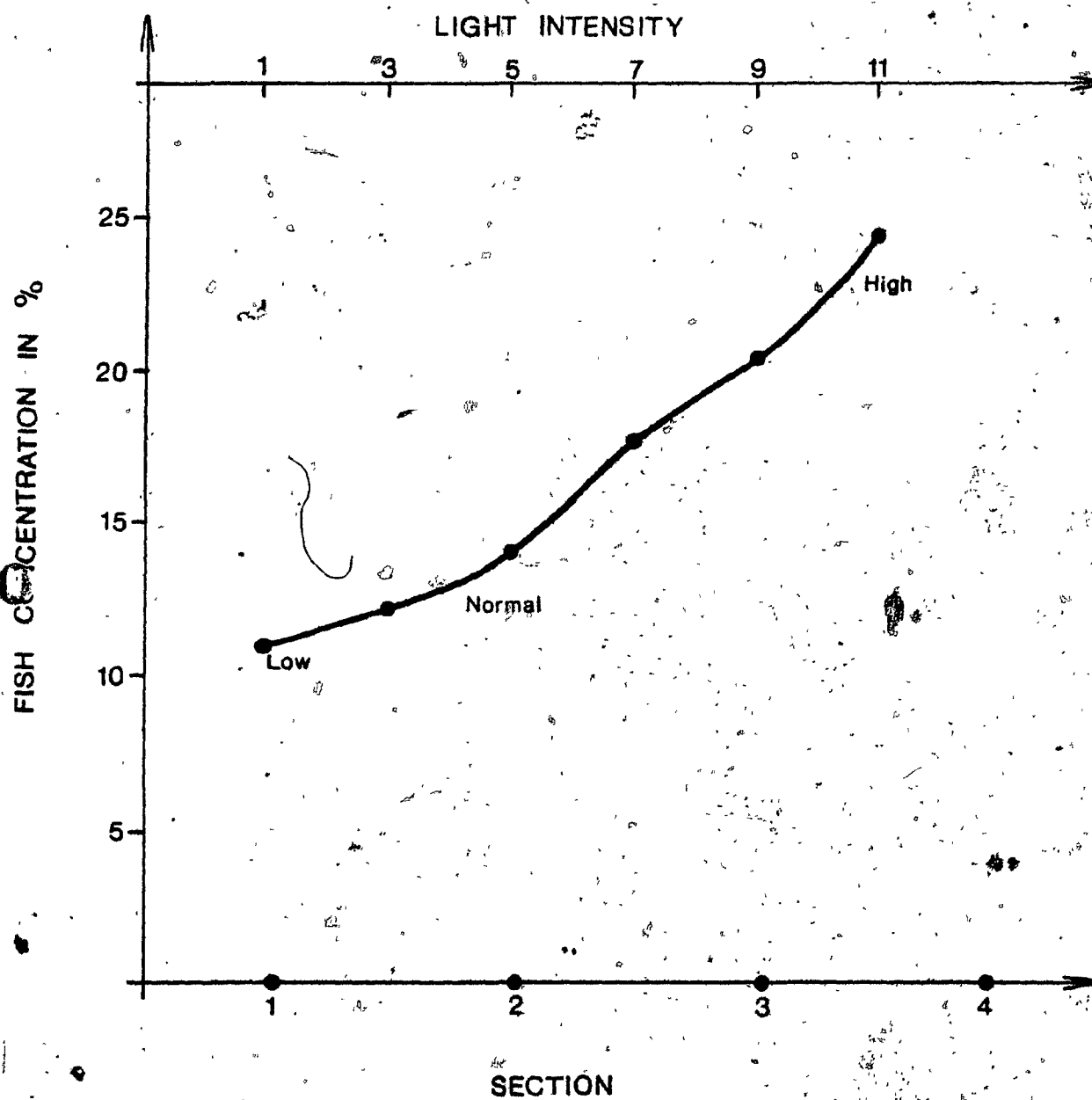


FIGURE 5.4
EFFECT OF LIGHT INTENSITY

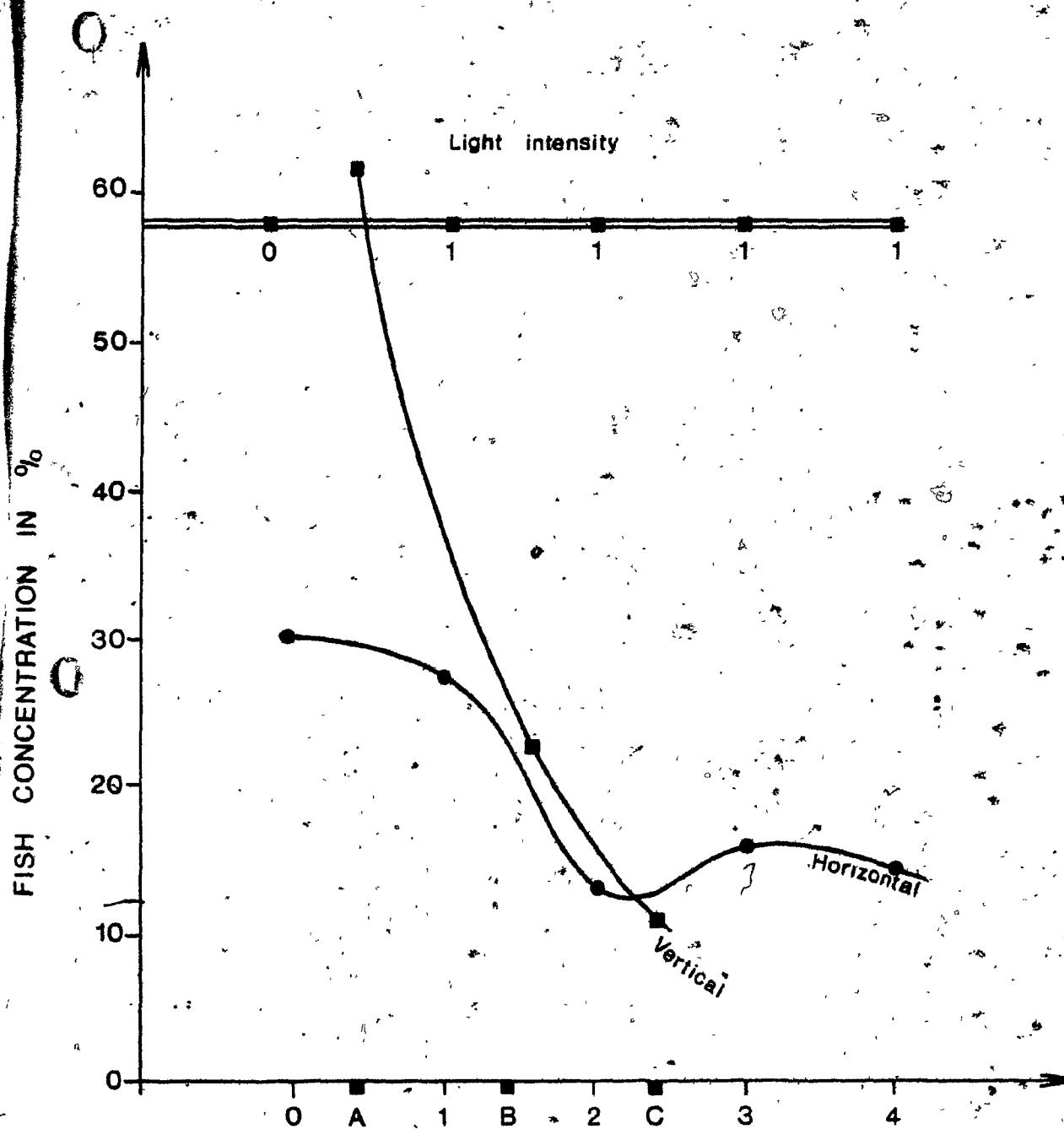


FIGURE 5.5
EFFECT OF LOW LIGHTS - HORIZONTAL AND VERTICAL DISTRIBUTIONS

useful, these relationships must be understood and defined experimentally.

5.5.2 Test Results

The thermal gradient in the channel of the avoidance apparatus used in this study depends on the temperature of the laboratory, the temperature of the incoming water and the flow in the channel. It had been found that for influent temperatures between 13-16°C the difference throughout the channel was 0.2° - 0.5°C. The sensitivity of fish to this temperature differential was found to be more than expected according to results reported in the literature (Fry et al, 1971).

Four tests (No: 3,4,5,6 at 12°, 13°, 22°, 23°C) were conducted to determine the effects of temperature on the behavior of the fish and the results are shown graphically in Figures 5.6 and 5.7. It must be reiterated that because of the length of the channel, there was a temperature gradient horizontally along the channel. The maximum temperature differential in the channel for all water temperatures used in the experiment was of the order of 0.5°C. For fish reared at 15°C (3 months or more) it appears there was a pronounced avoidance reaction to high temperatures, especially for temperatures higher than 16°C. In the range between 16° - 23°C a small temperature difference had a profound effect on the distribution curve. For temperatures lower than acclimation the effect was much smaller. In a high temperature environment the fish preferred the coldest section, while with low temperatures the vertical disposition changed,

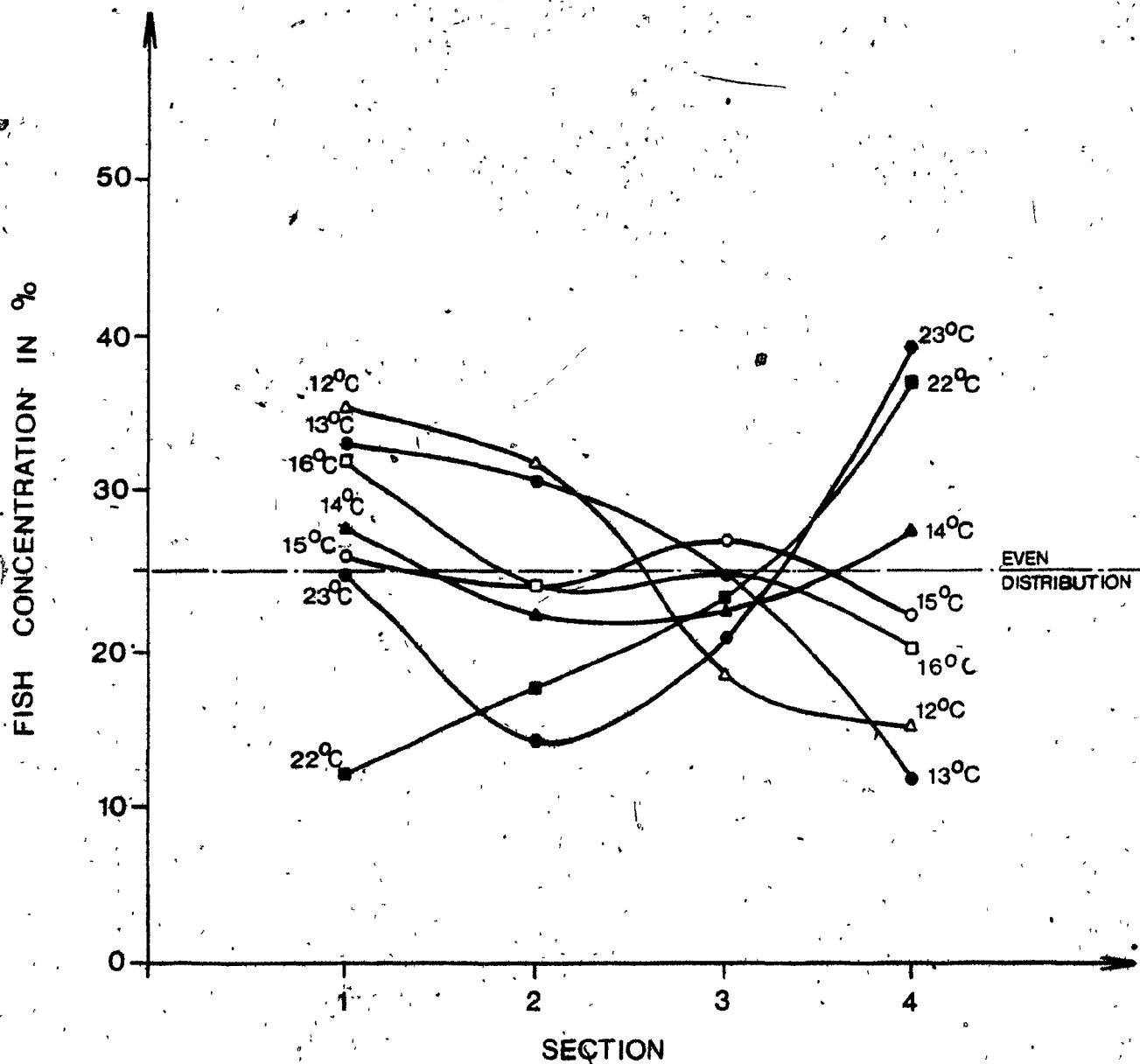


FIGURE 5.6

EFFECT OF TEMPERATURE - HORIZONTAL DISTRIBUTION

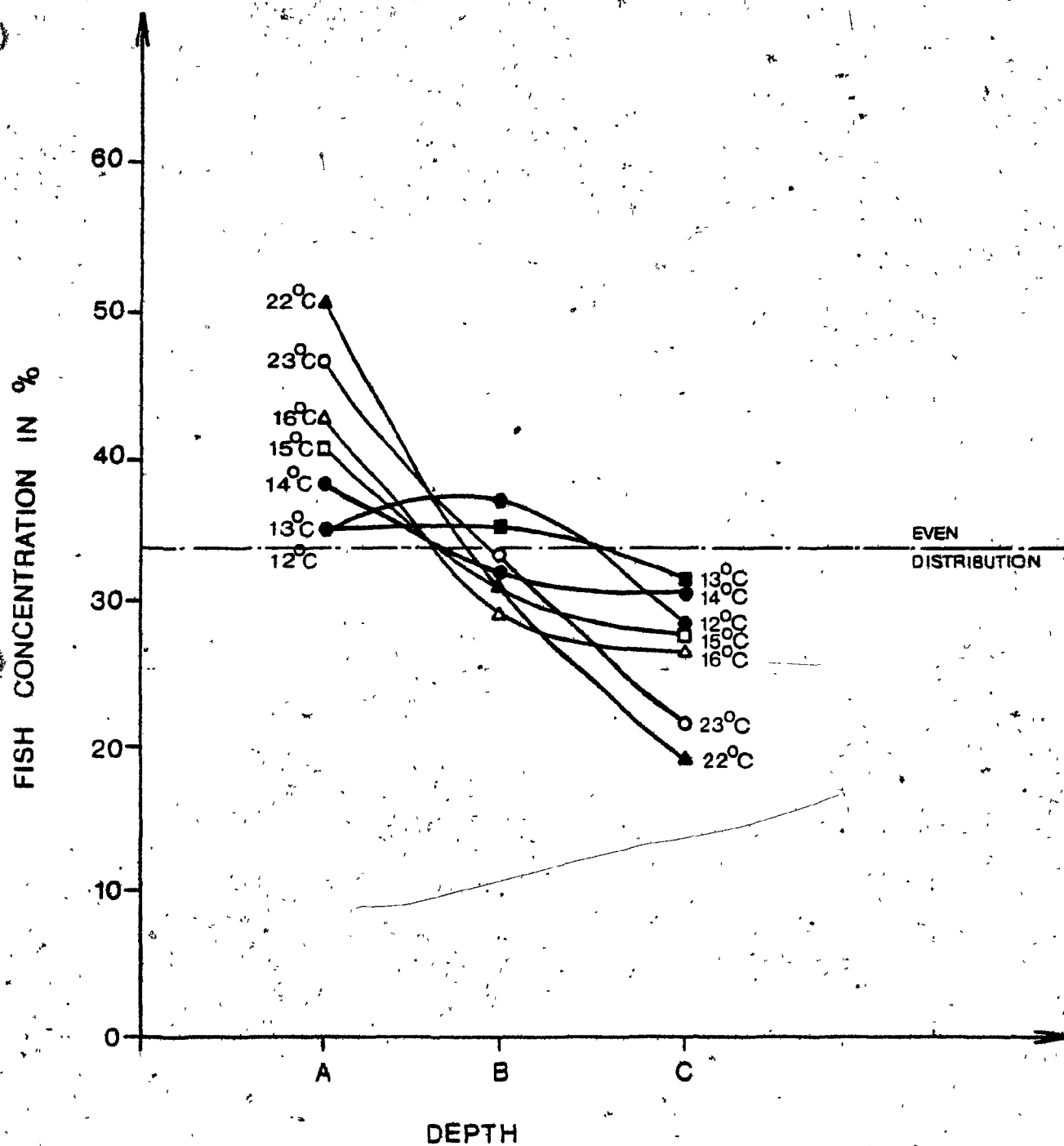


FIGURE 5.7
EFFECT OF TEMPERATURE - VERTICAL DISTRIBUTION

indicating that the temperature was not the most comfortable for them. For example, with an influent temperature of 22°C a high concentration of fish was observed in the downstream end section (the coldest), and at 12°C a movement from depth A (deepest) to depth B was observed. The analysis of variance for the temperature tests (ANOVA 20 and 21 in Appendix C3) indicated that in the horizontal distribution the responses from the 22°C and 23°C tests belonged to a different group than the responses from the rest of the temperatures tested (Duncan's tests).

Three additional tests at (14°C, 15°C, 16°C) showed that the "temperature preferendum", compared with the even distribution, was at 15°C. Based on the results of the testing an experimental acclimation temperature of 15°C was therefore adopted for all subsequent experiments.

5.6 Final Adjustments of the Foregoing Parameters

The general disposition of the fish and jet effects on this disposition were evaluated (as shown in Table 5.1), and these observations were used in interpreting subsequent data. As a result of preliminary testing, ambient noise, light and temperature were selected for the avoidance experimentation as follows:

A) NOISE LEVEL: 64 dBA

Remark: Noise was found to have a minimal effect on the disposition of fish within the channel.

B) LIGHT INTENSITY: 3

Remark: Constant intensity fluorescent lights were set up along the length of the channel and a 12-hour photo period was established for the duration of the experiments.

C) TEMPERATURE: 15°C

It was concluded from the preliminary testing that these values would not bias the subsequent research program.

CHAPTER VI

"TESTS FOR OPTIMAL NUMBER OF FISH,
INFLUENCE OF VIDEO ANALYSIS,
VARIABILITY, ADAPTABILITY AND CONTINUITY"

CHAPTER VI

6.0 Introduction

The aims of this chapter's testing were:

- (a) To define the optimal number of fish per experiment which would give accurate results using the "Design H" avoidance apparatus;
- (b) To define the influence of the number of readings on the analysis of video cassette data, and to establish a standard number of readings which would yield a desired level of accuracy within prescribed confidence limits;
- (c) To evaluate the variability and adaptability of the experiments;
- (d) To evaluate the feasibility of conducting sequential experiments with different toxicant concentrations but using the same batch of fish.

6.1 Tests to define the optimal number of fish per experiment6.1.1 General

In the past, the majority of avoidance apparatus were designed to study one fish at a time (in some cases, up to a maximum of five). The number of aquatic organisms used per experiment influences the accuracy of the observed behavioral reactions. If one excludes the effect of "schooling phenomena", then in general the larger the number of fish used, the more unbiased and accurate will be the results produced. The cost per experiment is increased significantly, but the resulting close resemblance to the natural situation justifies the higher costs.

6.1.2 Experiments - Results

Five two-hour experiments (Nos. 45,46,47,48,49) using clear water, were conducted with 10,30,50,75 and 100 fish respectively, in order to define the optimal number of fish per experiment. The temperature was constant in all five experiments (15°C). The acclimation period for each experiment was two days.

Table 6.1a shows the results of the horizontal and vertical distribution of fish in these five experiments. Table 6.1b shows their avoidance behavior and the difference in accuracy, assuming that in experiments with a clear water, 50% of fish will show avoidance reactions.

Figures 6.1 and 6.2 illustrate the horizontal and vertical distributions using experiments with different numbers of fish in clear water.

6.1.3 Interpretation of results

- A. The difference in avoidance reaction in clear water (without pollutants), using different numbers of fish is relatively small, $\pm 8\%$.
- B. Two additional tests showed that the difference became significantly greater when pollutant concentrations were injected (max. 20%).
- C. It appears that variation in the number of fish used led to major differences in the horizontal and vertical distribution throughout the channel.
- D. The effort to produce horizontal and vertical distributions approaching the ones derived from the general disposition of the fish and the 50% avoidance reaction in clean water led to the selection of an optimal number of fish which would be used per experiment.

Horizontal Distribution					No. of Fish
No. of Experiment	% in Sections 1-4				
	1	2	3	4	
45	57.4	17.8	13.8	11.0	10
46	48.6	23.7	16.7	11.0	30
47	42.2	28.8	18.0	11.0	50
48	38.1	26.5	20.3	15.1	75
49	33.2	25.7	23.1	18.0	100

Vertical Distribution				
No. of Experiment	% in Depths A-C			No. of Fish
	A	B	C	
45	76.4	12.9	10.7	10
46	67.0	20.1	12.9	30
47	58.7	27.8	13.5	50
48	52.0	29.1	18.9	75
49	46.0	28.	26	100

TABLE 6.1a Horizontal and vertical distributions in clear water using different number of fish per experiment.

Avoidance Reaction				
No. of Experiment	No. of Fish	Percent in Side B	Difference in % *	Remarks
45	10	58	+8	least accurate
46	30	55	+5	less accurate
47	50	53	+3	accurate
48	75	51	+1	highly accurate
49	100	49	-1	highly accurate

TABLE 6.1b Avoidance reaction in clear water using different no. of fish

* considering the 50% as accurate evaluation

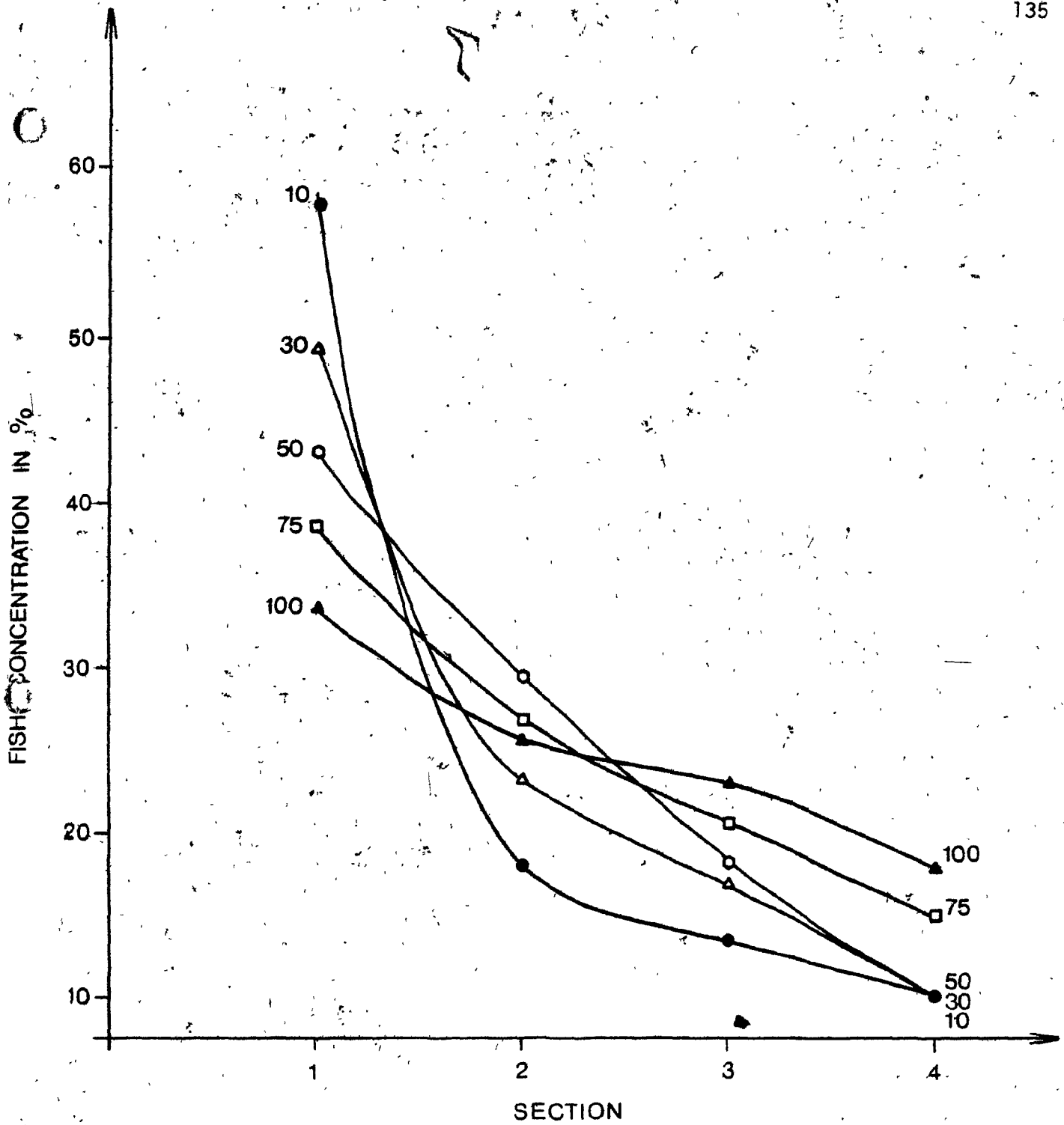


FIGURE 6.1
HORIZONTAL DISTRIBUTION WITH DIFFERENT NUMBER OF FISH
IN CLEAR WATER

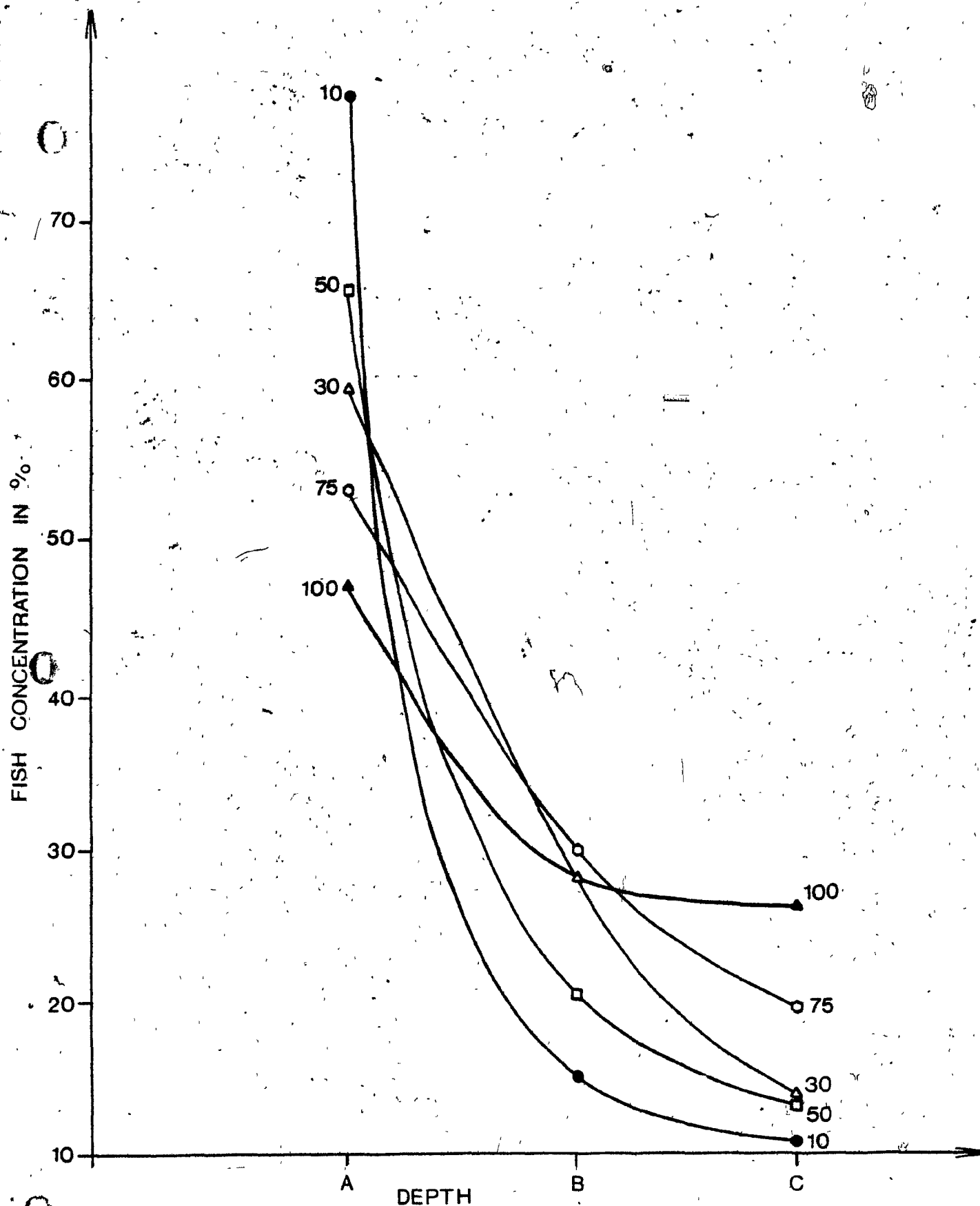


FIGURE 6.2

VERTICAL DISTRIBUTION WITH DIFFERENT NUMBER OF FISH
IN CLEAR WATER

E. For the avoidance apparatus of Design H, and using trout between 3-4 " , the optimal number of fish for avoidance experiments was found to be 100, taking into consideration the prohibitive expense involved in using more than 100 organisms per test.

6.2 Tests to define the influence of the number of readings in a video cassette

6.2.1 Standard procedure

During each experiment, a continuous two-hour recording was made on four video cassettes, one for each section of the apparatus. The analysis of these tapes involved replaying them and tabulating results from a certain number of still frames which were taken to represent the whole tape; each of these still frames is referred to as "a reading". The purpose of experiment "K" was to define the influence of the number of readings on the accuracy of results. More specifically, the effect on the accuracy of the results was studied using a tape cassette representation with 3,6,12,24,48 and 96 readings.

6.2.2 Experiments - Results

Experiment "K" was a normal two-hour test with a plating industry pollutant concentration of 1:2000 LC50 injected from Side A of the channel. The rest of the parameters remained constant. The avoidance reaction to that concentration using 24 readings for the analysis of the tape cassette was 58%. After the initial experiment, further analyses of the four tapes were made, taking readings according to the following schedule:

1. 3 Readings (every 40 minutes)
2. 6 Readings (every 20 minutes)
3. 12 Readings (every 10 minutes)
4. 24 Readings (every 5 minutes)
5. 48 Readings (every 2-1/2 minutes)
6. 96 Readings (every 1-1/3 minutes)-considered to be the true value.

The results of the horizontal and vertical distribution appear in Table 6.2a, as well as in Figures 6.3 and 6.4. The avoidance reaction, using different numbers of readings is illustrated in Table 6.2b. Figure 6.5 illustrates the divergence from most accurate value ("true value") in relation to the number of readings.

6.2.3 Interpretation of Results

Accepting a 3% divergence from the true value, the video cassette analysis, based on 24 readings, provided a very good approximation. Sometimes 12 readings (every 10 minutes) gave only 5% divergence from the true value which was also a satisfactory estimate for tests not requiring a higher degree of accuracy. Three or six readings were not at all satisfactory for analysis (10% divergence). The 48 reading analysis was extremely close to the true value (0.5%) but considering that this doubles the time needed for counting, it was not appropriate for a system which is not fully computerized.

From the horizontal and vertical distribution curves, it is apparent that sufficient accuracy is obtained with 24 or 48 readings.

Horizontal Distribution

No. of Readings	% in Section 1-4			
	1	2	3	4
3	27.6	38.4	13	20.7
6	43.8	29.5	9.6	17
12	41.2	31.2	11.3	16.3
24	42.1	29.6	12.5	15.6
48	41.1	30.1	12.3	16.5
96	40.8	30.4	12.6	16.2

Vertical Distribution

No. of Readings	% at Depth A-C			Accuracy
	A	B	C	
3	46	29	25	not accurate
6	39.4	34.2	26.4	
12	40	34.4	25.6	transition zone
24	38.9	32.9	28.2	accurate
48	38.8	34.6	26.6	
96	38.8	33.7	27.5	

TABLE 6.2a Horizontal and vertical distribution of fish using different number of readings for the video analysis.

Avoidance Reaction			
No. of Readings	Side A in % Polluted	Side B in % Non-polluted	Divergence in %
3	40	60	8
6	47.2	52.8	8.5
12	45.6	54.4	4.8
24	42	58	3.4
48	43.2	56.8	0.6
96	43.5	56.5 True value	0

TABLE 6.2b Avoidance reactions
using different number
of readings for the
video analysis.

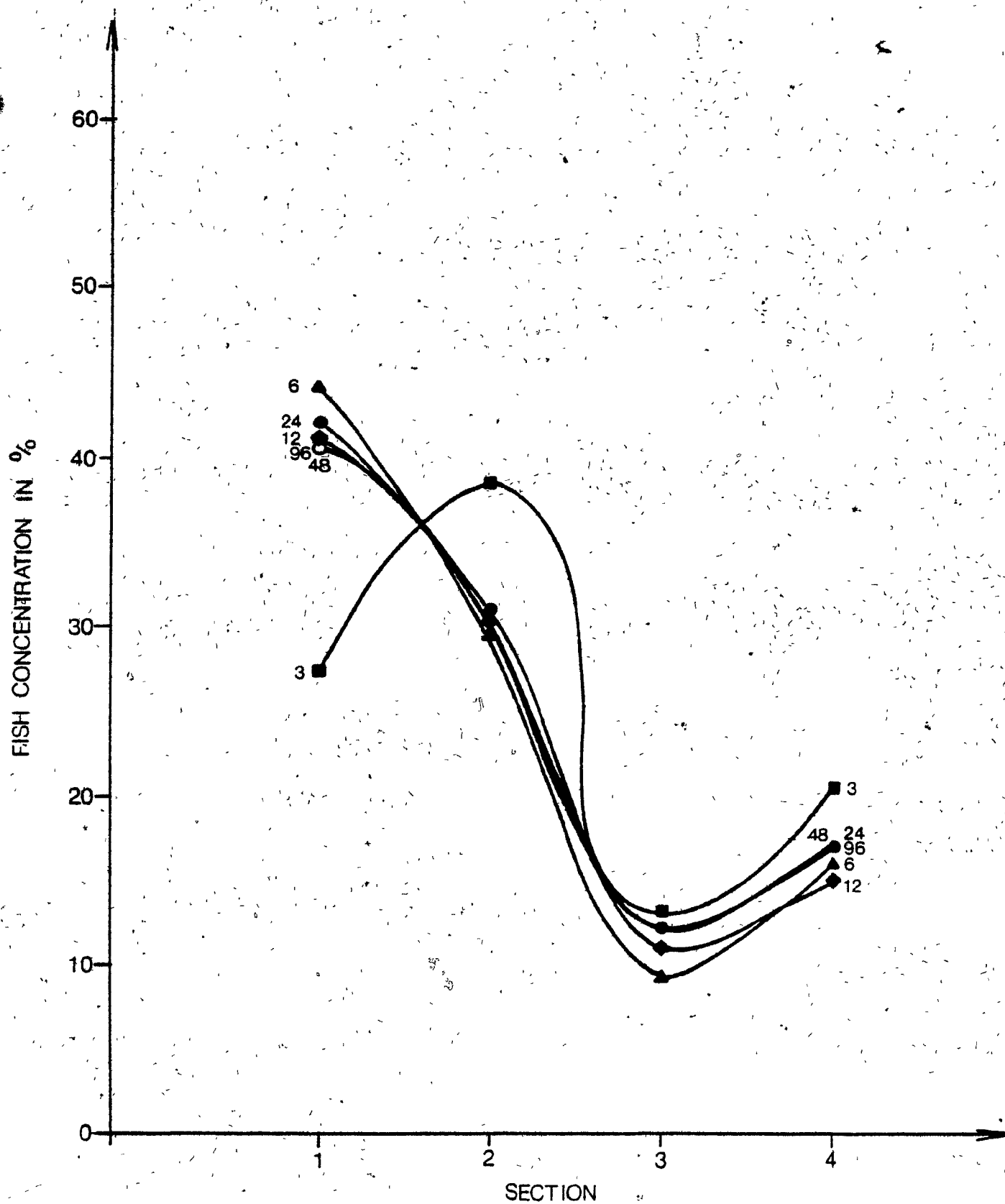


FIGURE 6.3
HORIZONTAL DISTRIBUTION USING DIFFERENT NUMBER
OF READINGS FOR THE VIDEO ANALYSIS

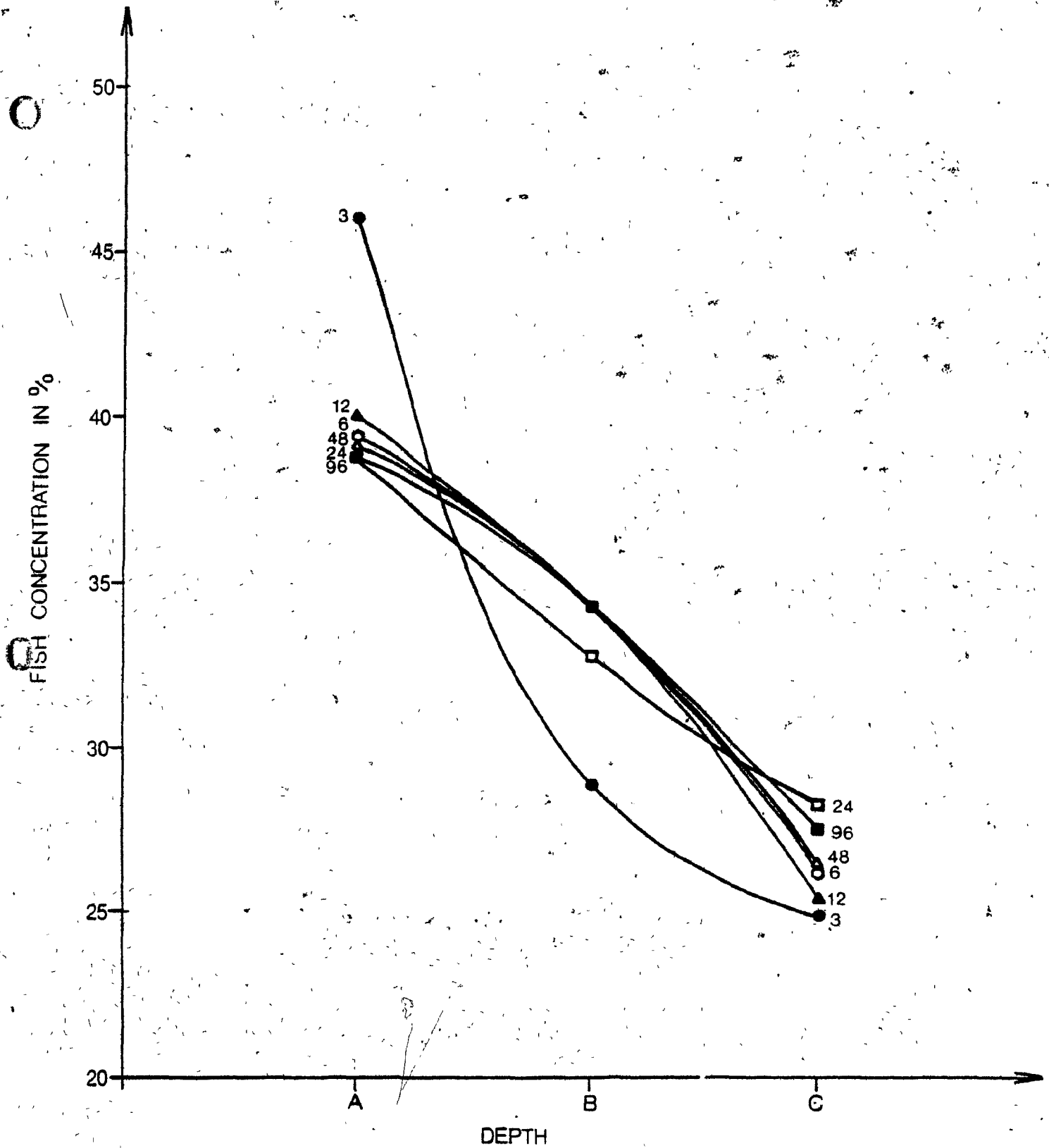


FIGURE 6.4
VERTICAL DISTRIBUTION USING DIFFERENT NUMBER
OF READINGS FOR THE VIDEO ANALYSIS

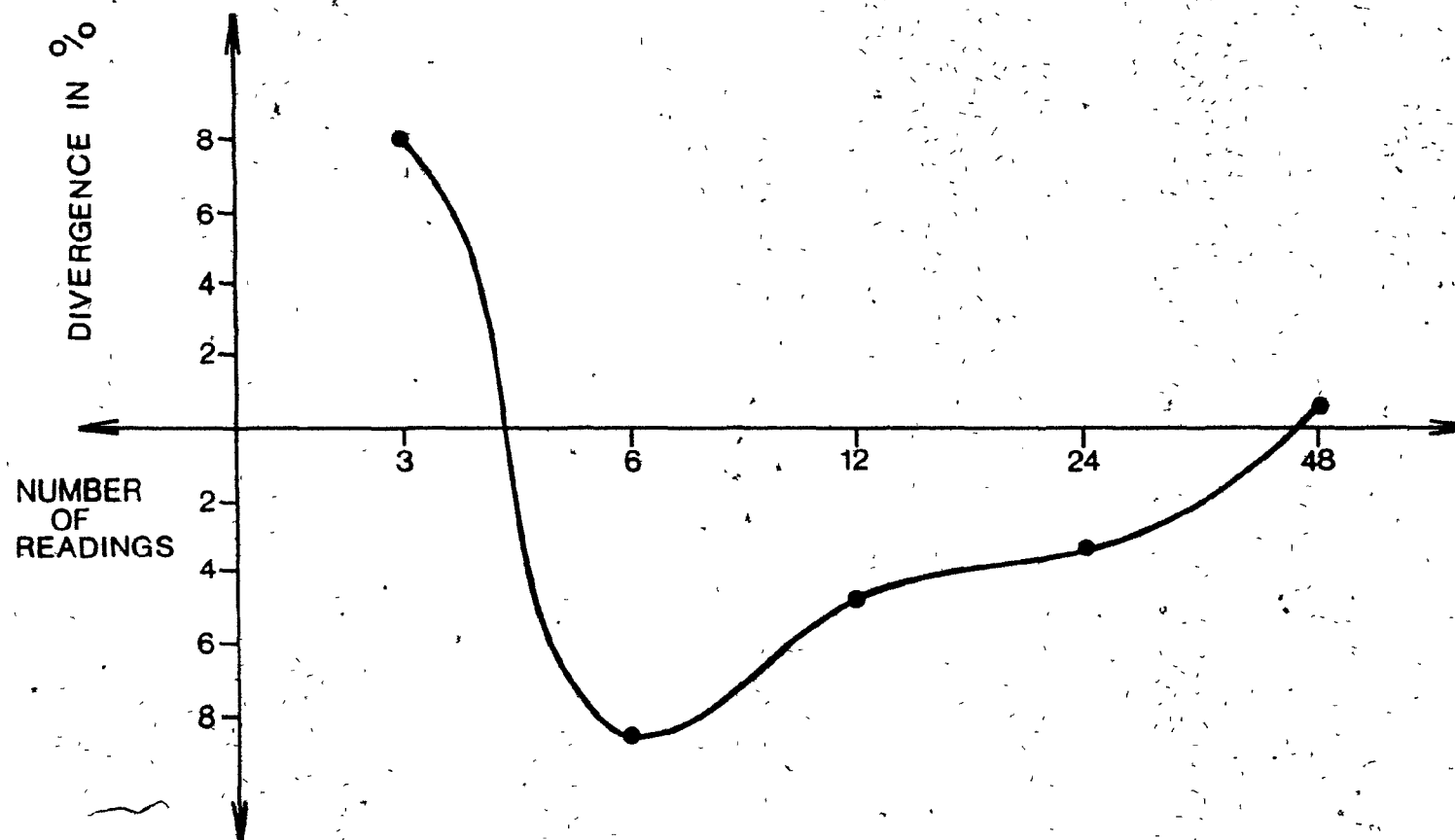


FIGURE 6.5 DIVERGENCE FROM THE "TRUE VALUE"
ACCORDING TO THE NUMBER OF READINGS.
96 IS TAKEN TO BE THE "TRUE VALUE".

Conclusions

1. Twenty-four readings from a ~~two-hour~~ video cassette (one every 5 minutes) were judged to be appropriate for the analysis of avoidance experiments considering the sufficiently high degree of accuracy ($\pm 3\%$) obtained, and the time consumption required for the analysis.
2. Twelve readings (one every 10 minutes) were suitable for tests in which the desired degree of accuracy was not great (e.g., a situation in which the general structure of an avoidance curve was being determined versus the greater accuracy needed when studying reactions to a specific pollutant concentration).

6.3 Variability tests

6.3.1 Tests of consistency

To evaluate the consistency of a series of experiments, factors which can influence the results must be analyzed, and the magnitude of their contribution to the variability of the tests must be estimated.

In this study, factors influencing the variability of the results included:

1. Season of the year
2. Differences between batches of fish
3. Differences between morning, afternoon and evening experiments.

A number of experiments was done to evaluate the effect of these factors, and results were compared with reference experiments in order to provide an estimate of their contribution to the variability and reproducibility of the tests.

6.3.2 Experiments and results

A. Experiments

To evaluate the effect of repeating the same test at different times, and with different batches of fish, Experiments No. 34 and 35 were conducted; data from two reference tests were used to evaluate the results.

All tests were done using injection system B, with a plating industry pollutant concentration of 1:200 LC50, at 15°C and with 6 gpm of flow through the channel. Table 6.3 illustrates the horizontal and

Horizontal Distribution					
No. of Experiment	Conditions	Section in %			
		1	2	3	4
34	Batch No. 1 Summer morning	23	30	24	23
35	Batch No. 1 Summer afternoon	23	38	19	19
R ₁	Batch No. 2 Winter morning	27	33	21	18
R ₂	Batch No. 2 Winter afternoon	28	32	25	15
Vertical Distribution					
No. of Experiment	Conditions	Depth in %			
		A	B	C	
34	Batch No. 1 Summer morning	32.7	37.2	30.1	
35	Batch No. 1 Summer afternoon	33.5	33	33.5	
R ₁	Batch No. 2 Winter morning	30	32.5	37.5	
R ₂	Batch No. 2 Winter afternoon	31	32	37	
Avoidance Reaction					
No. of Experiment	Conditions	Side B (Non-polluted)			
34	Batch No. 1, summer morning	75%			
35	Batch No. 1, summer afternoon	77%			
R ₁	Batch No. 2, winter morning	81%			
R ₂	Batch No. 2, winter afternoon	80%			

TABLE 6.3 Horizontal and vertical distribution and avoidance reaction for variability tests.

vertical distribution of the fish and their avoidance reactions at various times and using different batches of fish.

B. Results

(a) In the avoidance reaction the tests results varied:

from morning to afternoon : max 2%

from batch to batch : max 4%

from 2 days to 6 months : max 2%

Therefore, the maximum difference would not exceed 6% if all three factors happened to influence the tests simultaneously.

(b) The effect of these variations on the horizontal distribution was relatively small; most of the variability could be attributed to differences between batches of fish (max. diff. 8%).

The vertical distribution was fairly stable around the even distribution (max. diff. 7.4%).

6.4 Adaptability tests

6.4.1 Tests of persistency

It is well known that the acclimation period of aquatic organisms before any kind of toxicity testing is very important. In-depth knowledge about how and when fish will adapt to a polluted environment is not available at present. A series of four continuous tests of two hours (total eight hours) was conducted to define the persistency of the fish response with time, i.e., their adaptability to the pollutant. Duration of exposure is a major variable in toxicity studies. Hence in testing pollutants, the evaluation of acute, subacute

and chronic effects and the estimation of the relationship among them becomes extremely important.

6.4.2 Experiments and results

Four continuous experiments (Nos. 36, 37, 38 and 39) were conducted from 11 A.M. to 7 P.M. on a summer day (No. 36 from 11-1, No. 37 from 1-3, No. 38 from 3-5 and No. 39 from 5-7 P.M.). The channel flow was 6 gpm and the temperature was 15°C. The pollutant concentration of 1:750 LC50 (plating industry effluent) was injected from Side A of the channel with Injection System A for 8 hours continuously, at a constant rate. Table 6.4 illustrates the horizontal distribution, the vertical distribution and the avoidance reaction of fish during these four continuous experiments. The results from Table 6.4 (which includes a reference test used for comparison), led to the following conclusions:

- A. There was a 6-7% avoidance reaction difference between the 2 and 8-hour tests in low pollutant concentrations (Figures 6.6 and 6.7).
- B. The vertical distribution seemed stable. In the horizontal distribution a tendency for fish to shift towards the second section was noted with the passage of time. This was mainly due to the characteristics of the Injection System A.

Horizontal Distribution					Vertical Distribution		
No. of Experiment	Sections in %				Depth in %		
	1	2	3	4	A	B	C
36	18.6	34.3	31.7	15.2	40	36	24
37	21.2	31.2	21.3	26.	-	-	-
38	18.8	46.1	20.7	14.3	30	42	28
39	19.5	49.8	27.7	5	37	30	23

Avoidance Reaction			
No. of Experiment	Side B (non-polluted)	Difference from the reference value	Timing in hours
36	66.5%	-2.0%	0-2
37	68.5%	+0.5%	2-4
38	73.5%	5.0%	4-6
39	75%	6.5%	6-8
Ref.	68.5%	0	0-2

TABLE 6.4 Horizontal, vertical distribution and avoidance reaction of adaptability tests.

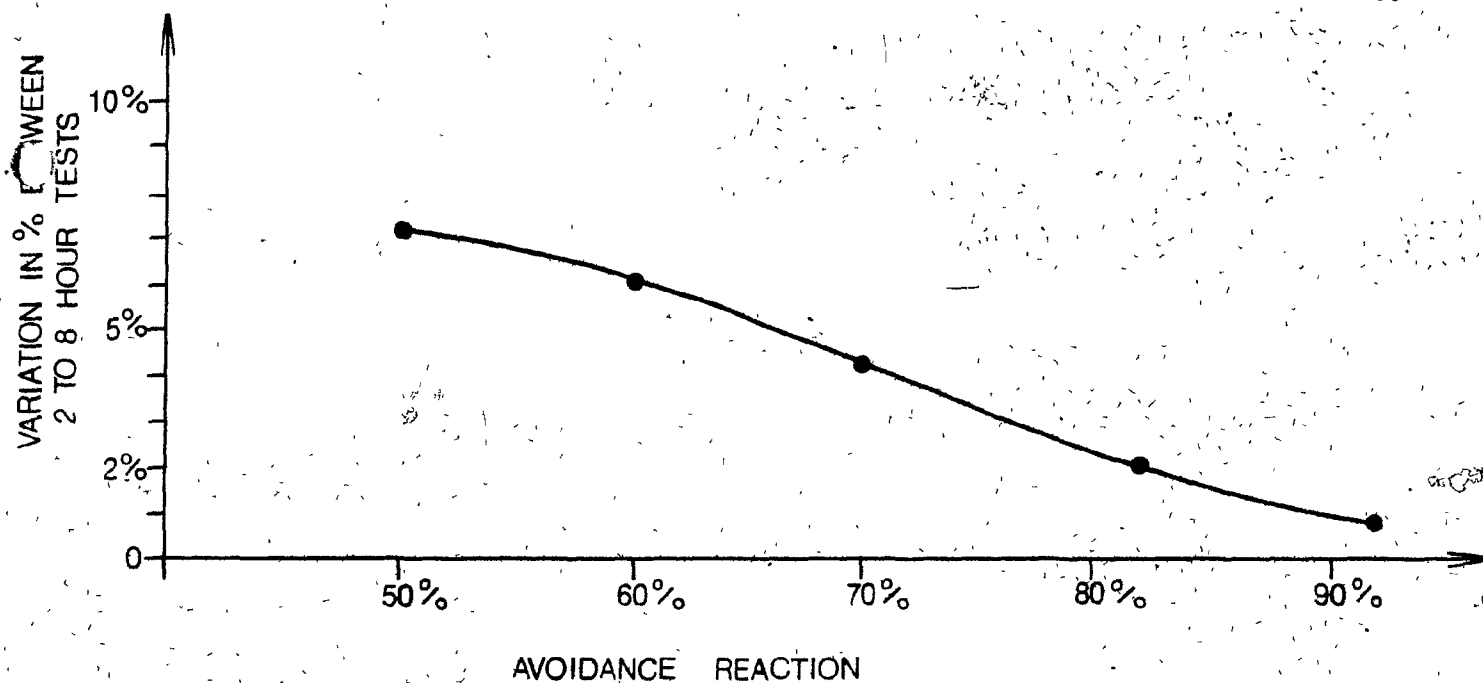


FIGURE 6.6

DIFFERENCES OF AVOIDANCE REACTION BETWEEN 2 AND 8 HOUR TESTS

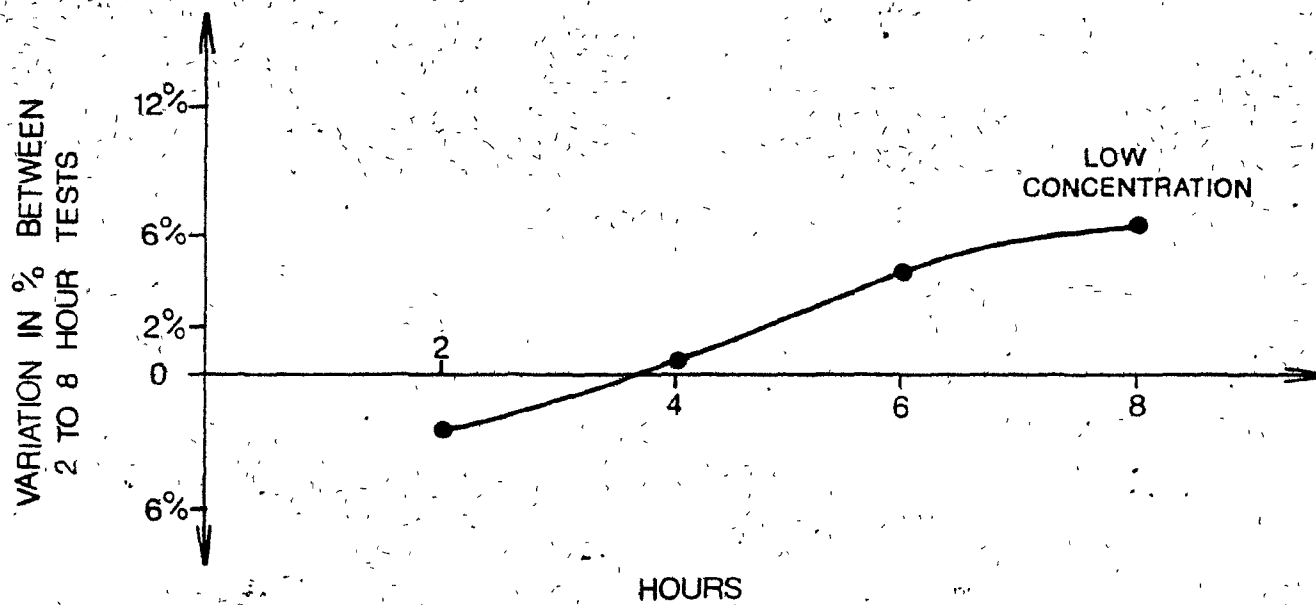


FIGURE 6.7

DIFFERENCES OF AVOIDANCE REACTION ON TIME IN LOW POLLUTANT CONCENTRATION

6.5 Continuity tests

6.5.1 Principal objectives

The objective of this part of the study was to see whether a continuous test with pollutant concentrations being increased at time intervals would produce results similar to those from the more usual individual tests, each of which requires adequate preparation and acclimation periods.

It was hoped that the time needed for preparation and analysis of a set of experiments could be reduced by conducting the experiments with different concentrations in a continuous fashion while still obtaining reliable results.

If this could be done the analysis of avoidance reactions against a specific pollutant would take only a few hours instead of the usual two weeks required for the traditional procedure.

6.5.2 Experiments and results

A. Experiments

Three 3½-hour experiments (Nos. 40, 41 and 42) were conducted using increasing concentrations of the plating industry effluent (to be used extensively in later experiments). The 6 concentrations were changed every 30 minutes according to Table 6.5.

A fourth 6-hour experiment (No. 43) was done later, again using 6 different pollutant concentrations but with a ½-hour acclimation period between them (non-polluted interval), as shown in Table 6.6.

Time Interval in hours	No. of Half Hour	Ratio of LC50	Flowmeter Reading	Injection System
0- $\frac{1}{2}$	1st	0	0	B
$\frac{1}{2}$ -1	2nd	1:2000	10	B
1-1 $\frac{1}{2}$	3rd	1:500	30	B
1 $\frac{1}{2}$ -2	4th	1:250	50	B
2-2 $\frac{1}{2}$	5th	1:100	110	B
2 $\frac{1}{2}$ -3	6th	1:75	150	B
3-3 $\frac{1}{2}$	7th	1:50	198	B

TABLE 6.5 Time intervals of continuous tests with increasing pollutant concentrations (Exp.40,41,42)

Time interval in hours	No. of Half Hour	Ratio of LC50	Flowmeter Reading	Injection System
0- $\frac{1}{2}$	1st	0	0	-
$\frac{1}{2}$ -1	2nd	1:2000	10	B
1-1 $\frac{1}{2}$	3rd	0	0	-
1 $\frac{1}{2}$ -2	4th	1:500	30	B
2-2 $\frac{1}{2}$	5th	0	0	-
2 $\frac{1}{2}$ -3	6th	1:250	50	B
3-3 $\frac{1}{2}$	7th	0	0	-
3 $\frac{1}{2}$ -4	8th	1:100	110	B
4-4 $\frac{1}{2}$	9th	0	0	-
4 $\frac{1}{2}$ -5	10th	1:75	150	B
5-5 $\frac{1}{2}$	11th	0	0	-
5 $\frac{1}{2}$ -6	12th	1:50	198	B

TABLE 6.6 Time intervals of continuous Experiment No.43

B. Results

Because the same set of experiments was done independently following the standard procedure (preparation, acclimation, etc., Chapter X), the results obtained from the four continuity tests were plotted for comparison in Figure 6.8 against the actual avoidance curve (Figure 10.7 Chapter X). Figure 6.8 shows that the avoidance curves produced by the four continuous tests were totally different from the avoidance curve produced by the independent tests. The continuous test gives unpredictable results because there are many uncontrollable variables in this type of experiment. Beyond a certain, toxicant concentration (80% avoidance) the curves are similar because they have reached the highest avoidance level. The continuous test was judged to be completely inapplicable. No theoretical assumptions could be made to relate the continuous with the normal tests because of the following facts:

- (a) Observations were begun immediately after the injection of each new toxicant concentration, without a prior acclimation period.
 - (b) The same fish were exposed to all toxicant concentrations and their behavior may have been affected by their previous exposure to lower concentrations of toxicants.
 - (c) The entire experiment seemed uncontrollable with many bias factors.
- In the final analysis, the results of the continuous tests with increasing pollutant concentrations were discarded as being meaningless.

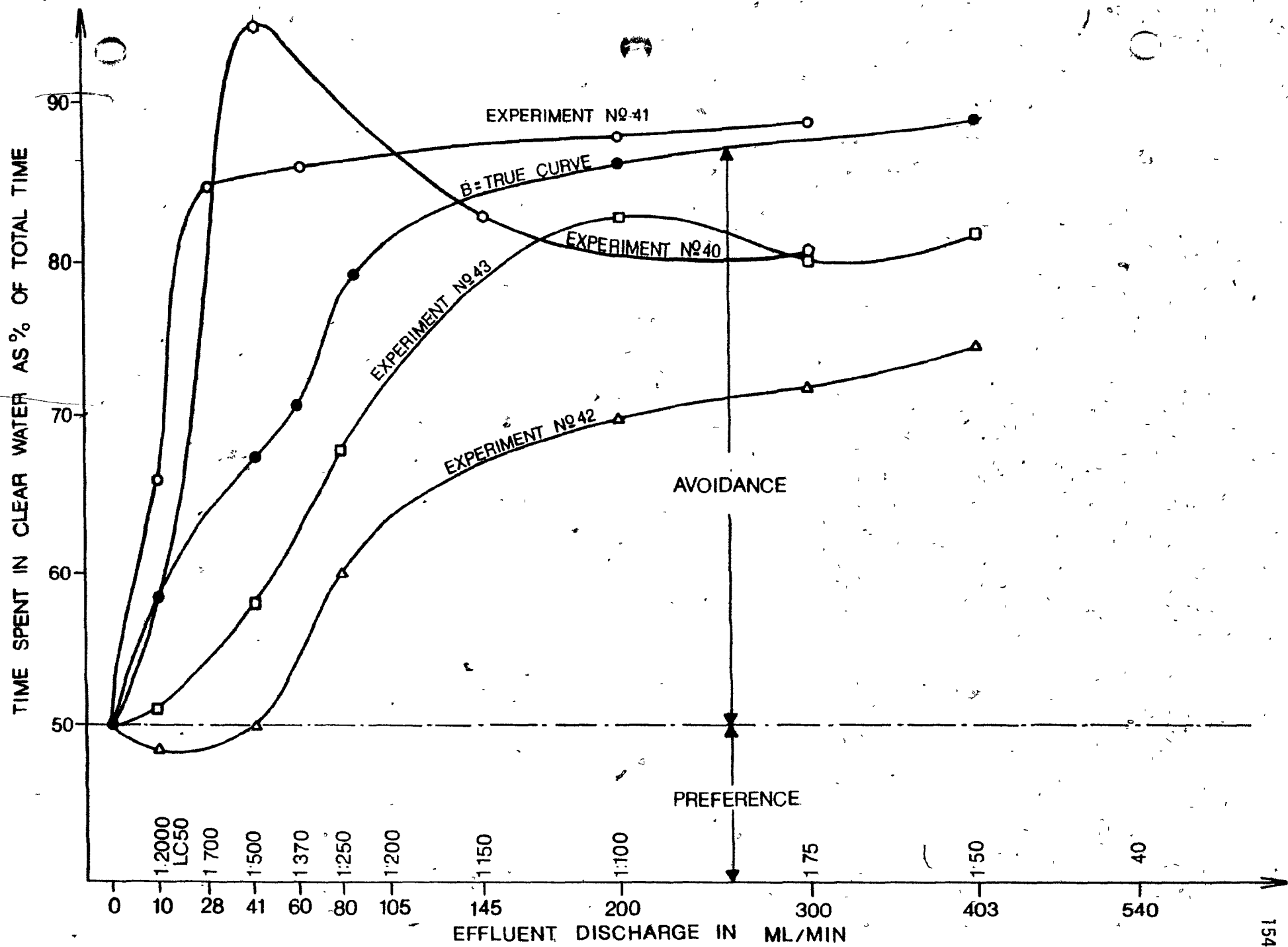


FIGURE 6.8
AVOIDANCE CURVES FROM PLATING INDUSTRY EFFLUENT AND THE CONTINUITY TESTS

CHAPTER VII
"EFFECT OF DSS"

CHAPTER VII

7.1 D.S.S. (Dodecyl sulfate de sodium)

DSS (dodecyl sulfate de sodium -- in French) or SDS (sodium or lauryl dodecyl sulfate - in English) (introduced first by G.Laroche (1970)), has been used widely as a reference compound in industrial pollution bioassays because of its reliable LC50 value in static toxicity tests (96 hr LC50 = 5-7 mg/l).

DSS is a white-cream powder which is found in commercial toothpaste and is commonly used for electrophoreses. Its chemical formula is $\text{CH}_3(\text{CH}_2)_{11}\text{OSO}_3\text{Na}$, and it has a molecular weight of 288.38. It has a neutral pH and low toxicity. A highly purified DSS (99%) (BDH Cat. No. B30176), was used as a toxic material to gain insight into the behavioral response of fish when exposed to the material at sublethal concentrations.

7.2 Experiments - Results

Seventeen tests (No. 11,12,13,14,15,16,17,18,19,20,21,29,30, 31,32,33,33') were conducted with DSS at nine different concentrations (1:400 LC50, 1:200 LC50, 1:100 LC50, 1:40 LC50, 1:32 LC50, 1:8 LC50, 1:4 LC50, 1:1.3 LC50, 2:1 LC50) with effluent injection from both sides A and B (injection System C) to evaluate its effect on the horizontal and vertical distribution of the fish and also to establish the relationship between avoidance and effluent concentration. The results appear in Tables 7.1 and 7.2.

As shown by Figure 7.1 the horizontal distribution of the fish did not clearly indicate a significant behavioral change due to the DSS. In the analysis of variance of the horizontal distribution

(ANOVA 22 in Appendix C3), the hypothesis test

$H_0 : \text{conc A} = \text{conc B} = \text{-----} = \text{conc Z}$

$H_A : \text{conc A} \neq \text{conc B} \neq \text{-----} \neq \text{conc Z}$

had an F value = 0.01 and $PR > F = 1.00 > 0.05$.

The hypothesis was accepted, which means that there was no significant difference in the horizontal distribution of the different DSS concentrations injected. Again in Figure 7.2, there is no basis to conclude that the fish were exhibiting a vertical behavior response. This latter point is significant because the effluent was mixed quickly with the water and tended to indicate that complete mixing had been achieved. From the analysis of variance of the vertical distribution (ANOVA 23 in Appendix C3), results virtually identical to those for the horizontal distribution were obtained. In other words there was no significant difference between the various DSS concentrations injected.

The Duncan's tests for both horizontal and vertical distribution showed that for all sections and depths, the different DSS concentrations belonged to the same group (not significantly different).

There was, however, a clearly defined behavioral response to the DSS as shown in Figure 7.3. This curve was found by calculating the amount of time the fish spent in their acclimation water and plotting this value against the concentration of DSS.

Horizontal Distribution						Vertical Distribution		
No. of Experiment	Ratio of LC50	Section in %				Depth in %		
		1	2	3	4	A	B	C
11, 12 29	1:400	41	25	21	13	42.5	19	38.5
13	1:200	40	30	20	10	32	25	43
14	1:100	35	37	19	9	30	26	44
15, 30	1:40	33	32	28	7	38	20	42
16, 31	1:32	37	29	23	11	36	23	41
18	1:8	43	25	23	7	35	22	43
12, 21 33	1:4	31.5	32.5	26	10	33	25	42
32	1:1.3	32.5	31.5	25	11	32	26	42
33 ¹	2:1	35	32	23	10	34	24	42

TABLE 7.1 Effect of DSS on the horizontal and vertical distribution

Avoidance Reaction in %			
No. of Experiment	LC50 Ratio	Avoidance	Range of Avoidance Values
11,12,29	1:400	43	± 3
13	1:200	44	± 2.5
14	1:100	49	± 2.75
15,30	1:40	52.5	± 2.5
16,31	1:32	54	± 2.5
18	1:8	58	± 2.75
19,21,33	1:4	60.5	± 8
32	1:1.3	64	± 1.5
33 ¹	2:1	66	± 1.75

TABLE 7.2

Effect of DSS on the avoidance reaction

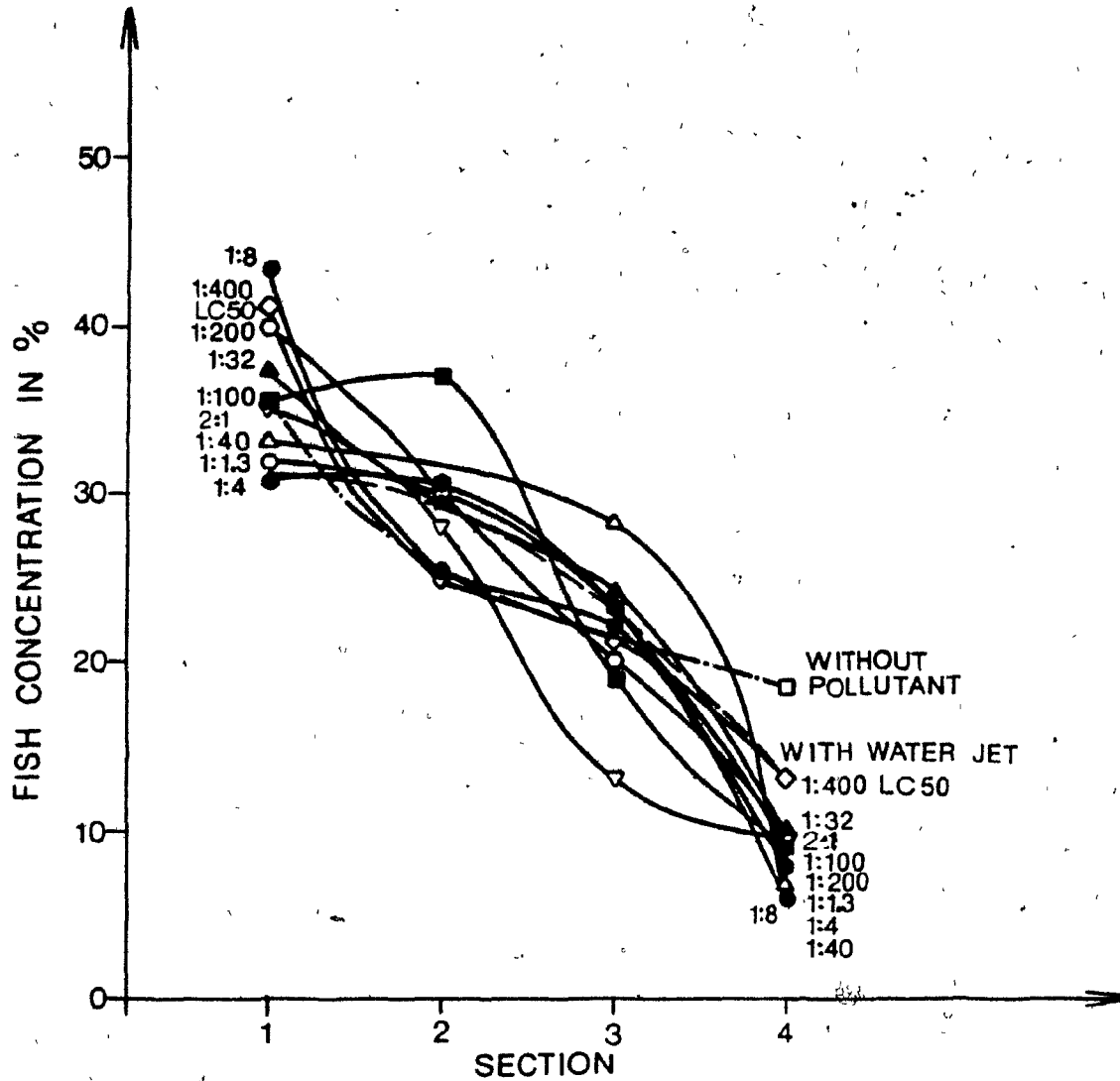


FIGURE 7.1
EFFECT OF DSS IN THE HORIZONTAL DISTRIBUTION

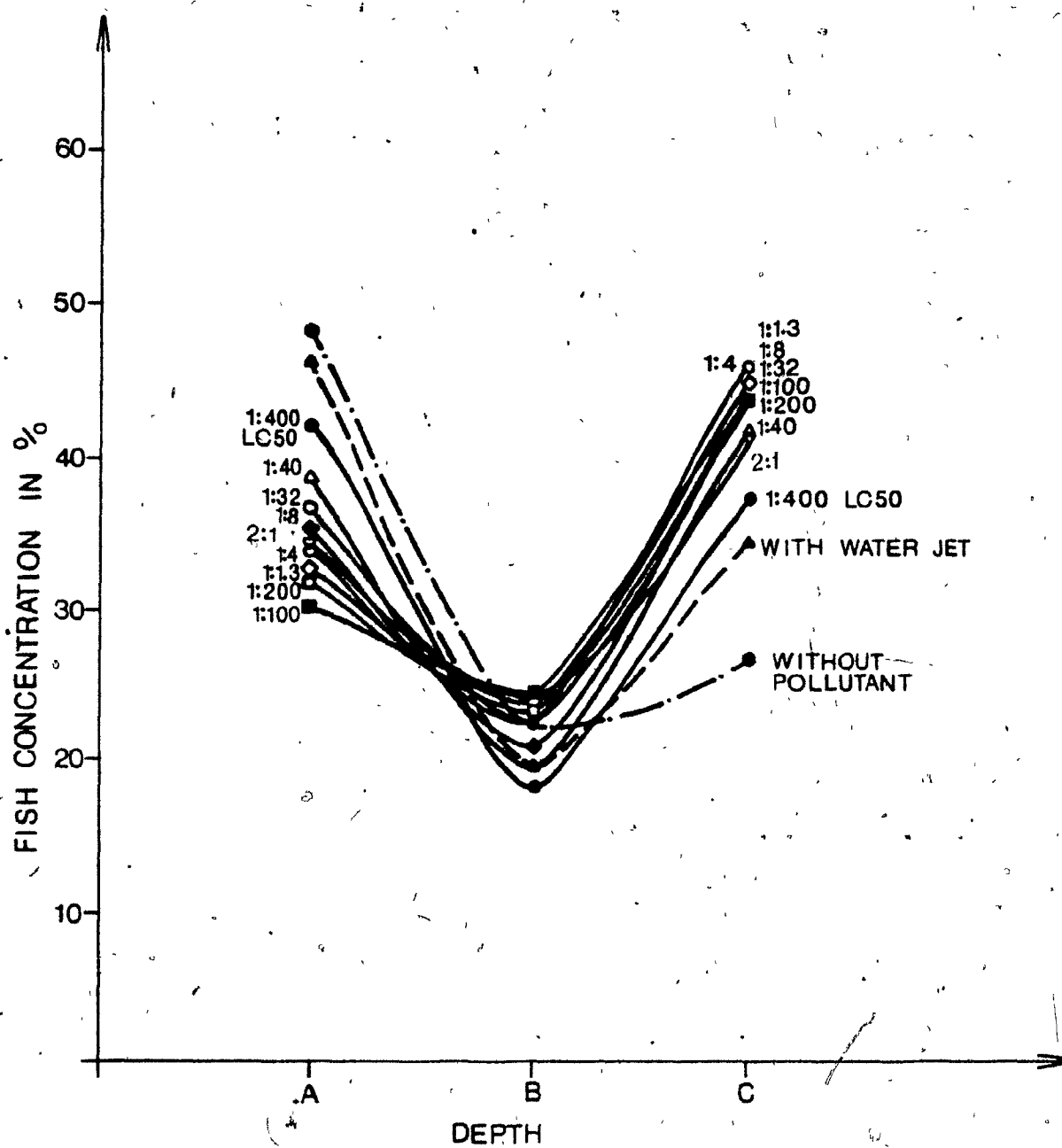


FIGURE 7.2
EFFECT OF DSS IN THE VERTICAL DISTRIBUTION

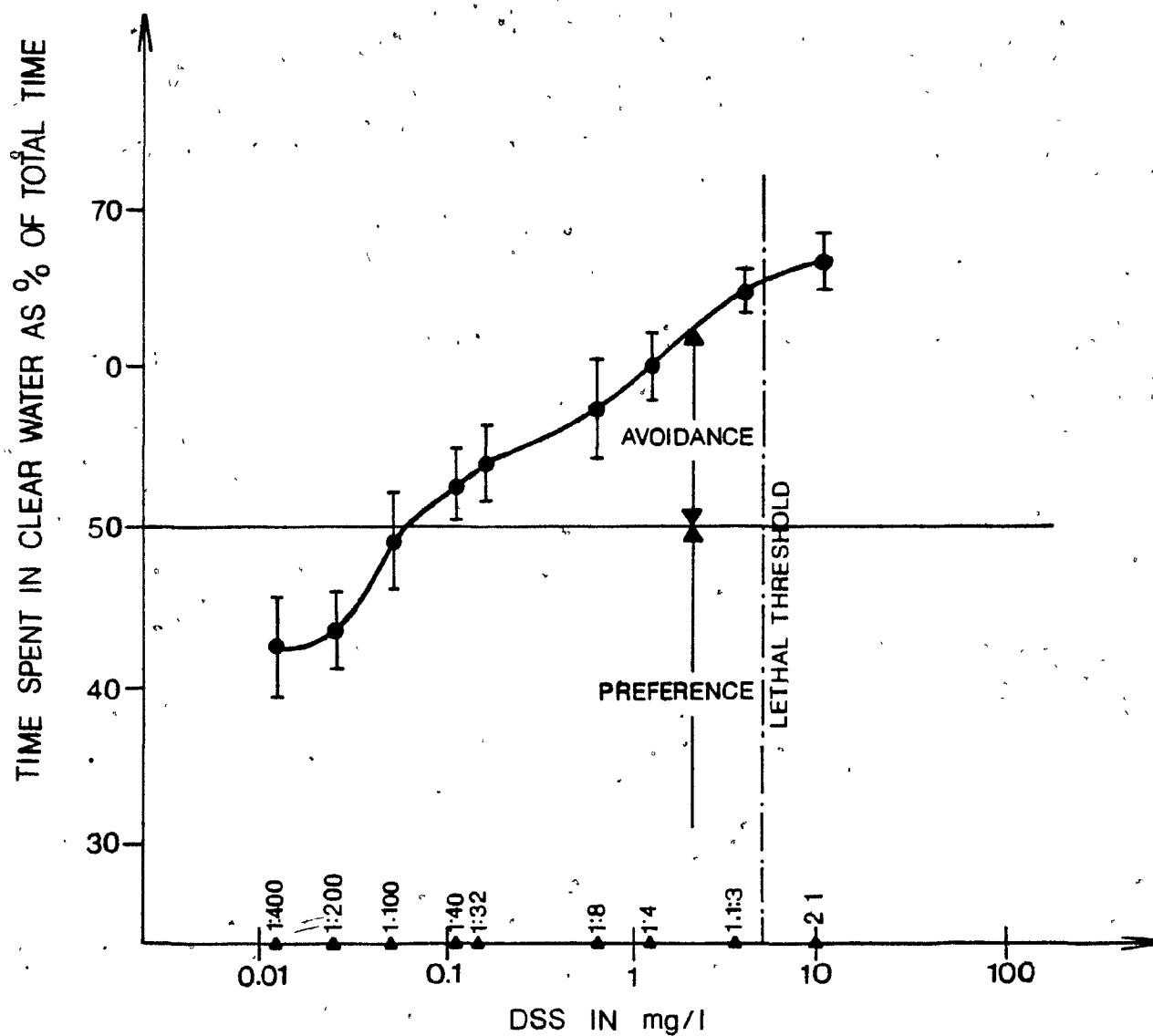


FIGURE 7.3
AVOIDANCE REACTION OF DSS

Preference for the effluent jet was observable until a concentration of 1:100 LC50 was reached. The finding that statistically significant preference occurred in very low concentrations, suggests that hormesis extends beyond cellular responses (Stebbing, 1982). Behavior as an expression of the sum of all biological responses may also exhibit a form of hormesis. Thereafter, an avoidance reaction occurred which clearly indicated that the fish found the DSS objectionable even at very low concentrations. When the fish were exposed to the LC50 concentration, a clear avoidance behavior was exhibited.

CHAPTER VIII

"EXPERIMENTS WITH POLYMERS AND MONOMERS"

CHAPTER VIII

8.0 Polyelectrolytes

Polyelectrolytes combine the properties of polymers and electrolytes. Essentially they are water soluble polymers with ionizable groups (PACKHAM, 1967). The term is often restricted to synthetic organic polymers, but this use is incorrect. Polyelectrolytes may be natural or synthetic and either inorganic or organic.

Synthetic polyelectrolytes - hereinafter called "Polymers" - are in widespread use for the treatment of water and wastewater. They are used as coagulant aids, as flocculants and flocculant aids and as aids in sludge dewatering.

Polyelectrolytes can be classified into four classes depending upon the residual charge of the polymer in aqueous solution, as follows:

1st class: Nonionic (no residual charge) e.g., polyalcohols, polyethers, polyamides, poly N-vinyl heterocycles.

2nd class: Anionic (negative residual charge) e.g., hydrolyzed polyacrylamides, polystyrene sulfurate, polyvinyl phosphorate, acrylamide/acrylic acid and copolymers.

3rd class: Cationic (positive residual charge) e.g., polyethylenimine, hydrochloride, polydimethyl-diallyl ammonium chloride or DMDAAC.

4th class: Amphoteric (both positive and negative residual charge), e.g., cationic polyacrylamide with minor hydrolysis of the amide group (Vorchheimer, 1981).

Although used in minute quantities (typically 5-20 mg/l), and usually assumed to be entirely adsorbed by the sludge, it has been found that under normal plant operations significant quantities of polymer may appear in the effluent (Gehr and Henry, 1982). This finding plus the increasing

demand for improved output and efficiency of water treatment plants prompted studies into the toxicity of these polymers, with regard to their possible breakdown or mutation during passage through a treatment process. The limited studies published in this area so far (Biesinger et al, 1976) have considered only basic static and dynamic tests, and the effect on the reproductive capacity of fish; no consideration has been given to the effects of polymer mutations (Bolker, 1974).

8.1 Monomers

Since the term polymer is derived from the Greek root MEROS (part), a number of prefixes may be used to form words commonly employed in polymer science. Thus, the word monomer literally means "one part" and represents the simple chemical building-block substance or substances from which the polymer is made or to which (in practice or in theory) it may be broken down.

Synthetic polymers are more likely to be called copolymers, because they are synthesized from mixtures of monomers polymerized together (Bolker 1974).

Because the breakdown of polymers into monomers has been observed during water (or wastewater) treatment processes where polymers are used it becomes interesting from the point of view of toxicity studies to investigate the effect of polymers vs. effect of the corresponding monomers, in order to have a global understanding of their toxic effects with time.

8.2 Toxicity of polymers and monomers

One of the advantages that natural polymers share with activated silica is that, in general, they are completely non-toxic.

As coagulant aids, they may be inferior to some of the synthetic products in flocculating power, but there is at least no doubt as to their complete safety from the point of view of public health.

Synthetic polyelectrolytes have been used quite widely in effluent treatment, mineral processing and other industrial processes but not much in the clarification of water for public supplies (Burke (1966), Garwood (1967), Hesner (1975)). This is especially so in the case of large communities because of the great concern about the toxicity of these compounds.

Toxicity tests with fish, though not as sensitive as tests with other biota, notably algae, are in frequent use because they can approximate closely the effects of pollutants on a stream.

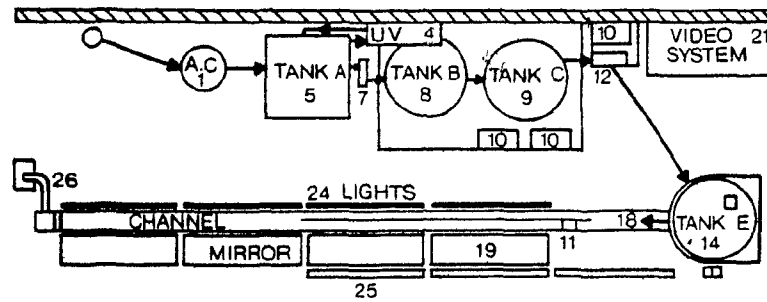
Very little is known about the toxicity of polymers and monomers and basically most of the existing information comes from static tests (LC50's) (Biesinger et al 1976).

Dynamic toxicity information is needed for a more unbiased and global evaluation of the toxic effects of polymers and monomers.

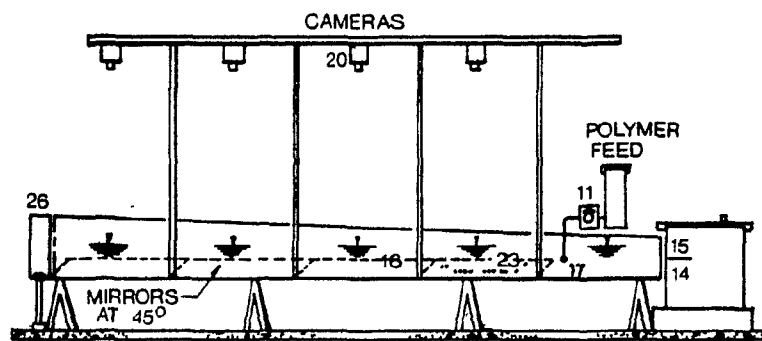
As far as can be ascertained, the present study is the first instance where toxicity avoidance tests have been introduced for the estimation of toxic levels of polymers and monomers. Using the newly developed avoidance apparatus (Design H), a series of experiments was conducted for two polymers and two monomers and avoidance curves for all four of them were constructed. The work of this Chapter was presented at the 11th International Conference of Water Pollution Research in Cape Town, South Africa, in the form of a poster presentation co-authored by Spraggs and Gehr (1982).

8.3 Experiments

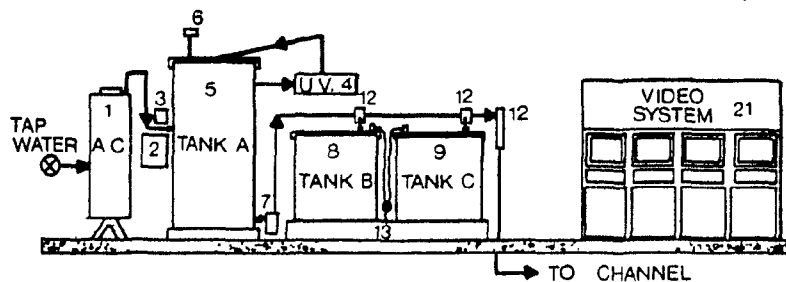
Twenty-three one-hour experiments were conducted for the evaluation of the effect of two polymers and their respective monomers, using the same avoidance apparatus described in Chapter III (Figures 8.1, 8.2).



PLAN VIEW
FIGURE 8.1



SIDE VIEW OF CHANNEL
FIGURE 8.2



SIDE VIEW OF FISH HANDLING APPARATUS
FIGURE 8.3

Number	Description	Number	Description
1	Activated Carbon Unit	14	Acclimation Tank E
2	Heating Control Unit	15	Front Gate to Channel
3	Automatic Temperature Control	16	Channel
4	Ultra Violet Light	17	Injection Point
5	Storage Reservoir (Tank A)	18	Centre Separation Unit
6	Heating Coil	19	Mirrors
7	Main Pump	20	Video Cameras
8	Fish Holding Tank B	21	Video System
9	Fish Holding Tank C	22	Thermometer
10	Small Water Coolers (Optional)	23	Channel Position Indicators
11	Polymer Storage & Pumping	24	Channel Lighting
12	Flowmeters	25	Sight and Sound Barriers
13	Large Cooler for Acclimation Tank & Channel	26	End Gate and Drainage

and 8.3). Minor modifications were made such as the establishment of a polymer feeding unit, of an extra proportioning pump and the use of the injection system C.

The polymers and monomers tested were:

- a) Polymer A (63% by weight of dimethyl amino ethyl acrylate quaternised with methyl chloride and 37% acrylamide).
- b) Polymer C (Dimethyldiallyl Ammonium Chloride)
- c) Monomer A (Acrylamide monomer)
- d) Monomer C (Dimethyldiallyl Ammonium Chloride Monomer).

8.3.1 Polymer A

Six tests were conducted (Experiment Nos. 61', 57, 58, 59, 60, 61) with 1:2500 (0.4 mg/l), 1:1500 (0.60 mg/l) 1:1000 (1.0 mg/l) 1:750 (1.33 mg/l), 1:500 (2 mg/l), 1:250 (4 mg/l) polymer concentrations respectively. The results of these tests appear in Table 8.1 and the avoidance curve for polymer A appears in Figure 8.4.

8.3.2 Polymer C

Five tests were conducted (Experiment Nos. 62, 63, 64, 65, 66) with 1:2500 (0.4 mg/l), 1:2000 (0.5 mg/l), 1:1500 (0.66 mg/l), 1:1000 (1.0 mg/l), 1:750 (1.33 mg/l) polymer concentrations respectively. The results of these tests appear in Table 8.1 and the avoidance curve is produced in Figure 8.5.

8.3.3 Monomer A

Six tests were conducted (Experiment Nos. 113,114,115, 116,117,118) with 1:3000 (0.3 mg/l), 1:1500 (0.66 mg/l), 1:750 (1.33 mg/l), 1:250 (4 mg/l), 1:100 (10 mg/l), 1:50 (20 mg/l) monomer concentrations respectively. The results of these tests appear in Table 8.2 and the avoidance curve is produced in Figure 8.6.

8.3.4 Monomer C

Six tests were conducted (Experiment Nos. 67,68,69,70, 71,72) with 1:3000 (0.3 mg/l), 1:2000 (0.5 mg/l), 1:1000 (1.0 mg/l), 1:750 (1.33 mg/l), 1:500 (2 mg/l), 1:250 (4 mg/l), monomer concentrations respectively. The results of these tests appear in Table 8.2 and the avoidance curve is produced in Figure 8.7.

Polymer	Number of Experiment	Pollutant Concentration		Avoidance Reaction in %	Final avoidance reaction (+5% for jet effect)	Range of avoidance values in %
		Dilution Ratio	mg/l			
A	61	1:2500	0.4	40	45	± 3.72
A	57	1:1500	0.66	38	43	± 1.77
A	58	1:1000	1.0	56	61	± 7.52
A	59	1:750	1.33	63	68	± 6.95
A	60	1:500	2	68	73	± 6.6
A	61	1:250	4	80	85	± 3.72
C	62	1:2500	0.4	49	54	± 2.76
C	63	1:2000	0.5	53	58	± 3.56
C	64	1:1500	0.66	61	66	± 4.34
C	65	1:1000	1.0	73	78	± 3.74
C	66	1:750	1.33	90	95	± 4.06

TABLE 8.1 Avoidance reaction of polymers

Monomer	Number of Experiment	Pollutant Concentration		Avoidance Reaction in %	Final avoidance reaction (+5% for jet effect)	Range of avoidance values in %
		Dilution Ratio	mg/l			
A	113	1:3000	0.3	48	53	± 4.75
A	114	1:1500	0.66	40	45	± 3.50
A	115	1:750	1.33	40	45	± 2.61
A	116	1:250	4	45	50	± 2.25
A	117	1:100	10	53	58	± 1.71
A	118	1:50	20	58	63	± 2.00
C	67	1:3000	0.3	45	50	± 3.31
C	68	1:2000	0.5	40	45	± 4.02
C	69	1:1000	1.0	49.5	54.5	± 4.79
C	70	1:750	1.33	45	50	± 4.78
C	71	1:500	2	44	49	± 2.31
C	72	1:250	4	42	47	± 3.39

TABLE 8.2 Avoidance reaction of monomers

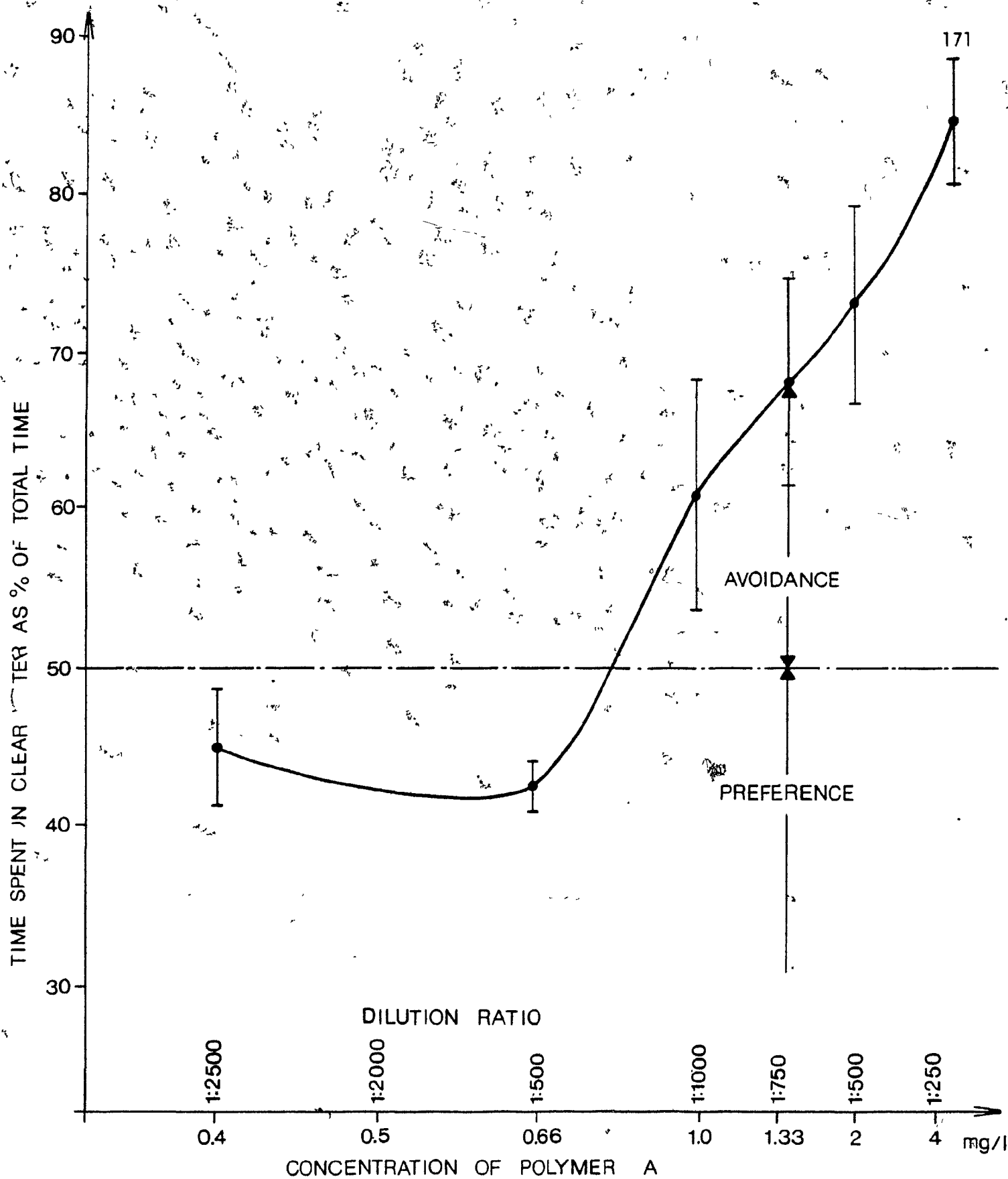


FIGURE 8-4
AVOIDANCE CURVE OF POLYMER A

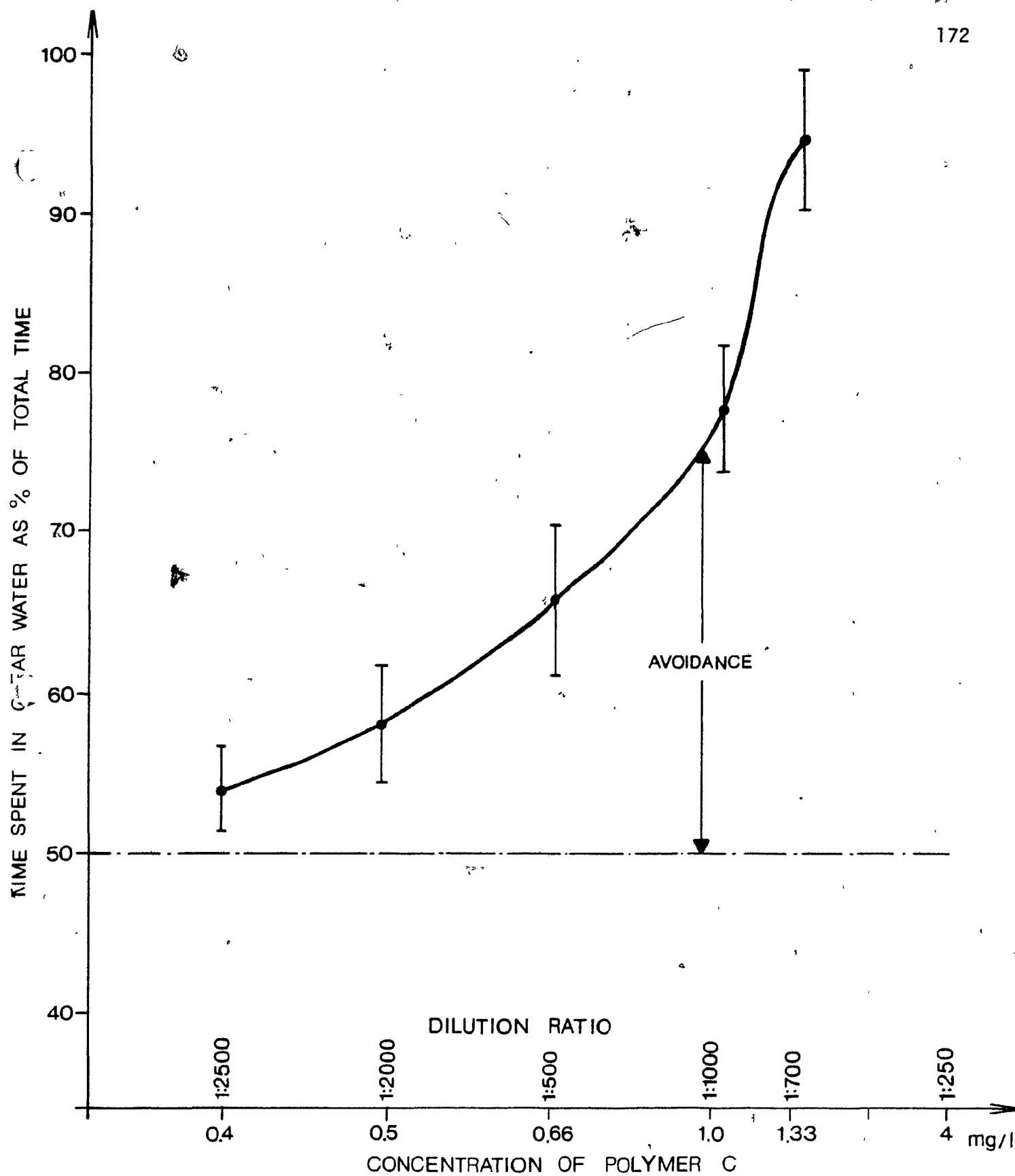


FIGURE 8.5
AVOIDANCE CURVE OF POLYMER C

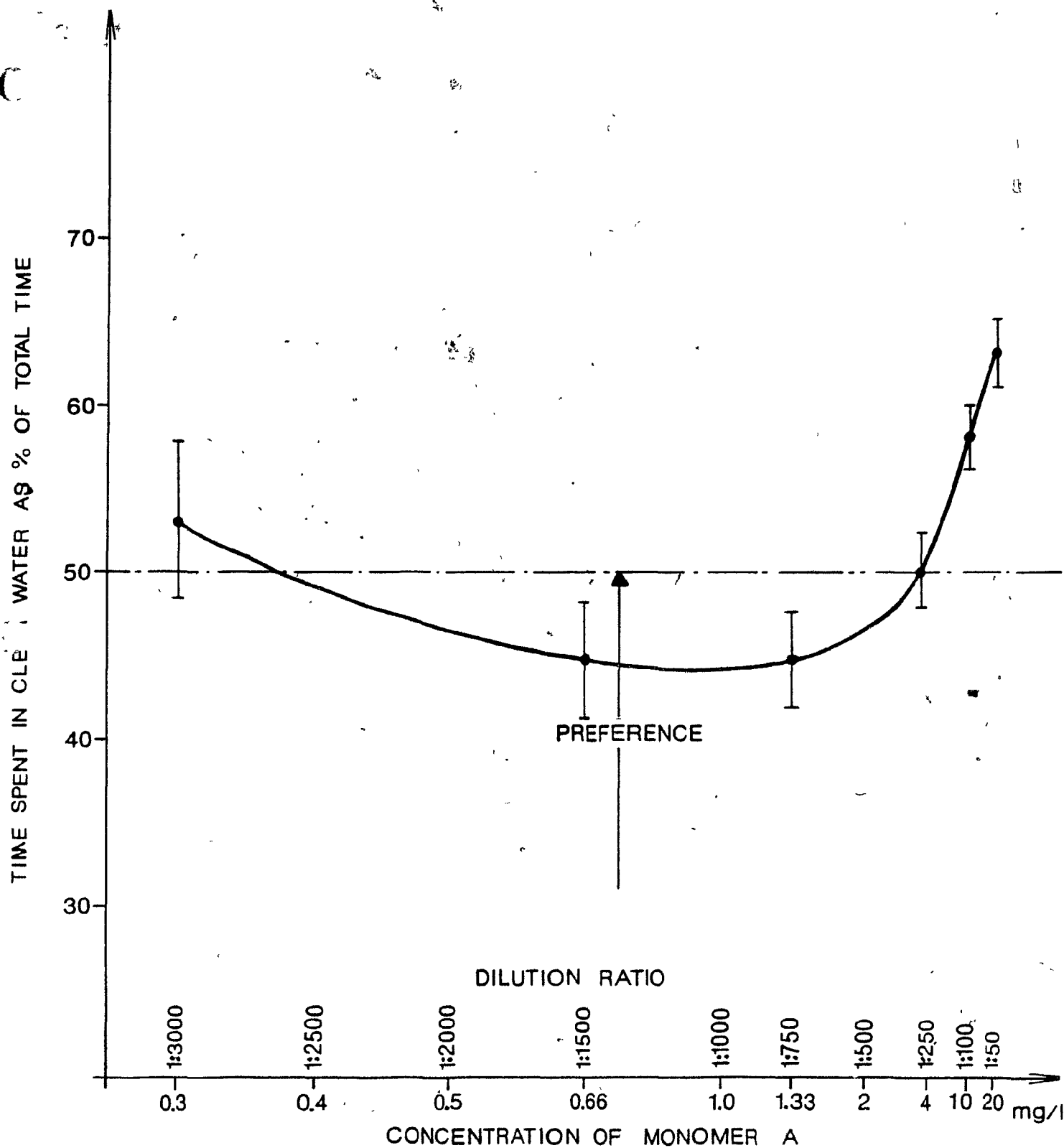


FIGURE 8.6
AVOIDANCE CURVE OF MONOMER A

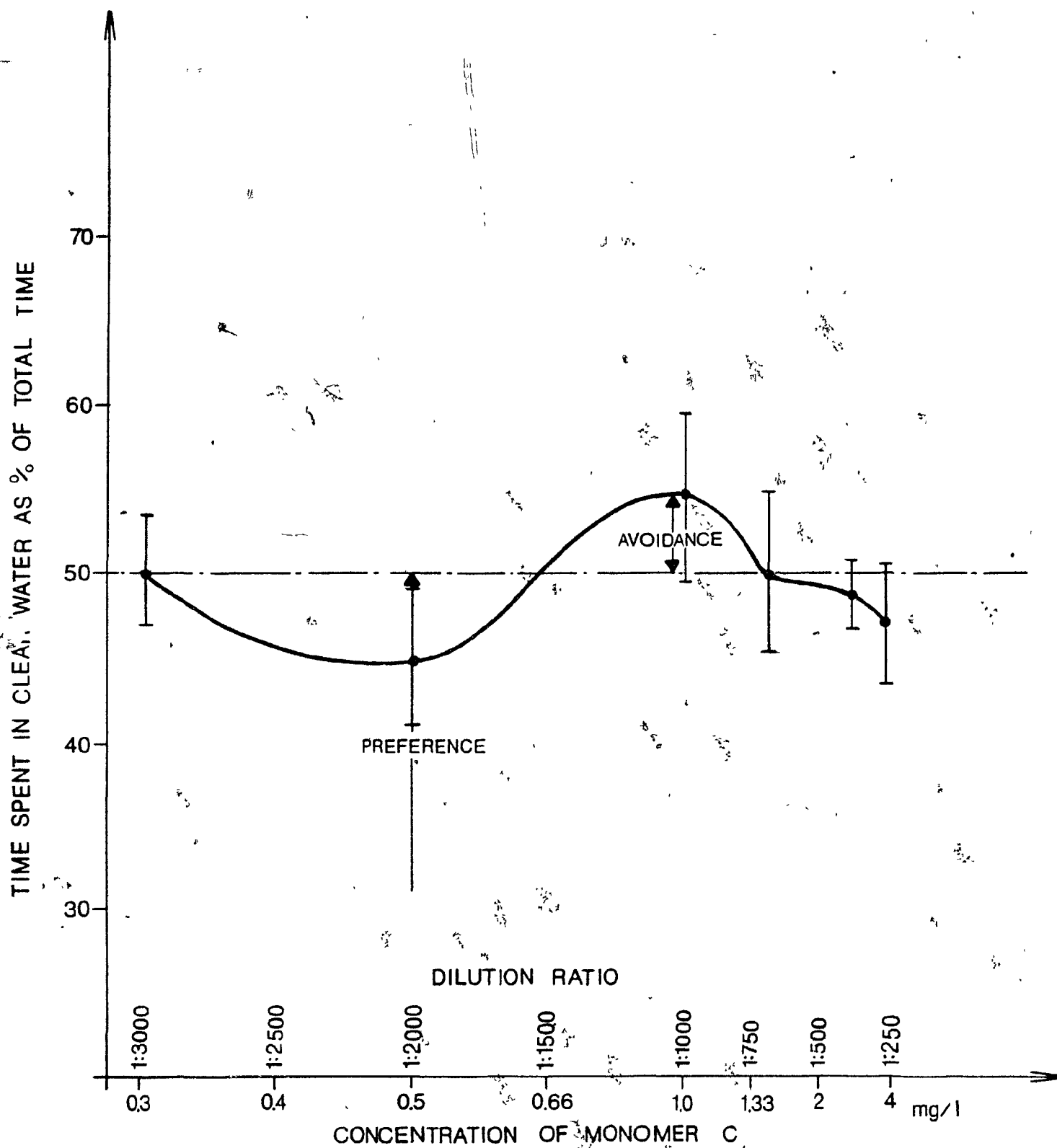


FIGURE 8.7
AVOIDANCE CURVE OF MONOMER C

8.4 Comparison of avoidance curves with static tests, algae tests and microtox results

Fish, algae and bacteria are markedly different organisms with variable reactions to different toxins. Each class of organisms can only contribute a small part to the overall toxicity picture. This picture should be limited according to the final disposal of the toxins (e.g., river, drinking water system), and the test organism chosen accordingly.

In situations where toxicity to man is the ultimate concern, care must be exercised about the application to humans of data obtained from tests employing lower species. However, it seems logical to assume that the reaction of motile vertebrate species, such as fish, will be of more direct relevance than the reactions of microorganisms.

For further evaluation of the significance of avoidance testing, other static tests (LC50's, algae and microtox) were performed using Salmo gairdneri (trout) for the LC50, Selenastrum capricornutum for the algae tests and fluorescing bacteria (Photobacterium phosphoreum) for the microtox. The static 96-hour LC50 test gives a good first approximation to toxicity levels, but does not actually represent true-life situations. In particular, the test considers death as the only possible toxic effect and is also restricted in terms of the time base i.e., the standard 96 hours.

The algal assay procedure states that "growth is limited by the substance that is present in minimal quantity in respect to the needs of the organism." Bottle algal tests consist of three steps: a) selection and measurement of appropriate parameters during the assay (e.g., biomass indicators such as total carbon); b) presentation and statistical evaluation of the measurements made during the assay; c) interpretation of the results. These measurements were made by adding a selected test algae to the test water and determining algal growth at appropriate intervals.

The microtox system is a true bioassay, based on the response of live, luminescent microorganisms. The microtox instrument (Beckman: Microtox Model 2055) determines the state of health of the test organisms by monitoring their light output. The bioassay organisms are handled like a simple chemical reagent. Response is monitored instrumentally and presented as a digital readout, or graphically on an optional stripchart recorder. Microtox gives a 5-minute response, compared with the 96-hour LC50, and also gives a reproducible, quantitative measure of toxicity. The final results of three static tests, two sets of 24 bottle algal tests and 30 response readings from microtox for polymer A and C and monomer A and C, plus the 50% avoidance level values from Figures 8.4, 8.5, 8.6 and 8.7 appear on Table 8.3. Additional information on the tests with polymers and monomers appears in Appendix C3 (page 396).

8.5 Discussion and Results

Results indicated that there was a significant behavioral change in the fish (Salmo gairdneri) for each of the polymers used. It is significant that the fish preference level for the two polymers tested was in the range which is lethal to them. However, in the case of monomers, the fish avoided the substance at a concentration considerably lower than that of the lethal limit. The results indicate that fish may not avoid a polymer, even though its presence is lethal.

The necessity for the dynamic avoidance tests is indicated clearly from the results of the three static tests (LC50's, algae and microtox). The static tests can give comparative data which are hierarchically compatible (i.e., monomer is always less toxic than polymer) but are not always quantitatively meaningful. Using the static tests independently would not yield information as to the behavioral reaction of fish when in contact with sublethal concentrations of the pollutant.

	Fish. 50% Avoidance Level	Fish. Static LC50	Microtox EC50	Algae 50% Inhibition
Monomer A	4	140	13,500	72
Polyelectrolyte A	0.8	0.4	250	2
Monomer C	0.66	900	22,500	220
Polyelectrolyte C	0.33	0.2	7,500	0.2

Note: The polyelectrolytes were commercially available products used in sewage and water treatment. Both were cationic. Polyelectrolyte A was an acrylamide copolymer and Monomer A was acrylamide. Polyelectrolyte C was polydimethyl-diallyl-ammonium chloride or DMDAAC, and Monomer C was thus DMDAAC Monomer.

TABLE 8.3 Concentration levels (in mg/l) for 50% toxicity of two polyelectrolytes and their respective monomers according to four assay procedures.

From the analysis of variance ANOVA 28, 30 and 31 in the Appendix C3, using a model with two independent variables (the concentration and the type of polymer or monomer) and one dependent variable (the % of fish in clear water) three hypotheses were tested:

a) H_0 : Polymer A = Polymer C = Monomer A = Monomer C

HA: Polymer A $\neq$ Polymer C $\neq$ Monomer A $\neq$ Monomer C

b) H_0 : Polymer A = Polymer C

HA: Polymer A $\neq$ Polymer C

and

c) H_0 : Monomer A = Monomer C

HA: Monomer A $\neq$ Monomer C

The first one was rejected having an F value = 15.63 and $PR > F = 0.0001 < 0.05$.

The second one was also rejected, having a F value = 40.99 and $PR > F = 0.0001 < 0.05$. The third one was accepted, having a F value = 0.61 and $PR > F = 0.45 > 0.05$.

These results indicated that, a) there was no significant difference between the avoidance reactions to the two monomers, b) there was a significant difference between the avoidance reactions to two polymers and, c) there was a significant difference between avoidance reactions to monomers and polymers.

Duncan's tests showed that for the different concentrations, the avoidance reactions to the monomers were not significantly different (i.e., they belonged to the same Group A) while the avoidance reactions to polymers were grouped into five different sets (A,B,C,D and E).

From the results obtained, the following conclusions were drawn:

- 1) All assays indicated significantly higher polymer toxicity than toxicity caused by a constituent monomer (Figure 8.8).
- 2) In wastewater treatment processes it is much more desirable from the avoidance point of view to have monomers instead of polymers in the treated water.

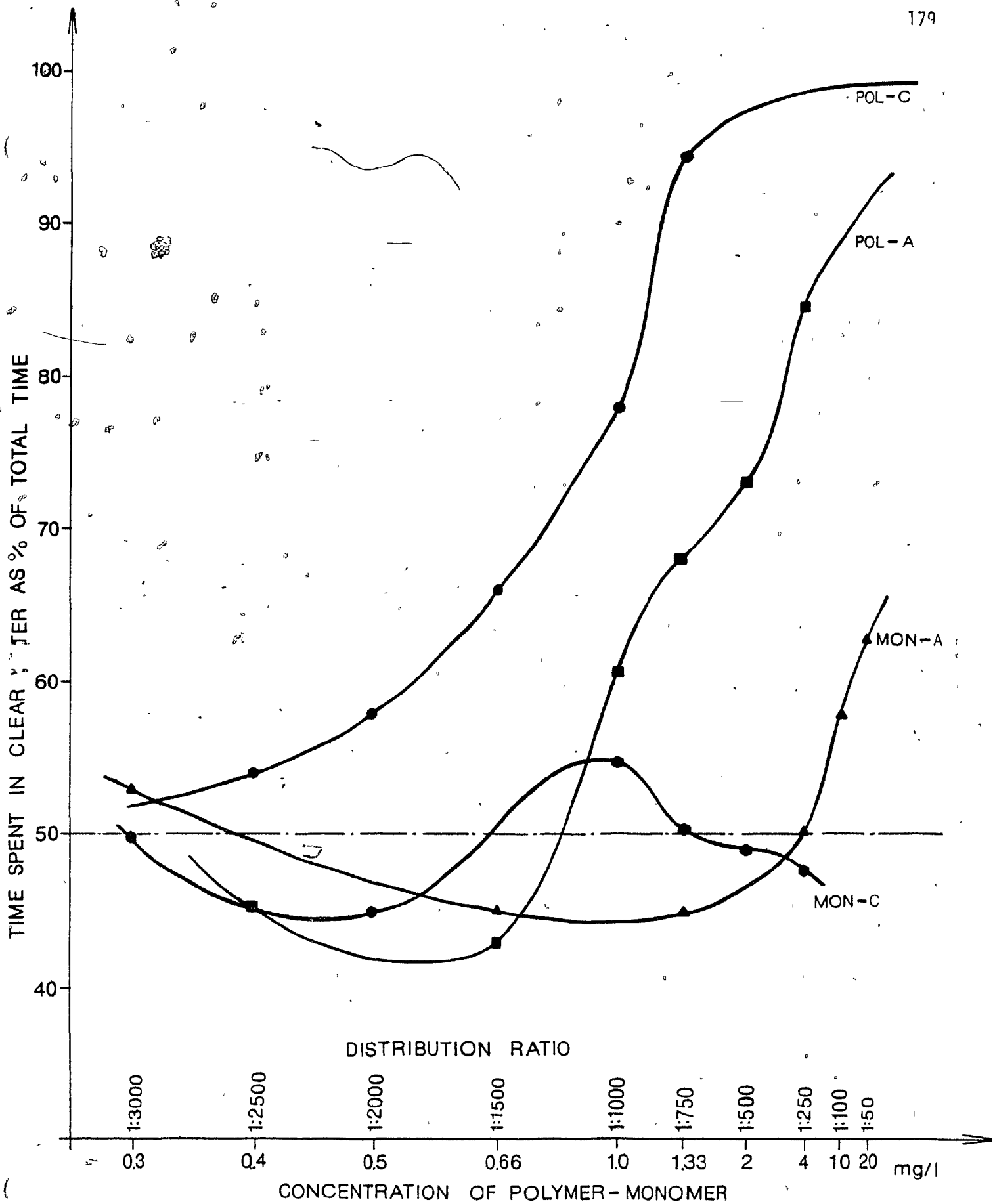


FIGURE 8.8
AVOIDANCE CURVES OF POLYMERS AND MONOMERS

- 3) The dynamic avoidance fish test conducted over a relatively short period was at least as sensitive as the acute static fish test. It could yield information such as preference for substances actually known to be toxic from results of the static tests.
- 4) Results from phosphorescent bacterial toxicity tests were inconsistent with those from fish and algae tests.
- 5) The LC50 values need to be re-examined, because the effects of light and time on the degradation of polymers was not considered.
- 6) The statistically significant preference result for low concentrations of polymer A and monomers A and C can be considered as examples of hormetic responses.
- 7) Histopathological and other additional tests would help to clarify the understanding of the behavioral changes caused by polymers and monomers.
- 8) From the magnitude of the concentration of polyelectrolytes showing significant toxicity, it appeared that discharges from waste treatment processes utilizing these polyelectrolytes could be hazardous to the biological community of a receiving water body.
- 9) Further studies are required to determine why both polymers and their constituent monomers showed marked differences in lethal and avoidance characteristics.

CHAPTER IX

"EXPERIMENTATION WITH ACTUAL INDUSTRIAL EFFLUENT"

CHAPTER IX

9.0 Experimentation with industrial effluent

The industrial effluent used in the study was taken from the effluent of a representative metal plating industry. (see App.A5). Toxic substances in the effluent are chromium (20 mg/l), nickel (50 mg/l) and small amounts of iron (4.5mg/l). The LC50 of the effluent was found to be an effluent-water mixture containing 60% effluent. Twenty-one tests (Nos. 75-88 inclusive, 89, 89', 90, 90', 91, 91', 92') were conducted to determine the effect of different concentrations of the effluent on the avoidance behavior and the distribution of the fish in the channel. Results of the tests are given as a function of the LC50 concentration and the actual concentration can be obtained by dividing by 0.60. For example a concentration of 1:250 LC50 yields an actual effluent concentration of 1:417 (dilution ratio).

Effluent discharge into the channel was regulated by two flowmeters. Table 9.1 and Figure 9.1 contain the information necessary to obtain the flowmeter setting for any desired concentration of effluent. The injection system was constructed in a manner that permitted effluent to be delivered either:

- (a) through wall jets situated 4" from the channel bottom (Injection System A) or 9" from the channel bottom (Injection System B); or
- (b) using horizontal or vertical multidiffusers (Injection Systems C and D described in Chapter IV).

Effluent could be injected from either side of the channel to negate the possibility of a side preference before testing began.

9.1 Time series analysis

After preliminary calculations were made from the 21 experiments with the industrial effluent, time series analysis was applied to the lateral

Flowmeter Reading in Millimeters	ml /min From the Calibration Curve	Corresponding LC50
0	0	0
10	10	1:2000 LC50
20	28	1:700 LC50
30	41	1:500 LC50
40	60	1:370 LC50
50	80	1:250 LC50
60	105	1:200 LC50
82	145	1:150 LC50
110	200	1:100 LC50
150	300	1:75 LC50
198	403	1:50 LC50
250	540	1:40 LC50

TABLE 9.1 Flowmeter calibration

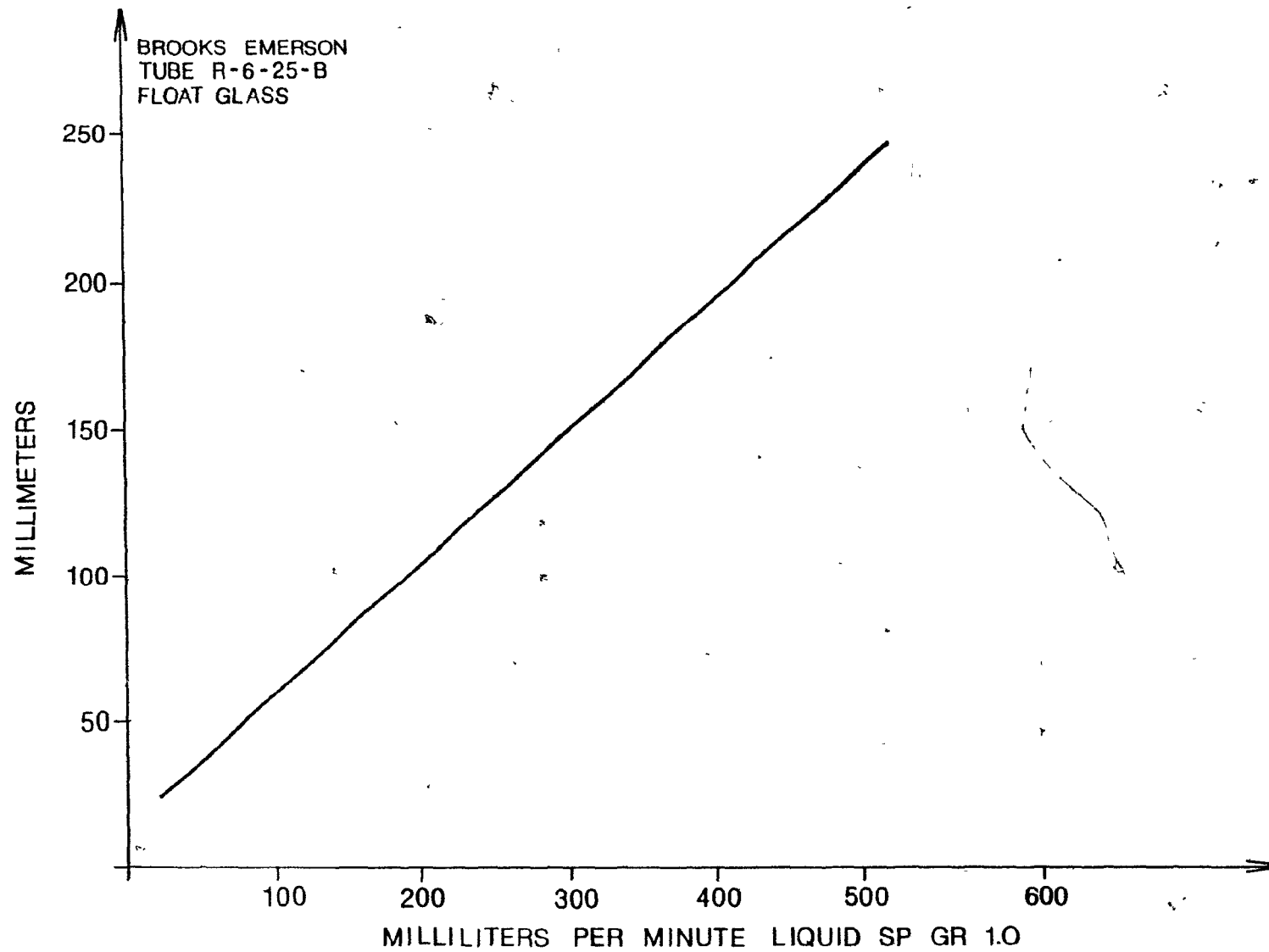


FIGURE 9.1 FLOWMETER CALIBRATION CURVE

Injection System	No. of Experiment	Corresponding LC50 ratio	% in clear water			
			Value of avoidance from computer output	Bias factors		Final avoidance value transferred to the curves
				Jet Effect	Algae Cases	
A	84	1:700	64	+5	-	69.0
A	85	1:500	70.8	+5	-	75.8
A	86	1:250	72.0	+5	-	77.0
A	87	1:200	80.27	+4	-5	79.2
A	88,89	1:150	77.57	+5	-	82.5
A	90,91	1:75	88.76	+3	-	91.7
B	80	1:700	57.59	+5	-	63.5
B	75	1:500	67.20	+5	-5	67.20
B	81	1:370	65.54	+5	-	70.5
B	76	1:250	79.10	+5	-5	79.10
B	77	1:150	80.35	+4	-	84.3
B	78,79	1:100	87.5	+4	-5	86.5
B	82	1:50	86.0	+3		89.0
B	83	1:40	97.0	+2	-5	94.0
A,B	1,2,22,24 25,26,74	0	45	+5	-	50
C	89'	1:250	76	+4	-	80
C	90'	1:250	77	+4	-	81
D	91'	1:250	71	+5	-	76
D	92'	1:250	70	+5	-	75

TABLE 9.2 Calculation of the final avoidance values from the times series analysis computer outputs of the Injection Systems A and B

distribution (sides A and B) to evaluate the stability of the responses with time, and to calculate the final avoidance values which would be transferred to the avoidance curves.

Program B in Appendix B2 plots the % of fish in clear water vs. time (in minutes); the mean value line of the readings for the lateral distribution excludes the first 20 minutes of each experiment, during which pollutant distribution was not yet stable.

These values were transferred to Table 9.2 and after the elimination of two bias factors: 1) the jet effect and 2) the influence of the algae cages (see App.7,) the final percentages of the avoidance reaction were calculated (Table 10.2). From these final values the avoidance curves for injection Systems A and B were derived (Figures 9.4 and 9.7), as described below in Sections 9.2 and 9.3 of this chapter.

Using the computer outputs of the time series analysis, the crossing time and the fluctuation around the mean for both Injection Systems A and B were derived, as described later in Section 9.5.

9.2 Injection System A

Eight tests (Nos. 84-91 inclusive) were conducted using Injection System A, with flowmeter readings of 0,20,30,50,60,82 and 150. Test results are shown graphically in Figures 9.2, 9.3 and 9.4 and in tabular form in Table 9.3.

The horizontal distribution shown in Figure 9.2 indicates that there was a massive shift of fish from Sections 3 and 4 to Section 2, at effluent concentrations 1:500 LC50. At the same time there was a change from the polluted side to the non-polluted side of the channel. The vertical distribution as shown in Figure 9.3 did not change significantly, indicating a movement of the fish to a similar elevation in the non-polluted side.

The distribution of the fish along the length of the channel (horizontal distribution) appeared to change dramatically as the effluent concentration was increased. With the higher concentrations there was a tendency for the fish to move into the clean water behind the separator in Section 2 of the channel. Logically, this behavior would be expected to occur because it was virtually the only location where the fish could avoid the effluent.

The analysis of variance for the horizontal distribution of injection System A (ANOVA 8 in Appendix C3) showed that, using a model with independent variables: a) the section of the channel and b) the concentration of industrial effluent, and with the percentage of fish in clear water as the dependent variable, the F value was 2.20. This tests how well the model as a whole accounts for the behavior, of the dependent variable.

The significance probability $PR > F$ for the F value was $0.074 > 0.05$ indicating that the F was not significant. In the same analysis of variance table, other statistics include the $R^2 = 0.52$ (which measures how much variation in the dependent variable can be accounted for by the model), the C.V (coefficient of variation) = 72.60 (used to describe the amount of variation in the population) and the standard deviation of the dependent variable = 18.12.

For each independent variable in the model, ANOVA also provides the degrees of freedom (dF); the sum of squares and an F value for testing the hypothesis that the group means are equal for a particular independent variable. In relation to this latter F value, probability values for the existence of Type I or Type IV statistical errors are also produced.

For Injection System A, testing the hypothesis

$$\begin{aligned} H_0 : \text{conc A} &= \text{conc B} = \dots = \text{conc Z} \\ H_A : \text{conc A} &\neq \text{conc B} \neq \dots \neq \text{conc Z} \end{aligned} \quad \begin{array}{l} (Z = \text{number of concentrations} \\ \text{used for Injection System A}) \end{array}$$

F was 0.00 with $PR > F = 1.0$; thus the hypothesis that there was no independent significant difference between the concentrations was therefore accepted.

The next step in the analysis of the horizontal distribution was the grouping by sections of the mean avoidance values for the different concentrations. To test whether they were significantly different or not, Waller-Duncan's K-Ratio T-Test, and Duncan's multiple range test were used.

With both tests, no statistically significant differences were found between Sections 1, 3 and 4 for any concentration. For the second section, there was a significant difference found between the 1:75 and 1:150 LC50 concentrations, when compared with the other five concentrations; this is clearly illustrated in Figure 9.2.

The analysis of variance for the vertical distribution (ANOVA 9 in the Appendix C3), uses the same model as for the horizontal distribution, except that "depth" replaces "section" as an independent variable. The F value for the model = 1.61 with $PR > F = 0.2205 > 0.05$, which indicates that F was not significant. Other statistical values were: $R^2 = 0.51$, C.V = 18.79, STD DEV = 6.26. For both independent variables (depth, concentration), F values for the hypothesis tests were calculated.

LATERAL			% Fish Distribution			
No. of Experiment	Flowmeter Reading (in mm)	Corresponding LC50	Side B Non-Polluted	Ranges of values for each experiment		
1,2,22,24 25,26,74	0	0	50	-		
84	20	1:700	61.0	±5		
85	30	1:500	75.8	±5.92		
86	50	1:250	77.0	±4.13		
87	60	1:200	79.2	±3.5		
88,89	82	1:150	82.5	±4.47		
90,91	150	1:75	91.7	±2.06		
HORIZONTAL			% Fish Distribution			
No. of Experiment	Flowmeter Reading (in mm)	Corresponding LC50	Section 1	Section 2	Section 3	Section 4
1,2,22,24 25,26,74	0	0	32.5	29.4	25.0	13.1
84	20	1:700	18.0	33.6	35.6	12.8
85	30	1:500	19.9	50.6	23.3	5.2
86	50	1:250	26.3	25.1	27.4	21.2
87	60	1:200	26.7	39.4	23.7	10.2
88,89	82	1:150	7.6	81.6	8.3	2.5
90,91	150	1:75	4.9	92.0	2.1	1.0
VERTICAL			% Fish Distribution			
No. of Experiment	Flowmeter Reading (in mm)	Corresponding LC50	Depth A	Depth B	Depth C	
1,2,22,24 25,26,74	0	0	46.5	20.7	32.8	
84	20	1:700	48.7	22.2	29.1	
85	30	1:500	37.8	29.6	32.6	
86	50	1:250	35.7	25.2	38.9	
87	60	1:200	38.7	28.1	33.2	
88,89	82	1:150	38.1	34.2	27.7	
90,91	150	1:75	33.7	36.4	30.5	

TABLE 9.3 Fish Disposition - Injection System A

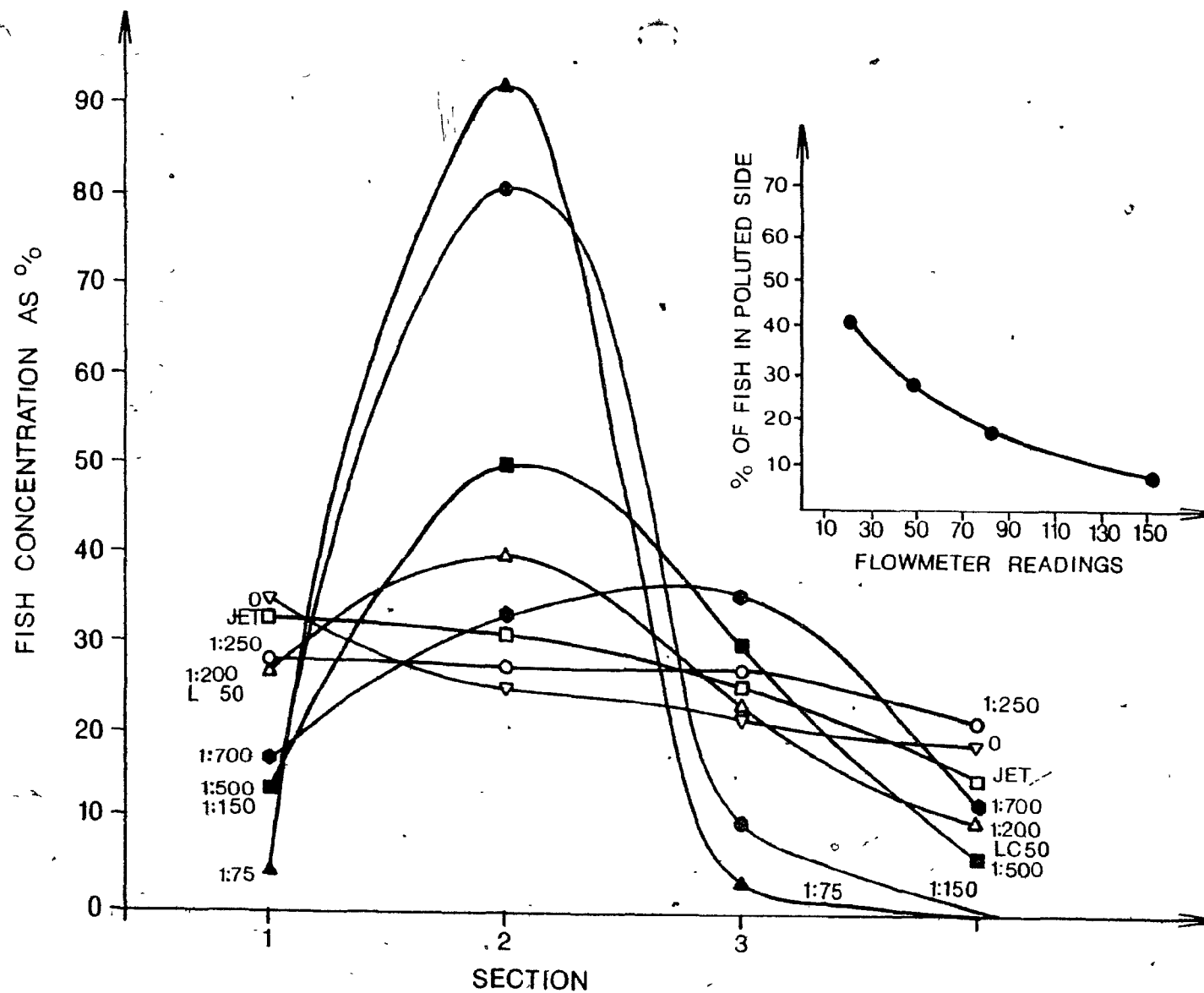


FIGURE 9.2
INJECTION SYSTEM A - HORIZONTAL DISTRIBUTION

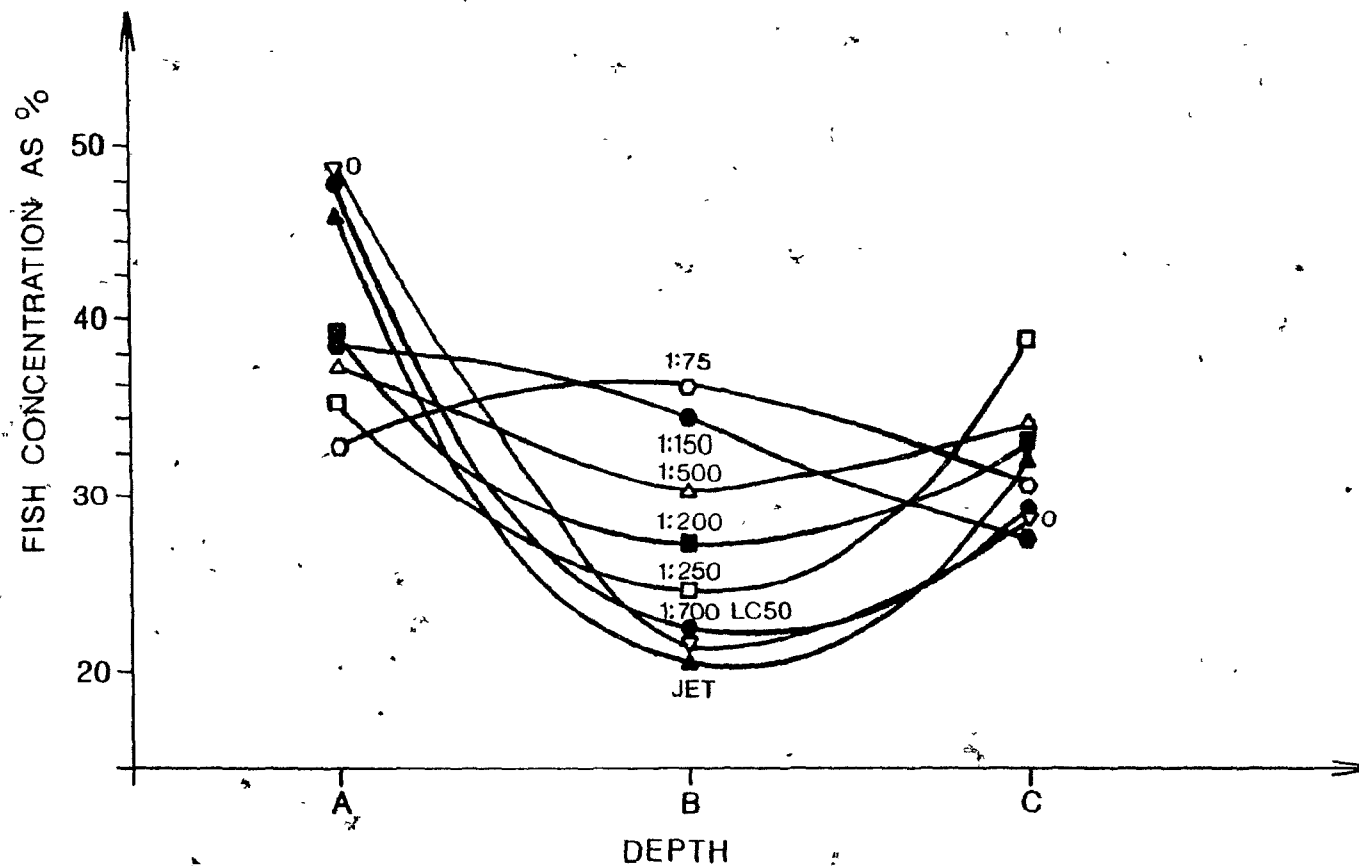


FIGURE 9.3
INJECTION SYSTEM A - VERTICAL DISTRIBUTION

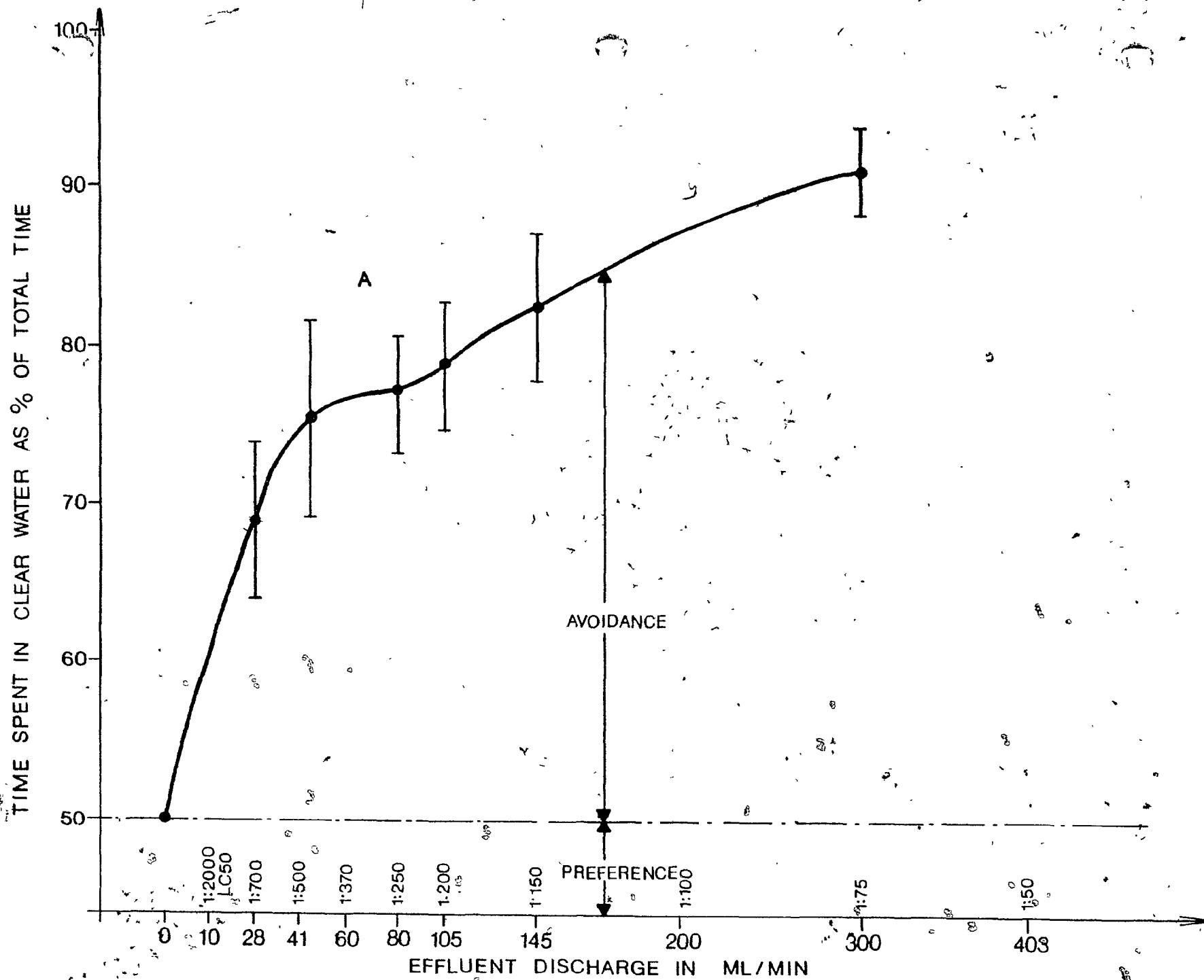


FIGURE 9.4
AVOIDANCE CURVE OF PLATING INDUSTRY EFFLUENT - INJECTION SYSTEM A

For the hypothesis

H_0 : Depth A = depth B = depth C

H_A : Depth A $\neq$ depth B $\neq$ depth C

The F value was 6.44 with $PR > F = 0.012 < 0.05$. The hypothesis was rejected, meaning that there was a difference between the three depths.

Testing the hypothesis of equal concentrations the results were the same as for the horizontal distribution.

Both Duncan's tests indicated no significant differences between the various concentrations at each depth. The test results shown in Figure 9.3 tend to indicate that while the vertical distribution changed, it is unclear that this was only due to the effluent. Part of the problem was the limited depth of the channel resulting in complete mixing of the effluent. Future work would have to include a deeper channel to determine better the vertical avoidance behavior.

Referring to Figure 9.4, it can be seen that avoidance began at very low concentrations and was already evident at a value of 1:700 LC50. Extrapolation of the avoidance curve would indicate that avoidance began as early as 1:2000 LC50. The final avoidance was of the order of 90% indicating that some of the fish did not avoid the effluent even at high concentrations. This could conceivably be explained by impairments of sensory organs in unresponsive fish.

9.3 Injection System B

Nine tests (Nos. 75-83 inclusive) were conducted using a wall jet located nine inches above the channel bottom (Injection System B), with flowmeter values varying between 0 and 250. The actual flowmeter settings and the corresponding concentration as a fraction of the LC50 can be obtained from Table 9.4.

As for Injection System A, the avoidance behavior shown in Figure 9.7 is clearly defined even at low concentrations of the effluent. However, from Figure 9.7 it can be noted again that some fish never avoided the effluent and remained in the discharge at high concentrations.

The horizontal distribution of the fish for Injection System B is given in Figure 9.5. Here again there is a preference for the second section of the channel behind the plexiglass divider but the preference is not as significant as for Injection System A.

The analysis of variance for the horizontal distribution of the Injection System B (ANOVA 10 in the Appendix C3) employed the same model as for Injection System A (Independent Variables were section and concentration, and the dependent variable was the percentage of fish in clear water). The F value for the model was 8.93 with the $PR > F = 0.001 < 0.05$ indicating that F was significant. Also, from the ANOVA 10, $R^2 = 0.80$, C.V. = 20.34, and $STD\ DE\sqrt{V} = 5.08$.

For the independent variable - "section", testing the hypothesis

H_0 : Section 1 = Section 2 = Section 3 = Section 4

H_A : Section 1 $\neq$ Section 2 $\neq$ Section 3 $\neq$ Section 4

An F value of 32.74 was obtained with $PR > F = 0.001$. The hypothesis was therefore rejected, i.e., there was a significant difference between the four sections of the channel, as for Injection System A.

The results of the Duncan's tests again showed that for Sections 1, 3 and 4 there was no significant difference at all concentrations. In the 2nd section, the range of the percentage of fish in the different concentrations divided them into two groups, A and B.

LATERAL			% Fish Distribution			
No. of Experiment	Flowmeter Reading (in mm)	Corresponding LC50	*Side B Non- Polluted	Ranges of values for each experiment		
1,2,22,24 25,26,74	0	0	50.0	-		
80	20	1:700	63.5	±3.78		
75	30	1:500	67.2	±4.72		
81	40	1:370	70.5	±5.69		
76	50	1:250	79.1	±3.68		
77	82	1:150	84.3	±2.57		
78,79	110	1:100	86.5	±4.95		
82	198	1:50	89.0	±1.12		
83	250	1:40	94.0	±0.28		
HORIZONTAL			% Fish Distribution			
No. of Experiment	Flowmeter Reading (in mm)	Corresponding LC50	Section 1	Section 2	Section 3	Section 4
1,2,22,24 25,26,74	0	0	32.5	29.4	25.0	13.1
80	20	1:700	24.6	32.2	30.8	12.4
75	30	1:500	26.8	43.4	21.9	7.9
81	40	1:370	27.2	37.6	23.8	11.4
76	50	1:250	36.6	38.7	17.2	7.5
77	82	1:150	36.4	33.8	17.7	12.1
78,79	110	1:100	30	30	25	15
82	198	1:50	30.4	27.4	21.1	21.1
83	250	1:40	31.1	34.6	25.0	9.3
VERTICAL			% Fish Distribution			
No. of Experiment	Flowmeter Reading (in mm)	Corresponding LC50	Depth A	Depth B	Depth C	
1,2,22,24 25,26,74	0	0	46.5	20.7	32.8	
80	20	1:700	42.3	31.7	26.0	
75	30	1:500	32.5	29.5	38.0	
81	40	1:370	28.2	29.4	42.4	
76	50	1:250	37.5	29.7	32.8	
77	82	1:150	39.5	27.7	32.8	
78,79	110	1:100	29.7	32.6	37.7	
82	198	1:50	37.7	32.5	29.8	
83	250	1:40	41.1	30.9	28.0	

TABLE 9.4 Fish Disposition - Injection System B

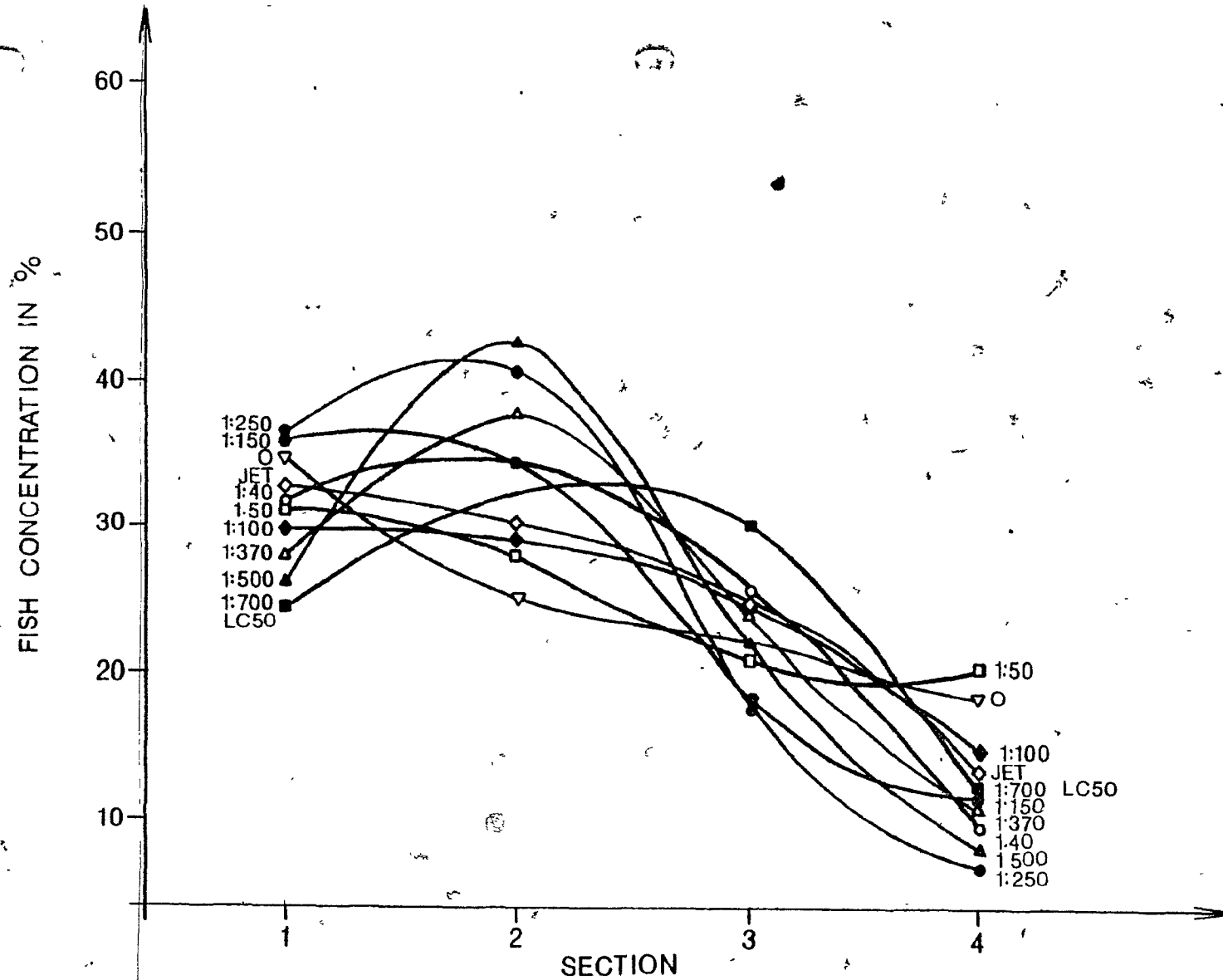


FIGURE 9.5
INJECTION SYSTEM B - HORIZONTAL DISTRIBUTION

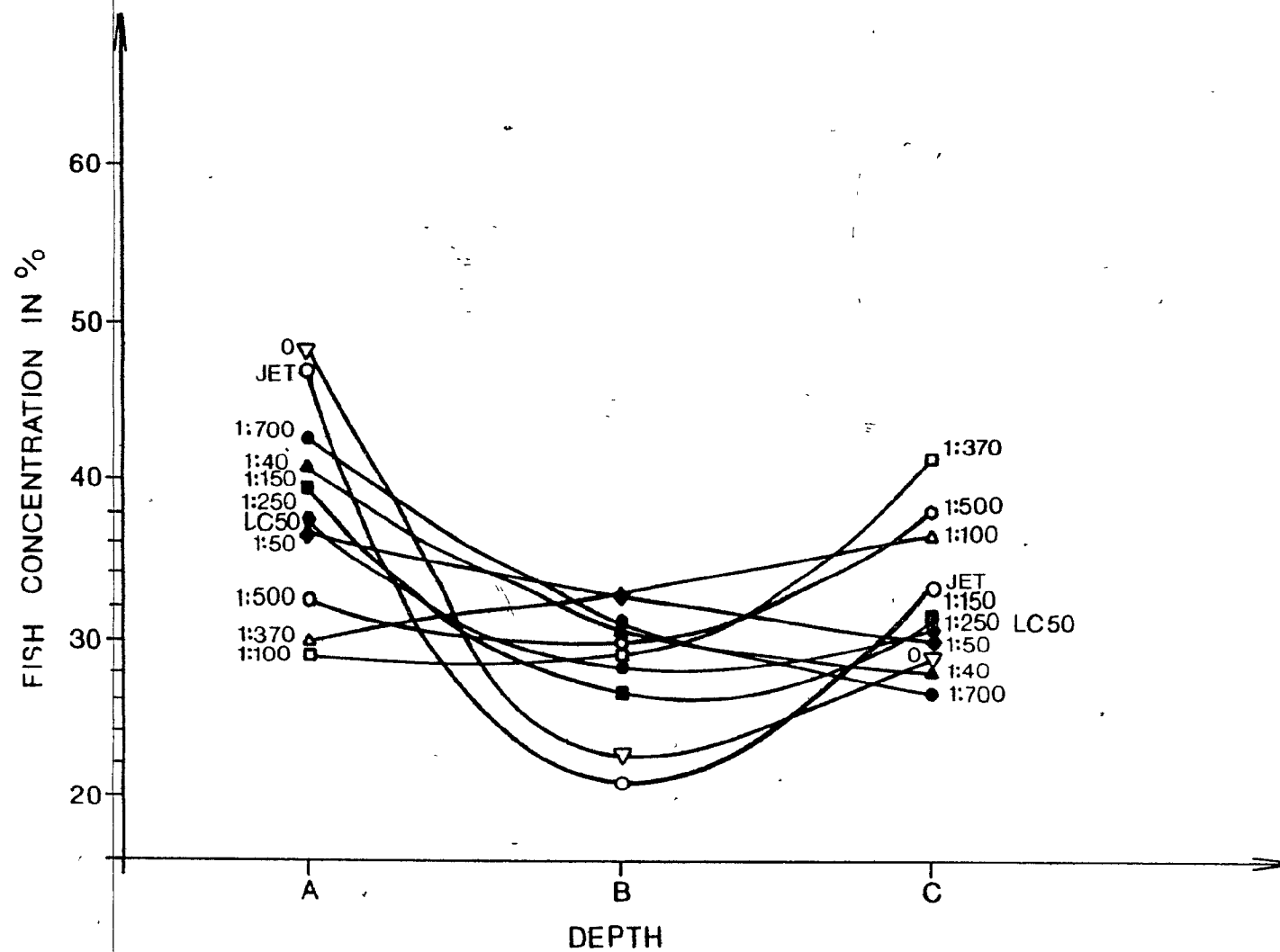


FIGURE 9.6
INJECTION SYSTEM B - VERTICAL DISTRIBUTION

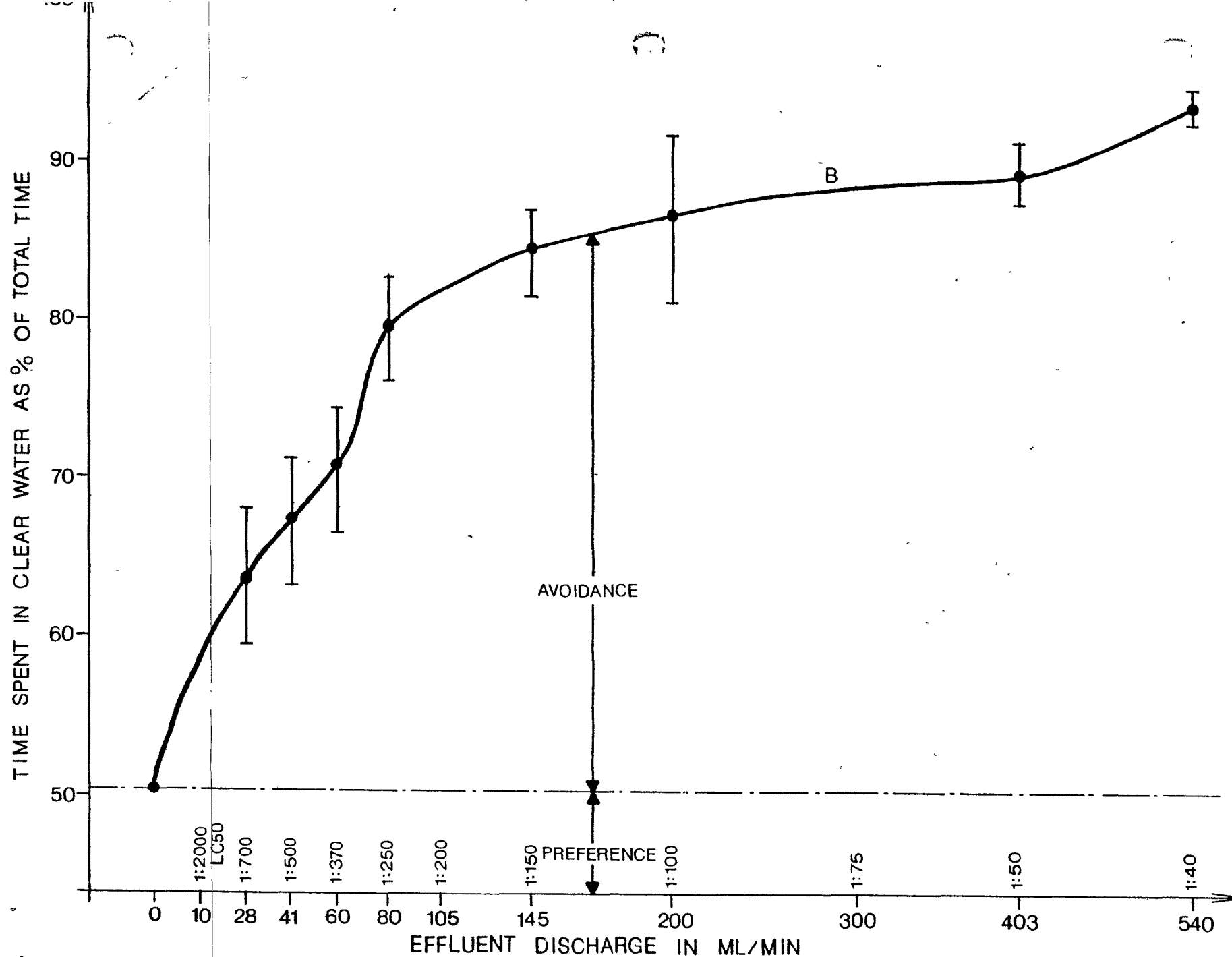


FIGURE 9.7
AVOIDANCE CURVE OF PLATING INDUSTRY EFFLUENT - INJECTION SYSTEM B

The vertical distribution given in Figure 9.6 indicates that there was no significant vertical preference or vertical avoidance for any concentrations tested. These results may have been influenced by the relatively shallow depth in the channel.

In the analysis of variance for the vertical distribution (ANOVA 11 in the Appendix C3) using the same model an F value of 0.71 was obtained with $PR > F = 0.70 > 0.05$, indicating that F is not significant. ($R^2 = 0.307$, C.V = 19.06 and STDDEV = 6.31)

For the independent variable - "depth" the hypothesis

H_0 : Depth A = Depth B = Depth C

H_A : Depth A $\neq$ Depth B $\neq$ Depth C

was accepted having an F value = 3.46 and $PR > F = 0.056 > 0.05$. This indicates that there was no significant difference between the three depths. For the Duncan's multiple range test, at each depth, all the concentrations were found to belong to the same group, i.e., they were not significantly different.

9.4 Injection Systems C and D

Knowing from the preceding experiments that the differences between Injection Systems A and B were small, four more representative experiments (Nos. 89', 90', 91', 92') were conducted using Injection System C for the first two (vertical centre multi-diffusers 4"-10" from the bottom) and Injection System D for the last two (horizontal multidiffusers, 9" from the bottom). These are shown diagrammatically in Figure 9.8. The results are presented in Table 9.5. Comparing the four injection systems for a 1:250 LC50 effluent concentration, the effect of the different injection systems in the Design H avoidance apparatus can be estimated (Table 9.6). The maximum difference between injection systems was 6% (C vs.D) which may be considered small, due to the shallow channel depth. Injection System C is similar both to Inj.Sys.A(max.diff-3%) and to Inj.Sys. B (max.diff +2%).

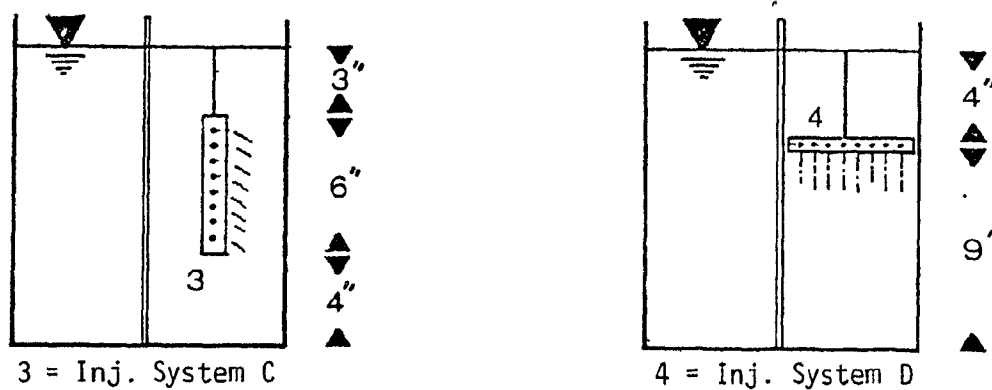


FIGURE 9.8 Injection Systems C and D

Injection System C LATERAL			% Fish Distribution	
No. of Experiment	Flowmeter Reading (in mm)	Corresponding LC50	Side A Polluted	Side B Non-polluted
89'	50	1:250	20.0	80.0
90'	50	1:250	19.0	81.0
Injection System D LATERAL			% Fish Distribution	
91'	50	1:250	24	76
92'	50	1:250	25	75

TABLE 9.5 Fish disposition - Injection Systems C and D

No. of Experiment	Flowmeter Reading (in mm)	Corresponding LC50	% Fish Distribution Side B Non-polluted	Injection System
86	50	1:250	77.0	A
76	50	1:250	79.10	B
89'	50	1:250	80.0	C
90'	50	1:250	81.0	C
91'	50	1:250	76.0	D
92'	50	1:250	75.0	D

TABLE 9.6 Avoidance values for the 1:250 LC50 concentration of the four injection systems A,B,C,D.

9.5. Avoidance Reaction Representation

In the avoidance curves (Figures 9.4 and 9.7), each experiment is represented by a single value, which is the percentage of total experimental time spent by the fish in clear water. Avoidance reactions have usually been portrayed in this fashion, and only rarely have attempts been made to use other parameters.

However, in order to have a clearer understanding of the meaning of this single value and to provide more insight for practical applications of the experimental setup used in the present study, it was felt worthwhile to elaborate two further parameters for the graphical representation of experimental avoidance data. These parameters are: a) the crossing time (T) of the mean avoidance line and b) the fluctuation (σ_e) around the mean avoidance line. The time T represents the adaptation time which elapses before the fish have achieved the mean avoidance value for the particular experiment. In calculating this parameter the four initial readings taken prior to a "stable" distribution of pollutant are excluded. When T is considered in conjunction with the σ_e (the fluctuation around the mean avoidance line) a more meaningful visualization of the output of the experiment is produced, as the following sketch shows (Figure 9.5).

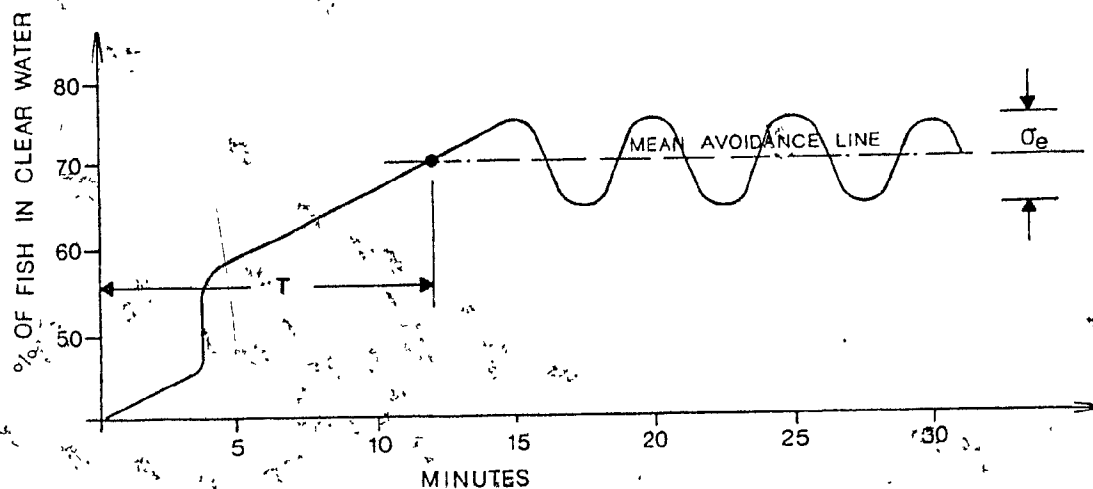


FIGURE 9.9
SKETCH DEFINING PARAMETERS T AND σ_e , ILLUSTRATING
A TYPICAL AVOIDANCE REACTION OVER TIME

The Avoidance Reaction Representation parameters σ_e and T give an indication of the quality of the experimental results. For example, for experiment 86 values of σ_e and T were 8.26 and 7.00 respectively. These values would be regarded as acceptable values. Values of σ_e and T greater than 18.00 and 40.00 respectively would be considered excessive, and would indicate instability and other experimental abnormalities such temperature fluctuations, flow variations, physical disturbances, etc. An example of this case would be experiment no. 85, where $\sigma_e = 20.00$ and $T = 41.00$. The results of this entire test was rejected and it is not included in this thesis.

Injection System	No. of Experiment	Corresponding LC50 ratio	Final Avoidance Value	T in minutes	σ_e
A	84	1:700	69	17	10.00
A	85	1:500	75.8	27	11.83
A	86	1:250	77	7	8.26
A	87	1:200	79.2	22	7.00
A	88,89	1:150	82.5*	25*	8.95*
A	90,91	1:75	91.7*	20*	4.11*
B	80	1:700	63.5	16	7.56
B	75	1:500	67.2	17	9.42
B	81	1:370	70.5	18	11.38
B	76	1:250	79.10	30	7.36
B	77	1:150	84.3	20	5.14
B	78,79	1:100	86.5*	28*	9.90*
B	82	1:50	89	5	2.23
B	83	1:40	94	15	0.53

* mean values

TABLE 9.7 T and σ_e values for the avoidance reaction representation of Injection Systems A and B

Table 9.7 illustrates the T and σ_e for the experiments with the plating industry effluent for both Injection Systems A and B. Figures 9.10 and 9.11 present the avoidance reaction representation (T, σ_e , final avoidance, dilution ratio) for the Injection Systems A and B respectively.

It appears that T fluctuates in a range between 5 and 30 minutes (80% between 15 and 25 minutes) and the σ_e between 0.5 and 11% (80% between 5 and 10%).

FINAL PERCENT OF FISH IN CLEAR WATER
EXCLUDING THE FIRST FOUR READINGS

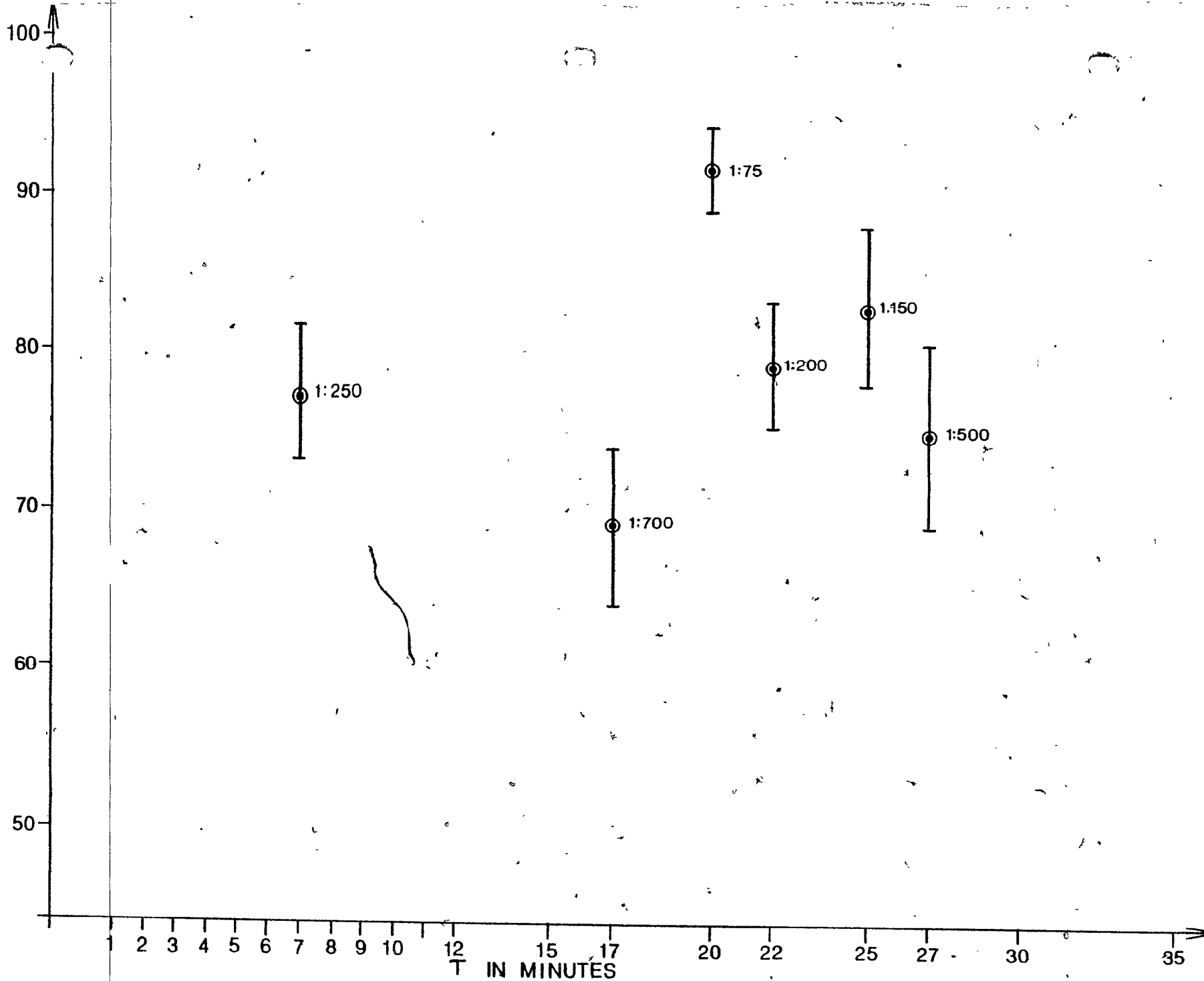


FIGURE 9.10
AVOIDANCE REACTION REPRESENTATION FOR INJECTION SYSTEM A

FINAL PERCENT OF FISH IN CLEAR WATER
EXCLUDING THE FIRST FOUR READINGS

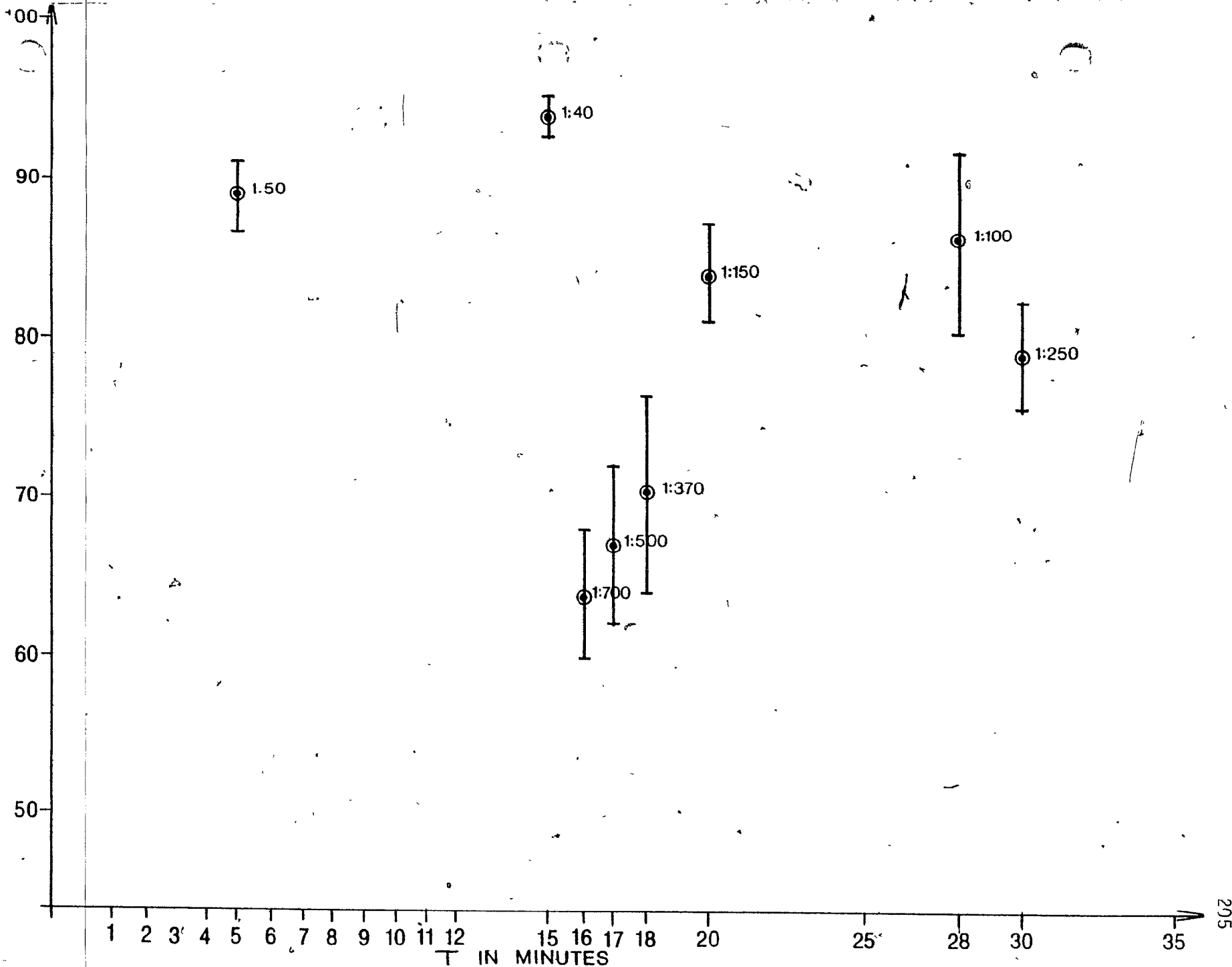


FIGURE 9.11
AVOIDANCE REACTION REPRESENTATION FOR INJECTION SYSTEM B

Avoidance curves were calculated from the results of channel regions 1 and 2, in which there was total separation of the polluted and the non-polluted sides by means of a plexiglass barrier. In Sections 3 and 4 of the channel there was no barrier separation but because the flow rate was low, both sides were polluted with (about) half of the injected concentration before the end of Section 3 (see App. A6 - Dye Studies). The following sketch shows schematically the pollutant concentration in the different sections of the channel (Figure 9.12).

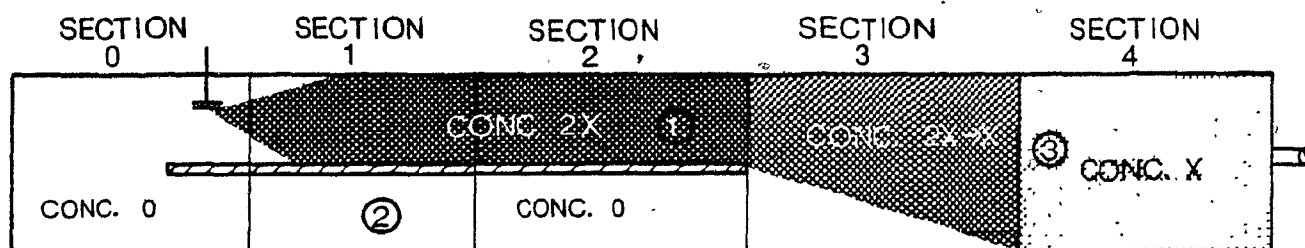


FIGURE 9.12

SKETCH SHOWING SCHEMATICALLY THE POLLUTION CONCENTRATION IN DIFFERENT SECTIONS OF THE CHANNEL - PLAN VIEW

CODE: REGION ① CONC 2x
 REGION ② CONC 0
 REGION ③ CONC 2x-x

The mean avoidance reaction for Sections 3 and 4 for all the experiments with the plating industry effluent for both A and B Injection Systems was calculated. The results are presented in Table 9.8 and illustrated in Figure 9.13. The avoidance level remained more or less constant between 50% and 60% regardless of the increment in the effluent concentration. This indicates that the side-by-side separation did not work at low flow velocities. It also suggests that the third region of the channel (which includes Sections 3 and 4 - Figure 9.12) is desirable in any avoidance apparatus, not for purposes of estimating the avoidance reaction, but as a transition zone between the 2x concentration and the zero concentration. In other words this region of the apparatus does not generate results, but rather functions as a necessary adaptation region before the fish choose to enter the polluted (region 1) or the non-polluted (region 2) sides.

Injection System	No. of Experiment	Flowmeter Reading (in mm)	Corresponding LC50	Side B in % less-polluted
A	84	20	1:700	57
B	80	20	1:700	56
A	85	30	1:500	59
B	75	30	1:500	53
A	-	40	1:370	-
B	81	40	1:370	56
A	86	50	1:250	60
B	76	50	1:250	54
A	87	60	1:200	58
B	77	82	1:150	55
A	88,89	82	1:150	61
B	78,79	110	1:100	51
A	90	150	1:75	57
B	82	198	1:50	56

TABLE 9.8 Avoidance of plating industry effluent in Sections 3 and 4.

TIME SPENT IN CLEAR WATER AS % OF TOTAL TIME

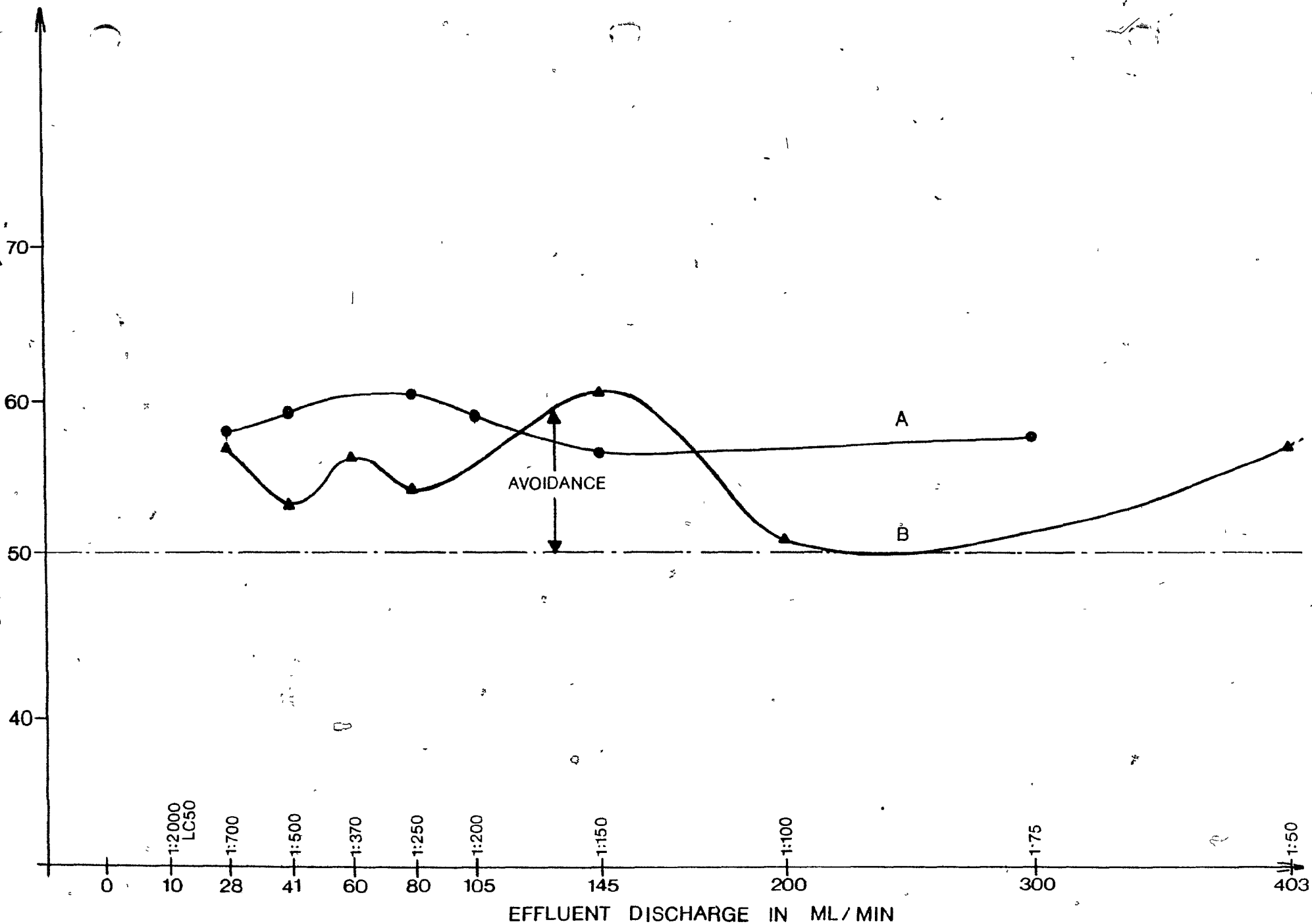


FIGURE 9.13
AVOIDANCE OF PLATING INDUSTRY EFFLUENT IN SECTIONS 3 AND 4

9.7 Relationships between avoidance curves and avoidance reaction representation of injection systems A and B

The comparison between the avoidance curves of Injection Systems A and B was made using a computer, using analysis of variance (ANOVA 3 in the Appendix C3). The F value for the model (with injection system and concentration as dependent variables and avoidance reaction as the independent variable) was 19.12 with $PR > F = 0.0001 < 0.05$. This indicates that F was significant ($R^2 = 0.95$, C.V. = 3.46 and Std. dev. = 2.82).

In the hypothesis testing of the independent variables, which was the most important part of this analysis, the hypothesis

H_0 : Injection System A = Injection System B

H_A : Injection System A $\neq$ Injection System B

was accepted, having an F value = 2.64 and $PR > F = 0.1387 > 0.05$. This means that there was no significant difference between the two injection systems, A and B. In Figure 9.14, the plotting of the avoidance curves for the two injection systems shows the same insignificant difference ($\pm$ max 4%). Figure 9.15 illustrates the avoidance reaction representation of Systems A and B together. 80% of these values for both systems were between 5 and 6%. The values of $\bar{x}$ were basically similar; with two exceptions (1:250 LC50, 1:500 LC50), they fell within a range between 5 and 30 minutes.

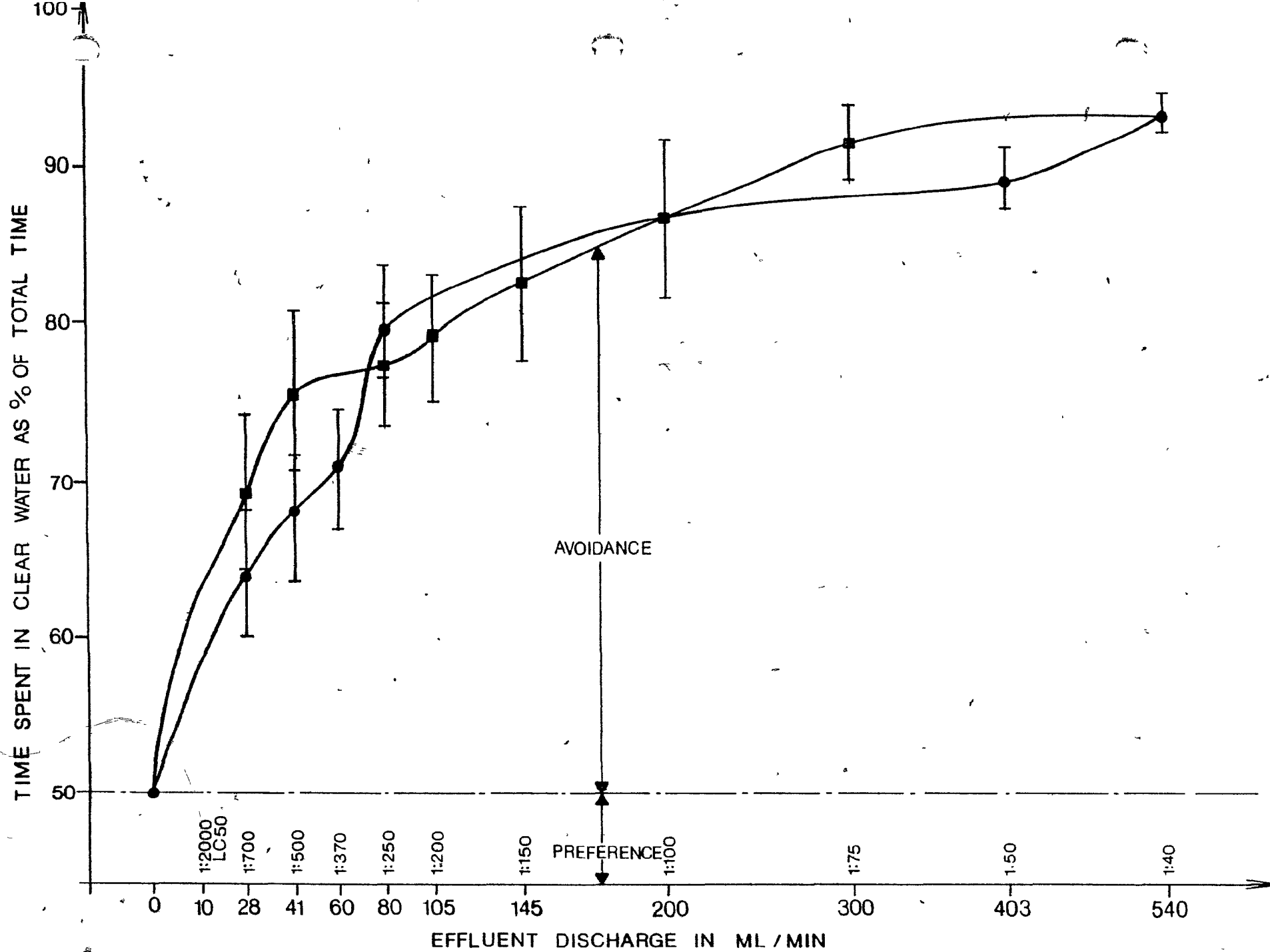


FIGURE 9.14
AVOIDANCE CURVE OF PLATING INDUSTRY EFFLUENT. INJECTION SYSTEM A AND B

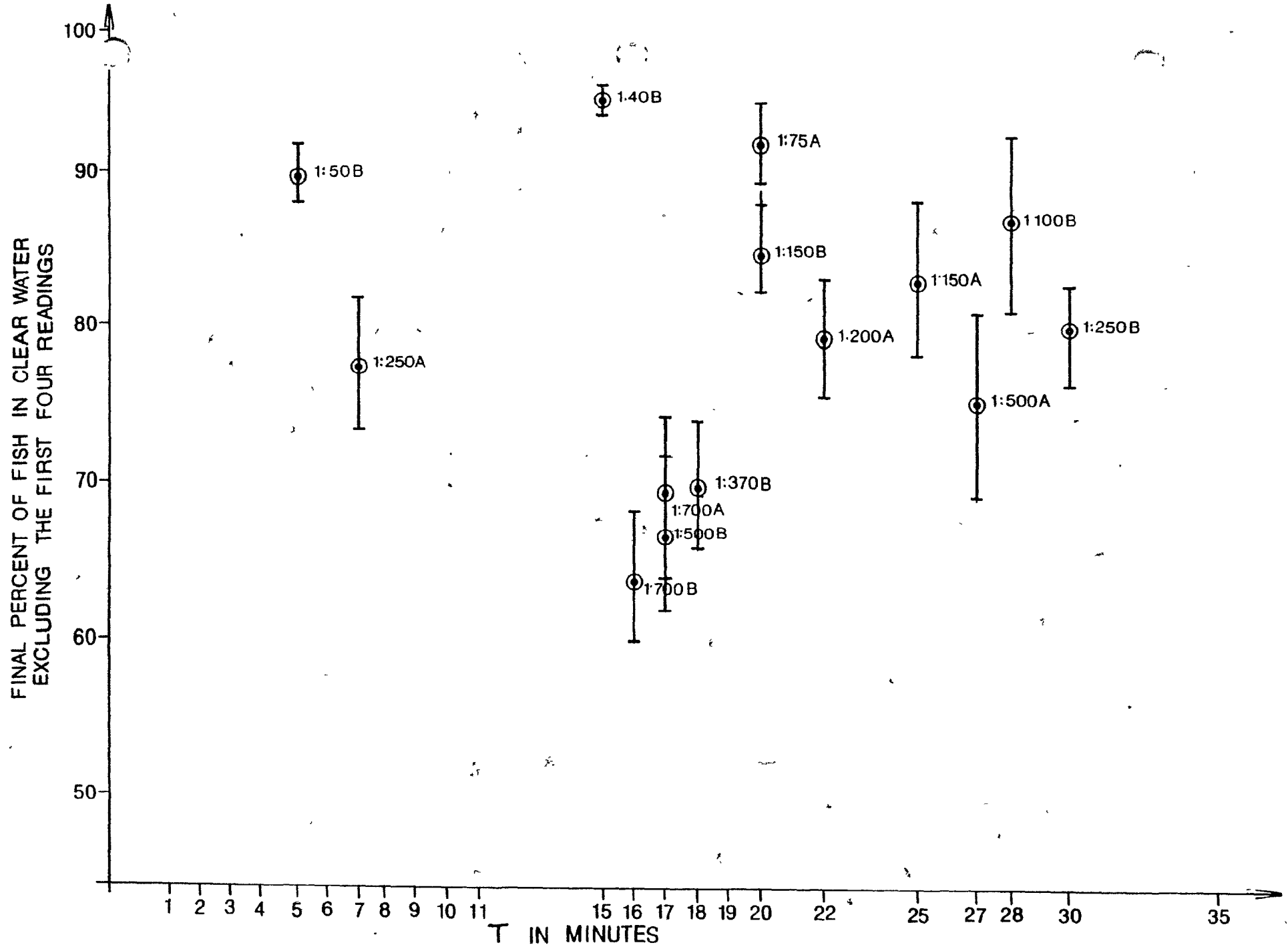


FIGURE 9.15
AVOIDANCE REACTION REPRESENTATIONS FOR THE INJECTION SYSTEMS A AND B

9.8 Discussion of Results

The sublethal threshold concentration for the avoidance reaction to the plating industry effluent used in this study (i.e., the concentration at which avoidance behavior was first noticed) was 1:3000 LC50. This means that a very small amount of effluent injected into a water body will produce an avoidance reaction, i.e. that reaching, in dilution ratios, between 1:75 and 1:50 LC50.

The tendency for fish to concentrate horizontally in the second section of the channel as the injected concentration increased can be attributed to the particular characteristics of the plating effluent, combined with the effects of the injection system used.

The influence of the effluent on the vertical distribution was relatively small, and did not follow any consistent pattern in relation to the injected concentration.

The difference in avoidance reaction between the Injection Systems A and B was not significant. There was a difference in the horizontal and vertical distribution which seemed to be, in this case, characteristic of the injection system.

The maximum difference in avoidance reaction between the four injection systems used was only 6% but it might be predicted to be greater in a system with a deeper experimental channel due to more extensive development of the vertical distribution difference.

The spectrum of concentrations injected which ranged between 1:3000 and 1:40 LC50 (50-94% avoidance reaction), was well below the lethal threshold. This illustrates a significant potential hazard to aquatic organisms at sublethal concentrations, which would be impossible to predict or estimate with the conventional static LC50 test.

Sections 0, 3 and 4 were found to play an important role in the operation of the avoidance apparatus. Section 3 had a shallow gradient tendency and served as a transition zone between Section 2 (which provided for physical separation between conc. "0" and conc. "2X") and Section 4 which had a constant conc. "X". Sections 0 and 4 were the entrance and the exit sections respectively, and were the necessary adaptation zones which allowed the fish to acclimatize to their new environment.

The avoidance reaction representation is proposed as a new manner of depicting experimental avoidance information and of permitting clearer understanding of the stability of the reactions with time.

CHAPTER X

"EXPERIMENTATION WITH THE COMPONENTS
OF THE INDUSTRIAL EFFLUENT"

10.0 The components of the industrial effluent

Following chemical analysis of the industrial effluent used in the previous chapter, it was found that the three principal metals in the effluent were: Nickel (50 mg/l), chromium (20 mg/l) and iron (4.5 mg/l). In order to ascertain the contribution of the individual metals to the avoidance behavior of the fish exposed to the raw effluent, each metal was tested separately at concentrations equivalent to the ones that were found in the chemical analysis (e.g., for flowmeter reading 150 corresponding to 1:75 LC50, the theoretical concentration of Cr is 0.444 mg/l because $0.444 \times 75 \times 0.60 = 20 \text{ mg/l}$).

A system of four siphons (S1, S2, S3 and S4), was installed throughout the channel in the positions shown in Figure 10.1.

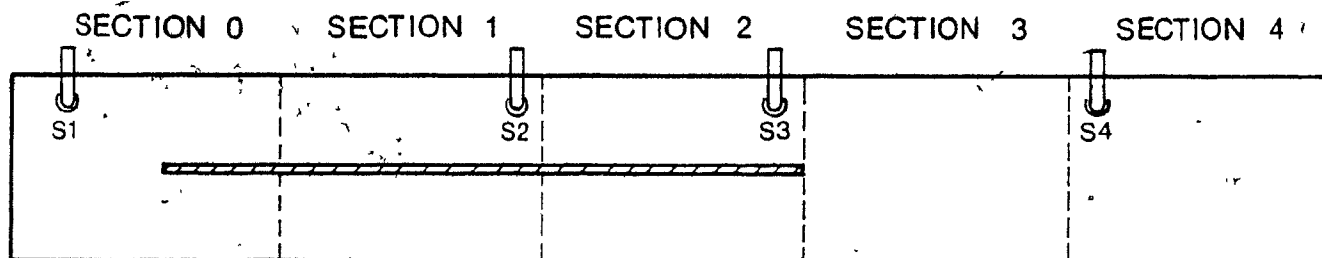


FIGURE 10.1
SIPHON-SAMPLING POSITIONS THROUGHOUT THE CHANNEL

Siphon 1 was the control and Siphons 2, 3 and 4 were situated at the end of the 1st and 2nd sections and at the beginning of the 4th section respectively. Three times per experiment, 200 ml of water from the polluted side of the channel were removed through each siphon and analyses were made for Ni, Cr, or Fe, using atomic absorption.

The Injection System C was used for all the experiments with Cr, Ni, and Fe, employing a small proportional pump similar to the one used for the experiments with polymers and monomers, at a rate of injection of 0.00892 gpm with different metal concentrations in each experiment. Knowing that the flow in each section was 3 gpm, the dilution factor of $3/0.00892 = 336$ was calculated. Twenty-one experiments were conducted (seven per metal) with flowmeter readings varying between 10 and 150 (Table 9.1 and Figure 9.1). These are described in the following sections.

10.1 Time Series Analysis

After twenty-one experiments with Cr, Ni and Fe, time series analysis was applied to the lateral distribution (Sides A and B), in order to evaluate the stability of the responses with time, and to calculate the final avoidance values.

From the output of Program B, values for the avoidance reaction were transferred to Table 10.1, and after the elimination of the jet influence (+5%), the final avoidance values were calculated (Table 10.1). Utilizing these values, the avoidance curves and the avoidance reaction representations were derived (Figures 10.4, 10.7, 10.10 and 10.12, 10.13, 10.14 respectively) as described in Sections 10.2, 10.3, 10.4 and 10.6 below.

10.2 Experiments with Chromium

Chromium (Cr^{+6}) was used for these experiments (Fisher, Certified Atomic Absorption Standard Reference Solution Cr 1,000 mg/l, solute: potassium Dichromate). Normally in plating industry effluents, chromium exists in two ionic forms - trivalent (Cr^{+3}) and hexavalent (Cr^{+6}). Chromium⁺⁶ is known

No. of Experiment	Corresponding Effluent LC50 ratio	% in clear water			
		Value of Avoidance from Computer Output	Bias factor jet influence in %	Final Avoidance Value	Chemical Used
92	1:2000	41	+5	46	Cr
93	1:500	42	+5	47	Cr
94	1:370	43	+5	48	Cr
95	1:250	44	+5	49	Cr
96	1:150	45	+5	50	Cr
97	1:100	47	+5	52	Cr
98	1:75	49	+5	54	Cr
99	1:2000	54	+5	59	Ni
100	1:500	58	+5	63	Ni
101	1:370	60	+5	65	Ni
102	1:250	65	+5	70	Ni
103	1:150	81	+5	86	Ni
104	1:100	85	+5	90	Ni
105	1:75	-	+5	-	Ni
106	1:2000	48	+5	53	Fe
107	1:500	48.5	+5	53.5	Fe
108	1:370	49	+5	54	Fe
109	1:250	49	+5	54	Fe
110	1:150	50	+5	55	Fe
111	1:100	51	+5	56	Fe
112	1:75	51	+5	56	Fe

TABLE 10.1 Calculation of the final avoidance values, for Cr, Ni, Fe experiments

to be a carcinogen and is generally believed to be the more toxic of the two ionic species. However, there is sufficient evidence currently available (Cherry, K. F. 1982) to warrant considering both forms of chromium as highly toxic. The recommendation of the EPA "Blue Book" in 1973 was that mixed aquatic population should be protected where the concentration of total chromium in water does not exceed 0.05 mg/l at any time or place.

Using atomic absorption measurements, the total chromium was measured for the effluent used in this study and found to be 20 mg/l. Based on this concentration, seven experiments (Nos. 92, 93, 94, 95, 96, 97 and 98) were conducted with seven flowmeter readings ranging between 10 and 150 (1:2000 LC50 to 1:75 LC50).

Tables 10.2 and 10.3 show the results of the experiments for the different flowmeter readings. The avoidance reaction, the theoretical concentrations of Cr in the channel, the mean values of the measurements of samples taken with the siphon technique and the dilution factors are presented in Table 10.2. Table 10.3 gives the horizontal and vertical distribution of the fish within the channel. Figures 10.2, 10.3 and 10.4 illustrate in graphical form the horizontal and the vertical distributions and the avoidance reactions to Cr. An analysis of variance for the horizontal distribution was done (ANOVA 12 in Appendix C3) using a model in which the section and the concentration were considered as independent variables and the percentage of fish in clear water as a dependent variable. This analysis yielded an F value = 6.26 with $PR > F = 0.002 < 0.05$, indicating that F was significant. Other statistics calculated were $R^2 = 0.74$,

					Cr			
					mg/l	mg in 4 litres	mg/l	in %
No. of Experiment	Flowmeter Reading	ml/min From the Calibration Curve	in gpm	Dilution Factor	Theoretical Concentration in Channel	Concent. req'd in 4 liters	Mean value of Measurements of samples	Avoidance Reaction Side B Non-polluted
92	10	10	0.0022	1364	0.0147	19.75	0.016	46
93	30	41	0.009	333	0.06	80.64	0.066	47
94	40	60	0.0139	227	0.089	118.27	0.123	48
95	50	80	0.0176	170	0.118	158.53	0.143	49
96	82	145	0.0319	94	0.212	284.93	0.283	50
97	110	200	0.0441	68	0.294	395.14	0.343	52
98	150	300	0.0661	45	0.444	596.74	0.416	54

TABLE 10.2 Experiments and Avoidance Reaction for Cr

Horizontal Distribution			% Fish Distribution			
No. of Experiment	Flowmeter Reading	Corresponding Effluent LC50 ratio	Section 1	Section 2	Section 3	Section 4
92	10	1:2000	11	36	36	17
93	30	1:500	14	53	20	13
94	40	1:370	29	37	21	13
95	50	1:250	23	41	23	13
96	82	1:150	16	56	17	11
97	110	1:100	9	53	26	13
98	150	1:75	20	48	20	12

Vertical Distribution			% Fish Distribution		
No. of Experiment	Flowmeter Reading	Corresponding Effluent LC50 ratio	Depth A	Depth B	Depth C
92	10	1:2000	50	40	10
93	30	1:500	44	32	24
94	40	1:370	50	35	15
95	50	1:250	43	31	26
96	82	1:150	33	33	34
97	110	1:100	35	30	35
98	150	1:75	27	28	45

TABLE 10.3 Horizontal and vertical distribution for Cr experiments

C.V. = 33.94, Std. Dev. = 8.56.

Testing the hypothesis

H_0 : Section 1 = Section 2 = Section 3 = Section 4

H_A : Section 1 $\neq$ Section 2 $\neq$ Section 3 $\neq$ Section 4

the F value was 20.82 with $PR > F = 0.0001 < 0.05$. These results rejected the hypothesis and indicated that there were significant differences between the sections of the channel. The same results depicted graphically in Figure 10.2 indicate a trend for the fish to cluster in the second section of the channel. This effect together with that of nickel (illustrated below in Figure 10.5) can explain the marked concentration of fish in the second section observed during testing of the raw effluent, as described earlier and shown in Figure 9.2.

Both Duncans' tests indicated that in Sections 1, 3 and 4, the percentages of fish in clear water for the different concentrations were not significantly different (i.e., they belonged to the same group A); in Section 2 two groups A and B were found, with the percentage of fish at concentrations 1:100 and 1:150 being different from the percentages in the remaining concentrations.

In the vertical distribution (Figure 10.3) a trend was observed for fish to move from Depth A to Depth C with increasing concentrations of Cr. The analysis of variance of the vertical distribution for Cr (ANOVA 13 in the Appendix C3), using the same model as for the horizontal distribution, produced an F value = 0.70 with $PF > F = 0.64 > 0.05$, indicating that F was not significant ($R^2 = 0.33$, C.V. = 31.97 and Std. Dev. = 10.68).

The hypothesis testing for the independent variable ~ "depth":

H_0 : Depth A = Depth B = Depth C

H_A : Depth A $\neq$ Depth B $\neq$ Depth C

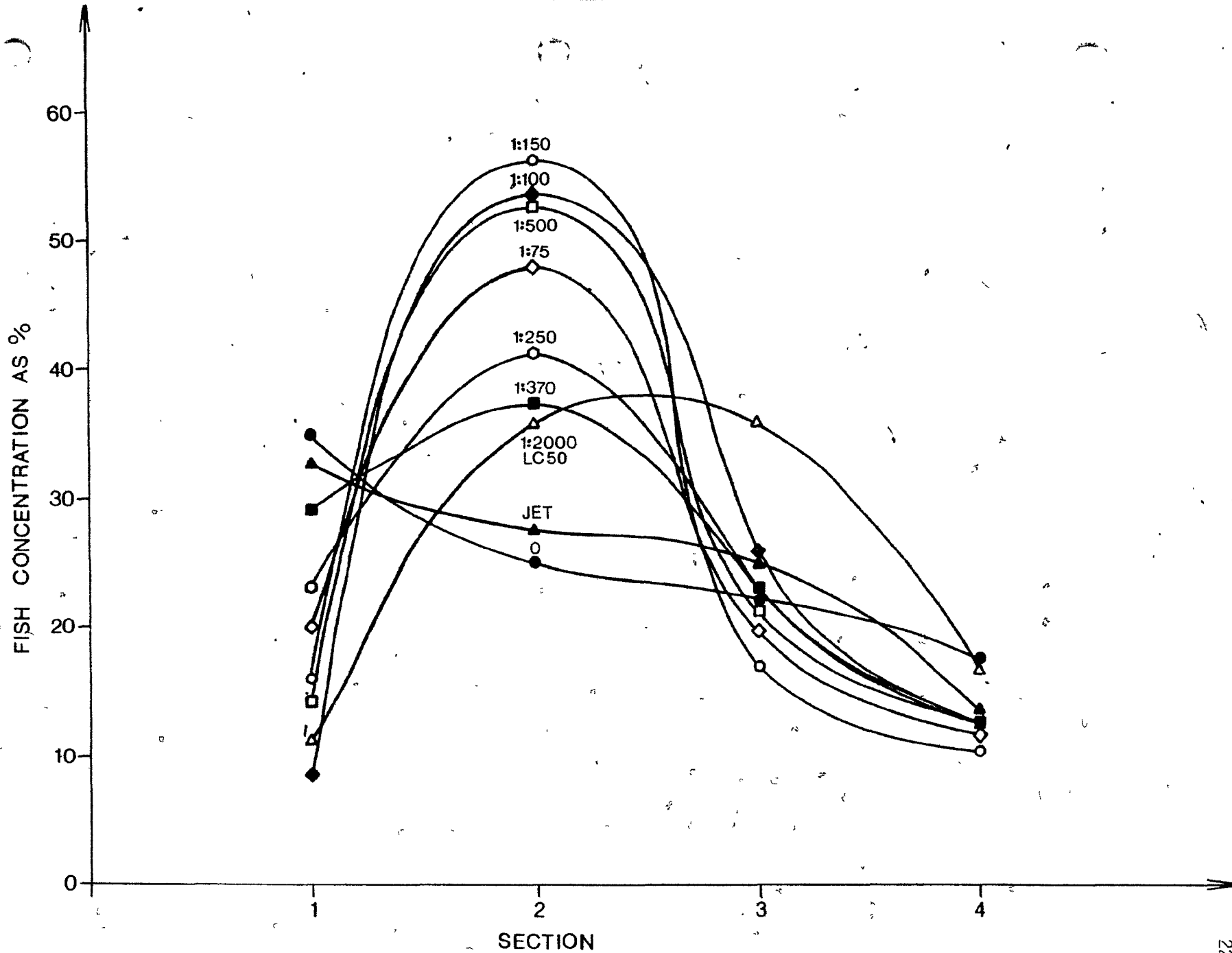


FIGURE 10.2
HORIZONTAL DISTRIBUTION OF CHROMIUM EXPERIMENTS

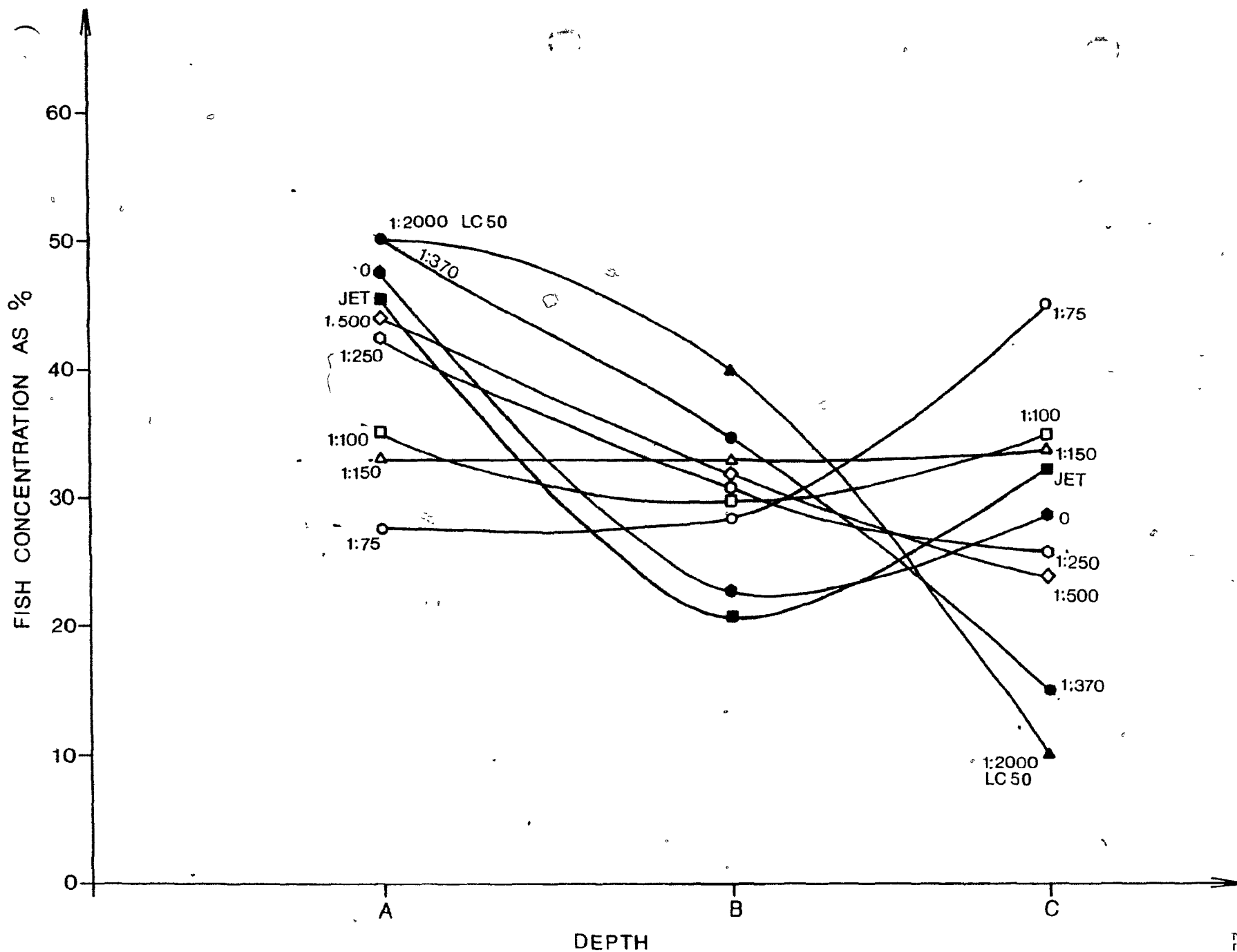


FIGURE 10.3
VERTICAL DISTRIBUTION OF CHROMIUM EXPERIMENTS

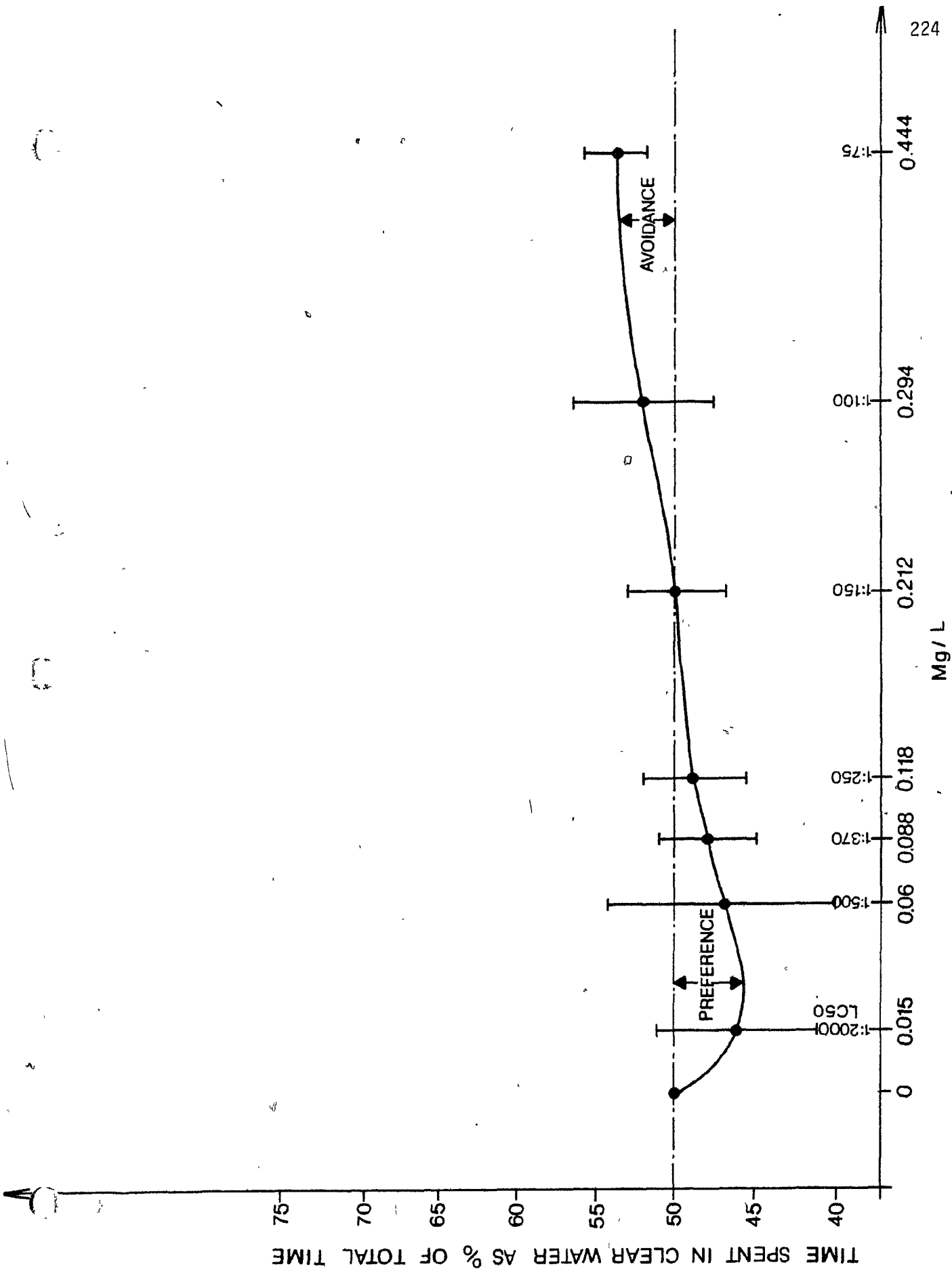


FIGURE 10.4
AVOIDANCE CURVE OF CHROMIUM

produced an F value = 3.49 with $PR > F = 0.058 > 0.05$. This indicated that there was no significant difference between the three depths A, B, and C. For both Duncan's tests at each depth, all the means were found to belong to the same Group A, meaning that there was no significant difference in the percentage of fish in clear water at the different concentrations.

In Figure 10.4 the Cr avoidance reaction shows that an increase in the Cr concentration from 0.016 mg/l to 0.444 mg/l (equivalent to the Cr present in the actual effluent) did not have much influence; the avoidance reactions for these levels of Cr remained within the 46 to 54 preference avoidance zone. The preference at low concentrations is an example of a hormetic response.

The contribution of Cr to the avoidance reaction of fish exposed to the actual industrial effluent will be discussed in Section 10.7 of this chapter.

10.3 Experiments With Ni

Nickel is toxic to a wide variety of aquatic species; it is also a human carcinogen. Threshold concentrations for a publicly owned treatment works (POTW) process inhibition are as low as 1 mg/l in activated sludge, and 2 mg/l in anaerobic digestion processes, with possible inhibition of nitrification at values of 0.5 mg/l (Cherry 1982). Inhibition in the growth of certain aquatic bacteria has been found with concentrations of nickel of 0.02 mg/l. The recommendation of the EPA "Blue Book" in 1973 was that once a 96-hour LC_{50} has been determined using the receiving water in question, and the most important species in the locality as the test organism, a concentration of nickel safe to aquatic life in the water should be estimated by multiplying the 96-hour LC_{50} by an application factor of 0.02. Nickel used for these

experiments was Fisher, Certified Atomic Absorption Stand. Ref. Solution Ni 1000 mg/l with solute nickel metal and solvent dilute nitric acid. Nickel was found at a concentration of 50mg/l in the actual effluent, having the highest pollutant concentration of all the heavy metals.

Six experiments (Nos. 99, 100, 101, 102, 103 and 104) were conducted with various Ni concentrations between 0.03 and 1.08mg/l, and with flowmeter readings from 10 to 150. These corresponded to a range of dilution ratios between 1:2000 and 1:75 LC50 (Table 9.1).

Tables 10.4 and 10.5 show the results of these experiments, with the data presented in a format identical to that for the earlier chromium data.

Figures 10.5, 10.6 and 10.7 illustrate the horizontal and vertical distribution and the avoidance reaction to Ni. The analysis of variance for the horizontal distribution of Ni (ANOVA 14 in the Appendix C3) calculated an F value = 5.13 with $PR > F = 0.0016 < 0.05$, indicating that F was significant. Other statistics were $R^2 = 0.719$, C.V. = 30.61 and Std. Dev. = 7.57.

Testing the hypothesis:

$H_0 = \text{Section 1} = \text{Section 2} = \text{Section 3} = \text{Section 4}$

$H_A = \text{Section 1} \neq \text{Section 2} \neq \text{Section 3} \neq \text{Section 4}$

for the independent variable - "section", an F value = 15.32 was obtained with $PR > F = 0.0001$. The hypothesis was rejected, meaning that there was a significant difference between the 4 sections of the channel.

Figure 10.5 shows that, as with Cr, there was a preference by the fish for the second section of the channel, which explains the enormous concentration in Section 2 observed during experiments with the actual raw effluent.

					Ni			
					mg/ℓ	mg in 4 litres	mg/ℓ	in %
No. of Experiment	Flowmeter Reading	ml/min From the Calibration Curve	in gpm	Dilution Factor	Theoretical Concentration in Channel	Concentration required in 4 liters	Mean value of Measurements of samples	Avoidance Reaction Side B Non-polluted
99	10	10	0.0022	1364	0.0359	48.28	0	59
100	30	41	0.009	333	0.147	197.77	0.083	63
101	40	60	0.0139	227	0.215	290.11	0.11	65
102	50	80	0.0176	170	0.288	387.39	0.08	70
103	82	145	0.0319	94	0.521	700.59	0.470	86
104	110	200	0.0441	68	0.721	968.47	0.75	90
105	150	300	0.0661	45	1.088	1463.47	-	-

TABLE 10.4 Experiments and Avoidance Reaction of Ni

Horizontal Distribution			% Fish Distribution			
No. of Experiment	Flowmeter Reading	Corresponding Effluent LC50 ratio	Section 1	Section 2	Section 3	Section 4
99	10	1:2000	13	40	28	19
100	30	1:500	18	38	32	12
101	40	1:370	13	52	20	15
102	50	1:250	20	40	29	11
103	82	1:150	8	33	31	28
104	110	1:100	10	41	33	16
105	150	1:75	-	-	-	-
Vertical Distribution			% Fish Distribution			
No. of Experiment	Flowmeter Reading	Corresponding Effluent LC50 ratio	Depth A	Depth B	Depth C	
99	10	1:2000	40	49	11	
100	30	1:500	57	25	18	
101	40	1:370	50	33	17	
102	50	1:250	47	43	10	
103	82	1:150	46	36	18	
104	110	1:100	44	39	17	
105	150	1:75	-	-	-	

TABLE 10.5 Horizontal and vertical distribution of Ni experiments

In the Duncan's multiple range test the means for all sections belonged to the same Group A, meaning that there was no significant difference between the percentage of fish in clear water at different concentrations.

In the Duncan's k-ratio t-test the only exception found was in Section 1 where the 0 concentration was different from the rest of the pollutant concentrations. The vertical distribution (Figure 10.6) shows an increasing preference for Depth A and a decreasing preference for Depth C.

The analysis of variance for the vertical distribution (ANOVA 15 in the Appendix C3), calculated an F value = 4.26, $PR > F = 0.0124 < 0.05$ which indicated that the F was significant. ($R^2 = 0.73$, C.V. = 28.54 and Std.Dev = 9.51)

Testing the hypothesis:

H_0 : Depth A = Depth B = Depth C

H_A : Depth A $\neq$ Depth B $\neq$ Depth C

for the independent variable - "depth", the F value was 17.03, $PR > F = 0.003 < 0.05$; the hypothesis was rejected, meaning that there was a significant difference between the three depths.

In both Duncan's tests for each depth, the entire set was found to belong to the same Group A. This implied that there was no significant difference between the percentage of fish in clear water calculated for different concentrations.

In Figure 10.7, the nickel avoidance curve reached 35% at a flowmeter reading of 110 (1:100 LC50).

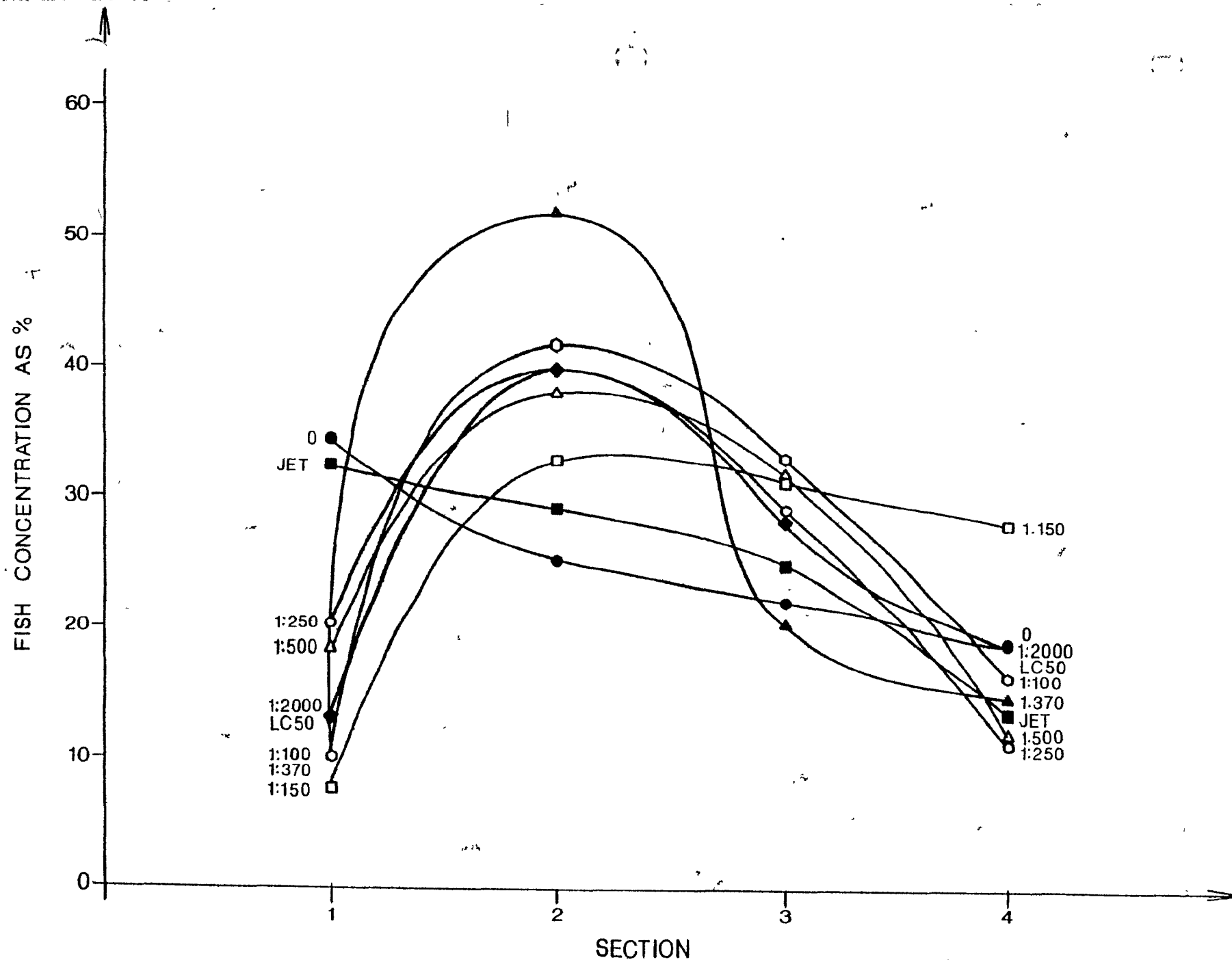


FIGURE 10.5
HORIZONTAL DISTRIBUTION OF NICKEL EXPERIMENTS

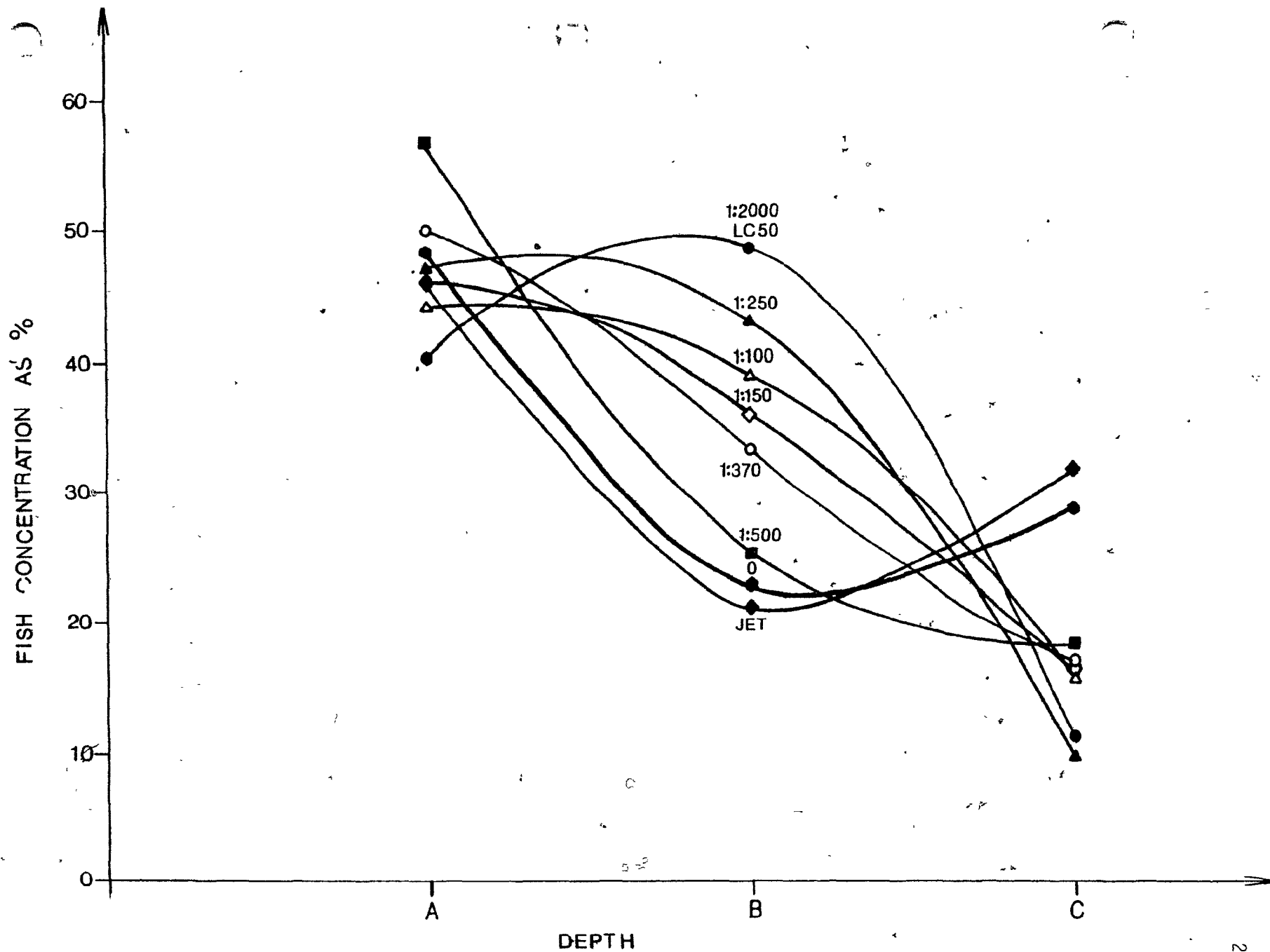


FIGURE 10.6
VERTICAL DISTRIBUTION OF NICKEL EXPERIMENTS

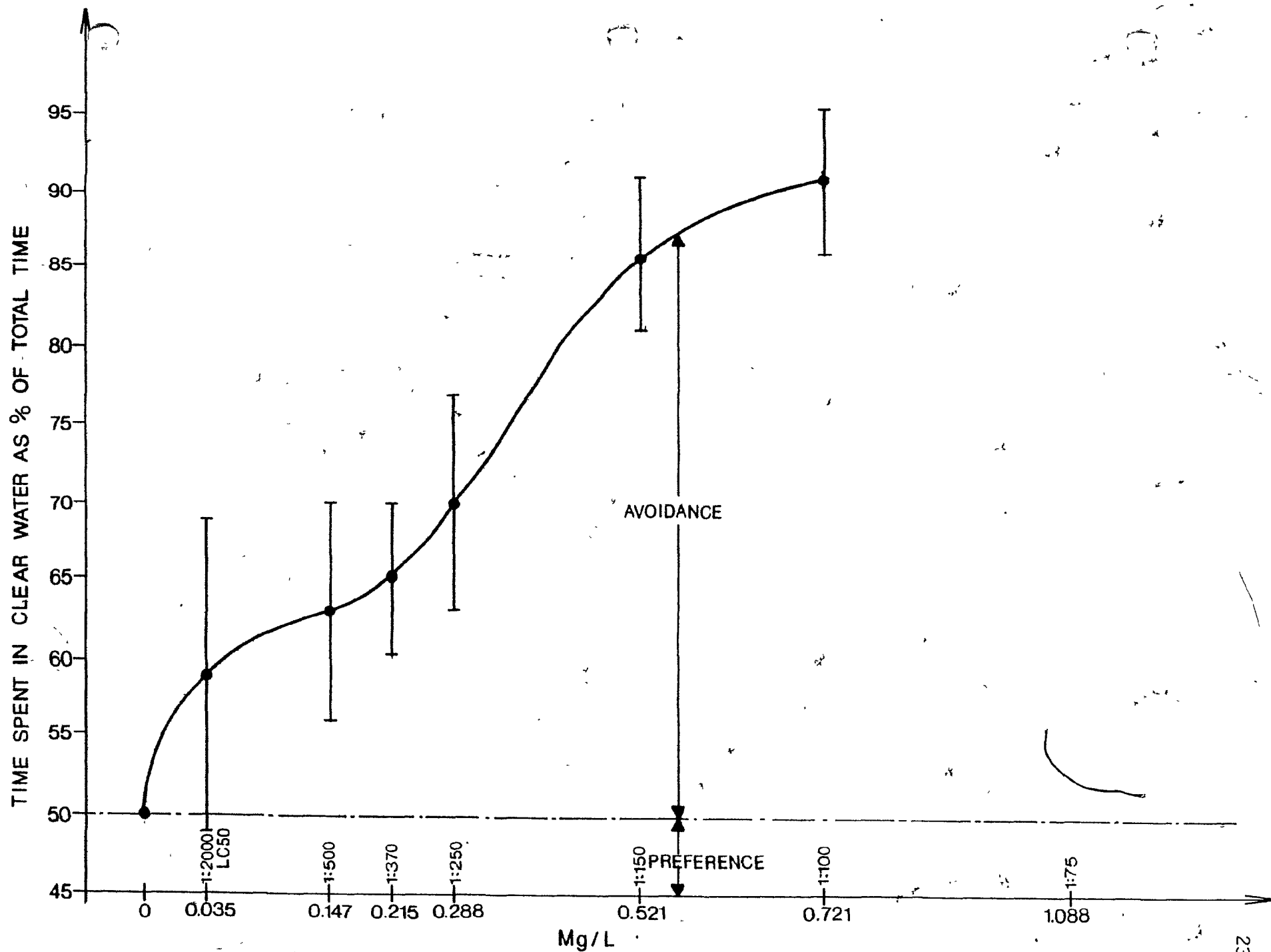


FIGURE 10.7
AVOIDANCE CURVE OF NICKEL

The increasing avoidance reaction with increasing nickel concentrations is apparent. It was concluded that Ni was the main contributor to the avoidance reaction of fish exposed to the actual effluent. Section 10.7 will discuss in detail the role of Ni in the avoidance behavior observed during the earlier study of the actual plating industry effluent.

10.4 Experiments with Fe

The concentration of Fe in the actual effluent was very small (4.5 mg/l) when compared to that of Ni and Cr. Although it was anticipated that the contribution of Fe to the overall effluent reaction would be small, seven experiments were conducted using Fe (Fisher Certified Atomic Absorption Standard, Iron Reference solution 1000mg/l, solute: Ferric chloride, solvent: dilute hydrochloric acid). Concentrations ranged between 0.0029 and 0.089 mg/l with flowmeter readings between 10 and 150 (1:2000 LC50 to 1:75 LC50).

The results appear in Tables 10.6 and 10.7, following the same format as with Ni and Cr.

Figures 10.8, 10.9 and 10.10 illustrate the horizontal and the vertical distribution throughout the channel and the avoidance reaction to Fe.

In the horizontal distribution (Figure 10.8) there was a marked tendency for fish to concentrate in the third section of the channel, but this appeared to have had little impact on the horizontal distribution during testing of the actual effluent.

					Fe			
					mg/l	mg in 4 litres	mg/l	in %
No. of Experiment	Flowmeter Reading	ml /min From the Calibration Curve	in gpm	Dilution Factor	Theoretical Concentration in Channel	Concentration required in 4 liters	Mean value of Measurements of samples	Avoidance Reaction Side B Non-polluted
106	10	10	0.0022	1364	0.0029	3.94	0	53
107	30	41	0.009	333	0.012	16.14	0	53.5
108	40	60	0.0132	227	0.017	23.68	0.01	54
109	50	80	0.0176	170	0.024	31.62	0.02	54
110	82	145	0.0319	94	0.043	57.19	0.04	55
111	110	200	0.0441	68	0.059	79.06	0.05	56
112	150	300	0.0661	45	0.089	119.47	0.07	56

TABLE 10.6 Experiments and Avoidance Reaction of Fe

Horizontal Distribution			% Fish Distribution			
No. of Experiment	Flowmeter Reading	Corresponding Effluent LC50 ratio	Section 1	Section 2	Section 3	Section 4
106	10	1:2000	6	11	55	28
107	30	1:500	4	32	52	12
108	40	1:370	6	30	53	11
109	50	1:250	13	23	54	10
110	82	1:150	22	19	47	12
111	110	1:100	19	27	50	4
112	150	1:75	21	20	54	5
Vertical Distribution			% Fish Distribution			
No. of Experiment	Flowmeter Reading	Corresponding Effluent LC50 ratio	Depth A	Depth B	Depth C	
106	10	1:2000	39	42	19	
107	30	1:500	34	36	30	
108	40	1:370	36	37	27	
109	50	1:250	39	32	29	
110	82	1:150	36	39	25	
111	110	1:100	34	37	29	
112	150	1:75	33	31	36	

TABLE 10.7 Horizontal and vertical distribution of Fe experiments

The analysis of variance for the horizontal distribution of Fe (ANOVA 16 in the Appendix C3) derived an F value = 6.60, $PR>F = 0.0001 < 0.05$, which indicated that F was significant. Other statistics were $R^2 = 0.75$, C.V. = 40.08, and Std. Dev. = 10.02.

The hypothesis test for equal sections was rejected with an F value = 22.01 and $PR>F = 0.0001 < 0.05$. This reflects the observed difference between the four sections of the channel, with a heavy concentration in Section 3.

The Duncan's tests showed that the fluctuation in the percentage of fish in clear water with variation in the injected polluted concentrations is not significant. The vertical distribution (Figure 10.9) showed a very small fluctuation around the even distribution, presumably because of the very small amounts of injected iron.

The analysis of variance for the vertical distribution of Fe (ANOVA 17 in Appendix C3) derived an F value = 0.80, $PR>F = 0.62 > 0.5$ which indicated that F is not significant. Other statistics were $R^2 = 0.33$, C.V. = 19.90 and Std. Dev. = 6.63.

The hypothesis test for the independent variable "depth":

H_0 : Depth A = Depth B = Depth C

H_A : Depth A $\neq$ Depth B $\neq$ Depth C

was accepted with an F value = 3.59 and $PR>F = 0.055 > 0.05$, which showed that there was no significant difference between the three depths of the channel.

Duncan's multiple range test showed that at every depth the percentage of fish in clear water produced by the different pollutant

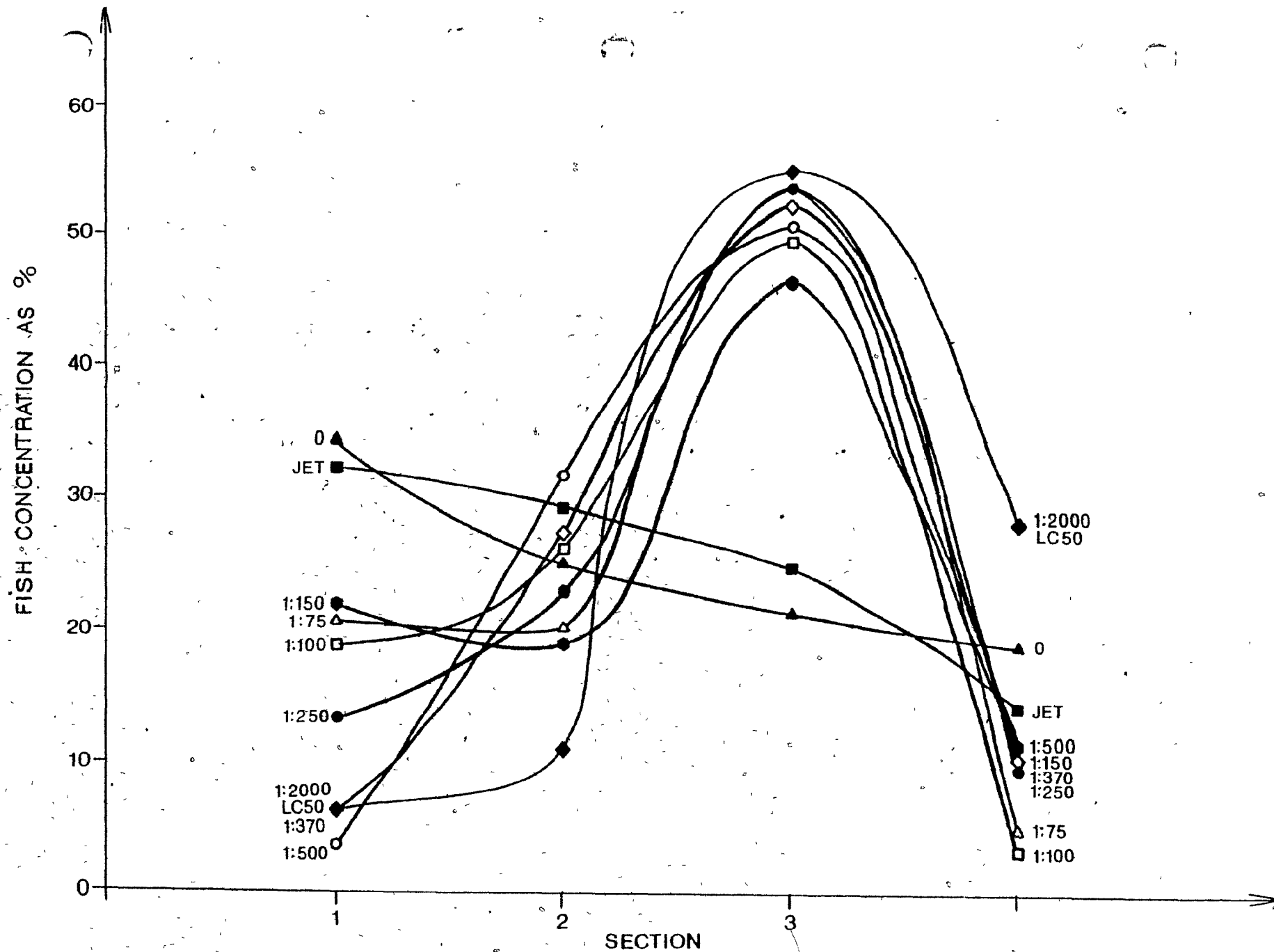


FIGURE 10.8
HORIZONTAL DISTRIBUTION OF IRON EXPERIMENTS

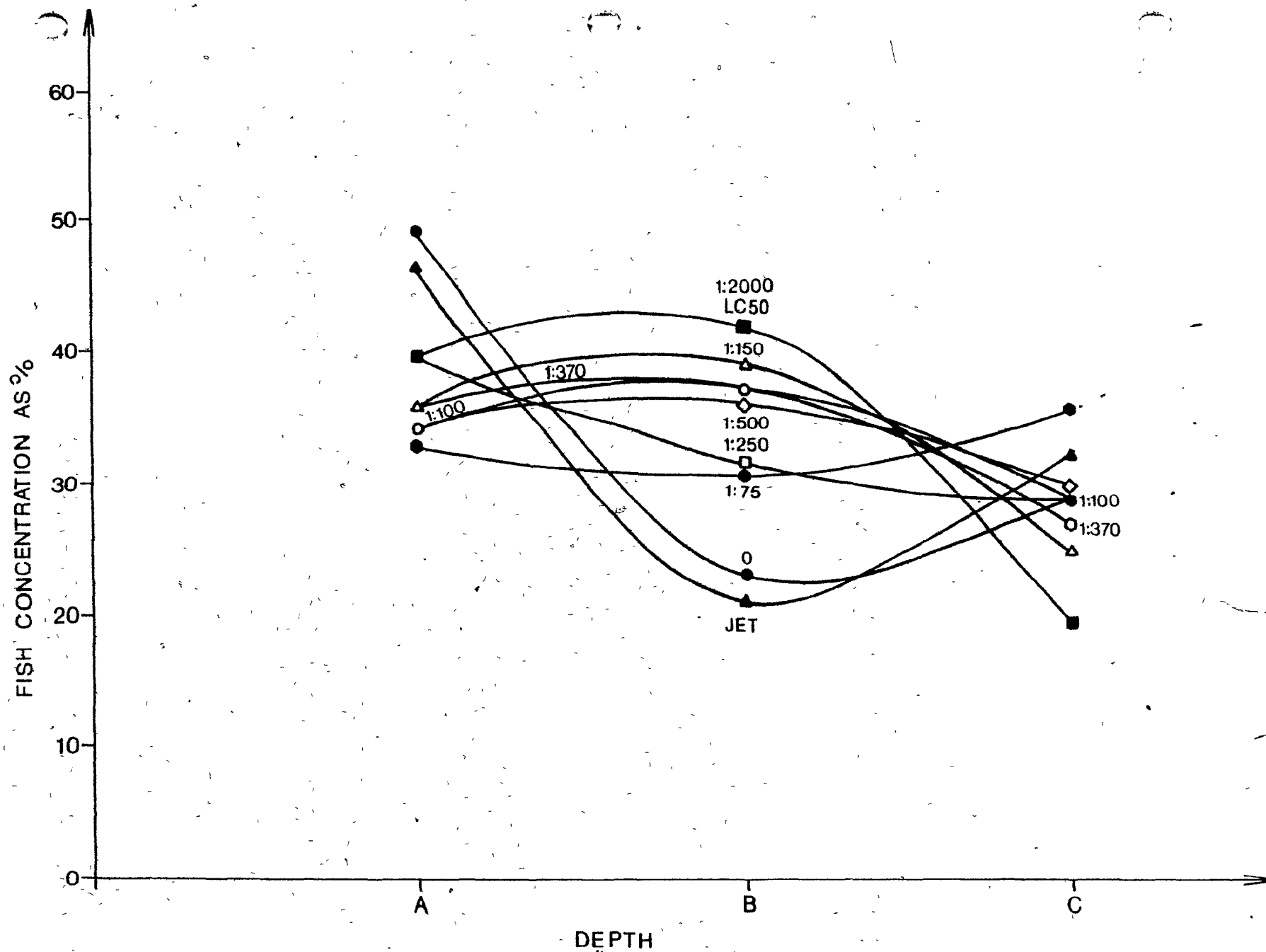


FIGURE 10.9
VERTICAL DISTRIBUTION OF IRON EXPERIMENTS

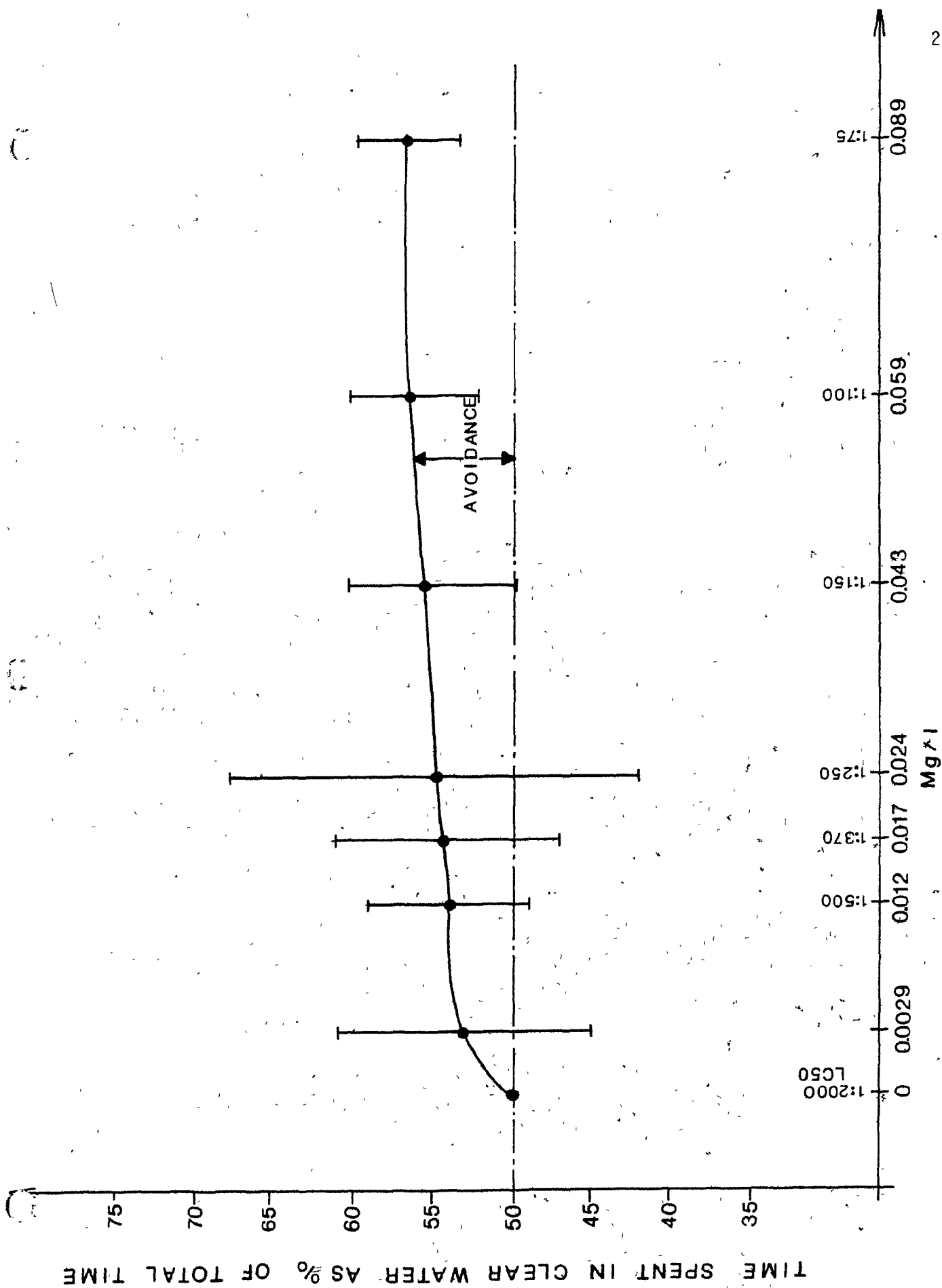


FIGURE 10.19
AVOIDANCE CURVE OF IRON

concentrations belonged to the same Group A, which indicated no significant difference between concentrations.

The avoidance reaction of Fe (Figure 10.10) remained stable between 50-55% because of the low level of injected Fe concentrations.

10.5 Avoidance Reaction Representation

For better visualization of the experimental results, the parameters T and σ_e have been introduced in Chapter IX.

Table 10.8 illustrates the T and σ_e values of the experiments with Cr, Ni and Fe produced by the time series analysis program outputs.

Figures 10.11, 10.12 and 10.13 illustrate the avoidance reaction representation of the Cr, Ni, and Fe experiments, plotting the T (crossing time in minutes) vs. the final percentage of fish in clear water excluding the first four readings. The plots give also the σ_e interval and the dilution ratio for each experiment. The T values vary between 7 and 35 minutes and the σ_e varies between 5-26%.

10.6 Synergistic and Antagonistic Phenomena

For a long period, a major barrier to progress was the inadequacy of methods for predicting toxicity of mixtures of pollutants. A reasonably accurate system was tested and proven during the 1960's by the British Water Pollution Research Laboratory in Stevenage (Brown 1968). It works at both lethal and sublethal levels and measures the degree of pollution, expressing the simultaneous effects of several pollutants by means of a single number ("toxic units").

For the prediction of lethal levels, the diagram in Figure 10.14, modified by Gaddum (1948) and summarized and explained by Brown (1968), seems adequate.

No. of Experiment	Chemical Used	Corresponding Effluent LC50 ^a ratio	Final Avoidance Value	T in Minutes	σ_e
92	Cr	1:2000	46	12	8.62
93	Cr	1:500	47	30	13.18
94	Cr	1:370	48	10	4.93
95	Cr	1:250	49	8	6.08
96	Cr	1:150	50	20	6.28
97	Cr	1:100	52	8	8.28
98	Cr	1:75	54	20	4.14
99	Ni	1:2000	59	35	18.54
100	Ni	1:500	63	35	13.93
101	Ni	1:370	65	18	11.07
102	Ni	1:250	70	25	13.55
103	Ni	1:150	86	28	9.48
104	Ni	1:100	90	7	10.88
105	Ni	1:75	-	-	-
106	Fe	1:2000	53	20	15.53
107	Fe	1:500	53.5	21	10.85
108	Fe	1:370	54	8	14.70
109	Fe	1:250	54	28	26.25
110	Fe	1:150	55	30	10.3
111	Fe	1:100	56	8	7.76
112	Fe	1:75	56	32	7.20

TABLE 10.8 T and σ_e values for the avoidance reaction representation of experiments with Cr, Ni and Fe

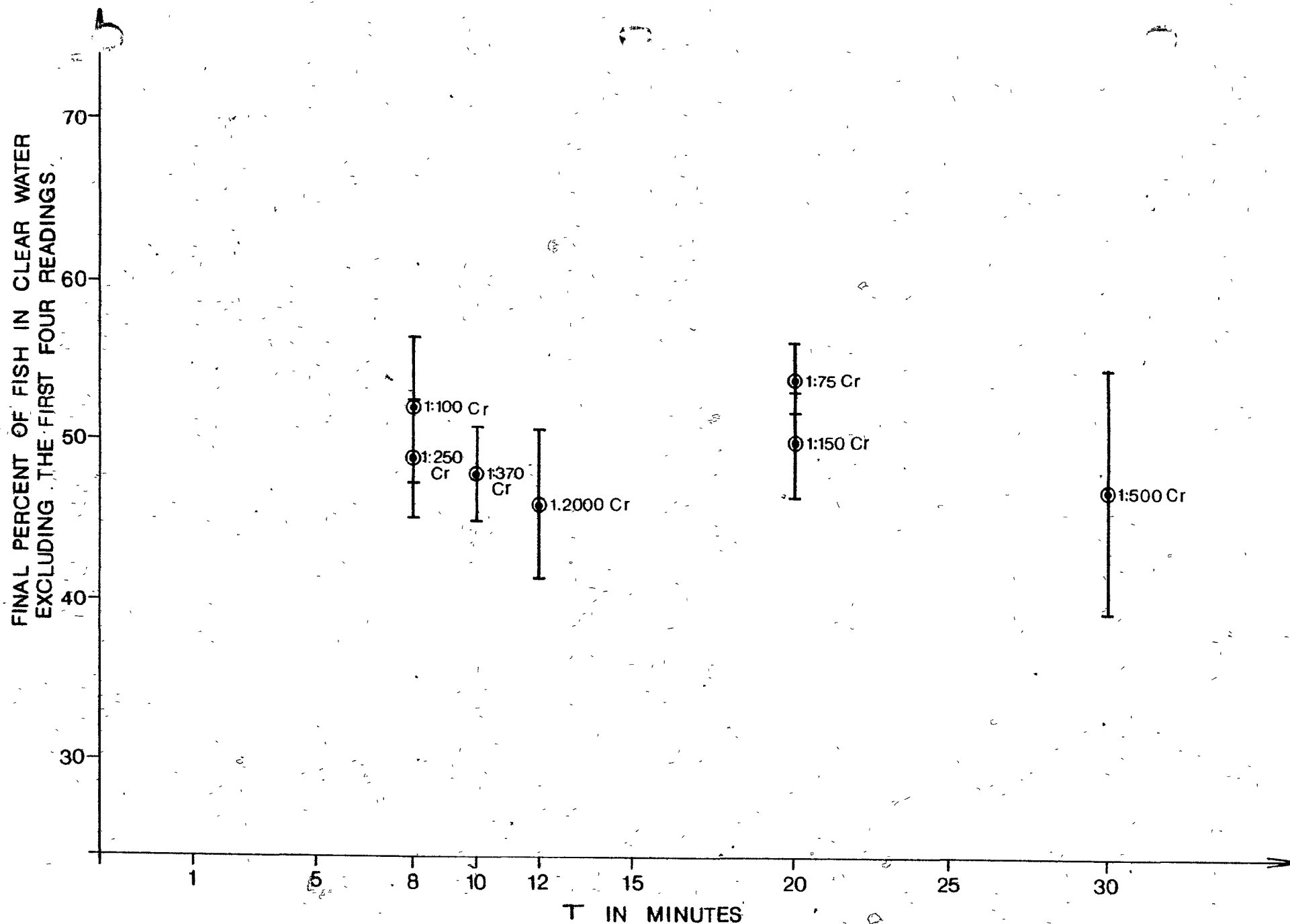


FIGURE 10.11
AVOIDANCE REACTION REPRESENTATION OF THE CHROMIUM EXPERIMENTS

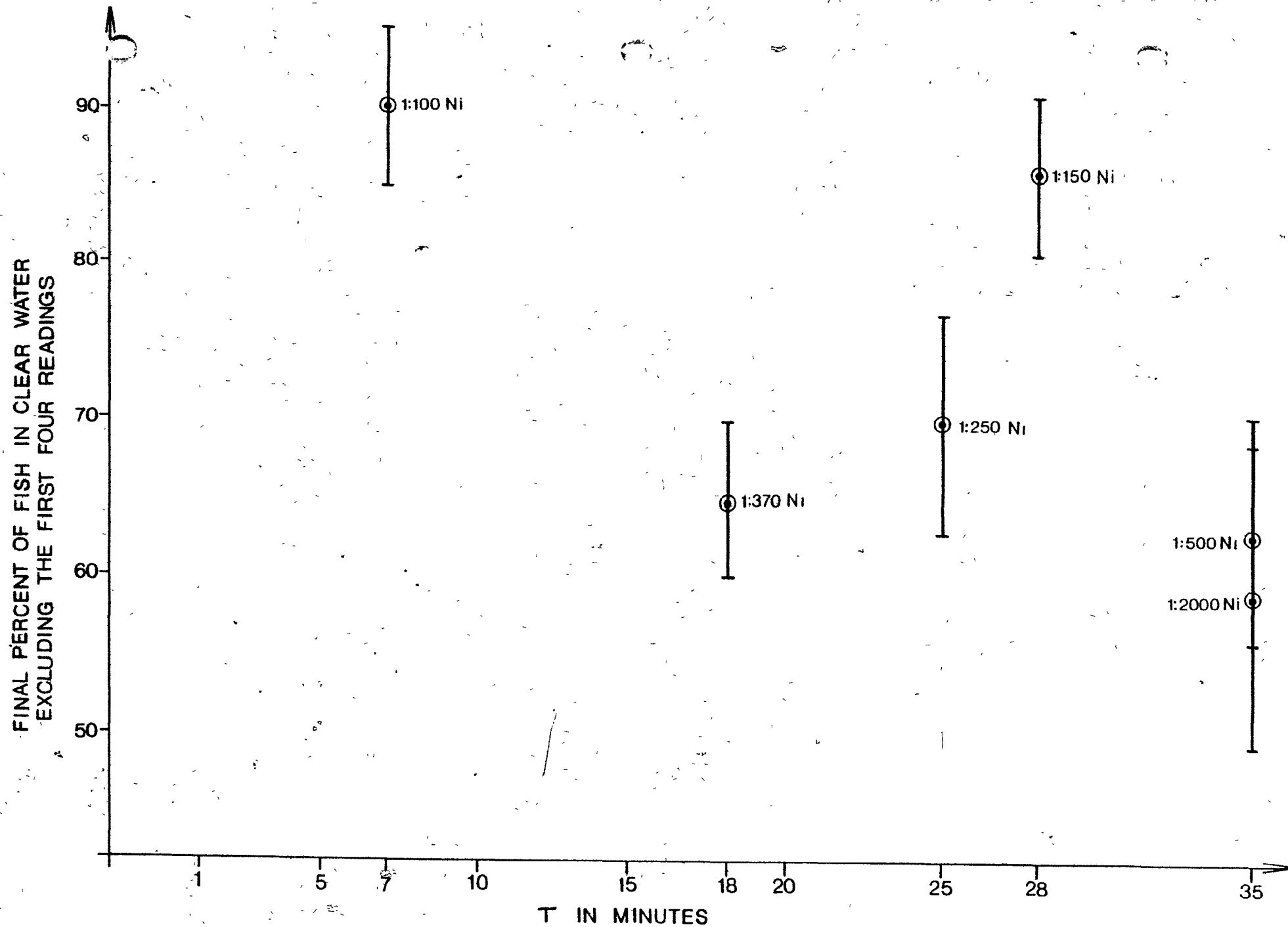


FIGURE 10.12
AVOIDANCE REACTION REPRESENTATION OF THE NICKEL EXPERIMENTS

FINAL PERCENT OF FISH IN CLEAR WATER
EXCLUDING THE FIRST FOUR READINGS

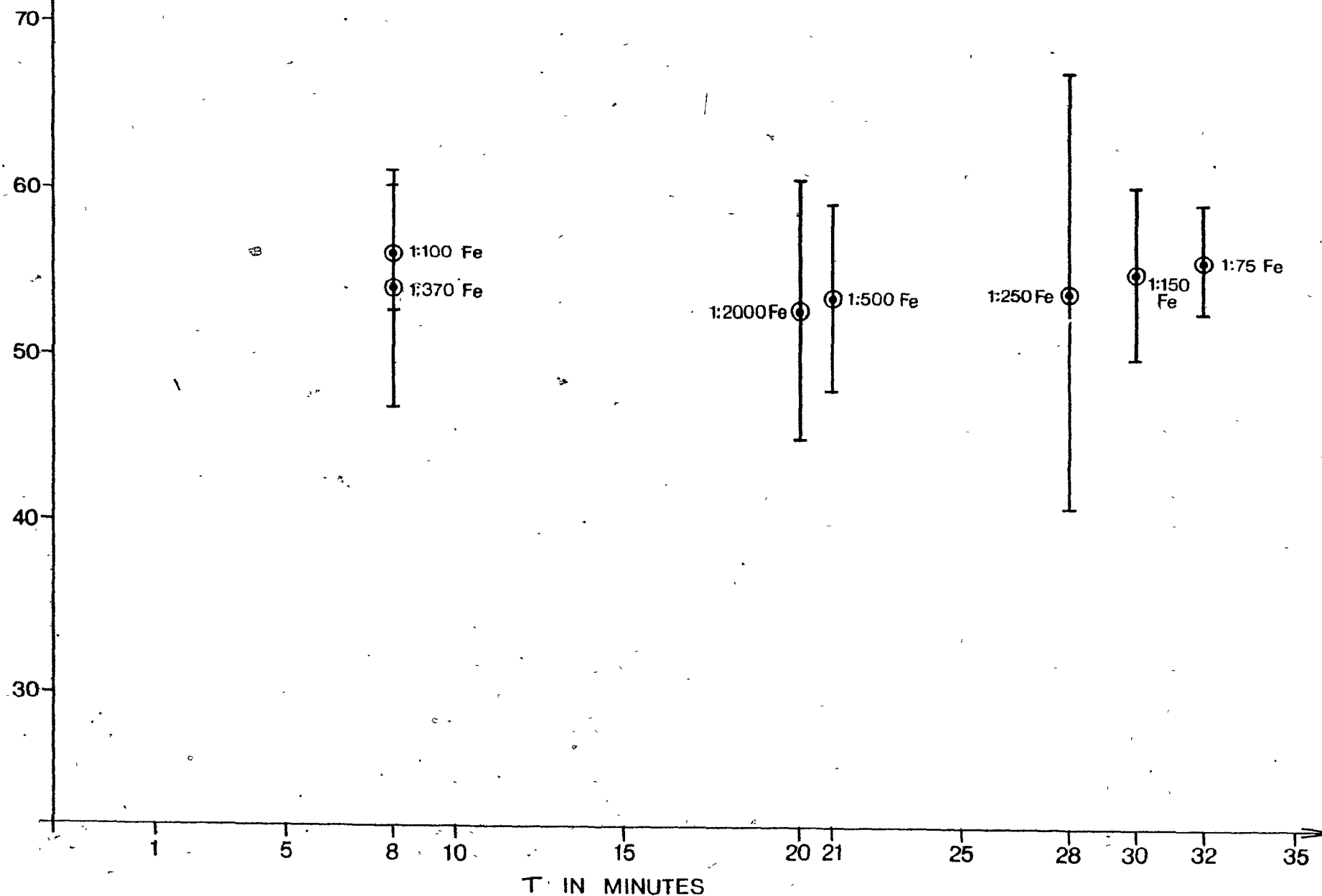


FIGURE 10.13
AVOIDANCE REACTION REPRESENTATION OF THE IRON EXPERIMENTS

The strength of a given toxicant measured in any suitable chemical unit is expressed as a fraction or proportion of its lethal threshold concentration (or another reasonable approximation such as 96-h LC50's), measured in the same units. Thus strength may be calculated as follows:

$$\text{toxic units} = \frac{\text{actual concentration in solution}}{\text{lethal threshold concentration}}$$

A very simple approach, which has been used in classifying the interactions of two toxicants, is to expose fish to half the concentration of toxicant A necessary to produce a given response and half the concentration of toxicant B necessary for the same response. If this combination just causes the response, the actions of A and B are exactly additive.

If it causes more than the given response, they are more than additive. If the combination does not produce the response the toxicants are either less than additive, show no interaction, or are antagonistic and further experimentation is necessary.

For the prediction of sublethal levels and especially avoidance reactions the same basic idea of additive (exactly, less or more) and antagonistic interactions can be applied, using various concentrations of different toxicant components of an industrial effluent (measured in toxic units) and comparing them with the avoidance response of the actual industrial effluent itself.

Section 10.7 of the present chapter discusses the synergism and antagonism phenomena for the industrial effluent used in the study (plating industry) and for its components (chromium, nickel, iron).

10.7 Relationship between the actual effluent and its components

10.7.1 Analysis of Variance

Each one of the basic components of this effluent (Cr, Ni, Fe) contributes a certain proportion to the final avoidance. To define the role of each component four analyses of variance tests were performed: ANOVA 4, 5, 6 and 7.

The results of these tests are illustrated in Tables 10.9, 10.10, 10.11 and 10.12.

Figure 10.15 illustrates the avoidance of the industrial effluent using the Injection Systems A and B, and also the avoidance of Ni, Cr, and Fe, using the Injection System C (Injection Systems A and B could not be used with pure metals because the small flows required for these tests could not be accurately controlled through these systems).

Figure 10.16 illustrates the avoidance of the industrial effluent and its components using the Injection System C. This system was not, in fact, used for the industrial effluent. Results from experiments on the industrial effluent, using Systems A and B, were adjusted to reflect behavior through System C, according to Table 9.6.

FACTORIAL EXPERIMENTAL ANOVA	<u>RESPONSE</u> Avoidance (Dependent variable) Model output <u>TREATMENTS</u> Pollutant, concentration (Independent variables) Factorial experiment 3 x 7 F = 99.91, $PR > F = 0.001$ — <u>F significant</u> $R^2 = 0.95$, C.V. = 6.51, Std.dev. = 4.46
HYPOTHESIS TESTING	$H_0 : \text{Act. effluent (ISA*)} = \text{Act. effluent (ISB**) } = \text{Cr}$ $H_A : \text{Act. effluent (ISA)} \neq \text{Act. effluent (ISB)} \neq \text{Cr}$ <u>Hypothesis rejected</u> <u>Result:</u> Significant difference in avoidance reaction between actual effluent (Inj. Syst. A and B) versus Cr $F = 97.37$ $RR > F = 0.0001 < 0.05$
DUNCAN'S TESTS	For all the concentrations 1:2000 - 1:50 LC50 <u>Two significantly different groups (A) and (B)</u> (A) Act. effluent (ISA, ISB) (B) Cr

TABLE 10.10 ANOVA 5 - TESTING WHETHER THE AVOIDANCE OF THE ACTUAL INDUSTRIAL EFFLUENT (ISA AND ISB) IS SIGNIFICANTLY DIFFERENT FROM THE AVOIDANCE OF Cr.

* ISA = Injection System A

** ISB = Injection System B

FACTORIAL EXPERIMENTAL ANOVA	<u>RESPONSE</u> Avoidance (Dependent variable) Model output <u>TREATMENTS</u> Pollutant, concentration (Independent variables) Factorial experiment 3 x 7 $F = 15.51$, $PR > F = 0.0001 \rightarrow F$ significant $R^2 = 0.91$, C.V. = 5.43, Std.dev. = 5.23
HYPOTHESIS TESTING	HO : Act. effluent (ISA*) = Act. effluent (ISB**) = Ni HA : Act. effluent (ISA) $\neq$ Act. effluent (ISB) $\neq$ Ni $F = 2.31$ $PR > F = 0.14 > 0.05$ <u>Hypothesis accepted</u> <u>Result:</u> No significant difference between avoidance reaction of actual effluent (Injection Systems A and B) versus Ni
DUNCAN'S TESTS	For all the concentrations 1:2000 - 1:75 LC50 Only one Group (A) ↙ Actual effluent (ISA, ISB), Ni

TABLE 10.11 ANOVA 6 - TESTING WHETHER THE AVOIDANCE OF THE ACTUAL INDUSTRIAL EFFLUENT (ISA AND ISB) IS SIGNIFICANTLY DIFFERENT FROM THE AVOIDANCE OF Ni

* ISA = Injection System A

** ISB = Injection System B

FACTORIAL EXPERIMENTAL ANOVA	<u>RESPONSE</u> Avoidance (Dependent variable) <u>TREATMENTS</u> Pollutant concentration (Independent variables) Factorial experiment 3 x 7 Model output $F = 27.42$, $PR > F = 0.0001 \rightarrow$ <u>F significant</u> $R^2 = 0.91$, C.V. = 7.45, St.dev. = 5.23
HYPOTHESIS TESTING	$H_0 : \text{Act. effluent (ISA*)} = \text{Act. effluent (ISB**) } = \text{Fe}$ } $F = 48.32$ $H_A : \text{Act. effluent (ISA)} \neq \text{Act. effluent (ISB)} \neq \text{Fe}$ } $PR > F = 0.0001 < 0.5$ <u>Hypothesis rejected</u> <u>Result:</u> Significant difference in avoidance reaction between actual effluent (Inj. Syst. A and B) versus Fe
DUNCAN'S TESTS	For all the concentrations 1:2000 - 1:50 LC50 Two significantly different Group (A) and (B)

TABLE 10.12 ANOVA 7 - TESTING WHETHER THE AVOIDANCE OF THE ACTUAL INDUSTRIAL EFFLUENT (ISA AND ISB) IS SIGNIFICANTLY DIFFERENT FROM THE AVOIDANCE OF Fe.

* ISA = Injection System A

** ISB = Injection System B

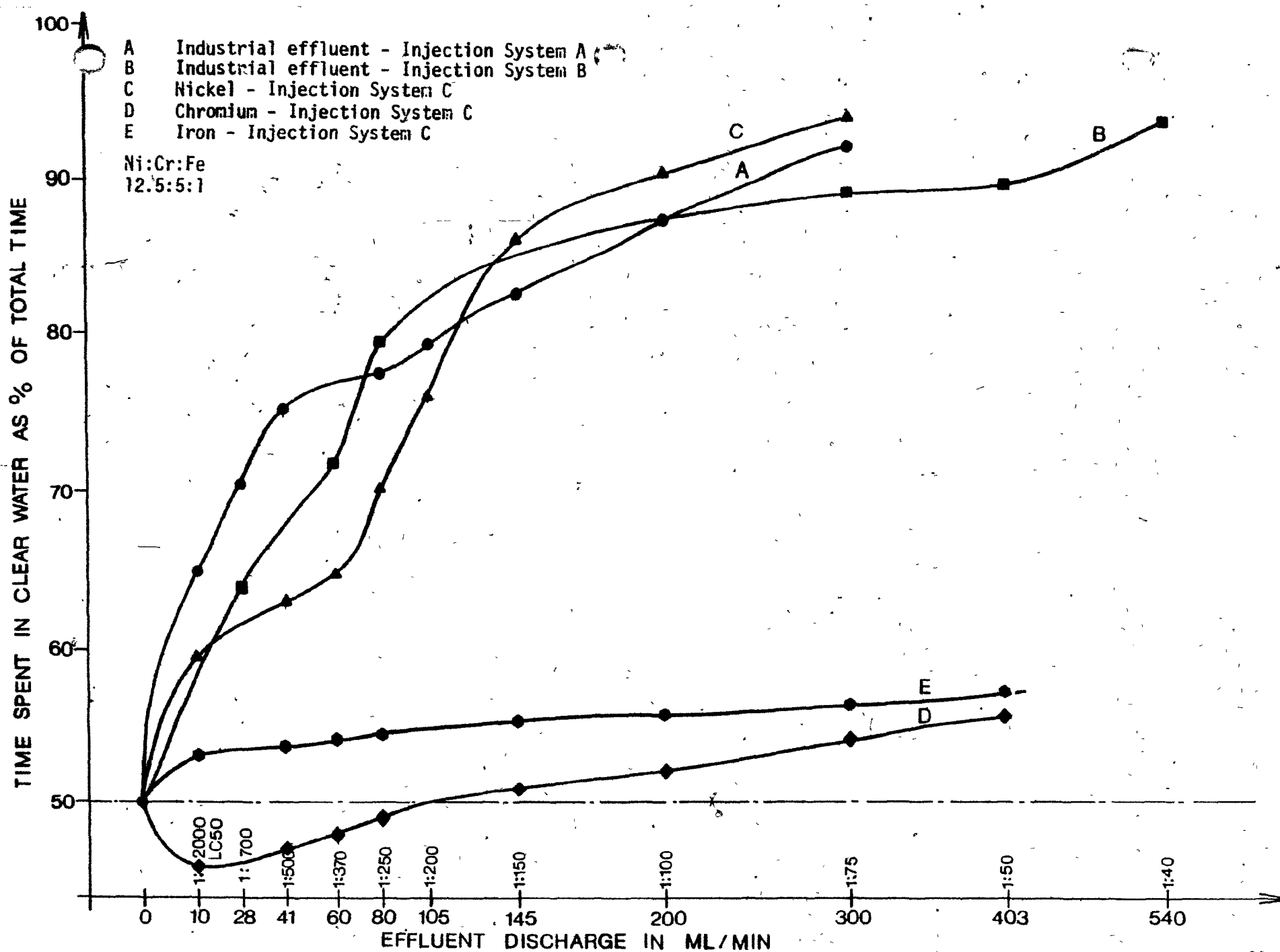


FIGURE 10.15
 AVOIDANCE OF INDUSTRIAL EFFLUENT (INJECTION SYSTEM A AND B) AND ITS COMPONENTS (Cr, Ni, Fe - Injection System C)

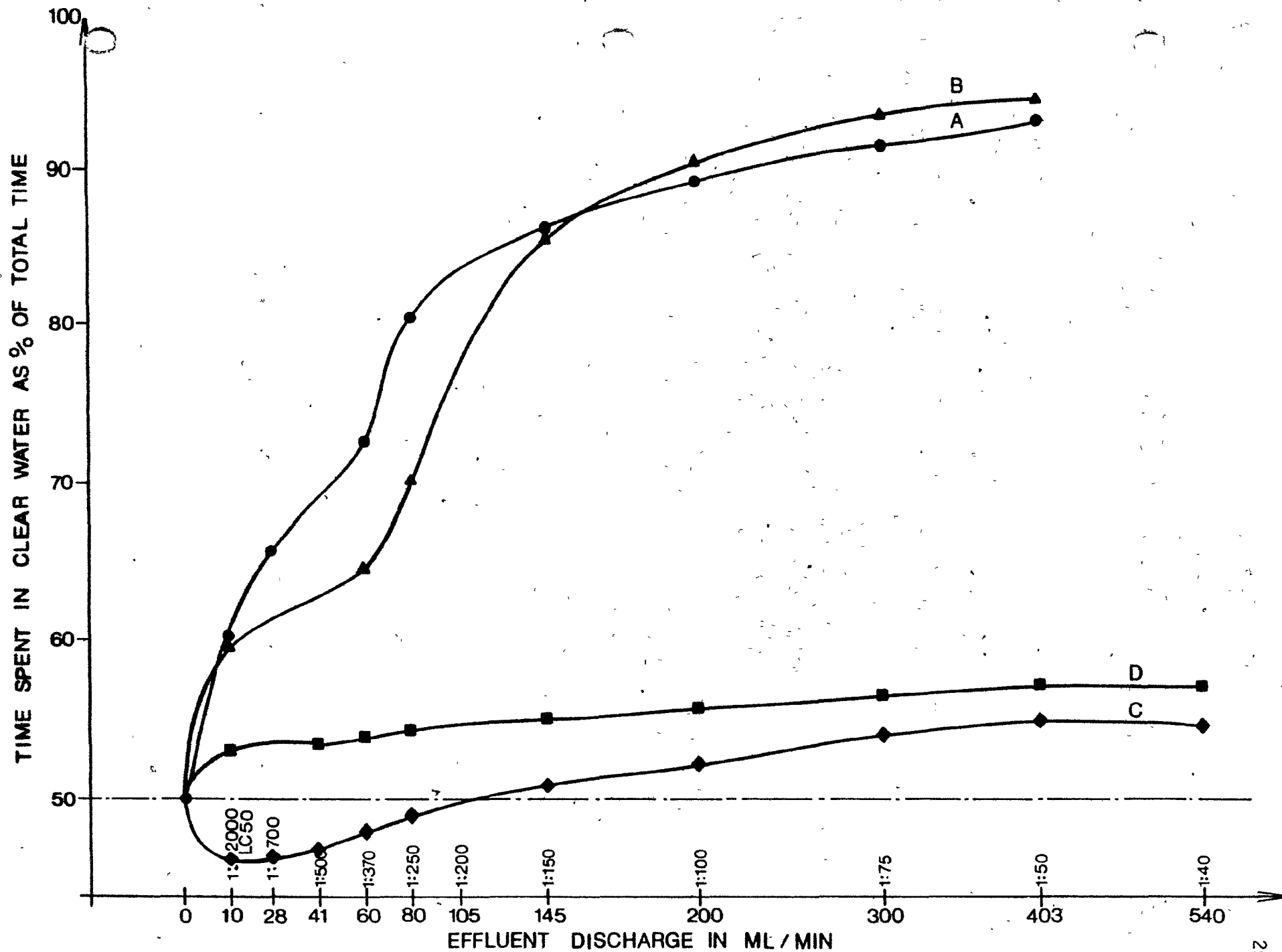


FIGURE 10.16
AVOIDANCE OF INDUSTRIAL EFFLUENT AND ITS COMPONENTS (INJECTION SYSTEM C)

10.7.2 Synergism and Antagonism

From ANOVA 5, 6 and 7 and Figures 10.15 and 10.16 the evaluation of synergism and antagonism phenomena of the three components of the industrial effluent are derived, for each concentration injected. From Table 10.9, in order to interpret the synergism or antagonism phenomena for each concentration, one compares the avoidance of the industrial effluent using the Injection System C (Column 3) with : a) the dominant avoidance reaction to Ni (Column 5), b) the additive avoidance of the three components - Cr, Ni, Fe (Column 7) and c) the additive of avoidance of the two dominant components - Cr, Ni (Column 8).

Table 10.13 (Column 9) illustrates that, at very low concentrations (e.g., 1:2000 LC50) the avoidance response of Cr, Ni and Fe was exactly additive. At concentrations between 1:700 LC50 and 1:150 LC50 synergism among the three heavy metals was observed in a decreasing range from more than additive to less than additive.

Finally at higher concentrations between 1:100 and 1:40 LC50 antagonistic reactions were observed, implying that the presence of Fe and Cr in the effluent contributed to the observations that the final effluent avoidance was less than the avoidance to the Ni alone.

In general, it appears that Ni is the dominant component of the industrial effluent and is the main contributor to the avoidance reaction. Cr plays a secondary role and the presence of Fe is almost insignificant.

A full spectrum of concentrations of the main components of the industrial effluent (from very low ones to lethal ones), provides a global understanding of the synergism and antagonism phenomena of the main components, while allowing speculation about possible significant effects of other elements present in the effluent at very low or trace concentrations.

Effluent Concentration of LC50	Avoidance of industrial effluent In. system B	Avoidance of industrial effluent In. system C	Proportional Avoidance of Cr	Proportional Avoidance of Ni	Proportional Avoidance of Fe	Additive Avoidance of Ni, Cr, Fe	Additive Avoidance of Ni and Cr	Remarks about synergism and antagonism	
(1)	(2) in %	(3) in % (2) + 1.5	(4) Cr in %	(5) Ni in %	(6) Fe In %	(7) Ni+Cr+Fe in %	(8) Ni+Cr in %	(9) Compare (3) with (5), (7), (8)	(10)
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
1:2000	56.0	57.5	46.0	59	53.0	58	62.0	Exactly additive	Synergism
1:700	63.5	65.0	46.5	61	53	60.5	64.0	Synergism More than additive	
1:500	67.20	68.70	47.0	64	53.5	64.5	67.5	Synergism More than additive	
1:370	70.50	72.0	48.0	65	54.0	67	69.0	Synergism More than additive	
1:250	79.10	80.6	49.0	70	54.0	73	74.0	Synergism More than additive	
1:200	82.0	83.5	50.0	75	54.5	79.5	79.5	Synergism More than additive	
1:150	84.3	85.8	51.0	85	55.0	91.0	90.0	Less than additive	Antagonism
1:100	86.5	88.0	52.0	90	56.0	98.0	96	Antagonism	
1:75	89.0	90.5	54.0	94	56.0	> 100	> 100	Antagonism	
1:50	89.0	90.5	55.0	95	57.0	> 100	> 100	Antagonism	
1:40	94.0	95.5	55.0	96	57.0	> 100	> 100	Antagonism	

TABLE 10.13 Synergism and antagonism in the avoidance reaction of the industrial effluent and its components

PART C

CHAPTER XI

IMPLEMENTATION OF THE RESULTS

CHAPTER XI

11.1 Introduction

The first contribution of this study has been the development of a new avoidance apparatus, able to evaluate avoidance reactions in the laboratory simulating as closely as possible the situation prevailing in natural bodies of water. The further development of this apparatus will improve its efficiency and capabilities and will eliminate its constraints.

The tabulation of the avoidance results includes the preliminary tests, the horizontal and vertical distribution, the avoidance reaction and the avoidance reaction representation for all the pollutants used in this study. The section dealing with implementation of these results leads to predictions and suggestions for safe toxicant levels for these specific pollutants and identifies untapped areas to be clarified by future research investigations.

11.2 Contributions and Further Development of the Avoidance Apparatus of Design H

11.2.1 Contributions

1. The capacity for vertical, horizontal and lateral analysis of the position of tests organisms.
2. The use of a large tank (10 meters) long which eliminates end effects, and permits the use of a large number of test organisms (100 or more fish).
3. The inclusion of engineering parameters.
4. The ability to test any kind of pollutant, to store results for later re-examination and to use advanced video and computer techniques for data acquisition.

5. The creation of a test environment which closely simulates natural conditions.

11.2.2 Further Development

1. To include other engineering parameters (e.g., different hydraulic and hydraulic-dynamic conditions) and to provide the necessary adjustable devices for that purpose.
2. To use mini-computers and special interfaces to analyse automatically the video signals of the fish movements.
3. To drop one end of the channel, thereby converting the existing system, which simulates a river, into a simulated lake system.
4. To attempt to hook the data acquiring devices (other than the video recorders) to an external real-time computer for detailed data acquisition and system control.

11.3 General Recommendations for Future Experiments Using Apparatus H

A detailed description of guidelines for avoidance experiments was discussed in Chapter IV. Based on the experience of the present study, the following general recommendations are made:

1. The acclimation period and temperature are very important factors, and have to be fully controlled in this experimental system.
2. The feeding procedure for the test organisms and the cleaning of the holding facilities can alter the behavior of the fish if a precise daily schedule is not adhered to.
3. The physical properties pertaining to the quality of the treated water have a great influence on the results; it is therefore desirable that they be measured in every experiment.
4. The analysis of the video tapes immediately after each experiment allows for adjustments and modifications to be made, and influences the planning of the subsequent test.

11.4 Specific Suggestions and Recommendations for Future Research Investigations

1. To study and select an optimal discharge system (flow rate) which will minimize toxicity avoidance by aquatic organisms.
2. To evaluate the influence of other engineering parameters (e.g., different hydraulic or hydrodynamic conditions).
3. To study diffusion-dispersion equations further, and to relate these to avoidance reaction responses.
4. To establish a data base for mathematical modelling of avoidance studies.

5. To establish a method of measuring fish avoidance as a function of toxicant dilutions found in natural bodies of water, and to investigate the possibility of controlling any experimental or real situation by using a real time acquisition system.
6. To evaluate the chronic effects of toxicants on fish avoidance.
7. To simulate a lake system and study the relationship between depth and avoidance reaction. This extension would allow for a more complete understanding of the behavioral changes in the vicinity of stratified jet discharges.
8. To study the effects of density stratification on avoidance behavior.
9. To investigate more concrete ways of relating avoidance reactions to safe concentrations.
10. To establish a small pilot plant to determine the feasibility of using avoidance as a measure for treatability of a particular effluent.
11. The results of this study indicate that avoidance could be used as a measure of environmental acceptability. Therefore, it is recommended that the present study be extended to develop a standard procedure for avoidance testing in environmental protection programs. This study should include the development of the equipment and experimental procedures to be followed.

11.5 Safe Toxicant Levels for the Pollutants used in This Study

An inventory of all the existing information in the literature for lethal and sublethal levels of DSS, Chromium, Nickel and Iron appears in Table 11.1. As can be seen from that table, for a variety of different experimental conditions and species used by different investigators, each lethal or sublethal limit varies within a certain range.

Clarke in 1974, using this information, classified Chromium, Nickel and Iron as toxic components of inorganic chemical industrial effluents, basing his classification on their lowest recorded lethal concentrations, and giving for each metal suggested receiving water criteria for both fresh water and marine aquatic life.

His suggestions for safe level concentrations appear in Table 11.2. To this information can now be added that derived from the present avoidance study (Table 11.3), which applies specifically and only to rainbow trout. Table 11.3 lists the pollutants used in column 1, the species used - (rainbow trout) in column 2 and the range of the 96 hr. acute lethal LC50 in column 3. The maximum allowable toxicant concentration, the safe concentration and the application factors from the most recent and most strict published references are presented in columns 4, 5 and 6 respectively. Columns 7 and 8 contain values for avoidance threshold and avoidance reaction derived from this study. Finally column 9 gives proposed adjustments of the safe level concentrations for the pollutants studied. These adjustments include the MATC, S.C and AF for each pollutant, and effluent limits for the principal chemicals of the raw industrial effluent.

POLLUTANT	SPECIES	RESULTS	Exposure Time*	Temp °C	Test** Conditions	References
USS Dodecyl sodium sulfate	Rainbow Trout Zebra fish Flay fish	96h-LC50 4.62 mg/l 96h-LC50 7.97 mg/l 96h-LC50 8.10 mg/l	F F F	15 25 25	ALK 200-225 HD 350-375 pH 8.0-8.3	Fogels (1977)
Chromium (VI)	Fathead minnow juvenile juvenile life cycle	96h-LC50 36.2 mg/l 96h-LC50 36.9 mg/l MATC 1.0-4.0 mg/l	S F F	25 25 16-29	pH 7.7 HD 209	Pickering (1980)
Chromium trioxide	<u>Colisa</u> <u>fasciatus</u>	96h-LC50 40 mg/l	S	25	pH 7.3 HD 120	Shrivastava (1979)
Chromium (potassium chromate)	Goldfish Goldfish shiner Bluegill Channel catfish Rainbow trout	24h-LC50 109-354 mg/l 24h-LC50 104-151 mg/l 24h-LC50 214-280 mg/l 24h-LC50 50-72 mg/l 24h-LC50 59-141 mg/l	S S S S S	5-30 5-30 5-30 5-30 5-18	DO 6-11 HD 36 pH 5.8	Cairns J. Jr. (1978)
Chromium	Rainbow trout	96h-LC50 11.2 mg/l 96h-LC50 9.0 mg/l 96h-LC50 7.05 mg/l	- - -	- - -	- PCB previous exposure 0.01 mg/l PCB pr. exp. 0.1 mg/l	Bills T.D. et al (1977)
Chromium (chromium nitrate)	Rainbow trout	96h-LC50 24.09 mg/l	F		ALK 82-132 DO 4.8-9.0 pH 6.4-8.3	Hale, J.G. (1977)

POLLUTANT	SPECIES	RESULTS	Exposure Time*	Temp °C	Test** Conditions	References
Chromium (sodium dichromate)	Rainbow trout	60-D-MATC 51-105 mg/l	F	10	A-3.3, ALK-30.1 DO-9.1, HD-33.4, pH-6.7-7.0	Santer, S. et al (1976)
	Lake trout	60-D MATC 105-194 mg/l	F	10	A-3.6, ALK-31.5 DO-9.5, HD-34.0 pH-6.8-7.1	
	Channel catfish	60-D MATC 150-305 mg/l	F	22	A-4.0, ALK-33.7 DO-8.1, HD-36.2 pH-7.0-7.4	
	Bluegill	60-D MATC 599-1122 mg/l	F	25	A-6.6, ALK-33.0 DO-6.6, HD-38.3 pH-6.7-7.1	
	White sucker	60-D MATC 990-538 mg/l	F	17	A-3.2, ALK-34.6 DO-8.3, HD-38.8 pH-6.9-7.2	
Nickel (nickel nitrate)	Rainbow trout	96h-LC50 35.5 mg/l	F	-	ALK-82-132 DO-4.8-5.0 pH-6.4-8.3	Hale, J.G. (1977)
Nickel sulfate	Coho salmon juvenile	14-D LC50 11.2 mg/l	F	15	pH-7.6-8.2 HD-6.5-7.8	Becker, C.D. (1980)
Iron (FeCl ₃)	<u>Poecilia reticulata</u>	48h-LC50 73 mg/dm ³	S	-	-	Furmanska, M (1979)
Iron	Brook trout	22 months 12 mg/l	F	10-11	pH 7-7.3 ALK 74-113 HD 128-228	Sykora, J.É. et al (1975)
Chromium	Rainbow Trout	96h-LC50 100 mg/l	S	-	-	EPA E.R.S. Water Quality Criteria (1973)

POLLUTANT	SPECIES	RESULTS		Exposure Time*	Temp °C	Test** Conditions	References
Nickel	Rainbow trout	48h-LC50	25 mg/l	S			Field river study EPA (1973) EPA (1973) Water Quality Criteria
		96h-LC50	135.5 mg/l				
Nickel	Rainbow trout	20 day	1.6 mg/l	S			
		96h-LC50	0.3-96.2 mg/l	S			
		chronic	0.4-0.8 mg/l	S			
Chromium Hexavalent	minnow	96h-LC50	17.6 mg/l	S			
	Bluegill	96h-LC50	133 mg/l	S			
		Safe Conc.	0.3 mg/l	S			
Iron	Rainbow trout	96h-LC50	0.4 mg/l	S	-	low oxygen low pH	
Chromium (+6)	Variety of aquatic life	MATC	0.01 mg/l	S			Cherry K.F. (1982)
	Chinook salmon	sublethal threshold	0.002 mg/l	S			
Nickel	Aquatic bacteria	sublethal threshold	0.02 mg/l	S			
Nickel	Rainbow trout	96h-LC50	26-27 mg/l	S		pH 7.9 ALK 85 HAR 125	Anderson (unpublished)
Chromium (trivalent)	<u>Gasterosteus aculeatus</u>	Incip. lethal conc.	1.2 mg/l	S		soft water	Clarke, R. (1974)
	<u>Salmo gairdneri</u>	96h-LC50	76 mg/l	S		hard water	
Chromium (hexavalent)	<u>Pimephales promelas</u>	96h-LC50	17.6 mg/l	S		soft water	
	<u>Lepomis macrochirus</u>	96h-LC50	133 mg/l	S		hard water	
Iron	certain fish	24 hr	0.1 mg/l	S			
	Tinca	1 week	10,000 mg/l	S			

POLLUTANT	SPECIES	RESULTS	Exposure Time*	Temp °C	Test** Conditions	References
Nickel	<u>Garterosteus</u>	Inc. lethal level 0.8 mg/l	S		soft water	Clarke, R. (1974)
	<u>Fundulus heteroclitus</u>	96h-LC50 46.2 mg/l	S		soft water	

TABLE 11.1 Results Collected from the Literature for Lethal and Sublethal Toxicity of DSS, Cr, Ni and Fe

* F = flow through system
S = static tests

** ALK - alkalinity
HD - hardness
DO - dissolved oxygen
A - acidity

Toxicity Classes	Component	Lowest Recorded Toxic Concentration		SUGGESTED RECEIVING WATER CRITERIA				
				Fresh Water Aquatic Life		Marine Aquatic Life		
		Lethal	Sublethal	Application Factor	Acceptable Limit	Application Factor	Hazardous Above	Minimum Risk Below
Extremely Toxic	Chromium (Trivalent)	0.01 Daphni	0.33 Daphnia	-	0.05*	0.01*	0.1*	0.05*
Very Highly Toxic	Chromium (Hexavalent)	0.05 Daphnia	0.1 Daphnia	-	0.05*	0.01*	0.1*	0.05*
	Iron	0.1 fish	0.52 fish sprawn	-	-	-	0.3	0.05
Highly Toxic	Nickel	0.8 fish	0.03 Daphnia	0.02	-	0.02	0.1	0.002

* Total Chromium

(All concentrations in mg/l)

Source: Clarke, 1974.

TABLE 11.2 Classification of Chromium Iron and Nickel as Components of Inorganic Chemical Industrial Estimates Based on Lowest Recorded Lethal Concentrations and Their Suggested Receiving Water Criteria.

Pollutant	Species	96-hr LC50 in mg/l	MATC	Safe Concentration	Application Factor	Avoidance Threshold	Avoidance Reaction	Proposed Adjustments
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
D.S.S.	Rainbow Trout	5-7	0.07	0.06	0.01	0.08	10 mg/l 68%	MATC = 0.07 SC = 0.06 AF = 0.01
Chromium (Hexavalent)	Rainbow Trout	69	0.050	0.025	0.03	-	-	MATC = 0.05 SC = 0.025 AF = 0.03
Chromium (Trivalent)	Rainbow Trout	100	0.10	0.10	0.04	-	-	MATC = 0.2 SC = 0.1 AF = 0.04
Chromium (Total)	Rainbow Trout	17-118	0.050	0.050	0.03	0.2	0.5 mg/l 55%	MATC = 0.050 SC = 0.025 AF = 0.03
Nickel	Rainbow Trout	25-40	0.04	0.025	0.01	0.01	1 mg/l 95%	MATC = 0.02 SC = 0.01 AF = 0.005- 0.008
Iron	Rainbow Trout	1-2	1.0	0.5	0.25	0.001	0.1 mg/l 55%	MATC = 0.02 SC = 0.01 AF = 0.1
Actual Plating Effluent	Rainbow Trout	60%	Dilution ratio 1:1380	Dilution ratio 1:2760	-	1:3000 LC50	1:40 LC50 93%	Effluent limits Cr(tot) = 1.0mg/l Ni = 1.5 mg/l Fe = 2 mg/l
Monomer A	Rainbow Trout	140	3	1.4	0.01	4.0	20 mg/l 65%	MATC = 3 SC = 1.4 AF = 0.005
Monomer C	Rainbow Trout	900	10	4.0	0.01	0.661	2 mg/l 50%	MATC = 10 SC = 4.0 AF = 0.005

Polymer A x	Rainbow Trout	0.4	0.06	0.04	0.1	0.8	4 mg/l 85%	MATC = 0.06 SC = 0.04 AF = 0.05
Polymer C	Rainbow Trout	0.2	0.04	0.02	0.1	1.33	1.35 mg/l 95%	MATC = 0.04 SC = 0.02 AF = 0.05

TABLE 11.3 Safe Toxic Levels and Proposed Adjustment for the Pollutants
of This Study for Freshwater Aquatic Life

CHAPTER XII

"SUMMARY AND CONCLUSIONS"

CHAPTER XI

12.1 Summary

The overall study is divided in three parts.

Part A includes Chapters I, II, III and IV.

Chapter I contains general introductory concepts and defines the role of toxicity avoidance studies in water pollution control, followed by the objectives and the scope of this study and a thesis outline.

Chapter II gives an extensive review of previous investigations describing analytically, all the avoidance apparatus which have been used so far in chemical avoidance tests, and also the trends and needs for new improved designs.

Chapter III describes in detail, the experimental set-up of the new avoidance apparatus (Design II) which has been designed and implemented in this study, including the water treatment and the holding facilities, the temperature control, the channel, the data acquisition process and cost analysis.

Chapter IV groups and describes the parameters of the problem in three categories: the fixed parameters, the variable parameters which can be fixed after experimentation, and the variable parameters. The second part of Chapter IV deals with the description of the statistical analysis used in this study and mainly the procedures to obtain and analyze results.

Part B contains the main body of the experimental work (Chapters V to X).

Chapter V, called preliminary testing, evaluates the general disposition of fish in the channel, the influence of a water acclimation jet, and the effects of noise, light and temperature.

Chapter VI contains the experiments defining the optimal number of fish per experiment, the influence of video analysis (optimal number of readings), and the tests of variability, adaptability and continuity.

Chapter VII contains the experimental work of the chemical toxicant DSS (dodecyl sulfate de sodium), commonly used as a reference pollutant.

Chapter VIII deals with the experimental work to define the toxicity of two polymers and their constituent monomers, comparing avoidance information with static tests.

Chapters IX and X contain the experimentation of the industrial effluent (plating industry) and its predominant chemical-toxicant components (Ni, Cr, Fe). Four different injection systems have been used and avoidance curves and avoidance reaction representation have been produced for the actual effluent and each of its components. Synergism and antagonism phenomena have been evaluated and prediction for safe toxicant levels have been estimated.

In Part C (Chapters XI to XII), the role of the avoidance reaction in relation to the lethal levels and the safe toxicant levels has been defined followed by the implementation of the results of this study (Chapter XI) and the prediction of safe level concentrations for all the pollutants used therein.

Chapter XII contains the summary and the conclusions of the overall study.

12.2 Conclusions

A. The new avoidance apparatus

1. A new avoidance apparatus (Design H) has been designed and implemented, using the advantages, and eliminating the disadvantages of previous avoidance systems while fulfilling the recent needs for toxicity avoidance studies, in the effort to link the gap between existing toxicity standards and actual damage under realistic environmental conditions.
2. The innovative characteristics of the Design H are:
the capacity for vertical, horizontal and lateral analysis of the position of test organisms; the use of a large tank which eliminates end effects and permits the use of a large number of test organisms; the inclusion of engineering parameters; the ability to test any kind of pollutant, to store results for later re-examination, to use advanced video and computer techniques for data acquisition and to create a test environment which closely simulates natural conditions.

B. Avoidance Experiments

1. Avoidance experiments belong to the most sensitive and unbiased sublethal behavioral effects of pollutants to the aquatic life. The relatively large number of parameters that influence the avoidance response, leads to the adjustment of some of them after experimentation and to the fixation of the values of others with carefully selected assumptions from the literature. Finally the variable parameters focus on the particular research to be studied.

2. Acclimation period, temperature, feeding procedure of the test organisms and water quality have to be fully controlled in every experimental system used for avoidance studies.

C. Preliminary Tests

1. From the preliminary testing of the avoidance apparatus Design H it was found that: noise has a minimal effect on the disposition of fish within the channel; a permanent set-up of fluorescent lights and a constant photo period are necessary; temperature has a minimal effect on the experimental results when the horizontal and vertical distribution are close to the even distributions; the general disposition of the fish and the effect of the jet needed to be evaluated before the introduction of the different pollutants into the system.

D. DSS

1. The toxicity avoidance of D.S.S. (dodecyl sulfate de sodium) starts at 1:100 LC50 (sublethal threshold) but reaches only 65% as the concentration enters the lethal range. This means that the sensitivity of fish to the presence of lethal concentrations is relatively low. Alternatively, the fish detect these lethal concentrations, but do not avoid them. Another possibility is that the fish sensors are numbed by lethal concentrations (or even exposures to sublethal concentrations). Consequently other sublethal tests must be used to evaluate sublethal thresholds and safe level concentrations. However the avoidance curve of DSS does predict hazardous effects to fish at concentrations in which the avoidance reaction is observed in only two-thirds of test fish, precisely because the fish do not avoid pollutant concentrations which are lethal to them.

E. Monomers - Polymers

1. The toxicity of polymers are significantly higher than the toxicity caused by constituent monomers.
2. In water treatment processes it is much more desirable from a toxicity point of view to have monomers than polymers.
3. The avoidance fish tests with polymers and monomers conducted over a relatively short period were, at least as sensitive as the acute static tests. They yield information such as preference for substances actually known to be toxic from results of the static tests.
4. From the magnitude of the concentration of polyelectrolytes showing significant toxicity, it appeared that discharges from waste treatment processes utilizing these polyelectrolytes could be hazardous to the biological community of the receiving water body.

F. Industrial Effluent (Plating Industry) and its Components (Ni,Cr,Fe)

1. The spectrum of plating industry effluent concentrations injected ranged between 1:3000 and 1:40 LC50 (well below the lethal threshold) and had an avoidance reaction between 50 and 94%. This illustrates a significant potential hazard to aquatic organisms at sublethal concentrations which would be impossible to predict or estimate with the conventional static LC50.
2. The sublethal threshold concentration for the avoidance reaction to the plating industry effluent was 1:3000 LC50. The avoidance reaction reaches 90% in dilution ratios between 1:15 and 1:50 LC50.

3. The difference in avoidance reaction between Injection Systems A and B was not significant, but the injection system influences the horizontal and the vertical distribution of the organism's position.
4. The horizontal and the vertical distribution of the organism's position tested, together with the avoidance curve, give a global three dimensional understanding of the avoidance behavior of the aquatic life to different toxicants.
5. Nickel is the dominant component of the industrial effluent used and the main contributor to the avoidance reaction. Chromium plays a secondary role and the presence of Iron is insignificant.
6. The comparison of the avoidance reaction of the industrial effluent: a) to Ni b) to the additive avoidance of Ni and Cr and c) to the additive avoidance to Ni, Cr and Fe, led to the estimation of the synergism and antagonism zones for the three heavy metals with the variation of the actual effluent's injected concentration. In very low concentrations responses were exactly additive, from 1:700 to 1:150 LC50 ranged from more additive to less than additive and in higher concentrations were antagonistic.
7. The avoidance reaction representation is proposed as a new manner of depicting experimental avoidance information and of permitting clearer understanding of the stability of the reactions with time.
8. Combining information from literature and findings from this study, adjustments of the safe level concentrations for the pollutant studied were proposed including values for maximum allowable toxicant concentration, safe concentrations and application factors.

12.3 General Conclusions

The new apparatus and procedure described herein is a viable tool for analyzing the behavioral responses of fish to potentially toxic effluent. The method is quick, sensitive and relatively easy to use if sufficient fish stock is available. Being a sublethal behavioral indicator it is probably more indicative of the in situ environmental impact than the existing legal standard. Secondly, the procedure could be used as a complementary test to the current LC50 for establishing realistic sublethal threshold effluent concentrations and safe level concentrations.

The avoidance to the industrial effluent was noticeable at concentrations as low as 1:3000 LC50 indicating that the sublethal threshold is achieved long before there is any chance of a lethal effect being observed.

Behavior as an expression of the sum of all biological responses may also exhibit a form of hormesis.

Avoidance behavioral changes were noticeable in a relatively short period of time, once the experiment was begun. Hence, because rapidity of results is an important feature of a surveillance program the procedure offers significant potential for relevant application in this field.

Further work is required to refine and extend the procedure, but the applicability for environmental impact analysis has been clearly established in this present study.

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APPENDICES

APPENDIX A

"INFORMATION ABOUT THE EXPERIMENTS"

- A1. Counting sheets
- A2. Information about the experiments
- A3. Grouping of the experiments
- A4. Routine water quality analysis
- A5. Chemical analysis of the effluent
- A6. Tests with dyes
- A7. Bias factors
- A8. Cost analysis of the avoidance apparatus - Design H

A 1 COUNTING SHEETS

EXPERIMENTAL DATA

DATE: _____

Starting Time: _____

No. of fish in the channel: _____

Duration of recording: _____

Duration of experiment: _____

Section 1, 2, 3, 4

Tape Number _____

Counter indication at the end _____

1. TEMPERATURE

PROBE TIME										

2. OTHER PARAMETERS

Time										
Noise										
Light										
Flow										
Diffuser Sysetem	A	B	C	D	E	F	G	H	I	None

3. EFFLUENT

Time	
Flow	
pH	
Side	
Colour	

4. POLLUTANT CONCENTRATIONS

Time	
Injected	
Section 1	
Section 2	
Section 3	
Section 4	

TAPE --- SECTION 2, 3 and 4

TIME SQUARES	77	154	231	308	385	462	539	616	693	770	847	924	1001	1078	1155	1232	1309	1386	1463	1540	1617	1694	1771	1848	Medium	Mean	St.D.	Remarks
1-6																												
7-12																												
13-18																												
TOTAL																												
E																												
F																												
G																												
TOTAL																												
SIDE A																												
SIDE B																												

A 2 INFORMATION ABOUT THE EXPERIMENTS

EXPERIMENTAL DATA ANALYSIS

Number of Experiment	GROUP	SUBGROUP		
Complete	Ready to go into the computer	Appear in the Results		
Readings	From-To	Actual Percentage	Percentage transfer into curves	
Bias Factors	A	B	C	Total
Horizontal Distribution	Vertical Distribution		Lateral Distribution	
Details - Special remarks				

INFORMATION ABOUT THE EXPERIMENTS

Number of Experiments	Complete	Incomplete	Ready for Computer Analysis	Possible Computer Analysis	Impossible Computer Analysis
1		X			X
2		X		X	
3		X		X	
4		X		X	
5		X		X	
6		X		X	
7		X			X
8		X			X
9		X		X	
10		X		X	
11		X*		X	
12		"		X	
13		"		X	
14		"		X	
15		"		X	
16		"		X	
17		"		X	
18		"		X	
19		"		X	
20		"		X	
21		"		X	
22		X		X	
23		X		X	
24		X		X	
25		X		X	
26		X		X	
27		X		X	
28		X		X	
29		X		X	
30		X		X	

X* - 3rd and 4th sections are missing

INFORMATION ABOUT THE EXPERIMENTS

Number of Experiments	Complete	Incomplete	Ready for Computer Analysis	Possible Computer Analysis	Impossible Computer Analysis
31		X		X	
32		X			X
33		X		X	
34	X		X		
35	X		X		
36	X		X		
37	X		X		
38	X		X		
39	X			X	
40	X			X	
41	X			X	
42	X			X	
43	X			X	
44	X			X	
45	X		X		
46	X		X		
47	X		X		
48	X		X		
49	X		X		
50	X				X
51	X				X
52		X			X
53		X		X	
54		X		X	
55		X		X	
56		X		X	
57		X		X	
58		X		X	
59		X		X	
60		X		X	
61		X		X	
62		X		X	

INFORMATION ABOUT THE EXPERIMENTS

Number of Experiments	Complete	Incomplete	Ready for Computer Analysis	Possible Computer Analysis	Impossible Computer Analysis
63		X		X	
64		X		X	
65		X		X	
66		X		X	
67	X		X		
68	X		X		
69	X		X		
70	X		X		
71	X		X		
72	X		X		
73	X		X		
74	X		X		
75	X		X		
76	X		X		
77		X		X	
78	X		X		
79	X		X		
80	X		X		
81	X		X		
82		X		X	
83		X		X	
84		X			X
85	X		X		
86		X		X	
87		X		X	
88	X				X
89	X		X		
90		X		X	
91		X		X	
92		X		X	
93	X		X		

INFORMATION ABOUT THE EXPERIMENTS

Number of Experiments	Complete	Incomplete	Ready for Computer Analysis	Possible Computer Analysis	Impossible Computer Analysis
94	X		X		
95	X		X		
96	X		X		
97		X		X	
98	X		X		
99	X		X		
100	X		X		
101		X		X	
102	X*		X		
103	X*		X		
104	X*		X		
105		X		X	
106	X		X		
107	X		X		
108	X		X		
109		X		X	
110	X		X		
111		X		X	
112	X		X		
113	X		X		
114	X		X		
115	X		X		
116	X		X		
117	X		X		
118	X		X		

A.3 GROUPING OF THE EXPERIMENTS

INFORMATION ABOUT THE EXPERIMENTS IN GROUPS

GROUP	SUBGROUP	NUMBER OF EXPERIMENTS			
		For Computer Analysis	For Possible Computer Analysis	Without Computer Analysis	TOTAL
A Preliminary	1 General Distribution	74	2, 22, 24, 25, 26	1	1, 2, 22, 24, 25, 26, 74
A Preliminary	2 Jet Influence		9, 10, 27, 28, 23		27, 28, 9, 10, 23
A Preliminary	3 Temperature		3, 4, 5, 6		3, 4, 5, 6
A Preliminary	4 Noise		3, 4, 5, 6, 7, 8, 9		3, 4, 5, 6, 7, 8, 9
A Preliminary	5 Light		3	7, 8	7, 8, 3
A Preliminary	6 DSS		11-21, 29, 30, 31, 33, 33	32	29, 30, 31, 32, 33 & 11-21, 3
B Specific Tests	1 Continuity		40, 41, 42, 43		40, 41, 42, 43
B Specific Tests	2 Adaptability	36, 37, 38	39		36, 37, 38, 39
B Specific Tests	3 Variability	34, 35			34, 35
B Specific Tests	4 Video Analysis		44 (K)		44 (K)
C Polymers	2 Polymer A		57, 58, 59, 60, 61, 61		57, 58, 59, 60, 61, 61
C Polymers	3 Polymer B		63, 64, 65, 66	62	62, 63, 64, 65, 66
C Polymers	4 Monomer C	67, 68, 69, 70, 71, 72			67, 68, 69, 70, 71, 72
B Specific Tests	6 Optimal No. of fish	45, 46, 47, 48, 49			45, 46, 47, 48, 49
C Polymers	1 Polymer Preliminary		53, 54, 55, 56		53, 54, 55, 56

A.3 GROUPING OF THE EXPERIMENTS

INFORMATION ABOUT THE EXPERIMENTS IN GROUPS

GROUP	SUBGROUP	NUMBER OF EXPERIMENTS			
		For Computer Analysis	For Possible Computer Analysis	Without Computer Analysis	TOTAL
D Industrial Effluent	1 Injection System A	85,89	86,87,90,91	84,88	84,85,86,87,88,89,90,91
D Industrial Effluent	2 Injection System B	75,76,78,79,80,81	77,82,83		75,76,77,78,79,80,81,82,83
D Industrial Effluent	3 Injection System C			89',90'	89', 90'
D Industrial Effluent	4 Injection System D			91',92'	91', 92'
E Components of Industrial Effluent	1 Nickel	99,100,102,103,104	101-	105	99,100,101,102,103,104,105
E Components of Industrial Effluent	2 Chromium	93,94,95,96,98	92,97		92,93,94,95,96,97,98
E Components of Industrial Effluent	3 Iron	106,107,108,110,112	109,111		106,107,108,109,110,111,112
C Polymer	Monomer A	113,114,115,116,117,118			113-118

A4 Routine Water Quality AnalysisA. Water Quality Control of the Water
entering the System (before treatment)

	<u>PPM</u>
Sodium	14.2
Calcium	23.5
Magnesium	8.0
Aluminium	<0.1
Iron	<0.1
Chromium	<0.1
Copper	<0.1
Nickel	<0.1
Lead	<0.1
Cadmium	<0.1
Zinc	<0.1
Potassium	-
Manganese	<0.1
TOC	8.0
TIC	17.0
Phenols	-
Total coliforms	0
Fecal coliforms	0

B. Water Quality Control before and after
the ultra violet lights

	PPM	
	Before Ultra Violet Lights	After Ultra Violet Lights
Phosphorus inorganic	<0.05	<0.05
Phosphorus total	0.02	0.01
Nitrite/Nitrate	0.39	0.39
N. Total KHELDAHL	<2.5	<2.5
N. Ammonium	0.6	0.2
Cyanide	-	-
Fluoride	112 CaCO ₃	95 CaCO ₃
Chromium	<0.1	<0.1
Copper	<0.1	<0.1
Nickel	<0.2	<0.1
Lead	<0.1	<0.1
Cadmium	<0.1	<0.1
Zinc	<0.1	<0.1

A5 Chemical Analysis of the Industrial Effluent
(Plating Industry Carry-All, Montreal, Canada)

	PPM
Sodium	100.0
Calcium	40.0
Magnesium	8.0
Aluminium	<0.1
Iron	4.5
Chromium	20
Copper	0.181
Nickel	50
Lead	<0.1
Cadmium	<0.1
Zinc	0.367
Potassium	1.01
Manganese	0.075
Total Solids	540.0
Suspended Solids	<5.0
Inorganic phosphorus	0.50
Total phosphorus	0.50
N. Total Kjeldahl	<2.5
N. Ammonium	<0.1

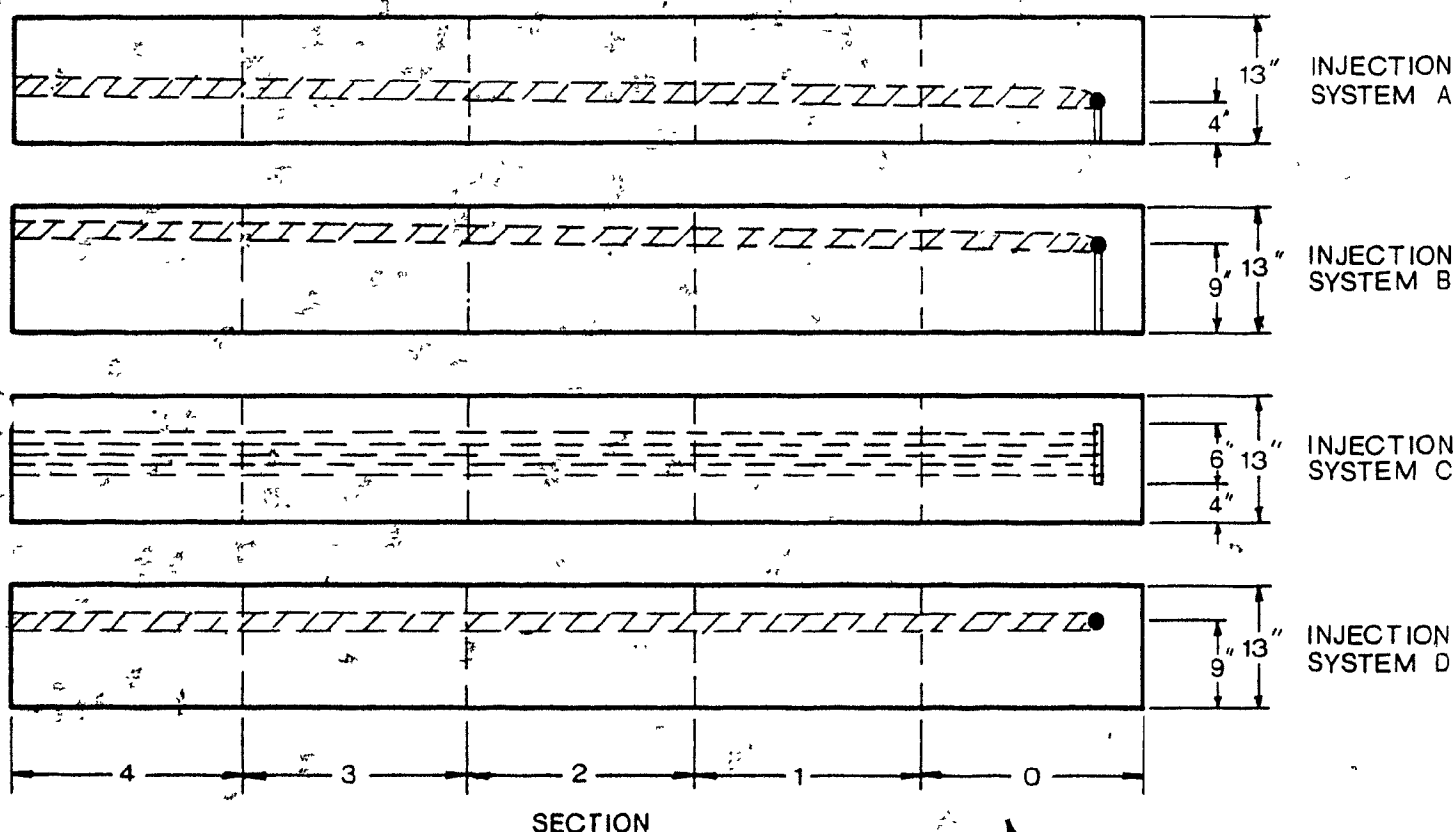
Plating industry information

1. Name	:	Carry- All
2. Location	:	171 St.Cyr, Montreal East
3. Size of the plant	:	90,000 sq.ft.
4. Type of plating	:	Electroplating nickel and chromium
5. Daily effluent	:	20-30 gpm.
6. Existing effluent treatment	:	pH adjustment

A 6. TESTS WITH DYES

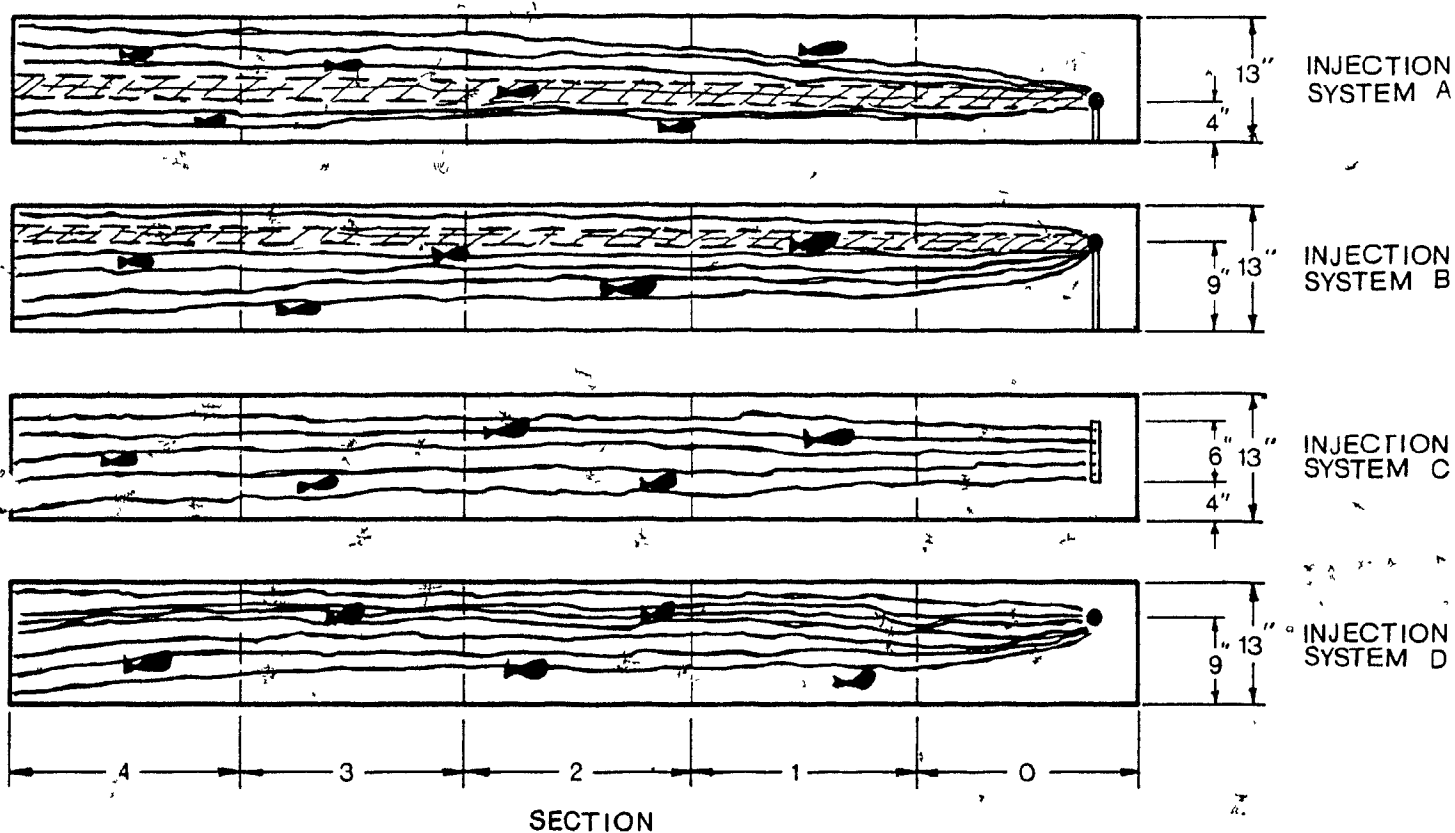
Preliminary tests with red fluorescent dyes have been done to define the pattern of the discharged plumes, using different injection systems with and without the presence of fish into the channel.

(a) without fish: The flow was laminar. The different injection systems produce patterns which are illustrated in Sketch A6.1.



SKETCH A6.1 SIDE VIEW OF THE CHANNEL TESTS WITH DYE WITHOUT FISH

(b) with fish: The flow was laminar. Due to the presence of fish, almost complete mixing was observed after the first section of the channel, as illustrated in Sketch A6.2.



SKETCH A6.2 SIDE VIEW OF THE CHANNEL
TEST WITH DYES WITH THE
PRESENCE OF FISH

A 7 BIAS FACTORS

A) Jet effect

The influence of the water jet has been evaluated or mentioned in Section 5.2, with five tests (Nos. 9,10,23,27,28) and with a small clear water flow from the one side of the channel.

It was concluded that there was a slight preference (max 5%) of the fish to align themselves with the jet. That slight preference has been incorporated in all subsequent results in which jets are used, according to the following rationale.

With the increase of pollutant concentration the amount of fish in the polluted side decreases and the effect of the jet becomes smaller. The adjusted percentages that represent more accurately the bias factor of the jet were estimated as follows:

- +5% up to 80% avoidance
- +4% from 80-85% avoidance
- +3% from 85-90% avoidance
- +2% from 90-100% avoidance

B) Algae cages

As part of an unrelated study five experiments (Nos.87,75,76, 78,83) were conducted with algae cages present in the channel.

The estimation of the effect of algae cages has been done considering the results of two tests (Nos. 78,79) with the same pollutant concentration in which only one (No. 78) had algae cages present during the experiment. The difference in avoidance reaction was 5%, and a conclusion was made to consider the presence of the cages as a bias factor with a max 5% contribution to the avoidance reaction on the side of the cages.

A 8 COST ANALYSIS OF THE AVOIDANCE APPARATUS - DESIGN H

(a) Installation cost (1981)

1. Activated carbon column (1)	= 2,000 \$
2. Ultra violet lights (2)	= 2,500 \$
3. 300 gal. polyethylene tanks (4)	= 1,000 \$
4. 500 gal. polyethylene tank (1)	= 300 \$
5. Plexiglass channel (30 ft. long)	= 5,000 \$
6. Mirrors (4)	= 500 \$
7. Front and end gate	= 200 \$
8. Cameras (Panasonic WV3320, WV3200) (4)	= 3,500 \$
9. T.V. Monitors (Panasonic CT1310MC, CT1309C) and recorders (Panasonic NV-8200, NV-8410) (4+4)	= 6,500 \$
10. Tele-thermometer (YSI Model 44) and probes (10)	= 500 \$
11. Heating installations (KTL-318-1 WMMR, HCP-60 Chromalox Canada)	= 5,000 \$
12. Coolers	= 2,000 \$
13. Piping	= 500 \$
14. Flowmeters (1358-V Brooks Instrument) (5) and air meters (2700V Brooks Instrument) (3)	= 500 \$
15. Labor	= 6,000 \$
16. Miscellaneous fittings	= 1,000 \$
TOTAL	<u>37,000 \$</u>

(b) Maintenance and operation

1. Video-tapes	=	25\$ each
2. Fish	=	300\$ per thousand
3. Fish food	=	30\$ per month
4. Computer costs	=	500\$ per month
5. Labor	=	1,000\$ per month
6. Miscellaneous	=	200\$ per month

(c) Cost per set of experiments

7-12 individual tests will yield the avoidance curve for one toxicant. Hence, considering 7-12 tests (duration 40 days) including the production of the report, the costs per set are as follows:

1. Installation amortization (considering 2 years pay-off period)	=	2,500\$
2. Fish	=	300\$
3. Tapes and materials	=	200\$
4. Computer cost	=	1,000\$
5. Labor	=	4,000\$
6. Supervision fees	=	1,000\$
7. Report	=	500\$
8. Miscellaneous	=	500\$

Total per set (40 days)	<u>10,000\$</u>

or, in general, \$7,000 per month

This cost, which appears high, can undoubtedly be reduced. With increased use, as well as automation, it is expected that the use of this refined and sensitive technique will be justified.

APPENDIX B
"COMPUTER PROGRAMS"

- B1. Computer Program A - Preliminary calculations
- B2. Computer Program B - Time Series Analysis
- B3. Computer Program C - Analysis of Variance (ANOVA)

NOTE: THE JJB HADJI HAS BEEN RUN UNDER RELEASE 79.5 OF SAS AT MCGILL UNIVERSITY COMPUTING CENTRE (01 240).

NOTE: SAS OPTIONS SPECIFIED ARE:

SORT=4

1 DATA A; INPUT SECTION PART GROUP Q1-Q24;
2 CARDS;

NOTE: DATA SET WORK.A HAS 28 OBSERVATIONS AND 27 VARIABLES. 86 OBS/TRK.
NOTE: THE DATA STATEMENT USED 0.09 SECONDS AND 196K.

31 PROC SORT; BY SECTION PART DESCENDING GROUP;

NOTE: 4 CYLINDERS DYNAMICALLY ALLOCATED PER SORT WORK DATA SET.
NOTE: DATA SET WORK.A HAS 28 OBSERVATIONS AND 27 VARIABLES. 86 OBS/TRK.
NOTE: THE PROCEDURE SORT USED 0.33 SECONDS AND 240K.

32 PROC MEANS NOPRINT; BY SECTION PART ;
33 VAR Q1-Q24;
34 OUTPUT OUT=MEANS
35 SUM=Q1-Q24;

NOTE: DATA SET WORK.MEANS HAS 10 OBSERVATIONS AND 26 VARIABLES. 89 OBS/TRK.
NOTE: THE PROCEDURE MEANS USED 0.14 SECONDS AND 196K.

36 DATA MEANS;
37 SET MEANS;
38 GROUP=4;

NOTE: DATA SET WORK.MEANS HAS 10 OBSERVATIONS AND 27 VARIABLES. 86 OBS/TRK.
NOTE: THE DATA STATEMENT USED 0.07 SECONDS AND 196K.

39 DATA; SET A MEANS;

NOTE: DATA SET WORK.DATA1 HAS 38 OBSERVATIONS AND 27 VARIABLES. 86 OBS/TRK.
NOTE: THE DATA STATEMENT USED 0.09 SECONDS AND 196K.

40 PROC SORT; BY SECTION PART DESCENDING GROUP;
41

NOTE: DATA SET WORK.DATA1 HAS 38 OBSERVATIONS AND 27 VARIABLES. 85 OBS/TRK.
NOTE: THE PROCEDURE SORT USED 0.22 SECONDS AND 240K.

41 DATA; SET;
42 RETAIN TOT_MEAN;
43 MEAN=MEAN(OF Q1-Q24);
44 STD=STD(OF Q1-Q24);
45 RANGE=RANGE(OF Q1-Q24);
46 IF GROUP =4 THEN DO;
47 TOT_MEAN=MEAN;
48 PERCENT=100; OUTPUT;
49 RETURN;
50 END;
51 PERCENT=MEAN/TOT_MEAN*100; OUTPUT;
52

B1 - PROGRAM A PRELIMINARY CALCULATIONS

NOTE: DATA SET WORK.DATA2 HAS 38 OBSERVATIONS AND 32 VARIABLES. 73 OBS/TRK.
NOTE: THE DATA STATEMENT USED 0.13 SECONDS AND 196K.

```
52 PROC FORMAT;
53 VALUE SECT
54 1=SECTION 1
55 2=SECTION 2
56 3=SECTION 3
57 4=SECTION 4
58 ;
59 VALUE PART
60 1= HORIZONTICALLY
61 2= VERTICALLY
62 3= LATERALLY
63 ;
64 VALUE GROUP
65 1=A
66 2=B
67 3=C
68 4=TOTAL
69 ;
```

NOTE: THE PROCEDURE FORMAT USED 0.12 SECONDS AND 200K.

```
70 PROC SORT; BY SECTION PART GROUP;
```

NOTE: DATA SET WORK.DATA2 HAS 38 OBSERVATIONS AND 32 VARIABLES. 73 OBS/TRK.
NOTE: THE PROCEDURE SORT USED 0.22 SECONDS AND 240K.

```
71 PROC PRINT;
72 BY SECTION PART;
73 ID PART GROUP;
74 VAR Q1-Q24 MEAN STD RANGE PERCENT ;
75 FORMAT SECTION SECT.
76 PART PART.
77 GROUP GROUP.
78 ;
```

NOTE: THE PROCEDURE PRINT USED 0.41 SECONDS AND 196K AND PRINTED PAGES 1 TO 3.

NOTE: SAS INSTITUTE INC.
SAS CIRCLE
BOX 8000
CARY, N.C. 27511

B1 - PROGRAM A PRELIMINARY CALCULATIONS (continued)

NOTE: THE JOB HADJI HAS BEEN RUN UNDER RELEASE 79.5 OF SAS AT MCGILL UNIVERSITY COMPUTING CENTRE (00340).

NOTE: SAS OPTIONS SPECIFIED ARE:
SORT=4

```
1 DATA TSA;  
2 INPUT PERCENT TIME;  
3 LABEL PERCENT=% OF FISH IN CLEAR WATER  
4 TIME=TIME IN MINUTES;  
5 CARDS;
```

NOTE: DATA SET WORK.TSA HAS 24 OBSERVATIONS AND 2 VARIABLES. 953 OBS/TRK.
NOTE: THE DATA STATEMENT USED 0.06 SECONDS AND 188K.

```
30 PROC PLOT DATA=TSA;  
31 PLOT PERCENT*TIME=**/VAX IS=5 TO 100 BY 5 HAXIS=5 TO 120 BY 5  
32 VREF=50.0 54.68 56.1 VREFCHAR='-' ;  
33 TITLE TIME SERIES ANALYSIS;  
34 TITLE2 % OF FISH IN CLEAR WATER VS TIME IN MINUTES;
```

NOTE: THE PROCEDURE PLOT USED 0.19 SECONDS AND 196K AND PRINTED PAGE 1.

```
35 PROC MEANS;  
36 VAR PERCENT;  
37 OUTPUT OUT=MEAN1;  
38 TITLE MEAN VALUES 1;
```

NOTE: DATA SET WORK.MEAN1 HAS 1 OBSERVATIONS AND 0 VARIABLES. 4766 OBS/TRK.
NOTE: THE PROCEDURE MEANS USED 0.17 SECONDS AND 188K AND PRINTED PAGE 2.

```
39 DATA ;SET TSA(FIRSTOBS=5);
```

NOTE: DATA SET WORK.DATA1 HAS 20 OBSERVATIONS AND 2 VARIABLES. 953 OBS/TRK.
NOTE: THE DATA STATEMENT USED 0.05 SECONDS AND 188K.

```
40 PROC MEANS;  
41 VAR PERCENT;  
42 OUTPUT OUT=MEAN2;  
43 TITLE MEAN VALUES 2;
```

NOTE: DATA SET WORK.MEAN2 HAS 1 OBSERVATIONS AND 0 VARIABLES. 4766 OBS/TRK.
NOTE: THE PROCEDURE MEANS USED 0.17 SECONDS AND 188K AND PRINTED PAGE 3.

NOTE: SAS USED 196K MEMORY.

NOTE: SAS INSTITUTE INC.
SAS CIRCLE
BOX 8000
CARY, N.C. 27511

B2 - PROGRAM B TIME SERIES ANALYSIS

21:04 MONDAY, AUGUST 30, 1982

STATISTICAL ANALYSIS SYSTEM

NOTE: THE FOLLOWING INFORMATION IS THE RELEASED BY SAS AT MCILL UNIVERSITY COMPUTING CENTRE (003400).

NOTE: THE FOLLOWING INFORMATION IS THE RELEASED BY SAS AT MCILL UNIVERSITY COMPUTING CENTRE (003400).

1. DATA SET: POLYMER
2. DATA SET: POLYMER
3. DATA SET: POLYMER
4. DATA SET: POLYMER
5. DATA SET: POLYMER
6. DATA SET: POLYMER
7. DATA SET: POLYMER
8. DATA SET: POLYMER
9. DATA SET: POLYMER
10. DATA SET: POLYMER

NOTE: SAS SET TO A VALUE AND INPUT STATEMENT

NOTE: DATA SET POLYMER HAS 40 OBSERVATIONS AND 3 VARIABLES: 680 OBS/TRK.

NOTE: THE DATA SET USED 3.00 SECONDS AND 496KB

41. DATA SET: POLYMER
42. DATA SET: POLYMER
43. DATA SET: POLYMER
44. DATA SET: POLYMER
45. DATA SET: POLYMER
46. DATA SET: POLYMER
47. DATA SET: POLYMER
48. DATA SET: POLYMER
49. DATA SET: POLYMER
50. DATA SET: POLYMER

NOTE: THE DATA SET USED 3.00 SECONDS AND 496KB

NOTE: THE DATA SET USED 3.00 SECONDS AND 496KB

NOTE: THE DATA SET USED 3.00 SECONDS AND 496KB

NOTE: THE DATA SET USED 3.00 SECONDS AND 496KB

NOTE: THE DATA SET USED 3.00 SECONDS AND 496KB

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NOTE: THE DATA SET USED 3.00 SECONDS AND 496KB

NOTE: THE DATA SET USED 3.00 SECONDS AND 496KB

NOTE: THE DATA SET USED 3.00 SECONDS AND 496KB

B3 - PROGRAM C ANALYSIS OF VARIANCE

APPENDIX C

"COMPUTER OUTPUTS"

- C1. Preliminary calculations
- C2. Time Series Analysis
- C3. ANOVA Tables and Duncan's Tests

SECTION=SECTION 1 PART=HORIZONTALLY

P A R T	P U R P O S E	P U R P O S E																				M E A N	S T D	R A N G E	P E R C E N T				
		0 1	0 2	0 3	0 4	0 5	0 6	0 7	0 8	0 9	1 0	1 1	1 2	1 3	1 4	1 5	1 6	1 7	1 8	1 9	2 0					2 1	2 2	2 3	2 4
HORIZONTICALLY A		5	4	2	5	13	4	6	10	8	4	5	9	7	9	20	12	15	11	20	16	17	13	13	17	10.2083	5.38903	18	40.033
HORIZONTICALLY B		15	4	6	9	6	9	6	5	5	5	6	5	16	9	11	7	13	7	15	9	12	7	7	12	8.5417	3.58717	12	33.497
HORIZONTICALLY C		7	3	5	5	2	5	5	8	6	2	9	5	8	8	4	8	8	10	11	7	11	10	7	6.7500	2.59179	9	26.471	
HORIZONTICALLY TOTAL		27	11	13	19	21	14	17	23	19	14	20	17	31	26	35	27	36	26	45	36	36	31	30	36	25.5000	9.05058	34	100.000

SECTION=SECTION 1 PART=VERTICALLY

PART	GROUP	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	MEAN	STD	RANGE	PERCENT
VERTICALLY A		20	10	12	7	16	10	12	18	17	10	12	10	18	18	19	15	17	12	20	17	19	16	16	20	15.0417	3.89514	13	58.226
VERTICALLY B		5	1	1	12	3	3	5	2	2	3	5	4	8	4	8	5	10	7	15	12	14	10	7	8	6.4167	4.06380	14	24.835
VERTICALLY C		2	0	0	1	3	1	1	1	6	1	2	6	6	4	5	8	9	6	7	6	5	6	9	10	4.3750	3.05013	10	16.935
VERTICALLY TOTAL		27	11	13	20	22	14	18	21	25	14	19	20	32	26	32	26	36	25	42	35	36	32	32	36	25.8333	8.80547	31	100.000

SECTION=SECTION 1 PART=LATERALLY

PART	GROUP	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	MEAN	STD	RANGE	PERCENT
LATERALLY B		12	3	2	8	4	1	2	9	3	3	4	3	5	7	3	2	5	3	9	5	7	6	6	8	4.8333	2.64845	11	20
LATERALLY C		14	8	9	11	13	12	10	13	15	12	15	15	30	21	28	24	30	20	31	29	25	24	24	25	19.3333	7.38192	23	80
LATERALLY TOTAL		26	11	11	19	17	13	12	18	18	15	19	18	35	28	31	26	35	23	40	34	32	30	30	33	24.1667	8.52447	25	100

12:36 THURSDAY, JANUARY 14, 1982

12:36 THURSDAY, JANUARY 14, 1982

12:36 THURSDAY, JANUARY 14, 1982

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12:36 THURSDAY, JANUARY 14, 1982³

P A R T	G R O U P	Q																		M E A N	S T D	R A N G E	P E R C E N T						
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18					19	20	21	22	23	24
HORIZONTAL	A	4	5	14	9	5	5	6	5	6	7	7	6	4	5	2	5	7	6	1	3	5	5	4	3	5.3750	2.51625	13	42.715
HORIZONTAL	B	4	8	10	10	7	7	12	4	3	4	4	1	1	1	3	4	4	0	0	0	4	3	2	3	4.1250	3.31417	12	32.781
HORIZONTAL	C	2	6	8	9	8	7	4	2	4	3	2	3	1	0	1	3	0	3	0	0	1	2	4	1	3.6333	2.71736	9	24.503
HORIZONTAL	TOTAL	10	19	32	28	20	19	22	11	13	14	13	10	6	6	12	11	9	1	3	10	10	10	7	12.5833	7.46538	31	100.000	

P A R T	G R O U P	C O U N T																M E A N	S T D	R A N G E	P E R C E N T									
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15					16	17	18	19	20	21	22	23	24
VERTICALLY A		10	19	30	25	18	18	17	10	12	13	12	7	5	4	0	0	0	0	0	0	0	0	0	0	0	10.91167	7.09511	29	85.902
VERTICALLY B		0	1	4	3	2	2	3	1	0	1	1	1	2	2	4	3	2	1	0	0	0	0	0	0	0	1.4167	1.13890	4	11.146
VERTICALLY C		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.3750	0.64690	2	2.951	
VERTICALLY TOTAL		10	20	34	28	20	20	21	11	12	14	13	10	6	6	6	6	12	11	10	1	3	10	10	10	10	12.7083	7.79928	53	100.000

PART	GRCLF	PERCENT																								MEAN	STD	RANGE	PERCENT
		0 1	0 2	0 3	0 4	0 5	0 6	0 7	0 8	0 9	0 10	0 11	0 12	0 13	0 14	0 15	0 16	0 17	0 18	0 19	0 20	0 21	0 22	0 23	0 24				
LATERALLY	B	5	13	15	16	11	9	12	4	5	5	9	5	4	4	2	5	5	6	1	2	3	5	5	4	6.7917	4.10704	15	53.795
LATERALLY	C	5	8	20	13	10	11	5	7	5	5	4	3	2	2	3	6	2	0	1	6	5	5	4	5.8333	4.41013	20	46.405	
LATERALLY	TOTAL	10	21	35	29	21	20	21	11	13	14	13	8	6	6	6	11	11	8	1	3	9	10	9	7	12.6250	8.12303	34	100.000

STATISTICAL ANALYSIS SYSTEM

12:36 THURSDAY, JANUARY 14, 1982

SECTION=SECTION 4 PART=HORIZONTALLY

PART	GROUP	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	MEAN	STD	RANGE	PERCENT
HORIZONTALLY	A	8	6	4	7	5	4	2	2	3	2	5	1	0	1	1	1	1	4	1	0	0	0	2	1	1	2.70833	2.62692	5	52.415
HORIZONTALLY	B	4	4	2	2	9	7	1	1	3	0	0	1	0	0	0	0	0	1	0	0	0	0	1	0	1	1.54167	2.35669	9	25.835
HORIZONTALLY	C	1	0	2	1	4	5	1	1	0	0	1	2	1	0	1	0	0	0	0	0	0	0	0	0	0	0.91667	1.28125	2	17.742
HORIZONTALLY	TOTAL	13	10	8	10	22	16	4	4	6	2	6	4	1	1	2	2	5	1	0	0	0	0	3	2	2	5.16667	5.55242	22	100.000

SECTION=SECTION 4 PART=VERTICALLY

PART	GROUP	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	MEAN	STD	RANGE	PERCENT
VERTICALLY	A	13	10	8	9	20	15	4	4	5	2	5	3	1	1	2	2	5	1	0	0	0	0	3	2	2	4.87500	5.17762	20	93.6
VERTICALLY	B	1	1	0	0	2	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0.29167	0.55013	2	5.6
VERTICALLY	C	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0.04167	0.20412	1	0.8
VERTICALLY	TOTAL	14	11	8	9	22	16	4	4	6	2	6	4	1	1	2	2	5	1	0	0	0	0	3	2	2	5.20833	5.62567	22	100.0

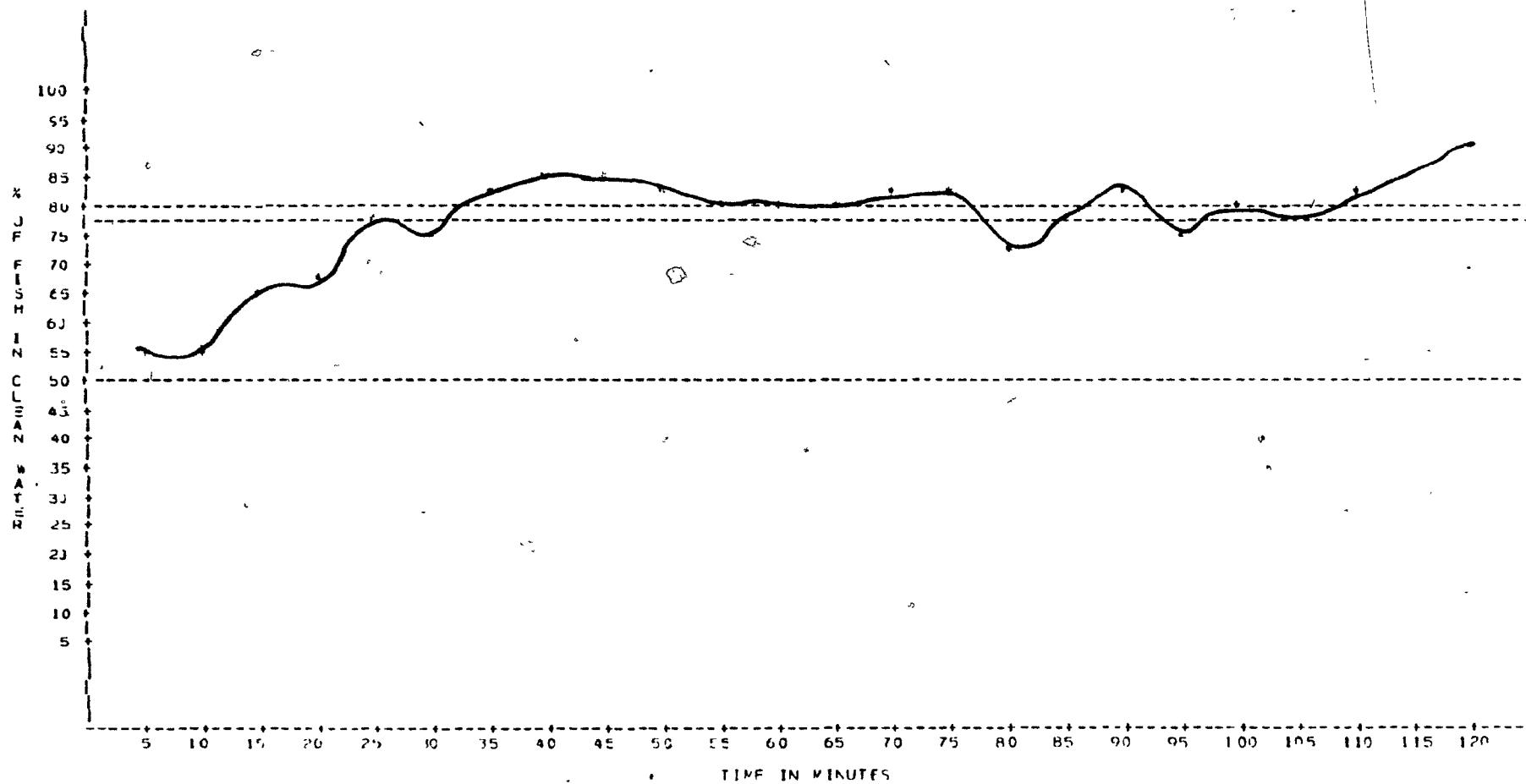
SECTION=SECTION 4 PART=LATERALLY

PART	GROUP	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	MEAN	STD	RANGE	PERCENT
LATERALLY	B	5	7	5	5	10	9	2	0	9	2	0	3	2	3	1	0	0	2	0	2	1	0	0	0	0	2.83333	3.18511	10	51.515
LATERALLY	C	5	4	3	6	11	5	2	4	2	0	3	3	1	1	0	2	2	0	0	0	0	0	1	1	1	2.66667	3.14364	11	48.485
LATERALLY	TOTAL	14	11	8	11	21	14	4	4	11	2	3	6	3	4	1	2	2	2	0	2	1	0	1	1	1	5.50000	5.25322	21	100.000

C2 - TIME SERIES ANALYSIS - EXP. 103

TIME SERIES ANALYSIS,
% OF FISH IN CLEAR WATER VS TIME IN MINUTES
PLOT OF PERCENT-TIME SYMBOL USED IS *

12:24 MONDAY, FEBRUARY 8, 1982



		MEAN VALUES 1					12:24 MONDAY, FEBRUARY 8, 1982		
VARIABLE	LABEL	N	MEAN	STANDARD DEVIATION	MINIMUM VALUE	MAXIMUM VALUE	STD ERROR OF MEAN	SUM	VARIANCE
PERCENT	% OF FISH IN CLEAN WATER	24	77.57500000	8.95001822	55.10000000	90.80000000	1.82691482	1861.80000	80.10292

		MEAN VALUES 2					12:24 MONDAY, FEBRUARY 8, 1982		
VARIABLE	LABEL	N	MEAN	STANDARD DEVIATION	MINIMUM VALUE	MAXIMUM VALUE	STD ERROR OF MEAN	SUM	VARIANCE
PERCENT	% OF FISH IN CLEAN WATER	20	81.03000000	4.00487860	73.40000000	90.80000000	0.89551808	1620.60000	16.03905

C3 - ANOVA 3 - AVOIDANCE REACTION OF INJECTION SYSTEMS A AND B

ANALYSIS OF VARIANCE FOR THE INJECTION SYSTEMS A AND BOF THE INDUS

TRIAL EFFLUENT 1
16:55 THURSDAY, AUGUST 5, 1982

GENERAL LINEAR MODELS PROCEDURE

CLASS LEVEL INFORMATION

CLASS	LEVELS	VALUES
INSYS	2	A B
CONC	10	1:100 1:150 1:200 1:250 1:370 1:40 1:50 1:500 1:700 1:75

NUMBER OF OBSERVATIONS IN DATA SET = 20

ANALYSIS OF VARIANCE FOR THE INJECTION SYSTEMS A AND BOF THE INDUS

TRIAL EFFLUENT 2
16:55 THURSDAY, AUGUST 5, 1982

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: AVOID

FINAL AVOIDANCE VALUE

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.	
MODEL	10	1522.50500000	152.25050000	19.12	0.0001	0.955041	3.4636	
ERROR	9	71.67250000	7.96361111					
CORRECTED TOTAL	19	1594.17750000						
					STD DEV		AVOID MEAN	
					2.82198709		81.47500000	
SOURCE	DF	TYPE I SS	F VALUE	PR > F	CF	TYPE IV SS	F VALUE	PR > F
INSYS	1	21.01250000	2.64	0.1387	1	21.01250000	2.64	0.1387
CONC	9	1501.49250000	20.95	0.0001	9	1501.49250000	20.95	0.0001

C3 - ANOVA 4 - AVOIDANCE REACTION OF Ni, Cr, Fe AND THE ACTUAL EFFLUENT (INJ.SYS. A AND B)

ANALYSIS OF VARIANCE OF THE INDUSTRIAL EFFLUENT AND ITS COMP/TS

14:18 MONDAY, AUGUST 16, 1982

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: AVOID

AVOIDANCE

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.	
MODEL	10	7098.83828571	709.88382857	21.69	0.0001	0.900362	8.5267	
ERROR	24	785.59142857	32.73297619					
CORRECTED TOTAL	34	7884.42971429						
					5.72127400		67.09714286	
SOURCE	DF	TYPE I SS	F VALUE	PR > F	CF	TYPE IV SS	F VALUE	PR > F
POLLUT	4	5514.91257143 *	42.12	0.0001	4	5514.91257143	42.12	0.0001
CUNC	6	1583.92571429	8.06	0.0001	6	1583.92571429	8.06	0.0001

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DUNCAN MULTIPLE RANGE TESTS
CONCENTRATION AS LC50 VALUE=1:100

14:18 MONDAY, AUGUST 16, 1982

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE AVOID
AVOIDANCE

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

KRATIO=100 DF=24 MS=32.73 F=21.69 LSD=15.2881

GROUPING	MEAN	N	POLLUT	CCNC	AVOID
A	90.000000	1	NI	1:100	90
A	87.000000	1	A	1:100	87
A	86.500000	1	B	1:100	86.5
B	56.000000	1	FE	1:100	56
B	52.000000	1	CR	1:100	52

DUNCAN MULTIPLE RANGE TESTS
CONCENTRATION AS LC50 VALUE=1:100

14:18 MONDAY, AUGUST 16, 1982

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE AVOID
AVOIDANCE

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=24 MS=32.73

GROUPING	MEAN	N	POLLUT	CCNC	AVOID
A	90.000000	1	NI	1:100	90
A	87.000000	1	A	1:100	87
A	86.500000	1	B	1:100	86.5
B	56.000000	1	FE	1:100	56
B	52.000000	1	CR	1:100	52

DUNCAN MULTIPLE RANGE TESTS
CONCENTRATION AS LC50 VALUE=1:150

14:18 MONDAY, AUGUST 16, 1982 5

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE AVOID
AVOIDANCE

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

KRATIO=100 DF=24 MS=32.73 F=21.65 LSD=15.2881

GROUPING	MEAN	N	POLLUT	CONC	AVOID
A	86.000000	1	NI	1:150	86
A	84.300000	1	B	1:150	84.3
A	82.500000	1	A	1:150	82.5
B	55.000000	1	FE	1:150	55
B	50.000000	1	CR	1:150	50

DUNCAN MULTIPLE RANGE TESTS
CONCENTRATION AS LC50 VALUE=1:150

14:18 MONDAY, AUGUST 16, 1982 6

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE AVOID
AVOIDANCE

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=24 MS=32.73

GROUPING	MEAN	N	POLLUT	CONC	AVOID
A	86.000000	1	NI	1:150	86
A	84.300000	1	B	1:150	84.3
A	82.500000	1	A	1:150	82.5
B	55.000000	1	FE	1:150	55
B	50.000000	1	CR	1:150	50

DUNCAN MULTIPLE RANGE TESTS
CONCENTRATION AS LC50 VALUE=1:2000

14:18 MONDAY, AUGUST 16, 1982

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE AVOID
AVOIDANCE

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

KRATIO=100 DF=24 MS=32.73 F=21.69 LSD=15.2881

GROUPING	MEAN	N	POLLUT	CONC	AVOID
A	69.000000	1	A	1:2000	69
B	59.000000	1	B	1:2000	59
B	59.000000	1	NI	1:2000	59
B	53.000000	1	FE	1:2000	53
B	46.000000	1	CR	1:2000	46

DUNCAN MULTIPLE RANGE TESTS
CONCENTRATION AS LC50 VALUE=1:2000

14:18 MONDAY, AUGUST 16, 1982

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE AVOID
AVOIDANCE

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=24 MS=32.73

GROUPING	MEAN	N	POLLUT	CONC	AVOID
A	69.000000	1	A	1:2000	69
B	59.000000	1	B	1:2000	59
B	59.000000	1	NI	1:2000	59
B	53.000000	1	FE	1:2000	53
B	46.000000	1	CR	1:2000	46

DUNCAN MULTIPLE RANGE TESTS
CONCENTRATION AS LC50 VALUE=1:250

14:18 MONDAY, AUGUST 16, 1982

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE AVOID
AVOIDANCE

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

KRATIO=100 DF=24 MS=32.73 F=21.65 LSD=15.2881

GROUPING	MEAN	N	POLLUT	CONC	AVOID
A	79.100000	1	B	1:250	79.1
A	77.000000	1	A	1:250	77
A	70.000000	1	NI	1:250	70
B	54.000000	1	FE	1:250	54
B	49.000000	1	CR	1:250	49

DUNCAN MULTIPLE RANGE TESTS
CONCENTRATION AS LC50 VALUE=1:250

14:18 MONDAY, AUGUST 16, 1982 10

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE AVOID
AVOIDANCE

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=24 MS=32.73

GROUPING	MEAN	N	POLLUT	CONC	AVOID
A	79.100000	1	B	1:250	79.1
A	77.000000	1	A	1:250	77
A	70.000000	1	NI	1:250	70
B	54.000000	1	FE	1:250	54
B	49.000000	1	CR	1:250	49

DUNCAN-MULTIPLE RANGE TESTS
CONCENTRATION AS LC50 VALUE=1:370

14:18 MONDAY, AUGUST 16, 1982 11

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE AVOID
AVOIDANCE

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

KRATIO=100 DF=24 MS=32.73 F=21.64 LSD=15.2881

GROUPING	MEAN	N	POLLUT	CONC	AVOID
A	76.300000	1	A	1:370	76.3
A	70.500000	1	B	1:370	70.5
A	65.000000	1	NI	1:370	65
B	54.000000	1	FE	1:370	54
B	48.000000	1	CR	1:370	48

DUNCAN MULTIPLE RANGE TESTS
CONCENTRATION AS LC50 VALUE=1:370

14:18 MONDAY, AUGUST 16, 1982 12

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE AVOID
AVOIDANCE

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=24 MS=32.73

GROUPING	MEAN	N	POLLUT	CONC	AVOID
A	76.300000	1	A	1:370	76.3
A	70.500000	1	B	1:370	70.5
A	65.000000	1	NI	1:370	65
B	54.000000	1	FE	1:370	54
B	48.000000	1	CR	1:370	48

DUNCAN MULTIPLE RANGE TESTS
CONCENTRATION AS LC50 VALUE=1:500

14:18 MONDAY, AUGUST 16, 1982 13

WALLER-DUNCAN, K-RATIO T TEST FOR VARIABLE AVOID
AVOIDANCE

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

KRATIO=100 DF=24 MS=32.73 F=21.69 LSD=15.2881

GROUPING	MEAN	N	POLLUT	CONC	AVOID
B B B B B	A	1	A	1:500	75.8
	A	1	B	1:500	67.2
	A	1	NI	1:500	63
	A	1	FE	1:500	53.5
	C	1	CR	1:500	47
	C	1			

DUNCAN MULTIPLE RANGE TESTS
CONCENTRATION AS LC50 VALUE=1:500

14:18 MONDAY, AUGUST 16, 1982 14

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE AVOID
AVOIDANCE

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=24 MS=32.73

GROUPING	MEAN	N	POLLUT	CONC	AVOID
B B B B B	A	1	A	1:500	75.8
	A	1	B	1:500	67.2
	A	1	NI	1:500	63
	C	1	FE	1:500	53.5
	C	1	CR	1:500	47
	C	1			

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DUNCAN MULTIPLE RANGE TESTS
CONCENTRATION AS LC50 VALUE=1:75

14:18 MONDAY, AUGUST 16, 1982 15

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE AVOID
AVOIDANCE

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

KRATIO=100 DF=24 MS=32.73 F=21.65 LSD=15.2881

GROUPING	MEAN	N	POLLUT	CONC	AVOID
A	94.000000	1	NI	1:75	94
A	91.700000	1	A	1:75	91.7
A	88.000000	1	B	1:75	88
B	56.000000	1	FE	1:75	56
B	54.000000	1	CR	1:75	54

DUNCAN MULTIPLE RANGE TESTS
CONCENTRATION AS LC50 VALUE=1:75

14:18 MONDAY, AUGUST 16, 1982 16

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE AVOID
AVOIDANCE

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=24 MS=32.73

GROUPING	MEAN	N	POLLUT	CONC	AVOID
A	94.000000	1	NI	1:75	94
A	91.700000	1	A	1:75	91.7
A	88.000000	1	B	1:75	88
B	56.000000	1	FE	1:75	56
B	54.000000	1	CR	1:75	54

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C3 - ANOVA 8 - HORIZONTAL DISTRIBUTION OF INJECTION SYSTEM A

ANALYSIS OF CURVES FOR THE HORIZONTAL AND VERTICAL DISTRIBUTION

17:22 MONDAY, AUGUST 16, 1982

GENERAL LINEAR MODELS PROCEDURE

CLASS LEVEL INFORMATION

CLASS	LEVELS	VALUES
DISTAN	4	SECT1 SECT2 SECT3 SECT4
CONC	7	0 1:150 1:200 1:250 1:500 1:700 1:75

NUMBER OF OBSERVATIONS IN DATA SET = 28

ANALYSIS OF CURVES FOR THE HORIZONTAL AND VERTICAL DISTRIBUTION

17:22 MONDAY, AUGUST 16, 1982

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: FISH

X IN CLEAR WATER

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.
MODEL	9	6501.44428571	722.38269841	2.20	0.0740	0.523659	72.6074
ERROR	18	5913.96000000	328.55333333		STD DEV		FISH MEAN
CORRECTED TOTAL	27	12415.40428571			18.12604020		24.96428571

SOURCE	DF	TYPE I SS	F VALUE	PR > F	CF	TYPE IV SS	F VALUE	PR > F
DISTAN	3	6501.23000000	6.60	0.0034	3	6501.23000000	6.60	0.0034
CONC	6	0.21428571	0.00	1.0000	6	0.21428571	0.00	1.0000

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT1

17:22 MONDAY, AUGUST 16, 1982

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE FISH
X IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

KRATIO=100 DF=18 MS=328.55 F=2.2 LSD=65.3775

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	32.500000	1	SECT1	0	32.5
A	26.700000	1	SECT1	1:200	26.7
A	26.300000	1	SECT1	1:250	26.3
A	19.500000	1	SECT1	1:500	19.5
A	18.000000	1	SECT1	1:700	18
A	7.600000	1	SECT1	1:150	7.6
A	4.500000	1	SECT1	1:75	4.5

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT1

17:22 MONDAY, AUGUST 16, 1982

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
X IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=18 MS=328.55

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	32.500000	1	SECT1	0	32.5
A	26.700000	1	SECT1	1:200	26.7
A	26.300000	1	SECT1	1:250	26.3
A	19.500000	1	SECT1	1:500	19.5
A	18.000000	1	SECT1	1:700	18
A	7.600000	1	SECT1	1:150	7.6
A	4.500000	1	SECT1	1:75	4.5

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT2

17:22 MONDAY, AUGUST 16, 1982

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

KRATIO=100 DF=18 MS=328.55 F=2.2 LSD=65.3775

GROUPING		MEAN	N	DISTAN	CCNC	FISH
	A	92.000000	1	SECT2	1:75	92
B	A	81.600000	1	SECT2	1:150	81.6
B	A	50.600000	1	SECT2	1:500	50.6
B	A	39.400000	1	SECT2	1:200	39.4
B	A	33.600000	1	SECT2	1:700	33.6
B	A	29.400000	1	SECT2	0	29.4
B	A	25.100000	1	SECT2	1:250	25.1

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT2

17:22 MONDAY, AUGUST 16, 1982

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=18 MS=328.55

GROUPING		MEAN	N	DISTAN	CCNC	FISH
	A	92.000000	1	SECT2	1:75	92
B	A	81.600000	1	SECT2	1:150	81.6
B	A	50.600000	1	SECT2	1:500	50.6
B	A	39.400000	1	SECT2	1:200	39.4
B	A	33.600000	1	SECT2	1:700	33.6
B	A	29.400000	1	SECT2	0	29.4
B	A	25.100000	1	SECT2	1:250	25.1

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT3

17:22 MONDAY, AUGUST 16, 1982

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

KRATIO=100 DF=18 MS=328.55 F=2.2 LSD=65.1775

GROUPING	MEAN	N	DISTAN	CCNC	FISH
A	35.600000	1	SECT3	1:700	35.6
A	27.400000	1	SECT3	1:250	27.4
A	25.000000	1	SECT3	0	25
A	23.700000	1	SECT3	1:200	23.7
A	23.300000	1	SECT3	1:500	23.3
A	8.300000	1	SECT3	1:150	8.3
A	2.100000	1	SECT3	1:75	2.1

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT3

17:22 MONDAY, AUGUST 16, 1982

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=18 MS=328.55

GROUPING	MEAN	N	DISTAN	CCNC	FISH
A	35.600000	1	SECT3	1:700	35.6
A	27.400000	1	SECT3	1:250	27.4
A	25.000000	1	SECT3	0	25
A	23.700000	1	SECT3	1:200	23.7
A	23.300000	1	SECT3	1:500	23.3
A	8.300000	1	SECT3	1:150	8.3
A	2.100000	1	SECT3	1:75	2.1

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT4

17:22 MONDAY, AUGUST 16, 1982

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE FISH
X IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

KRATIO=100 DF=18 MS=328.55 F=2.2 LSD=65.3775

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	21.200000	1	SECT4	1:250	21.2
A	13.100000	1	SECT4	0	13.1
A	12.800000	1	SECT4	1:700	12.8
A	10.200000	1	SECT4	1:200	10.2
A	5.200000	1	SECT4	1:500	5.2
A	2.500000	1	SECT4	1:150	2.5
A	1.600000	1	SECT4	1:75	1

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT4

17:22 MONDAY, AUGUST 16, 1982

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
X IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=18 MS=328.55

GROUPING	MEAN	N	DISTAN	CCNC	FISH
A	21.200000	1	SECT4	1:250	21.2
A	13.100000	1	SECT4	0	13.1
A	12.800000	1	SECT4	1:700	12.8
A	10.200000	1	SECT4	1:200	10.2
A	5.200000	1	SECT4	1:500	5.2
A	2.500000	1	SECT4	1:150	2.5
A	1.600000	1	SECT4	1:75	1

C3 - ANOVA 9 - VERTICAL DISTRIBUTION OF INJECTION SYSTEM A

ANALYSIS OF CURVES FOR THE HORIZONTAL AND VERTICAL DISTRIBUTION

17:31 MONDAY, AUGUST 16, 1982

GENERAL LINEAR MODELS PROCEDURE

CLASS LEVEL INFORMATION

CLASS	LEVELS	VALUES
DISTAN	3	DEPTH A DEPTH B DEPTH C
CONC	7	0 1:150 1:200 1:250 1:500 1:700 1:75

NUMBER OF OBSERVATIONS IN DATA SET = 21

ANALYSIS OF CURVES FOR THE HORIZONTAL AND VERTICAL DISTRIBUTION

17:31 MONDAY, AUGUST 16, 1982

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: FISH

X IN CLEAR WATER

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.
MODEL	8	505.92380952	63.24047619	1.61	0.2205	0.517626	18.7936
ERROR	12	471.46857143	39.28904762		STD DEV		FISH MEAN
CORRECTED TOTAL	20	977.39238095			6.26809761		33.35238095

SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F
DISTAN	2	505.79809524	6.44	0.0126	2	505.79809524	6.44	0.0126
CONC	6	0.12571429	0.00	1.0000	6	0.12571429	0.00	1.0000

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DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=DEPTH A

17:31 MONDAY, AUGUST 16, 1982 3

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

KRATIO=100 DF=12 MS=39.28 F=1.61 LSD=24.4934

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	48.700000	1	DEPTH A	1:700	48.7
A	46.500000	1	DEPTH A	0	46.5
A	38.700000	1	DEPTH A	1:200	38.7
A	38.100000	1	DEPTH A	1:150	38.1
A	37.800000	1	DEPTH A	1:500	37.8
A	35.700000	1	DEPTH A	1:250	35.7
A	33.700000	1	DEPTH A	1:75	33.7

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=DEPTH A

17:31 MONDAY, AUGUST 16, 1982 4

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=12 MS=39.28

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	48.700000	1	DEPTH A	1:700	48.7
A	46.500000	1	DEPTH A	0	46.5
A	38.700000	1	DEPTH A	1:200	38.7
A	38.100000	1	DEPTH A	1:150	38.1
A	37.800000	1	DEPTH A	1:500	37.8
A	35.700000	1	DEPTH A	1:250	35.7
A	33.700000	1	DEPTH A	1:75	33.7

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DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=DEPTHB

17:11 MONDAY, AUGUST 16, 1982

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE FISH
X IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

KRATIO=100 DF=12 MS=39.28 F=1.61 LSD=24.4934

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	36.400000	1	DEPTHB	1:75	36.4
A	34.200000	1	DEPTHB	1:150	34.2
A	29.600000	1	DEPTHB	1:500	29.6
A	28.100000	1	DEPTHB	1:200	28.1
A	25.200000	1	DEPTHB	1:250	25.2
A	22.200000	1	DEPTHB	1:700	22.2
A	20.700000	1	DEPTHB	0	20.7

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=DEPTHB

17:31 MONDAY, AUGUST 16, 1982

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
X IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=12 MS=39.28

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	36.400000	1	DEPTHB	1:75	36.4
A	34.200000	1	DEPTHB	1:150	34.2
A	29.600000	1	DEPTHB	1:500	29.6
A	28.100000	1	DEPTHB	1:200	28.1
A	25.200000	1	DEPTHB	1:250	25.2
A	22.200000	1	DEPTHB	1:700	22.2
A	20.700000	1	DEPTHB	0	20.7

DUNCAN MULTIPLE RANGE TESTS
SECTION OF DEPTH=DEPTHC

17:31 MONDAY, AUGUST 16, 1982 7

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

KRATIO=100 DF=12 MS=39.28 F=1.61 LSD=24.4934

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	38.900000	1	DEPTHC	1:250	39.9
A	33.200000	1	DEPTHC	1:200	33.2
A	32.800000	1	DEPTHC	0	32.8
A	32.600000	1	DEPTHC	1:500	32.6
A	30.500000	1	DEPTHC	1:75	30.5
A	29.100000	1	DEPTHC	1:700	29.1
A	27.700000	1	DEPTHC	1:150	27.7

DUNCAN MULTIPLE RANGE TESTS
SECTION OF DEPTH=DEPTHC

17:31 MONDAY, AUGUST 16, 1982 8

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=12 MS=39.28

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	38.900000	1	DEPTHC	1:250	38.9
A	33.200000	1	DEPTHC	1:200	33.2
A	32.800000	1	DEPTHC	0	32.8
A	32.600000	1	DEPTHC	1:500	32.6
A	30.500000	1	DEPTHC	1:75	30.5
A	29.100000	1	DEPTHC	1:700	29.1
A	27.700000	1	DEPTHC	1:150	27.7

C3 - ANOVA 10 - HORIZONTAL DISTRIBUTION OF INJECTION SYSTEM B

ANALYSIS OF CURVES FOR THE HORIZONTAL AND VERTICAL DISTRIBUTION

17:32 MONDAY, AUGUST 16, 1982

GENERAL LINEAR MODELS PROCEDURE

CLASS LEVEL INFORMATION

CLASS	LEVELS	VALUES
DISTAN	4	SECT1 SECT2 SECT3 SECT4
CCNC	9	0 1:100 1:150 1:250 1:370 1:40 1:50 1:500 1:700

NUMBER OF OBSERVATIONS IN DATA SET = 36

ANALYSIS OF CURVES FOR THE HORIZONTAL AND VERTICAL DISTRIBUTION

17:32 MONDAY, AUGUST 16, 1982

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: FISH

X IN CLEAR WATER

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.
MODEL	11	2542.00666667	231.09151515	8.63	0.0001	0.803649	20.7482
ERROR	24	621.07333333	25.87805556		STD DEV		FISH MEAN
CORRECTED TOTAL	35	3163.08000000			5.08704792		25.00000000

SOURCE	DF	TYPE I SS	F VALUE	PR > F	CF	TYPE IV SS	F VALUE	PR > F
DISTAN	3	2542.00666667	32.74	0.0001	3	2542.00666667	32.74	0.0001
CCNC	8	0.00000000	0.00	1.0000	8	0.00000000	0.00	1.0000

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DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT1

17:32 MONDAY, AUGUST 16, 1982

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE FISH
X IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

KRATIO=100 DF=24 MS=25.87 F=4.93 LSD=14.2063

GROUPING	MEAN	N	DISTAN	CCNC	FISH
A	36.600000	1	SECT1	1:250	36.6
A	36.400000	1	SECT1	1:150	36.4
A	32.500000	1	SECT1	0	32.5
A	31.100000	1	SECT1	1:40	31.1
A	30.400000	1	SECT1	1:50	30.4
A	30.000000	1	SECT1	1:100	30
A	27.200000	1	SECT1	1:370	27.2
A	26.600000	1	SECT1	1:500	26.6
A	24.600000	1	SECT1	1:700	24.6

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT1

17:32 MONDAY, AUGUST 16, 1982

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
X IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=24 MS=25.87

GROUPING	MEAN	N	DISTAN	CCNC	FISH
A	36.600000	1	SECT1	1:250	36.6
A	36.400000	1	SECT1	1:150	36.4
A	32.500000	1	SECT1	0	32.5
A	31.100000	1	SECT1	1:40	31.1
A	30.400000	1	SECT1	1:50	30.4
A	30.000000	1	SECT1	1:100	30
A	27.200000	1	SECT1	1:370	27.2
A	26.600000	1	SECT1	1:500	26.6
A	24.600000	1	SECT1	1:700	24.6

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT2

17:32 MONDAY, AUGUST 16, 1982

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

KRATIO=100 DF=24 MS=25.87 F=8.93 LSD=14.2063

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	43.400000	1	SECT2	1:500	43.4
B	38.700000	1	SECT2	1:250	38.7
B	37.600000	1	SECT2	1:370	37.6
B	34.600000	1	SECT2	1:40	34.6
B	33.600000	1	SECT2	1:150	33.6
B	32.200000	1	SECT2	1:700	32.2
B	30.000000	1	SECT2	1:100	30
B	29.400000	1	SECT2	0	29.4
B	27.400000	1	SECT2	1:50	27.4

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT2

17:32 MONDAY, AUGUST 16, 1982

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=24 MS=25.87

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	43.400000	1	SECT2	1:500	43.4
A	38.700000	1	SECT2	1:250	38.7
A	37.600000	1	SECT2	1:370	37.6
A	34.600000	1	SECT2	1:40	34.6
A	33.600000	1	SECT2	1:150	33.6
A	32.200000	1	SECT2	1:700	32.2
A	30.000000	1	SECT2	1:100	30
A	29.400000	1	SECT2	0	29.4
A	27.400000	1	SECT2	1:50	27.4

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT3

17:32 MONDAY, AUGUST 16, 1982 7

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

KRATIO=100 DF=24 MS=25.87 F=8.93 LSD=14.2063

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	30.800000	1	SECT3	1:700	30.8
A	25.000000	1	SECT3	0	25
A	25.000000	1	SECT3	1:40	25
A	25.000000	1	SECT3	1:100	25
A	23.800000	1	SECT3	1:370	23.8
A	21.900000	1	SECT3	1:500	21.9
A	21.100000	1	SECT3	1:50	21.1
A	17.700000	1	SECT3	1:150	17.7
A	17.200000	1	SECT3	1:250	17.2

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT3

17:32 MONDAY, AUGUST 16, 1982 7

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=24 MS=25.87

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	30.800000	1	SECT3	1:700	30.8
A	25.000000	1	SECT3	0	25
A	25.000000	1	SECT3	1:40	25
A	25.000000	1	SECT3	1:100	25
A	23.800000	1	SECT3	1:370	23.8
A	21.900000	1	SECT3	1:500	21.9
A	21.100000	1	SECT3	1:50	21.1
A	17.700000	1	SECT3	1:150	17.7
A	17.200000	1	SECT3	1:250	17.2

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DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT4

17:32 MONDAY, AUGUST 16, 1982

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE FISH
X IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

KRATIO=100 DF=24 MS=25.87 F=8.93 LSD=14.2063

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	21.100000	1	SECT4	1:50	21.1
A	15.000000	1	SECT4	1:100	15
A	13.100000	1	SECT4	0	13.1
A	12.400000	1	SECT4	1:700	12.4
A	12.100000	1	SECT4	1:150	12.1
A	11.400000	1	SECT4	1:370	11.4
A	9.300000	1	SECT4	1:40	9.3
A	7.900000	1	SECT4	1:500	7.9
A	7.500000	1	SECT4	1:250	7.5

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT4

17:32 MONDAY, AUGUST 16, 1982 10

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
X IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=24 MS=25.87

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	21.100000	1	SECT4	1:50	21.1
A	15.000000	1	SECT4	1:100	15
A	13.100000	1	SECT4	0	13.1
A	12.400000	1	SECT4	1:700	12.4
A	12.100000	1	SECT4	1:150	12.1
A	11.400000	1	SECT4	1:370	11.4
A	9.300000	1	SECT4	1:40	9.3
A	7.900000	1	SECT4	1:500	7.9
A	7.500000	1	SECT4	1:250	7.5

C3 - ANOVA 11 - VERTICAL DISTRIBUTION OF INJECTION SYSTEM B

ANALYSIS OF CURVES FOR THE HORIZONTAL AND VERTICAL DISTRIBUTION

17:33 MONDAY, AUGUST 16, 1982

GENERAL LINEAR MODELS PROCEDURE

CLASS LEVEL INFORMATION

CLASS	LEVELS	VALUES
DISTAN	3	DEPTH A DEPTH B DEPTH C
CONC	9	0 1:100 1:150 1:250 1:370 1:40 1:50 1:500 1:700

NUMBER OF OBSERVATIONS IN DATA SET = 27

ANALYSIS OF CURVES FOR THE HORIZONTAL AND VERTICAL DISTRIBUTION

17:33 MONDAY, AUGUST 16, 1982

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: FISH

X IN CLEAR WATER

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.
MODEL	10	283.86888889	28.38688889	0.71	0.7029	0.307748	19.0600
ERROR	16	638.53777778	39.90861111				
CORRECTED TOTAL	26	922.40666667					
						STD DEV	FISH MEAN
						6.31732626	33.14444444

SOURCE	DF	TYPE I SS	F VALUE	PR > F	CF	TYPE IV SS	F VALUE	PR > F
DISTAN	2	276.16222222	3.46	0.0564	2	276.16222222	3.46	0.0564
CONC	8	7.70666667	0.02	1.0000	8	7.70666667	0.02	1.0000

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DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=DEPTH A

17:13 MONDAY, AUGUST 16, 1982

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05

DF=16

MS=39.9

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	46.500000	1	DEPTH A	0	36.5
A	42.300000	1	DEPTH A	1:700	42.3
A	41.100000	1	DEPTH A	1:40	41.1
A	39.500000	1	DEPTH A	1:150	39.5
A	37.700000	1	DEPTH A	1:50	37.7
A	37.500000	1	DEPTH A	1:250	37.5
A	32.500000	1	DEPTH A	1:500	32.5
A	29.700000	1	DEPTH A	1:100	29.7
A	28.200000	1	DEPTH A	1:370	28.2

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=DEPTH B

17:33 MONDAY, AUGUST 16, 1982

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05

DF=16

MS=39.9

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	32.600000	1	DEPTH B	1:100	32.6
A	32.500000	1	DEPTH B	1:500	32.5
A	31.700000	1	DEPTH B	1:700	31.7
A	30.900000	1	DEPTH B	1:40	30.9
A	29.700000	1	DEPTH B	1:250	29.7
A	29.500000	1	DEPTH B	1:500	29.5
A	29.400000	1	DEPTH B	1:370	29.4
A	27.700000	1	DEPTH B	1:150	27.7
A	20.700000	1	DEPTH B	0	20.7

DUNCAN MULTIPLE RANGE TESTS
SECTION OF DEPTH=DEPTH

17:33 MONDAY, AUGUST 16, 1982

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05

DF=16

MS=39.9

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	42.400000	1	DEPTH	1:370	42.4
A	38.000000	1	DEPTH	1:500	38
A	37.700000	1	DEPTH	1:100	37.7
A	32.800000	1	DEPTH	1:250	32.8
A	32.800000	1	DEPTH	0	32.8
A	29.800000	1	DEPTH	1:50	29.8
A	28.000000	1	DEPTH	1:40	28
A	27.700000	1	DEPTH	1:150	27.7
A	26.000000	1	DEPTH	1:700	26

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C3 - ANOVA 12 - HORIZONTAL DISTRIBUTION OF Cr EXPERIMENTS

ANALYSIS OF CURVES FOR THE HORIZONTAL AND VERTICAL DISTRIBUTION

21:59 MONDAY, AUGUST 16, 1982¹

GENERAL LINEAR MODELS PROCEDURE

CLASS LEVEL INFORMATION

CLASS	LEVELS	VALUES
DISTAN	4	SECT1 SECT2 SECT3 SECT4
CONC	8	0 1:100 1:150 1:2000 1:250 1:370 1:500 1:75

NUMBER OF OBSERVATIONS IN DATA SET = 32

ANALYSIS OF CURVES FOR THE HORIZONTAL AND VERTICAL DISTRIBUTION

21:59 MONDAY, AUGUST 16, 1982²

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: FISH

% IN CLEAR WATER

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.
MODEL	10	4587.7650000	458.77650000	6.26	0.0002	0.748816	33.9450
ERROR	21	1538.92375000	73.28208333				
CONNECTED TOTAL	31	6126.68875000			8.58049551		25.21875000

SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F
DISTAN	3	4577.04625000	20.82	0.0001	3	4577.04625000	20.82	0.0001
CONC	7	10.71875000	0.02	1.0000	7	10.71875000	0.02	1.0000

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COPIE DE QUALITE INFERIEURE

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT1

21:59 MONDAY, AUGUST 16, 1982 3

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE FISH
X IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

KRATIO=100 DF=21 MS=73.28 F=6.26 LSD=25.2042

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	32.500000	1	SECT1	0	32.5
A	29.000000	1	SECT1	1:370	29
A	23.000000	1	SECT1	1:250	23
A	20.000000	1	SECT1	1:75	20
A	16.000000	1	SECT1	1:150	16
A	14.000000	1	SECT1	1:500	14
A	11.000000	1	SECT1	1:2000	11
A	9.000000	1	SECT1	1:100	9

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT1

21:59 MONDAY, AUGUST 16, 1982 4

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
X IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=21 MS=73.28

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	32.500000	1	SECT1	0	32.5
A	29.000000	1	SECT1	1:370	29
A	23.000000	1	SECT1	1:250	23
A	20.000000	1	SECT1	1:75	20
A	16.000000	1	SECT1	1:150	16
A	14.000000	1	SECT1	1:500	14
A	11.000000	1	SECT1	1:2000	11
A	9.000000	1	SECT1	1:100	9

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DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT2

21:59 MONDAY, AUGUST 16, 1982 5

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

KRATIO=100 DF=21 MS=73.28 F=6.26 LSD=25.2042

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	59.000000	1	SECT2	1:100	59
A	56.000000	1	SECT2	1:150	56
A	53.000000	1	SECT2	1:500	53
A	48.000000	1	SECT2	1:75	48
A	41.000000	1	SECT2	1:250	41
A	37.000000	1	SECT2	1:370	37
A	36.000000	1	SECT2	1:2000	36
A	29.400000	1	SECT2	0	29.4

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT2

21:59 MONDAY, AUGUST 16, 1982 6

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=21 MS=73.28

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	59.000000	1	SECT2	1:100	59
A	56.000000	1	SECT2	1:150	56
A	53.000000	1	SECT2	1:500	53
A	48.000000	1	SECT2	1:75	48
A	41.000000	1	SECT2	1:250	41
A	37.000000	1	SECT2	1:370	37
A	36.000000	1	SECT2	1:2000	36
A	29.400000	1	SECT2	0	29.4

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DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT3

21:59 MONDAY, AUGUST 16, 1982

7

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

KRATIO=100 DF=21 MS=73.28 F=6.26 LSD=25.2042

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	36.000000	1	SECT3	1:2000	36
A	26.000000	1	SECT3	1:100	26
A	25.000000	1	SECT3	0	25
A	23.000000	1	SECT3	1:250	23
A	21.000000	1	SECT3	1:370	21
A	20.000000	1	SECT3	1:500	20
A	20.000000	1	SECT3	1:75	20
A	17.000000	1	SECT3	1:150	17

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT3

21:59 MONDAY, AUGUST 16, 1982

8

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=21 MS=73.28

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	36.000000	1	SECT3	1:2000	36
A	26.000000	1	SECT3	1:100	26
A	25.000000	1	SECT3	0	25
A	23.000000	1	SECT3	1:250	23
A	21.000000	1	SECT3	1:370	21
A	20.000000	1	SECT3	1:500	20
A	20.000000	1	SECT3	1:75	20
A	17.000000	1	SECT3	1:150	17

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DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT4

21:59 MONDAY, AUGUST 16, 1982 9

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE FISH
X IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

KRATIO=100 DF=21 MS=73.28 F=6.26 LSD=25.2042

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	17.000000	1	SECT4	1:2000	17
A	13.100000	1	SECT4	0	13.1
A	13.000000	1	SECT4	1:500	13
A	13.000000	1	SECT4	1:370	13
A	13.000000	1	SECT4	1:250	13
A	13.000000	1	SECT4	1:100	13
A	12.000000	1	SECT4	1:75	12
A	11.000000	1	SECT4	1:150	11

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT4

21:59 MONDAY, AUGUST 16, 1982 10

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
X IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=21 MS=73.28

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	17.000000	1	SECT4	1:2000	17
A	13.100000	1	SECT4	0	13.1
A	13.000000	1	SECT4	1:500	13
A	13.000000	1	SECT4	1:370	13
A	13.000000	1	SECT4	1:250	13
A	13.000000	1	SECT4	1:100	13
A	12.000000	1	SECT4	1:75	12
A	11.000000	1	SECT4	1:150	11

C3 - ANOVA 13 VERTICAL DISTRIBUTION OF Cr EXPERIMENTS

ANALYSIS OF CURVES FOR THE HORIZONTAL AND VERTICAL DISTRIBUTION

22:00 MONDAY, AUGUST 16, 1982¹

GENERAL LINEAR MODELS PROCEDURE

CLASS LEVEL INFORMATION

CLASS	LEVELS	VALUES
DISTAN	3	DEPTH A DEPTH B DEPTH C
CONC	8	0 1:100 1:150 1:250 1:370 1:500 1:700 1:75

NUMBER OF OBSERVATIONS IN DATA SET = 24

ANALYSIS OF CURVES FOR THE HORIZONTAL AND VERTICAL DISTRIBUTION

22:00 MONDAY, AUGUST 16, 1982²

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLES: FISH

% IN CLEAR WATER

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.
MODEL	9	797.94750000	88.66033333	0.78	0.6409	0.332976	31.9760
ERROR	14	1598.46583333	114.17613095		STD DEV		FISH MEAN
CORRECTED TOTAL	23	2396.41333333			10.68532316		33.41666667

SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F
DISTAN	2	796.78083333	3.49	0.0589	2	796.78083333	3.49	0.0589
CCNC	7	1.16666667	0.00	1.0000	7	1.16666667	0.00	1.0000

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=DEPTH A

22:00 MONDAY, AUGUST 16, 1982 3

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
X IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=14 MS=114.17

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	50.000000	1	DEPTH A	1:370	50
A	50.000000	1	DEPTH A	1:700	50
A	46.500000	1	DEPTH A	0	46.5
A	44.000000	1	DEPTH A	1:500	44
A	43.000000	1	DEPTH A	1:250	43
A	35.000000	1	DEPTH A	1:150	35
A	35.000000	1	DEPTH A	1:100	35
A	27.000000	1	DEPTH A	1:75	27

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=DEPTH B

22:00 MONDAY, AUGUST 16, 1982 4

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
X IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=14 MS=114.17

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	40.000000	1	DEPTH B	1:700	40
A	35.000000	1	DEPTH B	1:370	35
A	33.000000	1	DEPTH B	1:150	33
A	32.000000	1	DEPTH B	1:500	32
A	31.000000	1	DEPTH B	1:250	31
A	30.000000	1	DEPTH B	1:100	30
A	28.000000	1	DEPTH B	1:75	28
A	20.700000	1	DEPTH B	0	20.7

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DUNCAN MULTIPLE RANGE TESTS
SECTION OF DEPTH=DEPTHC

22:00 MONDAY, AUGUST 16, 1982 5

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
X IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05

DF=14

MS=114.17

GROUPING	MEAN	N	DEPTAN	CONC	FISH
A	45.000000	1	DEPTHC	1:75	45
A	35.000000	1	DEPTHC	1:100	35
A	34.000000	1	DEPTHC	1:150	34
A	32.800000	1	DEPTHC	0	32.8
A	26.000000	1	DEPTHC	1:250	26
A	24.000000	1	DEPTHC	1:500	24
A	15.000000	1	DEPTHC	1:370	15
A	10.000000	1	DEPTHC	1:700	10

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DUNCAN MULTIPLE RANGE TESTS
SECTION OF DEPTH=DEPTHC

22:00 MONDAY, AUGUST 16, 1982 5

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
X IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05

DF=14

MS=114.17

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	45.000000	1	DEPTHC	1:75	45
A	35.000000	1	DEPTHC	1:100	35
A	34.000000	1	DEPTHC	1:150	34
A	32.800000	1	DEPTHC	0	32.8
A	26.000000	1	DEPTHC	1:250	26
A	24.000000	1	DEPTHC	1:500	24
A	15.000000	1	DEPTHC	1:370	15
A	10.000000	1	DEPTHC	1:700	10

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C3 - ANOVE 14 HORIZONTAL DISTRIBUTION OF N1 EXPERIMENTS

ANALYSIS OF CURVES FOR THE HORIZONTAL AND VERTICAL DISTRIBUTION

22:02 MONDAY, AUGUST 16, 1982¹

GENERAL LINEAR MODELS PROCEDURE

CLASS LEVEL INFORMATION

CLASS	LEVELS	VALUES
DISTAN	4	SECT1 SECT2 SECT3 SECT4
CONC	7	0 1:100 1:150 1:2000 1:250 1:370 1:500

NUMBER OF OBSERVATIONS IN DATA SET = 28

ANALYSIS OF CURVES FOR THE HORIZONTAL AND VERTICAL DISTRIBUTION

22:02 MONDAY, AUGUST 16, 1982²

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: FISH

X IN CLEAR WATER

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.	
MODEL	9	2649.06714286	294.34079365	5.13	0.0016	0.719372	30.6142	
ERROR	18	1033.40285714	57.41126984					
CORRECTED TOTAL	27	3682.47000000						
					STD DEV		FISH MEAN	
					7.57702249		24.75000000	
SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F
DISTAN	3	2638.56714286	15.32	0.0001	3	2638.56714286	15.32	0.0001
CONC	6	10.50000000	0.03	0.9998	6	10.50000000	0.03	0.9998

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DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT1

22:02 MONDAY, AUGUST 16, 1982 3

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

KRATIO=100 DF=18 MS=57.41 F=5.13 LSD=23.1892

GROUPING	MEAN	N	DISTAN	CONC	FISH
3	32.500000	1	SECT1	0	32.5
8	20.000000	1	SECT1	1:250	20
8	18.000000	1	SECT1	1:500	18
8	13.000000	1	SECT1	1:370	13
8	13.000000	1	SECT1	1:2000	13
8	10.000000	1	SECT1	1:100	10
8	8.000000	1	SECT1	1:150	8

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT1

22:02 MONDAY, AUGUST 16, 1982 4

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=18 MS=57.41

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	32.500000	1	SECT1	0	32.5
A	20.000000	1	SECT1	1:250	20
A	18.000000	1	SECT1	1:500	18
A	13.000000	1	SECT1	1:370	13
A	13.000000	1	SECT1	1:2000	13
A	10.000000	1	SECT1	1:100	10
A	8.000000	1	SECT1	1:150	8

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DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT2

22:02 MONDAY, AUGUST 16, 1982

5

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE FISH
X IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

KRATIO=100 DF=18 MS=57.41 F=5.13 LSD=23.1892

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	52.000000	1	SECT2	1:370	52
A	41.000000	1	SECT2	1:100	41
A	40.000000	1	SECT2	1:2000	40
A	40.000000	1	SECT2	1:250	40
A	38.000000	1	SECT2	1:500	38
A	33.000000	1	SECT2	1:150	33
A	29.400000	1	SECT2	0	29.4

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT2

22:02 MONDAY, AUGUST 16, 1982

6

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
X IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=18 MS=57.41

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	52.000000	1	SECT2	1:370	52
A	41.000000	1	SECT2	1:100	41
A	40.000000	1	SECT2	1:2000	40
A	40.000000	1	SECT2	1:250	40
A	38.000000	1	SECT2	1:500	38
A	33.000000	1	SECT2	1:150	33
A	29.400000	1	SECT2	0	29.4

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DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT3

22:02 MONDAY, AUGUST 16, 1982

7

WALLER-DUNCAN-K-RATIO T TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

KRATIO=100 DF=18 MS=57.41 F=5.13 LSD=23.1892

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	33.000000	1	SECT3	1:100	33
A	32.000000	1	SECT3	1:500	32
A	31.000000	1	SECT3	1:150	31
A	29.000000	1	SECT3	1:250	29
A	28.000000	1	SECT3	1:2000	28
A	25.000000	1	SECT3	0	25
A	20.000000	1	SECT3	1:370	20

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT3

22:02 MONDAY, AUGUST 16, 1982

8

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=18 MS=57.41

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	33.000000	1	SECT3	1:100	33
A	32.000000	1	SECT3	1:500	32
A	31.000000	1	SECT3	1:150	31
A	29.000000	1	SECT3	1:250	29
A	28.000000	1	SECT3	1:2000	28
A	25.000000	1	SECT3	0	25
A	20.000000	1	SECT3	1:370	20

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT4

22:02 MONDAY, AUGUST 16, 1982 9

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

KRATIO=100 DF=18 MS=57.41 F=5.13 LSD=23.1892

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	28.000000	1	SECT4	1:150	28
A	16.000000	1	SECT4	1:100	16
A	15.000000	1	SECT4	1:370	15
A	13.100000	1	SECT4	0	13.1
A	12.000000	1	SECT4	1:2000	12
A	12.000000	1	SECT4	1:500	12
A	11.000000	1	SECT4	1:250	11

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT4

22:02 MONDAY, AUGUST 16, 1982 10

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=18 MS=57.41

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	28.000000	1	SECT4	1:150	28
A	16.000000	1	SECT4	1:100	16
A	15.000000	1	SECT4	1:370	15
A	13.100000	1	SECT4	0	13.1
A	12.000000	1	SECT4	1:2000	12
A	12.000000	1	SECT4	1:500	12
A	11.000000	1	SECT4	1:250	11

C3 - ANOVA 15 VERTICAL DISTRIBUTION OF Ni EXPERIMENTS

ANALYSIS OF CURVES FOR THE HORIZONTAL AND VERTICAL DISTRIBUTION

22:02 MONDAY, AUGUST 16, 1982

GENERAL LINEAR MODELS PROCEDURE

CLASS LEVEL INFORMATION

CLASS	LEVELS	VALUES
DISTAN	3	DEPTH A DEPTH B DEPTH C
CONC	7	0 1:100 1:150 1:2000 1:250 1:370 1:500

NUMBER OF OBSERVATIONS IN DATA SET = 21

ANALYSIS OF CURVES FOR THE HORIZONTAL AND VERTICAL DISTRIBUTION

22:02 MONDAY, AUGUST 16, 1982

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: FISH		% IN CLEAR WATER						
SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.	
MODEL	8	3084.54952381	385.56869048	4.26	0.0124	0.739479	28.5486	
ERROR	12	1086.69714286	90.55809524		STD DEV		FISH MEAN	
CORRECTED TOTAL	20	4171.24666667			9.51620172		33.33333333	

SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F
DISTAN	2	3084.54952381	17.03	0.0003	2	3084.54952381	17.03	0.0003
CONC	6	0.00000000	0.00	1.0000	6	0.00000000	0.00	1.0000

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=DEPTH

22:02 MONDAY, AUGUST 16, 1982 3

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

KRATIO=100 DF=12 MS=90.55 F=4.26 LSD=31.594

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	57.000000	1	DEPTH	1:500	57
A	50.000000	1	DEPTH	1:370	50
A	47.000000	1	DEPTH	1:250	47
A	46.500000	1	DEPTH	0	46.5
A	46.000000	1	DEPTH	1:150	46
A	44.000000	1	DEPTH	1:100	44
A	40.000000	1	DEPTH	1:2000	40

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=DEPTH

22:02 MONDAY, AUGUST 16, 1982 4

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=12 MS=90.55

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	57.000000	1	DEPTH	1:500	57
A	50.000000	1	DEPTH	1:370	50
A	47.000000	1	DEPTH	1:250	47
A	46.500000	1	DEPTH	0	46.5
A	46.000000	1	DEPTH	1:150	46
A	44.000000	1	DEPTH	1:100	44
A	40.000000	1	DEPTH	1:2000	40

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=DEPTHB

22:02 MONDAY, AUGUST 16, 1982 5

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE FISH
X IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

KRATIO=100 DF=12 MS=90.55 F=4.26 LSD=31.594

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	49.000000	1	DEPTHB	1:2000	49
A	43.000000	1	DEPTHB	1:250	43
A	39.000000	1	DEPTHB	1:100	39
A	36.000000	1	DEPTHB	1:150	36
A	33.000000	1	DEPTHB	1:370	33
A	25.000000	1	DEPTHB	1:500	25
A	20.700000	1	DEPTHB	0	20.7

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=DEPTHB

22:02 MONDAY, AUGUST 16, 1982 6

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
X IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=12 MS=90.55

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	49.000000	1	DEPTHB	1:2000	49
A	43.000000	1	DEPTHB	1:250	43
A	39.000000	1	DEPTHB	1:100	39
A	36.000000	1	DEPTHB	1:150	36
A	33.000000	1	DEPTHB	1:370	33
A	25.000000	1	DEPTHB	1:500	25
A	20.700000	1	DEPTHB	0	20.7

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=DEPTHC

22:02 MONDAY, AUGUST 16, 1982 7

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

KRATIO=100 DF=12 MS=90.55 F=4.26 LSD=31.594

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	32.800000	1	DEPTHC	0	32.8
A	18.000000	1	DEPTHC	1:500	18
A	18.000000	1	DEPTHC	1:150	18
A	17.000000	1	DEPTHC	1:370	17
A	17.000000	1	DEPTHC	1:100	17
A	11.000000	1	DEPTHC	1:2000	11
A	10.000000	1	DEPTHC	1:250	10

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=DEPTHC

22:02 MONDAY, AUGUST 16, 1982 8

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=12 MS=90.55

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	32.800000	1	DEPTHC	0	32.8
A	18.000000	1	DEPTHC	1:500	18
A	18.000000	1	DEPTHC	1:150	18
A	17.000000	1	DEPTHC	1:370	17
A	17.000000	1	DEPTHC	1:100	17
A	11.000000	1	DEPTHC	1:2000	11
A	10.000000	1	DEPTHC	1:250	10

C3 - ANOVA 16 HORIZONTAL DISTRIBUTION OF Fe EXPERIMENTS

ANALYSIS OF CURVES FOR THE HORIZONTAL AND VERTICAL DISTRIBUTION

22:03 MONDAY, AUGUST 16, 1982¹

GENERAL LINEAR MODELS PROCEDURE

CLASS LEVEL INFORMATION

CLASS	LEVELS	VALUES
DISTAN		SECT1 SECT2 SECT3 SECT4
CONC	8	0 1:100 1:150 1:2000 1:250 1:370 1:500 1:75

NUMBER OF OBSERVATIONS IN DATA SET = 32

ANALYSIS OF CURVES FOR THE HORIZONTAL AND VERTICAL DISTRIBUTION

22:03 MONDAY, AUGUST 16, 1982²

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: FISH

% IN CLEAR WATER

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.
MODEL	10	6628.77750000	662.87775000	6.60	0.0001	0.758683	40.0803
ERROR	21	2108.44250000	100.40202381				
CORRECTED TOTAL	31	8737.22000000					
					STD DEV		FISH MEAN
					10.02008103		25.00000000

SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F
DISTAN	3	6628.77750000	22.01	0.0001	3	6628.77750000	22.01	0.0001
CONC	7	0.00000000	0.00	1.0000	7	0.00000000	0.00	1.0000

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT1

22103 MONDAY, AUGUST 16, 1982 3

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

KRATIO=100 DF=21 MS=100.4 F=6.6 LSD=29.3141

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	32.500000	1	SECT1	0	32.5
A	22.000000	1	SECT1	1:150	22
A	21.000000	1	SECT1	1:75	21
A	19.000000	1	SECT1	1:100	19
A	13.000000	1	SECT1	1:250	13
A	6.000000	1	SECT1	1:370	6
A	6.000000	1	SECT1	1:2000	6
A	4.000000	1	SECT1	1:500	4

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT1

22103 MONDAY, AUGUST 16, 1982 4

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=21 MS=100.4

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	32.500000	1	SECT1	0	32.5
A	22.000000	1	SECT1	1:150	22
A	21.000000	1	SECT1	1:75	21
A	19.000000	1	SECT1	1:100	19
A	13.000000	1	SECT1	1:250	13
A	6.000000	1	SECT1	1:370	6
A	6.000000	1	SECT1	1:2000	6
A	4.000000	1	SECT1	1:500	4

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT2

22:03 MONDAY, AUGUST 16, 1982

5

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

KRATIO=100 DF=21 MS=100.4 F=6.6 LSD=29.3141

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	32.000000	1	SECT2	1:500	32
A	30.000000	1	SECT2	1:370	30
A	29.400000	1	SECT2	0	29.4
A	27.000000	1	SECT2	1:100	27
A	23.000000	1	SECT2	1:250	23
A	20.000000	1	SECT2	1:75	20
A	19.000000	1	SECT2	1:150	19
A	11.000000	1	SECT2	1:2000	11

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT2

22:03 MONDAY, AUGUST 16, 1982

6

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=21 MS=100.4

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	32.000000	1	SECT2	1:500	32
A	30.000000	1	SECT2	1:370	30
A	29.400000	1	SECT2	0	29.4
A	27.000000	1	SECT2	1:100	27
A	23.000000	1	SECT2	1:250	23
A	20.000000	1	SECT2	1:75	20
A	19.000000	1	SECT2	1:150	19
A	11.000000	1	SECT2	1:2000	11

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT3

22103 MONDAY, AUGUST 16, 1982 7

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

KRATIO=100		DF=21	MS=100.4	F=6.6	LSD=29.3141	
GROUPING		MEAN	N	DISTAN	CONC	FISH
3 8 8 8 8 3 8 8 8 8 B	A	55.000000	1	SECT3	1:2000	55
	A	54.000000	1	SECT3	1:75	54
	A	54.000000	1	SECT3	1:250	54
	A	53.000000	1	SECT3	1:370	53
	A	52.000000	1	SECT3	1:500	52
	A	50.000000	1	SECT3	1:100	50
	A	47.000000	1	SECT3	1:150	47
	A	25.000000	1	SECT3	0	25

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT3

22103 MONDAY, AUGUST 16, 1982 8

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05		DF=21	MS=100.4			
GROUPING		MEAN	N	DISTAN	CONC	FISH
A A A A A A A A A A	A	55.000000	1	SECT3	1:2000	55
	A	54.000000	1	SECT3	1:75	54
	A	54.000000	1	SECT3	1:250	54
	A	53.000000	1	SECT3	1:370	53
	A	52.000000	1	SECT3	1:500	52
	A	50.000000	1	SECT3	1:100	50
	A	47.000000	1	SECT3	1:150	47
	A	25.000000	1	SECT3	0	25

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT4

22:03 MONDAY, AUGUST 16, 1982 9

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE FISH
X IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

KRATIO=100 DF=21 MS=100.4 F=6.6 LSD=29.3141

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	26.000000	1	SECT4	1:2000	28
A	13.100000	1	SECT4	0	13.1
A	12.000000	1	SECT4	1:500	12
A	12.000000	1	SECT4	1:150	12
A	11.000000	1	SECT4	1:370	11
A	10.000000	1	SECT4	1:250	10
A	5.000000	1	SECT4	1:75	5
A	4.000000	1	SECT4	1:100	4

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT4

22:03 MONDAY, AUGUST 16, 1982 10

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
X IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=21 MS=100.4

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	26.000000	1	SECT4	1:2000	28
A	13.100000	1	SECT4	0	13.1
A	12.000000	1	SECT4	1:500	12
A	12.000000	1	SECT4	1:150	12
A	11.000000	1	SECT4	1:370	11
A	10.000000	1	SECT4	1:250	10
A	5.000000	1	SECT4	1:75	5
A	4.000000	1	SECT4	1:100	4

C3 - ANOVA 17 VERTICAL DISTRIBUTION OF Fe EXPERIMENTS

ANALYSIS OF CURVES FOR THE HORIZONTAL AND VERTICAL DISTRIBUTION

22:04 MONDAY, AUGUST 16, 1982

GENERAL LINEAR MODELS PROCEDURE

CLASS LEVEL INFORMATION

CLASS	LEVELS	VALUES
DISTAN	3	DEPTHB DEPTHC
CONC	8	0 1:100 1:150 1:2000 1:250 1:370 1:500 1:75

NUMBER OF OBSERVATIONS IN DATA SET = 24

ANALYSIS OF CURVES FOR THE HORIZONTAL AND VERTICAL DISTRIBUTION

22:04 MONDAY, AUGUST 16, 1982

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: FISH

% IN CLEAR WATER

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.
MODEL	9	315.7308333	35.08120370	0.80	0.6253	0.338798	19.9027
ERROR	14	616.1825000	44.01303571		STD DEV		FISH MEAN
CORRECTED TOTAL	23	931.9133333			6.63423211		33.33333333

SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F
DISTAN	2	315.7308333	3.59	0.0553	2	315.7308333	3.59	0.0553
CONC	7	0.00000000	0.00	1.0000	7	0.00000000	0.00	1.0000

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=DEPTH

22:04 MONDAY, AUGUST 16, 1982 3

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=14 MS=44.01

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	46.500000	1	DEPTH	0	46.5
A	39.000000	1	DEPTH	1:2000	39
A	39.000000	1	DEPTH	1:250	39
A	36.000000	1	DEPTH	1:150	36
A	36.000000	1	DEPTH	1:370	36
A	34.000000	1	DEPTH	1:500	34
A	34.000000	1	DEPTH	1:100	34
A	33.000000	1	DEPTH	1:75	33

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=DEPTH

22:04 MONDAY, AUGUST 16, 1982 4

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=14 MS=44.01

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	42.000000	1	DEPTH	1:2000	42
A	39.000000	1	DEPTH	1:150	39
A	37.000000	1	DEPTH	1:370	37
A	37.000000	1	DEPTH	1:100	37
A	36.000000	1	DEPTH	1:500	36
A	32.000000	1	DEPTH	1:250	32
A	31.000000	1	DEPTH	1:75	31
A	20.700000	1	DEPTH	0	20.7

DUNCAN MULTIPLE RANGE TESTS
SECTION ON DEPTH=DEPTHC

22:04 MONDAY, AUGUST 16, 1982 5

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05

DF=14

MS=44.01

GROUPING	MEAN	N	DISTAN.	CONC	FISH
A	36.000000	1	DEPTHC	1:75	36
A	32.000000	1	DEPTHC	0	32
A	30.000000	1	DEPTHC	1:500	30
A	29.000000	1	DEPTHC	1:100	29
A	29.000000	1	DEPTHC	1:250	29
A	27.000000	1	DEPTHC	1:370	27
A	25.000000	1	DEPTHC	1:150	25
A	19.000000	1	DEPTHC	1:2000	19

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT1

21:09 TUESDAY, AUGUST 17, 1982

3

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
X IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05

DF=9

MS=12.72

GROUPING	MEAN	N	DISTAN	NOISE	FISH
A	31.000000	1	SECT1	73DBA	31
A	29.000000	1	SECT1	80DBA	29
A	28.000000	1	SECT1	64DBA	28
A	25.000000	1	SECT1	60DBA	25
GROUPING	MEAN	N	DISTAN	NOISE	FISH
A	30.000000	1	SECT2	60DBA	30
A	25.000000	1	SECT2	80DBA	25
A	23.000000	1	SECT2	64DBA	23
A	22.000000	1	SECT2	73DBA	22
GROUPING	MEAN	N	DISTAN	NOISE	FISH
A	26.000000	1	SECT3	64DBA	26
A	26.000000	1	SECT3	73DBA	26
A	21.000000	1	SECT3	80DBA	21
A	19.000000	1	SECT3	60DBA	19
GROUPING	MEAN	N	DISTAN	NOISE	FISH
A	27.000000	1	SECT4	60DBA	27
A	25.000000	1	SECT4	80DBA	25
A	23.000000	1	SECT4	64DBA	23
A	21.000000	1	SECT4	73DBA	21

C3 - ANOVA 19 VERTICAL DISTRIBUTION OF NOISE EXPERIMENTS

ANALYSIS OF CURVES FOR THE HORIZONTAL AND VERTICAL DISTRIBUTION

21:10 TUESDAY, AUGUST 17, 1982¹

GENERAL LINEAR MODELS PROCEDURE

CLASS LEVEL INFORMATION

CLASS	LEVELS	VALUES
DISTAN	3	DEPTH A DEPTH B DEPTH C
NOISE	4	60DBA 64DBA 73DBA 80DBA

NUMBER OF OBSERVATIONS IN DATA SET = 12

ANALYSIS OF CURVES FOR THE HORIZONTAL AND VERTICAL DISTRIBUTION

21:10 TUESDAY, AUGUST 17, 1982²

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: FISH

% IN CLEAR WATER

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.
MODEL	5	666.16666667	133.23333333	4.33	0.0513	0.783111	16.6358
ERROR	6	184.50000000	30.75000000				
CORRECTED TOTAL	11	850.66666667					
					STD DEV		FISH MEAN
					5.54526825		33.33333333

SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F
DISTAN	2	666.16666667	10.83	0.0102	2	666.16666667	10.83	0.0102
NOISE	3	0.00000000	0.00	1.0000	3	0.00000000	0.00	1.0000

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=DEPTH

21:10 TUESDAY, AUGUST 17, 1982

3

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

KRATIO=100 DF=6 MS=30.75 F=4.33 LSD=20.5224

GROUPING	MEAN	N	DISTAN	NOISE	FISH
A	50.000000	1	DEPTH	80DBA	50
A	44.000000	1	DEPTH	73DBA	44
A	41.000000	1	DEPTH	64DBA	41
A	35.000000	1	DEPTH	60DBA	35

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=DEPTH

21:10 TUESDAY, AUGUST 17, 1982

4

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=6 MS=30.75

GROUPING	MEAN	N	DISTAN	NOISE	FISH
A	50.000000	1	DEPTH	80DBA	50
A	44.000000	1	DEPTH	73DBA	44
A	41.000000	1	DEPTH	64DBA	41
A	35.000000	1	DEPTH	60DBA	35

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=DEPTHB

21:10 TUESDAY, AUGUST 17, 1982 5

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

KRATIO=100 DF=6 MS=30.75 F=4.33 LSD=20.5224

GROUPING	MEAN	N	DISTAN	NOISE	FISH
A	36.000000	1	DEPTHB	60DBA	36
A	34.000000	1	DEPTHB	73DBA	34
A	33.000000	1	DEPTHB	64DBA	33
A	30.000000	1	DEPTHB	80DBA	30

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=DEPTHB

21:10 TUESDAY, AUGUST 17, 1982 6

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=6 MS=30.75

GROUPING	MEAN	N	DISTAN	NOISE	FISH
A	36.000000	1	DEPTHB	60DBA	36
A	34.000000	1	DEPTHB	73DBA	34
A	33.000000	1	DEPTHB	64DBA	33
A	30.000000	1	DEPTHB	80DBA	30

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=DEPTHC

21:10 TUESDAY, AUGUST 17, 1982

7

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE FISH
X IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

KRATIO=100 DF=6 MS=30.75 F=4.33 LSD=20.5224

GROUPING	MEAN	N	DISTAN	NOISE	FISH
A	29.000000	1	DEPTHC	60DBA	29
A	26.000000	1	DEPTHC	64DBA	26
A	22.000000	1	DEPTHC	73DBA	22
A	20.000000	1	DEPTHC	80DBA	20

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=DEPTHC

21:10 TUESDAY, AUGUST 17, 1982

8

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
X IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=6 MS=30.75

GROUPING	MEAN	N	DISTAN	NOISE	FISH
A	29.000000	1	DEPTHC	60DBA	29
A	26.000000	1	DEPTHC	64DBA	26
A	22.000000	1	DEPTHC	73DBA	22
A	20.000000	1	DEPTHC	80DBA	20

C3 - ANOVA 20 HORIZONTAL DISTRIBUTION OF TEMPERATURE EXPERIMENTS

ANALYSIS OF CURVES FOR THE HORIZONTAL AND VERTICAL DISTRIBUTION

21:11 TUESDAY, AUGUST 17, 1982¹

GENERAL LINEAR MODELS PROCEDURE

CLASS LEVEL INFORMATION

CLASS	LEVELS	VALUES
DISTAN	4	SECT1 SECT2 SECT3 SECT4
TEMP	7	12C 13C 14C 15C 16C 22C 23C

NUMBER OF OBSERVATIONS IN DATA SET = 28

ANALYSIS OF CURVES FOR THE HORIZONTAL AND VERTICAL DISTRIBUTION

21:11 TUESDAY, AUGUST 17, 1982²

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: FISH

% IN CLEAR WATER

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.
MODEL	9	161.93506494	17.99278499	0.25	0.9808	0.110730	34.4927
ERROR	18	1300.49350649	72.24963925		STD DEV		FISH MEAN
CORRECTED TOTAL	27	1462.42857143			9.49997878		24.64285714

SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F
DISTAN	3	78.14285714	0.36	0.7822	3	87.17316017	0.40	0.7532
TEMP	6	83.79220779	0.19	0.9767	6	93.79220779	0.19	0.9747

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT1

21:11 TUESDAY, AUGUST 17, 1982

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
X IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=18 MS=72.24

GROUPING	MEAN	N	DISTAN	TEMP	FISH
A	35.000000	1	SECT1	12C	35
A	33.000000	1	SECT1	13C	33
A	32.000000	1	SECT1	16C	32
A	27.000000	1	SECT1	14C	27
A	26.000000	1	SECT1	15C	26
A	25.000000	1	SECT1	23C	25
A	12.000000	1	SECT1	22C	12

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT2

21:11 TUESDAY, AUGUST 17, 1982

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
X IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=18 MS=72.24

GROUPING	MEAN	N	DISTAN	TEMP	FISH
A	32.000000	1	SECT2	12C	32
A	30.000000	1	SECT2	13C	30
A	24.000000	1	SECT2	15C	24
A	24.000000	1	SECT2	16C	24
A	23.000000	1	SECT2	14C	23
A	17.000000	1	SECT2	22C	17
A	14.000000	1	SECT2	23C	14

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT3

21:11 TUESDAY, AUGUST 17, 1982 5

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05

DF=18

MS=72.24

GROUPING	MEAN	N	DISTAN	TEMP	FISH
A	27.000000	1	SECT3	15C	27
A	25.000000	1	SECT3	13C	25
A	24.000000	1	SECT3	16C	24
A	23.000000	1	SECT3	14C	23
A	23.000000	1	SECT3	22C	23
A	20.000000	1	SECT3	23C	20
A	18.000000	1	SECT3	12C	18

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT4

21:11 TUESDAY, AUGUST 17, 1982 6

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05

DF=18

MS=72.24

GROUPING	MEAN	N	DISTAN	TEMP	FISH
B	41.000000	1	SECT4	23C	41
B	38.000000	1	SECT4	22C	38
B	27.000000	1	SECT4	14C	27
B	23.000000	1	SECT4	15C	23
B	20.000000	1	SECT4	16C	20
B	15.000000	1	SECT4	13C	15
B	12.000000	1	SECT4	13C	12

C3 - ANOVA 21 VERTICAL DISTRIBUTION OF TEMPERATURE EXPERIMENTS

ANALYSIS OF CURVES FOR THE HORIZONTAL AND VERTICAL DISTRIBUTION

21:12 TUESDAY, AUGUST 17, 1982¹

GENERAL LINEAR MODELS PROCEDURE

CLASS LEVEL INFORMATION

CLASS	LEVELS	VALUES
DISTAN	3	DEPTH A DEPTH B DEPTH C
TEMP	7	12C 13C 14C 15C 16C 22C 23C

NUMBER OF OBSERVATIONS IN DATA SET = 21

ANALYSIS OF CURVES FOR THE HORIZONTAL AND VERTICAL DISTRIBUTION

21:12 TUESDAY, AUGUST 17, 1982²

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: FISH X IN CLEAR WATER

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.
MODEL	8	720.38095238	90.04761905	2.96	0.0441	0.663975	10.7268
ERROR	12	364.57142857	30.38095238			STD DEV	FISH MEAN
CORRECTED TOTAL	20	1084.95238095				5.51189191	32.95238095

SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F
DISTAN	2	702.09523810	11.55	0.0016	2	702.09523810	11.55	0.0016
TEMP	6	18.28571429	0.10	0.9949	6	18.28571429	0.10	0.9949

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=DEPTH

21:12 TUESDAY, AUGUST 17, 1982

3

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

KRATIO=100 CF=12 MS=30.38 F=2.96 LSD=19.4215

GROUPING	MEAN	N	DISTAN	TEMP	FISH
A	50.000000	1	DEPTH	22C	50
A	46.000000	1	DEPTH	23C	46
A	42.000000	1	DEPTH	16C	42
A	40.000000	1	DEPTH	15C	40
A	38.000000	1	DEPTH	14C	38
A	34.000000	1	DEPTH	13C	34
A	34.000000	1	DEPTH	12C	34

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=DEPTH

21:12 TUESDAY, AUGUST 17, 1982

4

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=12 MS=30.38

GROUPING	MEAN	N	DISTAN	TEMP	FISH
A	50.000000	1	DEPTH	22C	50
A	46.000000	1	DEPTH	23C	46
A	42.000000	1	DEPTH	16C	42
A	40.000000	1	DEPTH	15C	40
A	38.000000	1	DEPTH	14C	38
A	34.000000	1	DEPTH	13C	34
A	34.000000	1	DEPTH	12C	34

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=DEPTHB

21:12 TUESDAY, AUGUST 17, 1992 5

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE FISH
X IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

KRATIO=100 CF=12 MS=30.38 F=2.96 LSD=19.4215

GROUPING	MEAN	N	DISTAN	TEMP	FISH
A	36.000000	1	DEPTHB	12C	36
A	34.000000	1	DEPTHB	13C	34
A	33.000000	1	DEPTHB	23C	33
A	31.000000	1	DEPTHB	15C	31
A	30.000000	1	DEPTHB	22C	30
A	30.000000	1	DEPTHB	16C	30
A	28.000000	1	DEPTHB	14C	28

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=DEPTHB

21:12 TUESDAY, AUGUST 17, 1992 6

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
X IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=12 MS=30.38

GROUPING	MEAN	N	DISTAN	TEMP	FISH
A	36.000000	1	DEPTHB	12C	36
A	34.000000	1	DEPTHB	13C	34
A	33.000000	1	DEPTHB	23C	33
A	31.000000	1	DEPTHB	15C	31
A	30.000000	1	DEPTHB	22C	30
A	30.000000	1	DEPTHB	16C	30
A	28.000000	1	DEPTHB	14C	28

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=DEPTHC

21:12 TUESDAY, AUGUST 17, 1932

7

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

KRATIO=100 DF=12 MS=30.38 F=2.96 LSD=19.4215

GROUPING	MEAN	N	DISTAN	TEMP	FISH
A	32.000000	1	DEPTHC	13C	32
A	30.000000	1	DEPTHC	12C	30
A	29.000000	1	DEPTHC	15C	29
A	28.000000	1	DEPTHC	16C	28
A	26.000000	1	DEPTHC	14C	26
A	21.000000	1	DEPTHC	23C	21
A	20.000000	1	DEPTHC	22C	20

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=DEPTHC

21:12 TUESDAY, AUGUST 17, 1932

8

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=12 MS=30.38

GROUPING	MEAN	N	DISTAN	TEMP	FISH
A	32.000000	1	DEPTHC	13C	32
A	30.000000	1	DEPTHC	12C	30
A	29.000000	1	DEPTHC	15C	29
A	28.000000	1	DEPTHC	16C	28
A	26.000000	1	DEPTHC	14C	26
A	21.000000	1	DEPTHC	23C	21
A	20.000000	1	DEPTHC	22C	20

C3 - ANOVA 22 HORIZONTAL DISTRIBUTION OF DSS EXPERIMENTS

ANALYSIS OF CURVES FOR THE HORIZONTAL AND VERTICAL DISTRIBUTION

22:05 MONDAY, AUGUST 16, 1982¹

GENERAL LINEAR MODELS PROCEDURE

CLASS LEVEL INFORMATION

CLASS	LEVELS	VALUES
DISTAN	4	SECT1 SECT2 SECT3 SECT4
CONC	10	JET 0 1:1.3 1:100 1:200 1:32 1:4 1:40 1:400 1:8

NUMBER OF OBSERVATIONS IN DATA SET = 40

ANALYSIS OF CURVES FOR THE HORIZONTAL AND VERTICAL DISTRIBUTION

22:05 MONDAY, AUGUST 16, 1982²

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: FISH

% IN CLEAR WATER

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.
MODEL	12	3573.52800000	297.79400000	20.63	0.0001	0.901646	15.2291
ERROR	27	389.81200000	14.43748148				FISH MEAN
CORRECTED TOTAL	39	3963.34000000			3.79966860		24.95000000

SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F
DISTAN	3	3572.62800000	82.49	0.0001	3	3572.62800000	82.49	0.0001
CONC	9	0.90000000	0.01	1.0000	9	0.90000000	0.01	1.0000

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT1

22:05 MONDAY, AUGUST 16, 1982 3

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE FISH
X IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

KRATIO=100 DF=27 MS=14.43 F=20.63 LSD=10.1073

GROUPING		MEAN	N	DISTAN	CONC	FISH
3 8 8 8 8 8 8 8 8 8 3 8 8 8 8 3 8 3	A	43.000000	1	SECT1	1:8	43
	A	41.000000	1	SECT1	1:400	41
	A	40.000000	1	SECT1	1:200	40
	A	37.000000	1	SECT1	1:32	37
	A	35.000000	1	SECT1	1:100	35
	A	33.000000	1	SECT1	1:40	33
	A	32.500000	1	SECT1	JET	32.5
	A	32.500000	1	SECT1	0	32.5
	A	32.500000	1	SECT1	1:1.3	32.5
	A	31.500000	1	SECT1	1:4	31.5
	A					

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT1

22:05 MONDAY, AUGUST 16, 1982 4

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
X IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=27 MS=14.43

GROUPING	MEAN	N	DISTAN	CONC	FISH
8 A A A A A A A A A A A A A A A A A	43.000000 41.000000 40.000000 37.000000 35.000000 33.000000 32.500000 32.500000 32.500000 32.500000 31.500000	1 1 1 1 1 1 1 1 1 1 1	SECT1 SECT1 SECT1 SECT1 SECT1 SECT1 SECT1 SECT1 SECT1 SECT1 SECT1	1:8 1:400 1:200 1:32 1:100 1:40 JET 0 1:1.3 1:4	43 41 40 37 35 33 32.5 32.5 32.5 32.5 31.5

2

LSD=10.1073

1

MS=14.43

389

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT3

22:05 MONDAY, AUGUST 16, 1982

7

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

KRAT(0=100 DF=27 MS=14.43 F=20.63 LSD=10.1073

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	28.000000	1	SECT3	1:40	28
A	26.000000	1	SECT3	1:4	26
A	25.000000	1	SECT3	0	25
A	25.000000	1	SECT3	1:1.3	25
A	25.000000	1	SECT3	JET	25
A	23.000000	1	SECT3	1:32	23
A	23.000000	1	SECT3	1:8	23
A	21.000000	1	SECT3	1:400	21
A	20.000000	1	SECT3	1:200	20
A	19.000000	1	SECT3	1:100	19

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT3

22:05 MONDAY, AUGUST 16, 1982

8

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=27 MS=14.43

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	28.000000	1	SECT3	1:40	28
A	26.000000	1	SECT3	1:4	26
A	25.000000	1	SECT3	0	25
A	25.000000	1	SECT3	1:1.3	25
A	25.000000	1	SECT3	JET	25
A	23.000000	1	SECT3	1:32	23
A	23.000000	1	SECT3	1:8	23
A	21.000000	1	SECT3	1:400	21
A	20.000000	1	SECT3	1:200	20
A	19.000000	1	SECT3	1:100	19

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT4

22:05 MONDAY, AUGUST 16, 1982

9

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE FISH
X IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

KRATIO=100 DF=27 MS=14.43 F=20.63 LSD=10.1073

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	13.100000	1	SECT4	0	13.1
A	13.100000	1	SECT4	JET	13.1
A	13.000000	1	SECT4	1:400	13
A	11.000000	1	SECT4	1:32	11
A	11.000000	1	SECT4	1:1.3	11
A	10.000000	1	SECT4	1:4	10
A	10.000000	1	SECT4	1:200	10
A	9.000000	1	SECT4	1:100	9
A	7.000000	1	SECT4	1:8	7
A	7.000000	1	SECT4	1:40	7

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=SECT4

22:05 MONDAY, AUGUST 16, 1982

10

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
X IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=27 MS=14.43

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	13.100000	1	SECT4	0	13.1
A	13.100000	1	SECT4	JET	13.1
A	13.000000	1	SECT4	1:400	13
A	11.000000	1	SECT4	1:32	11
A	11.000000	1	SECT4	1:1.3	11
A	10.000000	1	SECT4	1:4	10
A	10.000000	1	SECT4	1:200	10
A	9.000000	1	SECT4	1:100	9
A	7.000000	1	SECT4	1:8	7
A	7.000000	1	SECT4	1:40	7

C3 - ANOVA 23 VERTICAL DISTRIBUTION OF DSS EXPERIMENTS

ANALYSIS OF CURVES FOR THE HORIZONTAL AND VERTICAL DISTRIBUTION

21:15 TUESDAY, AUGUST 17, 1982

GENERAL LINEAR MODELS PROCEDURE

CLASS LEVEL INFORMATION

CLASS	LEVELS	VALUES
DISTAN	3	DEPTH A DEPTH B DEPTH C
CONC	10	JET 0 1:1.3 1:100 1:200 1:32 1:4 1:40 1:400 1:8

NUMBER OF OBSERVATIONS IN DATA SET = 30

ANALYSIS OF CURVES FOR THE HORIZONTAL AND VERTICAL DISTRIBUTION

21:15 TUESDAY, AUGUST 17, 1982

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: FISH

% IN CLEAR WATER

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	L.V.
MODEL	11	1646.24466667	149.65860606	4.18	0.0036	0.718625	17.9525
ERROR	18	644.58200000	35.81011111				
CORRECTED TOTAL	29	2290.82666667					
					STD DEV		FISH MEAN
					5.98415500		33.3333333

SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F
DISTAN	2	1646.24466667	22.99	0.0001	2	1646.24466667	22.99	0.0001
CONC	9	0.00000000	0.00	1.0000	9	0.00000000	0.00	1.0000

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=DEPTH

21:15 TUESDAY, AUGUST 17, 1992

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE FISH
X IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

KRATIO=100 DF=18 MS=35.81 F=4.18 LSD=18.9974

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	48.200000	1	DEPTH	0	48.2
A	46.500000	1	DEPTH	JET	46.5
A	42.500000	1	DEPTH	1:400	42.5
A	38.000000	1	DEPTH	1:40	38
A	36.000000	1	DEPTH	1:32	36
A	35.000000	1	DEPTH	1:8	35
A	33.000000	1	DEPTH	1:4	33
A	32.000000	1	DEPTH	1:200	32
A	32.000000	1	DEPTH	1:1.3	32
A	30.000000	1	DEPTH	1:100	30

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=DEPTH

21:15 TUESDAY, AUGUST 17, 1992

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
X IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=18 MS=35.81

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	48.200000	1	DEPTH	0	48.2
A	46.500000	1	DEPTH	JET	46.5
A	42.500000	1	DEPTH	1:400	42.5
A	38.000000	1	DEPTH	1:40	38
A	36.000000	1	DEPTH	1:32	36
A	35.000000	1	DEPTH	1:8	35
A	33.000000	1	DEPTH	1:4	33
A	32.000000	1	DEPTH	1:200	32
A	32.000000	1	DEPTH	1:1.3	32
A	30.000000	1	DEPTH	1:100	30

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=DEPTH3

21:15 TUESDAY, AUGUST 17, 1932 5

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

KRATIO=100 DF=18 MS=35.81 F=4.18 LSD=18.9974

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	26.000000	1	DEPTHB	1:100	26
A	26.000000	1	DEPTHB	1:1.3	26
A	25.000000	1	DEPTHB	1:200	25
A	25.000000	1	DEPTHB	1:4	25
A	23.000000	1	DEPTHB	1:32	23
A	22.800000	1	DEPTHB	0	22.8
A	22.000000	1	DEPTHB	1:8	22
A	20.700000	1	DEPTHB	JET	20.7
A	20.000000	1	DEPTHB	1:40	20
A	19.000000	1	DEPTHB	1:400	19

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=DEPTHB

21:15 TUESDAY, AUGUST 17, 1932 6

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=18 MS=35.81

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	26.000000	1	DEPTHB	1:100	26
A	26.000000	1	DEPTHB	1:1.3	26
A	25.000000	1	DEPTHB	1:200	25
A	25.000000	1	DEPTHB	1:4	25
A	23.000000	1	DEPTHB	1:32	23
A	22.800000	1	DEPTHB	0	22.8
A	22.000000	1	DEPTHB	1:8	22
A	20.700000	1	DEPTHB	JET	20.7
A	20.000000	1	DEPTHB	1:40	20
A	19.000000	1	DEPTHB	1:400	19

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=DEPTHC

21:15 TUESDAY, AUGUST 17, 1982 7

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

KRATIO=100 DF=18 MS=35.81 F=4.18 LSD=18.9274

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	44.000000	1	DEPTHC	1:100	44
A	43.000000	1	DEPTHC	1:8	43
A	43.000000	1	DEPTHC	1:200	43
A	42.000000	1	DEPTHC	1:1.3	42
A	42.000000	1	DEPTHC	1:4	42
A	42.000000	1	DEPTHC	1:40	42
A	41.000000	1	DEPTHC	1:32	41
A	38.500000	1	DEPTHC	1:400	38.5
A	32.800000	1	DEPTHC	JET	32.8
A	29.000000	1	DEPTHC	0	29

DUNCAN MULTIPLE RANGE TESTS
SECTION OR DEPTH=DEPTHC

21:15 TUESDAY, AUGUST 17, 1982 8

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FISH
% IN CLEAR WATER

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=18 MS=35.81

GROUPING	MEAN	N	DISTAN	CONC	FISH
A	44.000000	1	DEPTHC	1:100	44
A	43.000000	1	DEPTHC	1:8	43
A	43.000000	1	DEPTHC	1:200	43
A	42.000000	1	DEPTHC	1:1.3	42
A	42.000000	1	DEPTHC	1:4	42
A	42.000000	1	DEPTHC	1:40	42
A	41.000000	1	DEPTHC	1:32	41
A	38.500000	1	DEPTHC	1:400	38.5
A	32.800000	1	DEPTHC	JET	32.8
A	29.000000	1	DEPTHC	0	29

C3 - ANOVA 28 AVOIDANCE REACTION OF POLYMERS AND MONOMERS

ANALYSIS OF VARIANCE FOR POLYMERS AND MONOMERS

17:09 FRIDAY, AUGUST 27, 1982 1

GENERAL LINEAR MODELS PROCEDURE

CLASS LEVEL INFORMATION

CLASS	LEVELS	VALUES
POLYMER	4	4A MC PA PC
CUNC	10	1:100 1:1000 1:150J 1:2070 1:250 1:2500 1:3000 1:50 1:500 1:750

NUMBER OF OBSERVATIONS IN DATA SET = 40

ANALYSIS OF VARIANCE FOR POLYMERS AND MONOMERS

17:09 FRIDAY, AUGUST 27, 1982 2

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: FISH

X IN CLEAR WATER

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.
MODEL	12	10165.47500000	847.12291667	6.29	0.0001	0.736561	19.1569
ERROR	27	3635.80000000	134.65925926				
CORRECTED TOTAL	39	13801.27500000					
					11.60427761		60.57500000

SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F
POLYMER	3	6338.82500000	15.69	0.0001	3	6338.82500000	15.69	0.0001
CUNC	9	3826.65000000	3.16	0.0099	9	3826.65000000	3.16	0.0099

17:04 FRIDAY, AUGUST 27, 1982 3

DUNCAN MULTIPLE RANGE TESTS
POLYMER OR MONOMER=MA

WALLER-DUNCAN CR-TEST T TEST FOR VARIABLE: FISH

IN CLEAR WATER 452235001 F=9.18 T VALUE=2.2448 L50=18.5774
GRATITUDE OF DEPTH 452235001 F=9.18 T VALUE=2.2448 L50=18.5774
MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

GROUP	MEAN	N	POLYMER	CCNC	FISH
A	61.000000	1	MA	1:50	63
A	58.000000	1	MA	1:100	58
A	51.000000	1	MA	1:3000	53
A	50.000000	1	MA	1:250	50
A	50.000000	1	MA	1:2500	50
A	48.000000	1	MA	1:500	48
A	47.000000	1	MA	1:2000	47
A	45.000000	1	MA	1:750	45
A	45.000000	1	MA	1:1500	45
A	44.000000	1	MA	1:1000	44

DUNCAN MULTIPLE RANGE TESTS
POLYMER OR MONOMER=MA

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE: FISH

IN CLEAR WATER 452235001 F=9.18 T VALUE=2.2448 L50=18.5774
GRATITUDE OF DEPTH 452235001 F=9.18 T VALUE=2.2448 L50=18.5774
MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

GROUP	MEAN	N	POLYMER	CCNC	FISH
A	63.000000	1	MA	1:50	63
A	58.000000	1	MA	1:100	58
A	53.000000	1	MA	1:3000	53
A	50.000000	1	MA	1:250	50
A	50.000000	1	MA	1:2500	50
A	48.000000	1	MA	1:500	48
A	47.000000	1	MA	1:2000	47
A	45.000000	1	MA	1:750	45
A	45.000000	1	MA	1:1500	45
A	44.000000	1	MA	1:1000	44

DUNCAN MULTIPLE RANGE TESTS
POLYMER OR MONOMER=MC

17:09 FRIDAY, AUGUST 27, 1982 5

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE: FISH
% IN CLEAR WATER
K-RATIO=100 CF=18 MSE=35.81 F=4.18 T-VALUE=2.2448 LSD=18.5974
MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

GROUPING	MEAN	N	POLYMER	CLNC	FISH
A	54.500000	1	MC	1:1000	54.5
A	51.000000	1	MC	1:1500	51
A	50.000000	1	MC	1:750	50
A	50.000000	1	MC	1:3000	50
A	49.000000	1	MC	1:500	49
A	47.000000	1	MC	1:250	47
A	46.000000	1	MC	1:100	46
A	45.000000	1	MC	1:2000	45
A	45.000000	1	MC	1:2500	45
A	44.000000	1	MC	1:50	44

DUNCAN MULTIPLE RANGE TESTS
POLYMER OR MONOMER=MC

17:09 FRIDAY, AUGUST 27, 1982 6

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE: FISH
% IN CLEAR WATER
ALPHA=0.05 CF=18 MSE=35.81
MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

GROUPING	MEAN	N	POLYMER	CLNC	FISH
A	54.500000	1	MC	1:1000	54.5
A	51.000000	1	MC	1:1500	51
A	50.000000	1	MC	1:750	50
A	50.000000	1	MC	1:3000	50
A	49.000000	1	MC	1:500	49
A	47.000000	1	MC	1:250	47
A	46.000000	1	MC	1:100	46
A	45.000000	1	MC	1:2000	45
A	45.000000	1	MC	1:2500	45
A	44.000000	1	MC	1:50	44

DUNCAN MULTIPLE RANGE TESTS
POLYMER CR MONOMER=PA

17:09 FRIDAY, AUGUST 27, 1982

7

WALLER-DUNCAN RATIO T TEST FOR VARIABLE: FISH
% IN CLEAR WATER
K=13 MSE=35.81 F=4.18 T VALUE=2.2448 LSD=18.9974
MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

GROUPING	MEAN	N	POLYMER	CONC	FISH
A	90.000000	1	PA	1:50	90
A	88.000000	1	PA	1:100	88
A	85.000000	1	PA	1:250	85
A	73.000000	1	PA	1:500	73
C	68.000000	1	PA	1:750	68
D	61.000000	1	PA	1:1000	61
J	48.000000	1	PA	1:3000	48
J	45.000000	1	PA	1:2500	45
J	43.000000	1	PA	1:1500	43
J	42.000000	1	PA	1:2000	42

DUNCAN MULTIPLE RANGE TESTS
POLYMER CR MONOMER=PA

17:09 FRIDAY, AUGUST 27, 1982

8

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE: FISH
% IN CLEAR WATER
ALPHA=0.05 CF=18 MSE=35.81
MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

GROUPING	MEAN	N	POLYMER	CONC	FISH
A	90.000000	1	PA	1:50	90
A	88.000000	1	PA	1:100	88
A	85.000000	1	PA	1:250	85
A	73.000000	1	PA	1:500	73
C	68.000000	1	PA	1:750	68
D	61.000000	1	PA	1:1000	61
J	48.000000	1	PA	1:3000	48
J	45.000000	1	PA	1:2500	45
J	43.000000	1	PA	1:1500	43
J	42.000000	1	PA	1:2000	42

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DUNCAN MULTIPLE RANGE TESTS
POLYMER GR MONOMER=PC

17:09 FRIDAY, AUGUST 27, 1982

9

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE: FISH
% IN CLEAR WATER
K-RATIO=100 DF=19 MSE=35.81 F=4.14 T VALUE=2.2448 LSD=12.5974
MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

GROUPING	MEAN	N	POLYMER	CONC	FISH
A	99.000000	1	PC	1:50	99
A	98.500000	1	PC	1:100	98.5
A	98.000000	1	PC	1:250	98
A	97.000000	1	PC	1:500	97
A	95.000000	1	PC	1:750	95
C	78.000000	1	PC	1:1000	78
C	66.000000	1	PC	1:1500	66
C	58.000000	1	PC	1:2000	58
C	54.000000	1	PC	1:2500	54
C	52.000000	1	PC	1:3000	52

DUNCAN MULTIPLE RANGE TESTS
POLYMER GR MONOMER=PC

17:09 FRIDAY, AUGUST 27, 1982

10

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE: FISH
% IN CLEAR WATER
ALPHA=0.05 CF=18 MSE=35.81
MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

GROUPING	MEAN	N	POLYMER	CONC	FISH
A	99.000000	1	PC	1:50	99
A	98.500000	1	PC	1:100	98.5
A	98.000000	1	PC	1:250	98
A	97.000000	1	PC	1:500	97
A	95.000000	1	PC	1:750	95
C	78.000000	1	PC	1:1000	78
C	66.000000	1	PC	1:1500	66
C	58.000000	1	PC	1:2000	58
C	54.000000	1	PC	1:2500	54
C	52.000000	1	PC	1:3000	52

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C3 - ANOVA 30 AVOIDANCE REACTION OF POLYMERS

ANALYSIS OF VARIANCE FOR POLYMERS AND MONOMERS
GENERAL LINEAR MODELS PROCEDURE
CLASS LEVEL INFORMATION

17:10 FRIDAY, AUGUST 27, 1982

CLASS	LEVELS	VALUES
POLYMON	2	PA PC
CONC	10	1:100 1:1000 1:1500 1:2000 1:250 1:2500 1:3000 1:50 1:500 1:750

NUMBER OF OBSERVATIONS IN DATA SET = 20

ANALYSIS OF VARIANCE FOR POLYMERS AND MONOMERS
GENERAL LINEAR MODELS PROCEDURE

17:10 FRIDAY, AUGUST 27, 1982

DEPENDENT VARIABLE: FISH

% IN CLEAR WATER

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.
MODEL	10	7930.82500000	793.08250000	27.96	0.0001	0.968812	7.4050
ERROR	9	255.31250000	28.36805556				
CORRECTED TOTAL	19	8186.13750000					

SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F
POLYMON	1	1162.81250000	40.99	0.0001	1	1162.81250000	40.99	0.0001
CONC	9	6768.01250000	26.51	0.0001	9	6768.01250000	26.51	0.0001

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DUNCAN MULTIPLE RANGE TESTS
POLYMER OR MONOMER=PA

17:10 FRIDAY, AUGUST 27, 1982

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE: FISH
% IN CLEAR WATER
K-RATIO=100 DF=18 MSE=35.81 F=4.18 T-VALUE=2.2448 LSD=18.9974
MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

GROUPING	MEAN	N	POLYMER	CONC	FISH
AAAAA	90.000000	1	PA	1:50	90
AAAAA	88.000000	1	PA	1:100	88
AAAAA	85.000000	1	PA	1:250	85
AAAAA	73.000000	1	PA	1:500	73
AAAAA	68.000000	1	PA	1:750	68
AAAAA	61.000000	1	PA	1:1000	61
AAAAA	48.000000	1	PA	1:3000	48
AAAAA	45.000000	1	PA	1:2500	45
AAAAA	43.000000	1	PA	1:1500	43
AAAAA	42.000000	1	PA	1:2000	42

DUNCAN MULTIPLE RANGE TESTS
POLYMER OR MONOMER=PA

17:10 FRIDAY, AUGUST 27, 1982

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE: FISH
% IN CLEAR WATER
ALPHA=0.05 DF=18 MSE=35.81
MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

GROUPING	MEAN	N	POLYMER	CONC	FISH
AAAAA	90.000000	1	PA	1:50	90
AAAAA	88.000000	1	PA	1:100	88
AAAAA	85.000000	1	PA	1:250	85
AAAAA	73.000000	1	PA	1:500	73
AAAAA	68.000000	1	PA	1:750	68
AAAAA	61.000000	1	PA	1:1000	61
AAAAA	48.000000	1	PA	1:3000	48
AAAAA	45.000000	1	PA	1:2500	45
AAAAA	43.000000	1	PA	1:1500	43
AAAAA	42.000000	1	PA	1:2000	42

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DUNCAN MULTIPLE RANGE TESTS
POLYMER CR MONOMER=PC

17:10 FRIDAY, AUGUST 27, 1982

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE: FISH
% IN CLEAR WATER
K-RATIO=100 DF=18 MSE=35.81 F=4.19 T-VALUE=2.2442 LSD=18.9974
MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

GROUPING	MEAN	N	POLYMER	CR	FISH
A	99.000000	1	PC	1:50	99
A	98.500000	1	PC	1:100	98.5
A	98.000000	1	PC	1:250	98
A	97.000000	1	PC	1:500	97
A	95.000000	1	PC	1:750	95
C	78.000000	1	PC	1:1000	78
C	66.000000	1	PC	1:1500	66
C	58.000000	1	PC	1:2000	58
C	54.000000	1	PC	1:2500	54
C	52.000000	1	PC	1:3000	52

DUNCAN MULTIPLE RANGE TESTS
POLYMER CR MONOMER=PC

17:10 FRIDAY, AUGUST 27, 1982

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE: FISH
% IN CLEAR WATER
ALPHA=0.05 DF=18 MSE=35.81
MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

GROUPING	MEAN	N	POLYMER	CR	FISH
A	99.000000	1	PC	1:50	99
A	98.500000	1	PC	1:100	98.5
A	98.000000	1	PC	1:250	98
A	97.000000	1	PC	1:500	97
A	95.000000	1	PC	1:750	95
C	78.000000	1	PC	1:1000	78
C	66.000000	1	PC	1:1500	66
C	58.000000	1	PC	1:2000	58
C	54.000000	1	PC	1:2500	54
C	52.000000	1	PC	1:3000	52

3 - ANOVA 31 AVOIDANCE REACTION OF MONOMERS

ANALYSIS OF VARIANCE FOR POLYMERS AND MONOMERS

17:11 FRIDAY, AUGUST 27, 1982

GENERAL LINEAR MODELS PROCEDURE

CLASS LEVEL INFORMATION

CLASS	LIVELS	VALUES
POLYMON	2	MA MC
CONC	10	1:100 1:1000 1:1500 1:2000 1:2500 1:3000 1:3500 1:4000 1:500 1:750

NUMBER OF OBSERVATIONS IN DATA SET = 20

ANALYSIS OF VARIANCE FOR POLYMERS AND MONOMERS

17:11 FRIDAY, AUGUST 27, 1982

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: FISH

% IN CLEAR WATER

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.
MODEL	10	123.2250000	12.32250000	0.33	0.9517	0.266584	12.4681
ERROR	9	339.0125000	37.66805556		STD DEV		FISH MEAN
CORRECTED TOTAL	19	462.2375000			6.13743070		49.22500000

SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F
POLYMON	1	23.11250000	0.61	0.4536	1	23.11250000	0.61	0.4536
CONC	9	100.11250000	0.30	0.9582	9	100.11250000	0.30	0.9582

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DUNCAN MULTIPLE RANGE TESTS
POLYMER OR MONOMER=MA

17:11 FRIDAY, AUGUST 27, 1982

WALLER-JUNCK K-RATIO T TEST FOR VARIABLE: FISH

% IN CLEAR WATER
K-RATIO=100 DF=18 MSE=35.81 F=4.18 T-VALUE=2.2448 LSD=12.9774
MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

GROUPING	MEAN	N	POLYMER	CONC	FISH
A	63.000000	1	MA	1:50	63
A	58.000000	1	MA	1:100	58
A	53.000000	1	MA	1:3000	53
A	50.000000	1	MA	1:250	50
A	50.000000	1	MA	1:2500	50
A	48.000000	1	MA	1:500	48
A	47.000000	1	MA	1:2000	47
A	45.000000	1	MA	1:750	45
A	45.000000	1	MA	1:1500	45
A	44.000000	1	MA	1:1000	44

DUNCAN MULTIPLE RANGE TESTS
POLYMER OR MONOMER=MA

17:11 FRIDAY, AUGUST 27, 1982

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE: FISH

% IN CLEAR WATER
ALPHA=0.05 DF=18 MSE=35.81
MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

GROUPING	MEAN	N	POLYMER	CONC	FISH
A	63.000000	1	MA	1:50	63
A	58.000000	1	MA	1:100	58
A	53.000000	1	MA	1:3000	53
A	50.000000	1	MA	1:250	50
A	50.000000	1	MA	1:2500	50
A	48.000000	1	MA	1:500	48
A	47.000000	1	MA	1:2000	47
A	45.000000	1	MA	1:750	45
A	45.000000	1	MA	1:1500	45
A	44.000000	1	MA	1:1000	44

DUNCAN MULTIPLE RANGE TESTS
POLYMER OR MONOMER=MC

17:11 FRIDAY, AUGUST 27, 1982

WALLER-DUNCAN K-RATIO T TEST FOR VARIABLE: FISH

% IN CLEAR WATER

KRATIO=100 CF=18 MSE=35.81 F=4.18 T VALUE=2.2448 LSD=12.5974
MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

GROUPING	MEAN	N	POLMON	CCNC	FISH
A	54.500000	1	MC	1:1000	54.5
A	51.000000	1	MC	1:1500	51
A	50.000000	1	MC	1:750	50
A	50.000000	1	MC	1:3000	50
A	49.000000	1	MC	1:500	49
A	47.000000	1	MC	1:250	47
A	46.000000	1	MC	1:100	46
A	45.000000	1	MC	1:2000	45
A	45.000000	1	MC	1:2500	45
A	44.000000	1	MC	1:50	44

DUNCAN MULTIPLE RANGE TESTS
POLYMER OR MONOMER=MC

17:11 FRIDAY, AUGUST 27, 1982

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE: FISH

% IN CLEAR WATER

ALPHA=0.05 CF=18 MSE=35.81

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

GROUPING	MEAN	N	POLMON	CCNC	FISH
A	54.500000	1	MC	1:1000	54.5
A	51.000000	1	MC	1:1500	51
A	50.000000	1	MC	1:750	50
A	50.000000	1	MC	1:3000	50
A	49.000000	1	MC	1:500	49
A	47.000000	1	MC	1:250	47
A	46.000000	1	MC	1:100	46
A	45.000000	1	MC	1:2000	45
A	45.000000	1	MC	1:2500	45
A	44.000000	1	MC	1:50	44

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APPENDIX D

"EFFLUENT LIMITS"

- D1. Canadian Guidelines for Plating Industry
Effluent Discharges
- D2. American Guidelines for Plating Industry
Effluent Discharges.

D1 Canadian guidelines for plating industry effluents
(Canadian Gazette, Part I - November 5, 1977)

SCHEDULE I.

Objectives for substances

Item	Substance	Max. total concentration in mg/l
1.	Total Suspended Matter	30
2.	Cadmium	1.5
3.	Chromium (Total)	1.0
4.	Copper	1.0
5.	Lead	1.5
6.	Zinc	2.0
7.	Nickel	2.0
8.	Cyanide (oxidizable)	0.1
9.	Cyanide (Total)	3.0

D2 American guidelines for plating industry effluents

**SUBPART A—ELECTROPLATING OF
COMMON METALS SUBCATEGORY****§413.10 Applicability: Description of the Electroplating
of Common Metals Subcategory**

The provisions of this subpart apply to dischargers of pollutants in process wastewaters resulting from the process in which a ferrous or nonferrous basis

material is electroplated with copper, nickel, chromium, zinc, tin, lead, cadmium, iron, aluminum, or any combination thereof.

§413.11 Specialized Definitions

For the purpose of this subpart:

(a) The term "sq m" ["sq ft"] shall mean the area plated expressed in square meters [square feet].

(b) The term "operation" shall mean any step in the electroplating process in which a metal is electrodeposited on a basis material and which is followed by a rinse; this includes the related operations of alkaline cleaning, acid pickle, stripping, and coloring when each operation is followed by a rinse.

§413.12 [Reserved]**§413.14 Pretreatment Standards for Existing Sources**

Except as provided in 40 CFR 403.7 and 40 CFR 403.13, any existing source subject to this subpart which introduces pollutants into a publicly owned treatment works must comply with 40 CFR Part 403 and this subpart shall augment the use of process wastewater or otherwise dilute the wastewater as a partial or total substitute for adequate treatment to achieved compliance with these standards

(b) For a source discharging less than 38,000 liters (10,000 gal.) per calendar day of electroplating process wastewater the following limitations shall apply:

**Subpart A. Common Metals Facilities Discharging Less Than 38,000 Liters per
Day PSES Limitations (mg/l)**

Pollutant or Pollutant Property	Maximum for Any 1 Day	Average of Daily Values for a Consecutive Monitoring Days Shall Not Exceed
CN, A	5.0	2.7
Pb	.6	.4
Cd	1.2	.7

(c) For plants discharging 38,000 liters (10,000 gal) or more per calendar day of electroplating process wastewater the following limitations shall apply:

**Subpart A. Common Metals Facilities Discharging 38,000 Liters or More per Day
PSES Limitations (mg/l)**

Pollutant or Pollutant Property	Maximum for Any 1 Day	Average of Daily Values for 4 Consecutive Monitoring Days Shall Not Exceed
CN, T	1.9	1.0
Cu	4.5	2.7
Ni	4.1	2.6
Cr	7.0	4.0
Zn	4.2	2.6
Pb	.6	.4
Cd	1.2	.7
Total Metals	10.5	6.8

d) Alternatively, the following mass-based standards are equivalent to and may be applied in place of those limitations specified under paragraph (c) of this section upon prior agreement between a source subject to these standards and the publicly owned treatment works receiving such regulated wastes:

**Subpart A. Common Metals Facilities Discharging 38,000 Liters or More per Day
PSES Limitations (mg/sq m-Operation)**

Pollutant or Pollutant Property	Maximum for Any 1 Day	Average of Daily Values for 4 Consecutive Monitoring Days Shall Not Exceed
CN, T	74	39
Cu	176	105
Ni	160	100
Cr	273	156
Zn	164	102
Pp	23	16
Cd	47	29
Total Metals	410	267

(e) For wastewater sources regulated under paragraph (c) of this section, the following optional control program may be elected by the source introducing treated process wastewater into a publicly owned treatment works with the concurrence of the control authority. These optional pollutant parameters are not eligible for allowance for removal achieved by the publicly owned treatment works under 40 CFR 403.7. In the absence of strong chelating agents, after reduction of hexavalent chromium wastes, and after neutralization using calcium oxide (or hydroxide) the following limitations shall apply:

**Subpart A. Common Metals Facilities Discharging 38,000 Liters or More per Day
PSES Limitations (mg/l)**

Pollutant or Pollutant Property	Maximum for Any 1 Day	Average of Daily Values for 4 Consecutive Monitoring Days Shall Not Exceed
CN, T	1.9	1.0
Pb	.6	.4
Cd	1.2	.7
TSS	20.0	13.4
pH	1	1

¹Within the range 7.5 to 10.0.

SUBPART A—ELECTROPLATING OF COMMON METALS SUBCATEGORY

§413.10 Applicability: Description of the Electroplating of Common Metals Subcategory

The provisions of this subpart apply to discharges of pollutants in process wastewaters resulting from the process in which a ferrous or nonferrous basis material is electroplated with copper, nickel, chromium, zinc, tin, lead, cadmium, iron, aluminum, or any combination thereof.

§413.11 Specialized Definitions

For the purpose of this subpart:

(a) The term "sq m" ("sq ft") shall mean the area plated expressed in square meters (square feet).

(b) The term "operation" shall mean any step in the electroplating process in which a metal is electrodeposited on a basis material and which is followed by a rinse; this includes the related operations of alkaline cleaning, acid pickle, stripping, and coloring when each operation is followed by a rinse.

§413.12 Effluent Limitations Guidelines Representing the Degree of Effluent Reduction Attainable by the Application of the Best Practicable Control Technology Currently Available

In establishing the limitations set forth in this section, EPA took into account all information it was able to collect, develop and solicit with respect to factors (such as age and size of plant, raw materials, manufacturing processes, products produced, treatment technology available, energy requirements and costs) which can effect the industry subcategorization and effluent levels established. It is, however, possible that data which would affect these limitations have not been available and, as a result, these limitations should be adjusted for certain plants in this industry. An individual discharger or other interested person may submit evidence to the Regional Administrator (or to the State, if the State has the authority to issue NPDES permits) that factors relating to the equipment or facilities involved, the process applied, or other such factors related to such dischargers are fundamentally different from the factors considered in the establishment of the guidelines. On the basis of such evidence or other available information, the Regional Administrator (or the State) will make a written finding that such factors are or are not fundamen-

tally different for that facility compared to those specified in the Development Document. If such fundamentally different factors are found to exist, the Regional Administrator or the State shall establish for the discharger effluent limitations in the NPDES permit either more or less stringent than the limitations established herein, to the extent dictated by such fundamentally different factors. Such limitations must be approved by the Administrator of the Environmental Protection Agency. The Administrator may approve or disapprove such limitations, specify other limitations, or initiate proceedings to revise these regulations.

Effluent Characteristic	Effluent Limitations	
	Maximum for Any 1 Day	Average of Daily Values for 30 Consecutive Days Shall Not Exceed
	(Metric units) Milligrams per Square Meters per Operation	
Copper	160	80
Nickel	160	80
Cr, Total	160	80
Cr, VI	16	8
Zinc	160	80
CN, Total	160	80
CN, A	16	8
Fluoride	6400	3200
Cadmium	96	48
Lead	160	80
Iron	320	160
Tin	320	160
Phosphorus	320	160
TSS	6400	3200
pH	Within the Range of 6.0 to 9.5	
	(English units) Pounds per Million Square Feet per Operation	
Copper	32.7	16.4
Nickel	32.7	16.4
Cr, Total	32.7	16.4
Cr, VI	3.3	1.6
Zinc	32.7	16.4
CN, Total	32.7	16.4
CN, A	3.3	1.6
Fluoride	1308	654
Cadmium	19.2	9.6
Lead	32.7	16.4
Iron	65.4	32.7
Tin	65.4	32.7
Phosphorus	65.4	32.7
TSS	1308	654
pH	Within the Range 6.0 to 9.5	

(a) The following limitations establish the quantity or quality of pollutants or pollutant properties, controlled by this section, which may be discharged by a point source subject to the provisions of this subpart after application of the best practicable control technology currently available:

(b) The post plating steps of chromating, phosphating and coloring, if followed by a rinse, may be included under the term "operation" for the purpose of calculating effluent discharges, providing such steps are an integral part of the plating line.

(c) Stripping, where followed by a rinse and conducted in conjunction with electroplating for the purpose of salvaging improperly plated parts, may be included under the term "operations" for the purpose of calculating effluent discharges.

(d) Electroless plating on non-metallic materials for the purpose of providing a conductive surface on the basis material, preceding the actual electroplating step, forming an integral step in the plating line and followed by a rinse may be included under the term "operation" for the purpose of calculating effluent discharges.

(e) For any point source subject to such effluent limitations with a total employment of less than 11 persons, with a discharge from the establishment of waste water generated from the metal finishing process of less than 7,800 liters per hour (2,061 gallons per hour) and with a production rate of less than 4.9 sq m per hour per employee (52.7 sq ft per hour per employee), the following limitations establish the quantity or quality of pollutants or pollutant properties, controlled by this section, which may be discharged:

Effluent Characteristic	Effluent Limitations	
	Maximum for Any 1 Day	Average of Daily Values for 30 Consecutive Days Shall Not Exceed
	(Metric units) Milligrams per Square Meters per Operation	
CN, A	16	8
CN, Total	160	80
Flow	Equalize	
pH	Within the range 6.0 to 9.0.	
	(English units) Pounds per Million Square Feet per Operation	
CN, A	3.3	1.6
CN, Total	32.7	16.4
Flow	Equalize	
pH	Within the range 6.0 to 9.0.	

(f) Pursuant to section 308 of the Act, a point sources subject to the provisions of this subpart shall maintain records of production expressed in sq m

or sq ft as defined in §413.11 for the purpose of determining compliance with the effluent limitations in §413.12(a). For the purpose of complying with the requirements of this paragraph, a discharger may establish a correlation between area plated and another parameter, such as ampere-hours used in plating.

Note.—At 41 FR 53018, Dec. 3, 1976, §413.12 was suspended indefinitely.

§413.14 Pretreatment Standards for Existing Sources

For the purpose of establishing pretreatment standards under section 307(b) of the Act for a source within the common metals subcategory, the provisions of Part 403 of this chapter shall apply. The following categorical pretreatment standards for an existing source within the common metals subcategory establish the concentration of pollutants or pollutant properties of effluent which may be introduced into a publicly owned treatment works by a source subject to the provisions of this subpart. After October 12, 1982:

(a) No User introducing wastewater pollutants into a publicly owned treatment works under the provisions of this subpart shall augment the use of process wastewater or otherwise dilute the wastewater as a partial or total substitute for adequate treatment to achieve compliance with this standard.

(b) For a source discharging less than 38,000 liters (10,000 gal) per calendar day of electroplating process wastewater the following limitations shall apply:

Pollutant or Pollutant Property	Pretreatment Standard (mg/l)	
	Maximum for Any 1 Day	Average of Daily Values for 30 Consecutive Monitoring Days Shall Not Exceed
CN, A	5.0	1.5
Pb	0.6	0.3
Cd	1.2	0.5

(c) For plants discharging 38,000 liters (10,000 gal) or more per calendar day of electroplating process wastewater the following limitations shall apply:

(d) Alternatively, the following mass-based standards are equivalent to and may be applied in place of those limitations specified under paragraph (c) of this section upon prior agreement between a source subject to these standards and the publicly owned treatment works receiving such regulated wastes:

Pollutant or Pollutant Property	Pretreatment Standard (mg/l)	
	Maximum for Any 1 Day	Average of Daily Values for 30 Consecutive Monitoring Days Shall Not Exceed
CN, T	0.8	
Cu	4.5	0.23
Ni	4.1	1.8
Cr	7.0	1.8
Zn	4.2	2.5
Pb	0.6	1.8
Cd	1.2	0.3
Total Metals	10.5	5.0

Pollutant or Pollutant Property	Pretreatment Standard (mg/l)	
	Maximum for Any 1 Day	Average of Daily Values for 30 Consecutive Monitoring Days Shall Not Exceed
CN, T	29	9
Cu	176	70
Ni	160	70
Cr	273	98
Zn	164	70
Pb	23	12
Cd	47	20
Total Metals	410	195

(e) For wastewater sources regulated under paragraph (c) of this section, the following optional control program may be elected, by the source introducing treated process wastewater into a publicly owned treatment works with the concurrence of the control authority. These optional pollutant parameters are not eligible for allowance for removal achieved by the publicly owned treatment works under 40 CFR 403.7. In the absence of strong chelating agents, after reduction of hexavalent chromium wastes, and after neutralization using calcium oxide (or hydroxide) the following limitations shall apply:

Pollutant or Pollutant Property	Pretreatment Standard (mg/l)	
	Maximum for Any 1 Day	Average of Daily Values for 30 Consecutive Monitoring Days Shall Not Exceed
CN, T	0.8	0.23
Pb	0.6	0.3
Cd	1.2	0.5
TSS	20.0	10.0
pH	Within the range 7.5 to 10.0.	

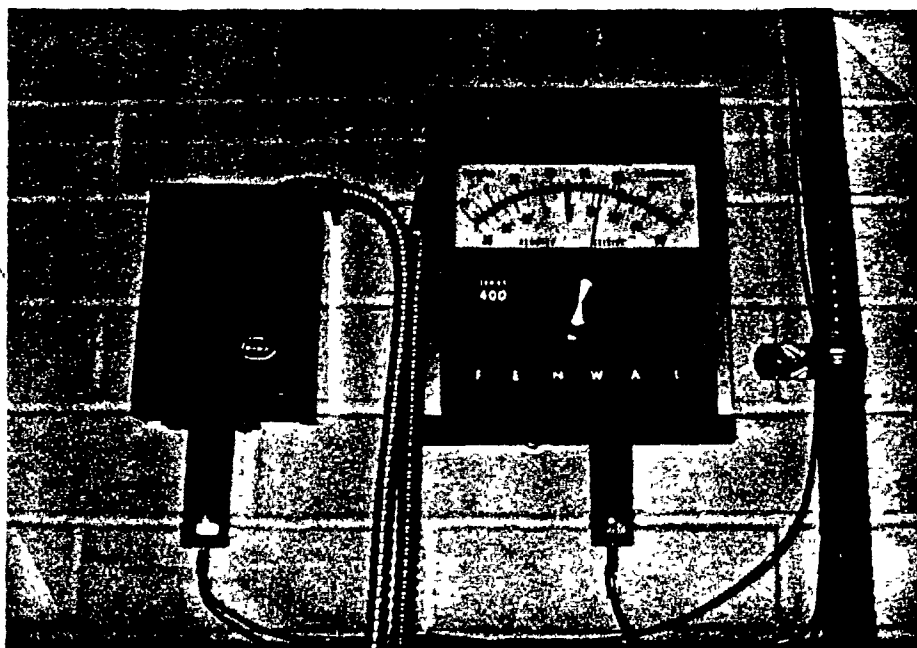
APPENDIX E

"PHOTOGRAPHS"

E1. Detailed Photographs of the Avoidance Apparatus used in this Study

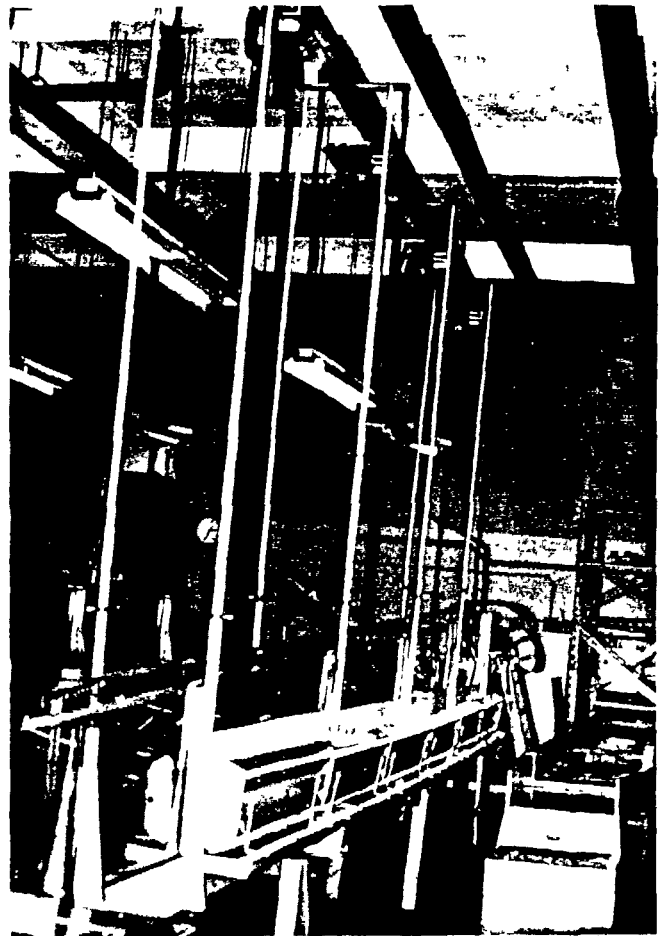
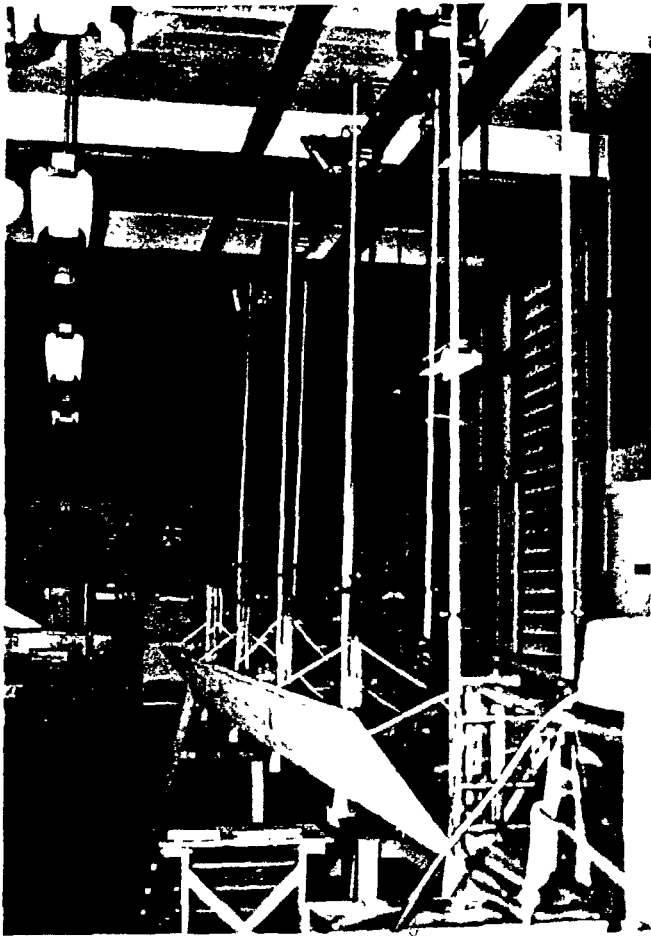
- A. Heating Control
- B. Flowmeters
- C. Channel
- D. Recorders
- E. Monitors

A. HEATING CONTROL



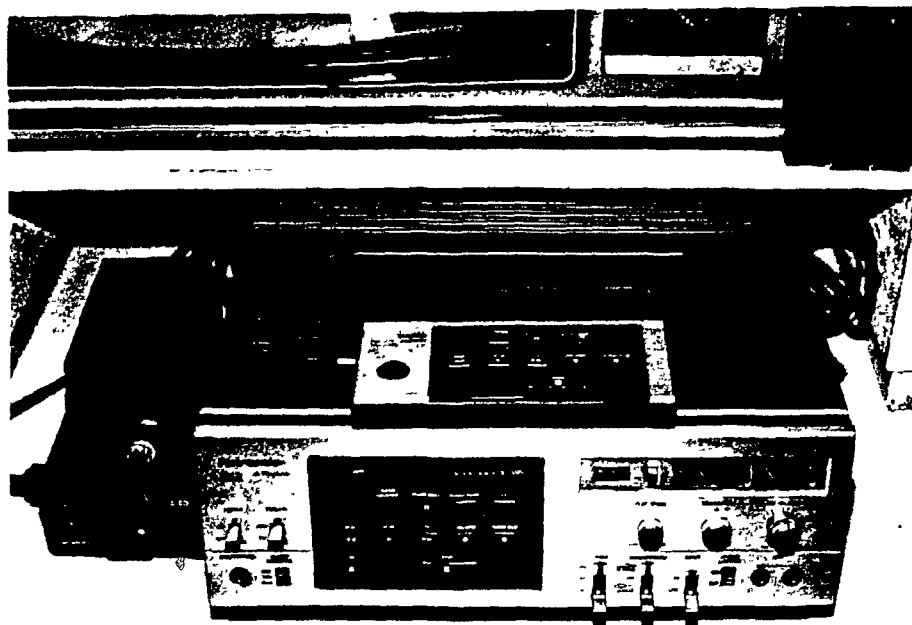
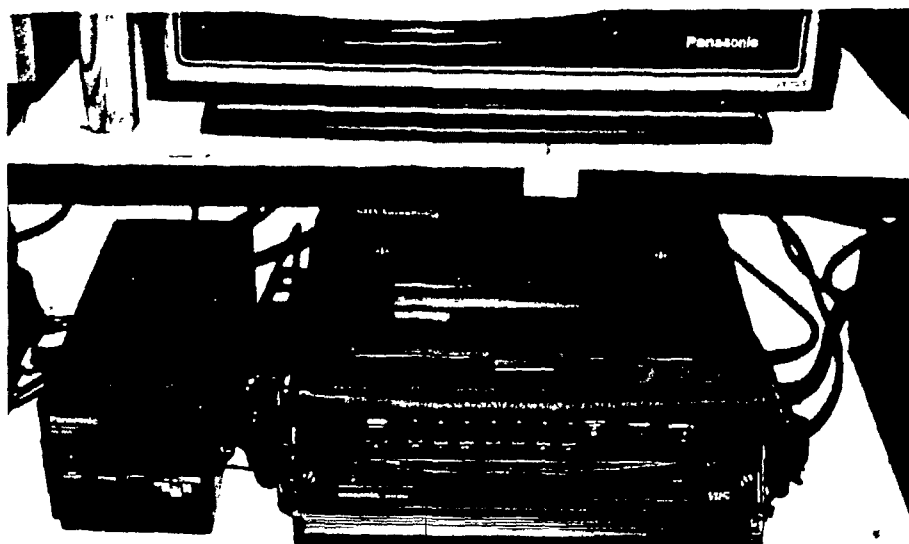
B. FLOWMETERS



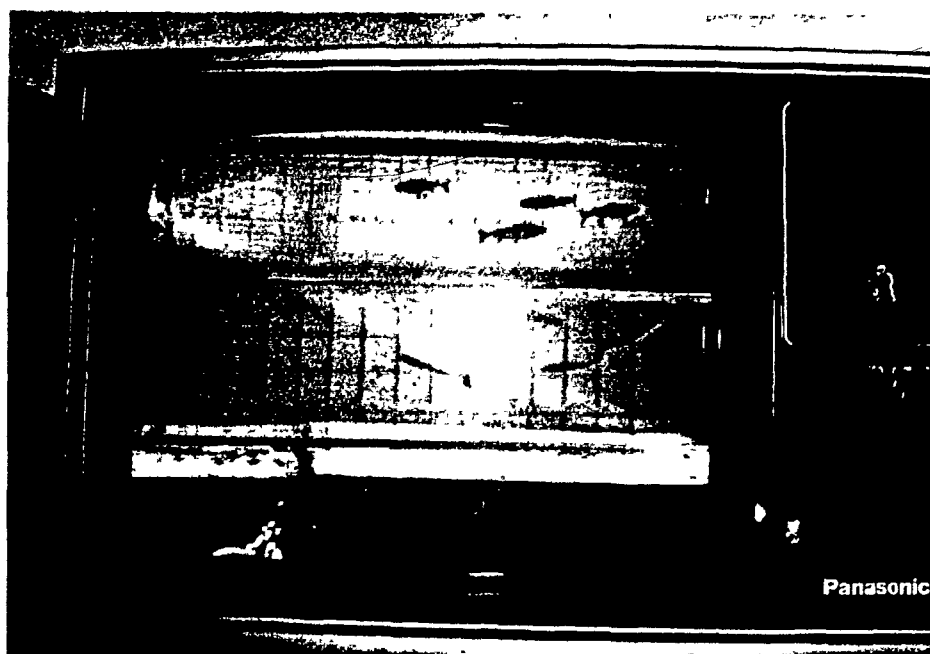
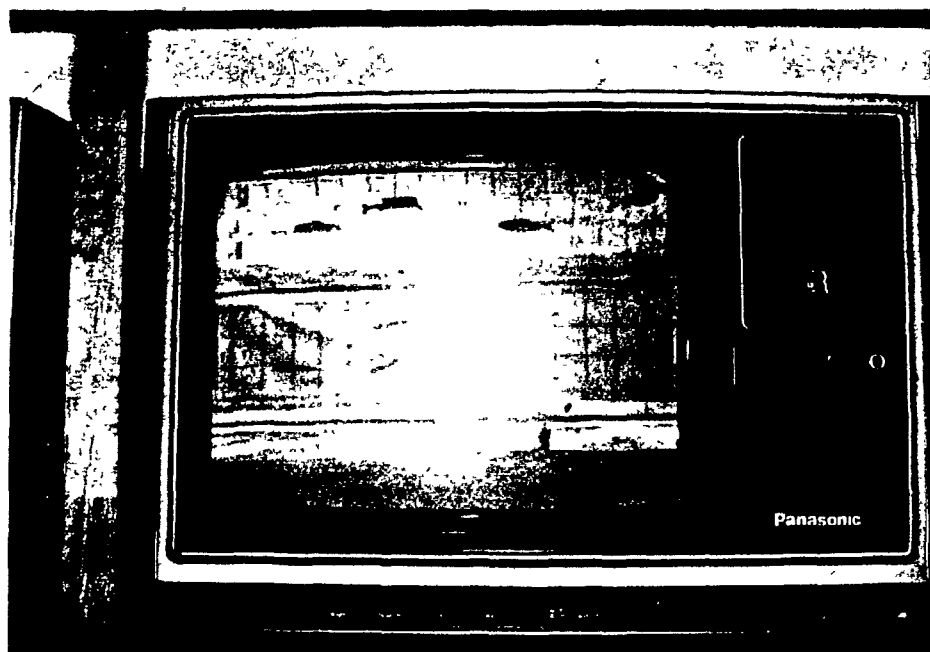


C. CHANNEL

D. RECORDERS



E. MONITORS



APPENDIX F
"TOXICITY INFORMATION"

- F1. List of Toxic Pollutants According to E.P.A.
- F2. Toxicity Classification of Major Effluent Components

F1 - List of Toxic Pollutants According to EPA

1. Acenaphtene
2. Acrolein
3. Acrylonitrile
4. Aldrin/Dieldrin
5. Antimony and Compounds
6. Arsenic and Compounds
7. Asbestos
8. Benzene
9. Bersidine
10. Beryllium and Compounds
11. Cadmium and Compounds
12. Carbon Tetrachloride
13. Chlordane (technical mixture and metabolites)
14. Chlorinated benzenes (other than dichlorobenzenes)
15. Chlorinated ethanes (including 1,2 dichloroethane, 1,1,1 - trichloroethane and hexachloroethane)
16. Chloroalkyl ethers (chloromethyl, chloroethyl, and mixed ethers)
17. Chlorinated naphthalene
18. Chlorinated phenols (other than those listed elsewhere; includes trichlorophenols and chlorinated cresols)
19. Chloroform
20. 2-Chlorophenol
21. Chromium and Compounds
22. Copper and Compounds
23. Cyanides
24. DDT and Metabolites
25. Dichlorobenzenes (1,2 - 1,3 - and 1,4 - dichlorobenzenes)

26. Dichlorobenzidine
27. Dichloroethylenes (1,1 - and 1,2 dichloroethylene)
28. 2,4 dichlorophenol
29. Dichloropropane and Dichloropropene
30. 2,4 Dimethylphenol
31. Dinitrotoluene
32. Diphenylhydrazine
33. Endosulfan and Metabolites
34. Endrin and Metabolites
35. Ethylbenzene
36. Fluoranthene
37. Haloethers
38. Halomethanes
39. Heptachlor and Metabolites
40. Hexachlorobutadiene
41. Hexachlorocyclohexane (all isomers)
42. Hexachlorocyclopentadiene
43. Isophorone /
44. Lead and Compounds
45. Mercury and Compounds
46. Naphthalene
47. Nickel and Compounds
48. Nitrobenzene
49. Nitrophenols (including 2,4 - dinitrophenol, dinitrocresol)
50. Nitrosamines
51. Pentachlorophenol
52. Phenol
53. Phthalate esters
54. Polychlorinated biphenyls (PCBs)
55. Polynuclear aromatic hydrocarbons
56. Selenium and compounds
57. Silver and Compounds
58. 2,3,7,8 tetrachlorodibenzo-p-dioxin
59. Tetrachloroethylene
60. Thallium and Compounds

- 61. Toluene
- 62. Toxaphene
- 63. Trichloroethylene
- 64. Vinyl chloride
- 65. Zinc and Compounds

F2.

A classification of the major effluent components, based on their lowest recorded lethal concentration, and their suggested receiving water criteria. All concentrations, except for pH, are in mg/L. (Clarke 1974)

Component	Lowest Recorded Toxic Concentration		Suggested Receiving Water Criteria ⁸			
	Lethal	Sublethal	Freshwater Aquatic Life Application Factor	Acceptable Limit	Marine Aquatic Life Application Factor	Hazardous above Minimal Risk below
EXTREMELY TOXIC (LOWEST LETHAL TOXICITY ≤ 0.01 mg/L)						
Polychlorinated Biphenyls	0.001	0.0009		0.000002		
Mercury	0.001	0.001		0.0002		
Silver	0.003	0.002			0.05	0.0001
Cadmium	0.005	0.0005		0.004, sw ^b 0.03, hw ^b	0.01	0.005 0.0002
Copper	0.008	0.0046	0.1		0.01	0.05 0.01
Chlorine (available)	0.01	0.001		0.003	0.1 ^c	0.01 ^c
Chromium (trivalent)	0.01	0.33		0.05 ^c	0.01 ^c	0.1 ^c 0.05 ^c
VERY HIGHLY TOXIC (LOWEST LETHAL TOXICITY 0.011-0.1 mg/L)						
Cyanide	0.05	0.003	0.05	0.005	0.1 ^c	0.01 ^c 0.005
Chromium (hexavalent)	0.05	0.1		0.05 ^c	0.01 ^c	0.1 ^c 0.05 ^c
Aluminum	0.07	0.32			0.01	1.5 0.2
Phenol	0.079	0.1	0.05	0.1		
Zinc	0.1	0.0056	0.005		0.01	0.1 0.02
Lead	0.1	0.03		0.03	0.02	0.05 0.005
Iron	0.1	0.52				0.3 0.05
HIGHLY TOXIC (LOWEST LETHAL TOXICITY 0.11 - 1.0 mg/L)						
Ammonia (unionized)	0.2		0.05	0.02	0.1	0.4 0.01
Oil and Grease - Crude Oil	0.3	0.01	0.05	No visible oil		No visible oil
Detergent - ABS ^c	0.4	0.37				
Detergent - LAS	0.6	0.027	0.05	0.2		
Nickel	0.8	0.03	0.02		0.02	0.1 0.002
MODERATELY HIGHLY TOXIC (LOWEST LETHAL TOXICITY 1.1 - 10 mg/L)						
Fluoride	2.3	1.5			0.1	1.5 0.5
EDC - ter	2.5	2.0				
Tin	6.0	0.35				
MODERATELY TOXIC (LOWEST LETHAL TOXICITY 10.1 - 100 mg/L)						
Barium Chloride (salt)	20.5 (13.5 ^d)	8.8 (5.8 ^d)			0.05 ^d	1.0 ^d 0.5 ^d
Oil and Grease - Gasoline	40		0.05	No visible oil		No visible oil
Carbon Dioxide	45	1-6				
Potassium Chloride (salt)	55 (29 ^d)	101 (53 ^d)				
Suspended Solids (inert)	82			25		
FAIRLY TOXIC (LOWEST LETHAL TOXICITY 101 - 1000 mg/L)						
Chloroform	107	20				
Sodium Sulphate (salt)	120 (39 ^d)					
Phosphate	126	0.01				0.05
Calcium Chloride (salt)	124 (52 ^d)	322 (116 ^d)				
Magnesium Chloride (salt)	476 (120 ^d)	324 (82 ^d)				
Trichloroethylene	660	55				
SLIGHTLY TOXIC (LOWEST LETHAL TOXICITY > 1000 mg/L)						
Sodium Chloride (salt)	1,270 (499 ^d)	1,730 (680 ^d)				
Oil and Grease - Kerosene	2,990		0.05	No visible oil		No visible oil
Total Dissolved Solids	5,000			No significant change		
Oil and Grease - No. 2 Cutting	14,500		0.05	No visible oil		No visible oil
pH						
Acid	6.2			6.5		6.5
Alkali	9.2			8.5		
				No change greater than 0.5		No change greater than 0.5

^a Application factor - the acceptable concentration for aquatic life can be estimated by multiplying the application factor by the 96 hr LC50 obtained from the most sensitive important species in the locality using the receiving water in question.

^b sw - soft water, hardness < 100 mg/L. hw - hard water, hardness > 100 mg/L.

^c values are for total chromium. ^d values for the metal ion.