AN ABSTRACT OF THE THESIS OF

JOHN ROBERT PALENSKY (Name) for the MASTER OF SCIENCE (Degree)

in FISHERIES (Major) presented on MAY 3, 1973 (Date)

Title: INFLUENCE OF 2, 4-DICHLOROPHENOL ON THE FLAVOR OF FISH

Abstract approved: Redacted for privacy

Dean L. Shumway

A laboratory investigation was performed to determine the influence of 2, 4-dichlorophenol on the flavor of fish. The study was conducted at the Oak Creek Fisheries Laboratory of the Department of Fisheries and Wildlife, Oregon State University.

The three experimental apparatus used in this study were designed to deliver 250 ml/min of contaminant solution to each of 14 exposure chambers. The dissolved oxygen concentration, pH, and water temperature could be controlled. At the completion of an experiment, test fish were evaluated for off-flavor by a panel of trained judges.

The flavor of rainbow trout, Salmo gairdneri, was impaired by exposure to 100 ppb of 2, 4-dichlorophenol for only 0. 25 hr. Trout exposed to 100 ppb required more than 33. 5 hr in clean water to lose the acquired off-flavor. Trout were exposed to 10 and 100 ppb of

2, 4-dichlorophenol at pH levels ranging from 6 to 9. The level of flavor-impairment in fish exposed at each concentration decreased as pH increased. Dissolved oxygen concentrations between 3. 9 and 10. 6 mg/l and weights of fish exposed per chamber from 133 to 1418 g had no effect on the degree of flavor-impairment of trout exposed to concentrations of 2, 4-dichlorophenol. Off-flavor levels obtained for trout exposed to 100 ppm of pyridine in combination with 1, 10, and 100 ppb of 2, 4-dichlorophenol suggested that the two compounds were interacting and that the flavor-imparting capacity of pyridine was being masked or lessened by the presence of 2, 4-dichlorophenol.

The estimated threshold concentrations of 2, 4-dichlorophenol for trout exposed for 96 hr at 15 C and for largemouth bass, Micropterus salmoides, and bluegill, Lepomis macrochirus, exposed for 48 hr at 20 C were determined as 1, 4, and 14 ppb, respectively.

Influence of 2, 4-Dichlorophenol on the Flavor of Fish

by

John Robert Palensky

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

June 1973

APPROVED:

Redacted for privacy

Associate Professor of Fisheries
in charge of major

Redacted for privacy

Head of Department of Fisheries and Wildlife

Redacted for privacy

Dean of Graduate School

Date thesis is presented May 3, 1973

Typed by Mary Jo Stratton for John Robert Palensky

ACKNOWLEDGEMENTS

I wish to express my sincere appreciation to the following people for their many contributions to this study and my graduate education.

To Mr. Dean L. Shumway, Associate Professor of Fisheries, Oregon State University, for his support, advice, and friendship throughout this study and helpful criticism during the preparation of this thesis.

To Mrs. Lois S. McGill, Professor of Food Science and Technology, Oregon State University, and her laboratory assistants, Mrs. Geraldine Stark and Mrs. Melba Carpenter, for their indispensable advice and help in preparing and evaluating the samples of fish.

To Messrs. Miles Potter, Lowell Moore, Steve Sasser, and Michael Wirsing who assisted with the conduct of experiments and tended the stock of experimental fish.

To the many students and staff members at Oregon State University who served as members of the panels that were used to evaluate the flavor of the samples of fish. Without the concentration and honest effort displayed by these people, this study could not have been conducted.

To my wife, Joan, for her help, encouragement, and patience.

This research was supported by Environmental Protection Agency Grant Number 18050 DDM.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INFLUENCE OF 2, 4-DICHLOROPHENOL ON THE FLAVOR OF FISH

INTRODUCTION

With industrial development and subsequent urbanization along our major waterways, our rivers and streams have become the prime vehicle for the dispersal and assimilation of man's wastes. While the assimilatory capacity is an important use of water, this use should not interfere with the quality of the fish stocks supported by these waters. Each year as man continues to increasingly tax the capacity of surface waters to handle his industrial and municipal wastes, the problem of insuring the viability of our fisheries becomes more urgent. Thus, water quality standards that will protect the quality and hence the value of these fisheries are needed.

The problem of taste and odor control in municipal water supplies and domestic and industrial wastes has been recognized for many years and extensive research undertaken to identify and abate those contaminants responsible. Baker (1961) discussed the current taste and odor control problem and outlined future research objectives. He made special reference to problems of taste and odor resulting from phenols, a group of organic compounds whose ability to impart objectionable tastes and odors to water supplies has been a matter of conjecture for nearly 40 years. Kinney (1961) states, "Fantastic is

the word to describe the role of phenols in pollution control. Few water constituents receive so much attention. Arguments, discourses, schools, studies, surveys, laws and regulations are directed towards it."

Although the value of many recreationally and commercially important species of fish has been reduced in recent years as a result of water-borne, fish-tainting substances, only a limited effort has been expended in identifying the sources of these substances. Contaminant sources that have been identified include paper mill effluents (Bandt, 1946; Fetterolf, 1964; Hasselrot, 1965; Shumway and Chadwick, 1971; and Tamura et al., 1954), oil refinery and associated petroleum industry wastes (Bandt, 1946; Fetterolf, 1964; Krishnaswami and Kupchanko, 1969; and Nitta et al., 1965), outboard motor exhausts (English et al., 1963), chemical-producing plants (Shumway, 1966), and waste waters from synthetic rubber factories and munitions plants (Bandt, 1946).

In only a few cases have workers identified and investigated the specific organic compounds responsible for off-flavor in fish. Those studies that have been conducted dealt primarily with various phenolic compounds. Böetius (1954) reported that oysters held at 1 ppb and 10 ppm of o-chlorophenol for about 3, 5 and 1 days, respectively, developed an objectionable taste. A minimum of 2 days at 1 ppm of o-chlorophenol was necessary to cause tainting in the flesh of eels.

Schulze (1961) found that 15 ppb of o-chlorophenol and 60 ppb of p-chlorophenol and m-chlorophenol caused a distinct change in the flavor of carp, Cyprinus carpio, while pure phenol in a near-toxic concentration (10 ppm) caused only a slight alteration of taste. Shumway (1966) showed that 2,4-dichlorophenol, the major constituent of a herbicide-producing plant's effluent, imparted an off-flavor to the flesh of coho salmon, Oncorhynchus kisutch, at concentrations near 1 ppb and that a concentration of 100 ppb of 2,4-dichlorophenol rendered the salmon nearly inedible.

Various factors may influence the capacity of phenols, and presumably most organic contaminants, to impart an off-flavor to the flesh of fish. Böetius (1954) suggested that differences in time required to produce an off-flavor in oysters and eels exposed to o-chlorophenol may be attributed to the different rates of water transport and to the natural inherent flavor differences between the two species. Böetius further suggested that the fat content of test animals may be important, owing to a strong tendency for phenols to be absorbed in fats.

The physical and chemical properties of water such as temperature, dissolved oxygen concentration, pH, and the presence of other compounds may be of great importance in determining the flavor-imparting capacity of phenols to fish. Mann (1962) demonstrated that even small amounts of detergents in the water increased the uptake of

phenols by fish. It has been known for some time that the odor of phenols in acid solutions is not nearly as intense as their odor in more basic solutions. In solutions of low pH, the dissociation of chlorophenols favors the formation of chlorophenates, which may be odorless (Schaafsma, 1935). The same relationship might also affect the ability of various phenols to impart an off-flavor to fish.

Unfortunately, there are very few studies reported in the literature which consider the influence of various water quality parameters on the uptake of phenols by fish. The objectives of this study were to determine under controlled laboratory conditions the rates of uptake and loss of a known flavor-causing chlorophenol, 2,4-dichlorophenol (2,4-DCP), and the influence of dissolved oxygen concentration and pH of the water on the flavor-imparting capacity of the compound to fish. My study was a part of a much larger investigation designed to determine the influence of a rather large number of organic compounds and effluents on the flavor of fish.

MATERIALS AND METHODS

Fish Facilities

Facilities for rearing and holding relatively large numbers of fish of several species were constructed for this study. Facilities for trout were located at the Averill Fisheries Laboratory approximately 1 mile east of Corvallis. The Averill site was selected because of the disease-free, year-round supply of well water with a nearly constant temperature of about 12 C.

Holding facilities for warmwater fish and temporary retention of trout were located at the Oak Creek Fisheries Laboratory northwest of Corvallis where the study was conducted.

Test Animals

Rainbow trout, Salmo gairdneri, largemouth bass, Micropterus salmoides, and bluegill, Lepomis macrochirus, were used as test fish in this study. Rainbow trout were the main test fish; bass and bluegill were used in only one experiment each.

During the initial few months of the study, rainbow trout of a suitable size were obtained from the Oregon Game Commission's Roaring River Hatchery located near Scio, Oregon, and transported to the Oak Creek Fisheries Laboratory in 55-gal. (208-liter) barrels lined with polyethylene bags. During transportation, water in the

barrels was kept cool by the addition of chlorine-free ice; the dissolved oxygen level was maintained near air saturation by aeration with compressed oxygen. Upon arrival at the laboratory the trout were placed either directly into the exposure chambers or into the temporary holding tanks.

Trout used in later tests were obtained as eyed eggs from the Trout Lodge Springs Hatchery in eastern Washington and were reared to the desired size at the Averill site. Both hatchery trout and Averill-reared trout were fed Oregon Moist Pellet, a food obtained from a local dealer. Although a few fish experienced a mild bacterial infection apparent on the dorsal and caudal fins, no major disease problems developed during the course of the study.

Largemouth bass used as test fish were seined from sloughs and ponds along the Willamette River. Bluegill were captured from local ponds by angling. Both species were held at the Oak Creek Fisheries Laboratory in the temporary retaining tanks until used. Food in the form of small live fish, angleworms, and Oregon Moist Pellet was provided the bass and bluegill.

Experimental Apparatus

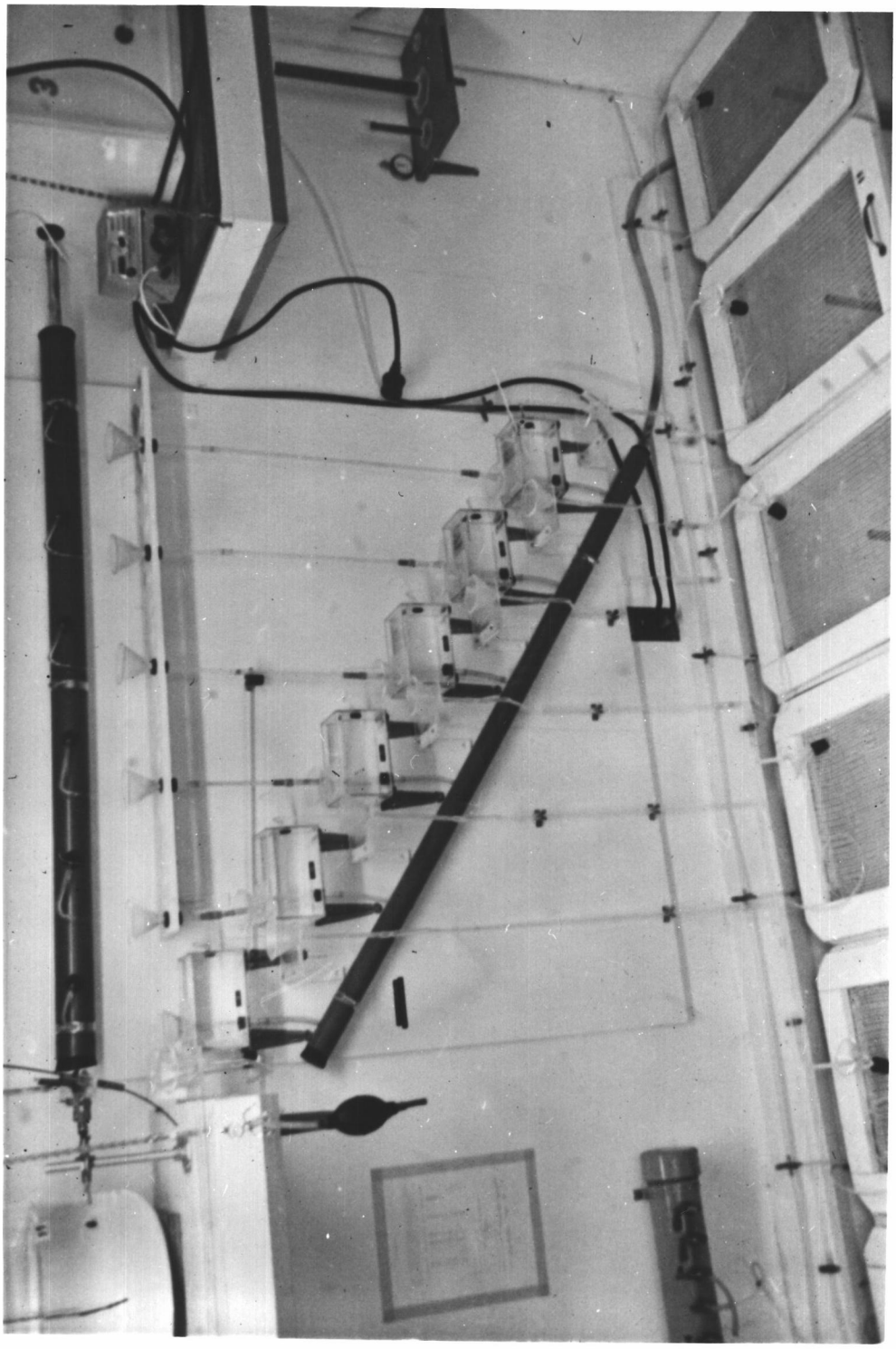
Three dilution apparatus, similar to one described by Chadwick et al. (1973), were built in a constant-temperature room at the Oak Creek Fisheries Laboratory. Each apparatus was designed to

continuously deliver 250 ml/min of water to each of a number of exposure chambers. The largest dilution apparatus delivered water to six exposure chambers and the other two sets supplied four chambers each. A photograph of one of the diluters is presented in Figure 1.

Each dilution apparatus consisted of four basic components; (1) a water-control box (constant-head box), (2) a toxicant introduction system, (3) the dilution portion (water manifold and mixing boxes), and (4) the exposure chambers. Well water (Appendix 1) from a storage tank flowed into a two-compartmented, wooden water-control box (Figure 2). The amount of water entering the water-control box was regulated by a plastic float valve located in the receiving compartment of the box. A small centrifugal pump connected the two compartments of the water-control box and pumped incoming water into the reservoir portion of the box. The excess water in the reservoir side flowed over a partition back to the receiving compartment, thus maintaining a constant water level in both the water-control box and the adjacent water manifold. The pump also served to remove excess dissolved gases and to agitate the water to insure a homogeneous water temperature in the water-control box. Water temperature was controlled at the water-control box by a thermoregulator unit attached to a 1500-watt, stainless-steel heating coil.

Water from the reservoir portion of the water-control box flowed to a distribution manifold made from a length of 2-in. (5.08 cm),

Figure 1. One of three dilution apparatus used to deliver contaminant solutions to exposure chambers.



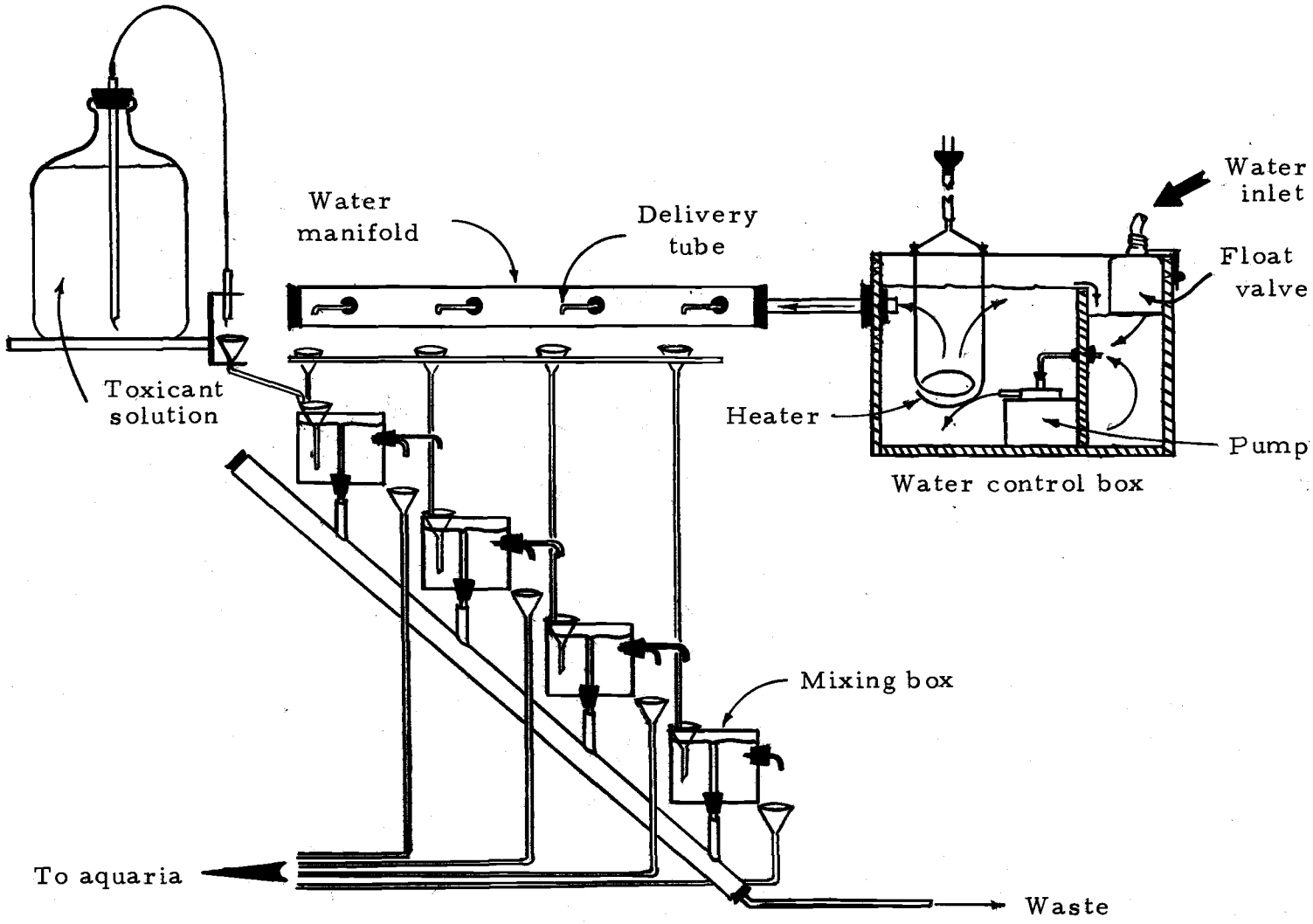


Figure 2. Schematic drawing of one of three dilution apparatus showing the water control box, toxicant introduction system, and the dilution portion of the apparatus.

non-toxic, plastic pipe stoppered at one end and fitted with adjustable delivery tubes to regulate the flow of water to the mixing boxes. Each mixing box, made of glass and cemented together with silicone rubber, was fitted with a constant-head overflow tube and two adjustable delivery tubes. The larger of the two delivery tubes discharged water to the exposure chamber and the other tube meted a small quantity of water into the next (lower) mixing box. The desired contaminant concentration was attained by adjusting the ratio of fresh water (from the water manifold) to contaminated water (from the preceding mixing box in the sequence) flowing into a mixing box. The lowest mixing box in the series received water from the water manifold only and was used as a control. Overflow water from the mixing boxes was discharged from the system.

Prepared stock solutions of 2, 4-DCP were introduced into the first mixing box from a Mariotte bottle (20-liter glass carboy). The rate of discharge of 2, 4-DCP solution from the Mariotte bottle was adjusted by raising or lowering the discharge orifice of the delivery tubing, thereby changing the effective head pressure. The concentration of 2, 4-DCP in the first mixing box was determined by the ratio of water from the water manifold to toxicant from the Mariotte bottle.

Water from the mixing boxes then flowed to 70-liter, fiberglass exposure chambers. Each chamber was fitted with a cover to prevent fish from jumping out and to reduce the light level, a centrally-located

stainless-steel standpipe for water-level control, a relief tube for removal of water samples, an air stone, and a mercury thermometer. Gas flowmeters were used to regulate the flow of compressed gas bubbled through each chamber.

Exposure

At the beginning of an experiment, stock solutions of 2, 4-DCP were prepared by adding appropriate amounts of reagent-grade 2, 4-DCP to distilled water. The mixture was then continuously stirred overnight to insure solution of the chemical. Early the following day, the temperature of the dilution water was raised to the desired level and the various water flows were set to attain the selected 2, 4-DCP concentrations. A flow of 250 ml/min was provided each exposure chamber.

The flow of contaminant from the Mariotte bottle was started about one-half hr before the standpipes were placed in the exposure chambers and the chambers permitted to fill. During that time, all water flows were again checked, and adjusted if necessary, to insure that proper dilution ratios were attained. Aeration within the exposure chambers was begun when the standpipes were secured and the chambers commenced filling. When the exposure chambers were completely full, test fish were placed in the chambers and the covers secured.

Water and toxicant flows were checked and recorded twice daily, once in the morning and again in the evening, throughout the duration of an experiment. Any flow that deviated more than 3 percent from the desired rate was adjusted. Water samples for the determination of dissolved oxygen concentration and pH were generally taken daily. In those experiments dealing with the influence of dissolved oxygen and pH, the dissolved oxygen concentration and pH were taken as often as necessary to insure that desired experimental conditions were met, usually three or four times each day.

At the end of the exposure period, the test fish were removed from the exposure chambers, weighed, and measured. The head, caudal fin, and entrails were removed and the samples of fish double-wrapped in plastic bags and coded. The samples of flesh were then either refrigerated and evaluated for off-flavor within 1 day or frozen for evaluation at a later date.

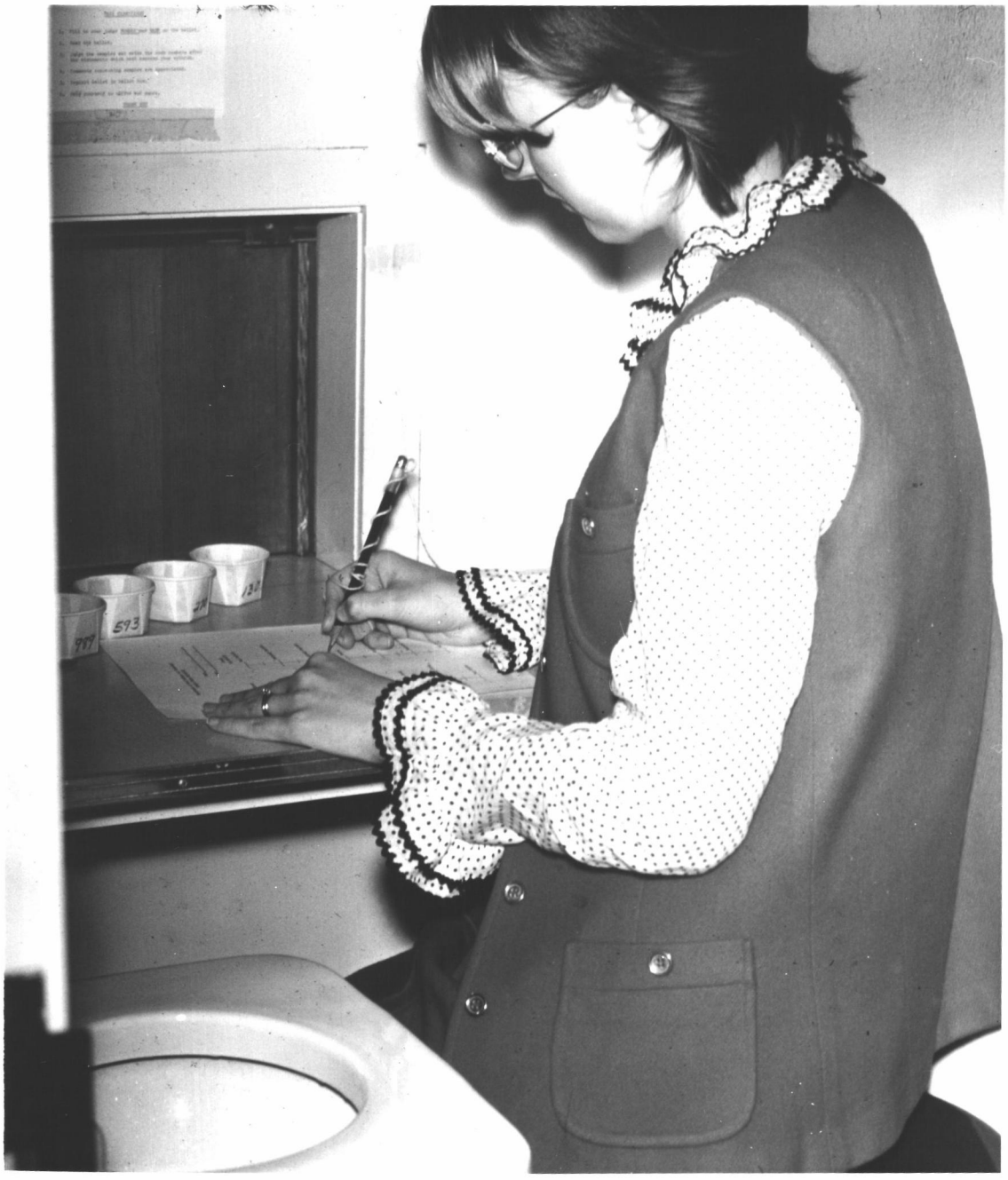
At the completion of an experiment, the mixing boxes, Mariotte bottles, and exposure chambers were washed with hot water and a mild laboratory soap and rinsed. The dilution system and Mariotte bottle were purged with ethyl alcohol or acetone to remove any 2, 4-DCP that might have sorbed to the glass. The water flows were again started and water allowed to flow through the system until the initiation of another experiment.

Organoleptic Evaluation

Test fish were prepared for organoleptic evaluation by personnel of the Sensory Evaluation Section of the Department of Food Science and Technology, Oregon State University. The Sensory Evaluation Section, under the direction of Mrs. Lois S. McGill, Professor of Food Science and Technology, also provided the facilities for the preparation and the evaluation of the samples of fish. Each sample of cleaned fish was wrapped in aluminum foil, placed in a pan, and cooked in an oven at approximately 210 C until done (about 30 to 40 min). No seasoning was added to the samples at any time.

After cooking, each sample of fish was removed from the oven, skinned and boned, the flesh of all fish within a sample lightly flaked and thoroughly mixed together, and a portion of flesh placed in each of a number of small, coded, paper cups. The number of cups was determined by the number of judges that would evaluate the samples of fish. The control, or uncontaminated sample of fish, was divided between coded cups and cups marked "reference." One cup from each group, including a cup from the group marked "reference," was placed on each of a number of small trays (one tray per judge). The tray of samples was then served immediately to a judge seated in an individual isolation booth (Figure 3). Each booth was illuminated by a red light used to eliminate possible bias due to differences in color between the samples.

Figure 3. Panel member (judge) seated in an isolation booth and evaluating samples of trout flesh.


977
593

The judges, all of whom had previous experience in evaluating the flavor of fish, were asked to smell the samples, and then to taste (masticate) and score the samples on a 7-point word-evaluation scale for intensity of off-flavor (Appendix 2). They were told that the sample marked "reference" contained flesh from the control fish. Judges were not required to taste samples with extremely intense or obnoxious odors. The word-evaluation scale for off-flavor shown in Appendix 2 was converted to a number scale of 0 to 6, with 0 representing the highest quality (no off-flavor), and 6 the lowest quality (extreme off-flavor).

In addition to evaluating the sample of flesh for off-flavor, the judges were asked to rate each sample for overall desirability. The 7-point hedonic scale shown in Appendix 2 was used. The ratings ranged from very desirable (0) to very undesirable (6). The results of the hedonic ratings, when compared with the results of the off-flavor ratings, were found less instructive and are not included in this paper.

Once the samples were evaluated, their ratings were compiled and the data treated statistically. A standard, two-way analysis of variance program (ANOVA) written for the CDC 3300 in FORTRAN language was used to test for experimental differences.

RESULTS

Rainbow trout were exposed to a control and to 2,4-DCP concentrations of 0.01, 0.1, 1, 10, and 100 ppb for 96 hr at 15 C. The mean off-flavor indices obtained for trout held at the above concentrations are 0.70, 1.25, 1.55, 1.75, 2.60, and 4.40, respectively. The results of this experiment are presented in Figure 4. The results indicate that a significant off-flavor was acquired by trout exposed to 2,4-DCP concentrations of 1 ppb and above, and that the intensity of the off-flavor significantly increased with each increase in exposure concentration above 1 ppb.

The estimated threshold concentration (ETC), defined as the lowest concentration of a material that significantly impairs the flavor of the flesh of exposed fish, was determined as illustrated in Figure 4. The mean off-flavor indices obtained were plotted against exposure concentration and a curve fitted by eye. The curve need not pass through the control point. After the curve was fitted to the data, a mean off-flavor index was determined for the flat or independent portion of the relationship and this value was than added to the least significant difference (LSD) value obtained by a two-way analysis of variance of the data. In Figure 4 the off-flavor index for the curve was 0.90; the $LSD_{.05}$ was 0.84. The sum of the two values, 1.74, was then located on the ordinate scale and a horizontal line drawn

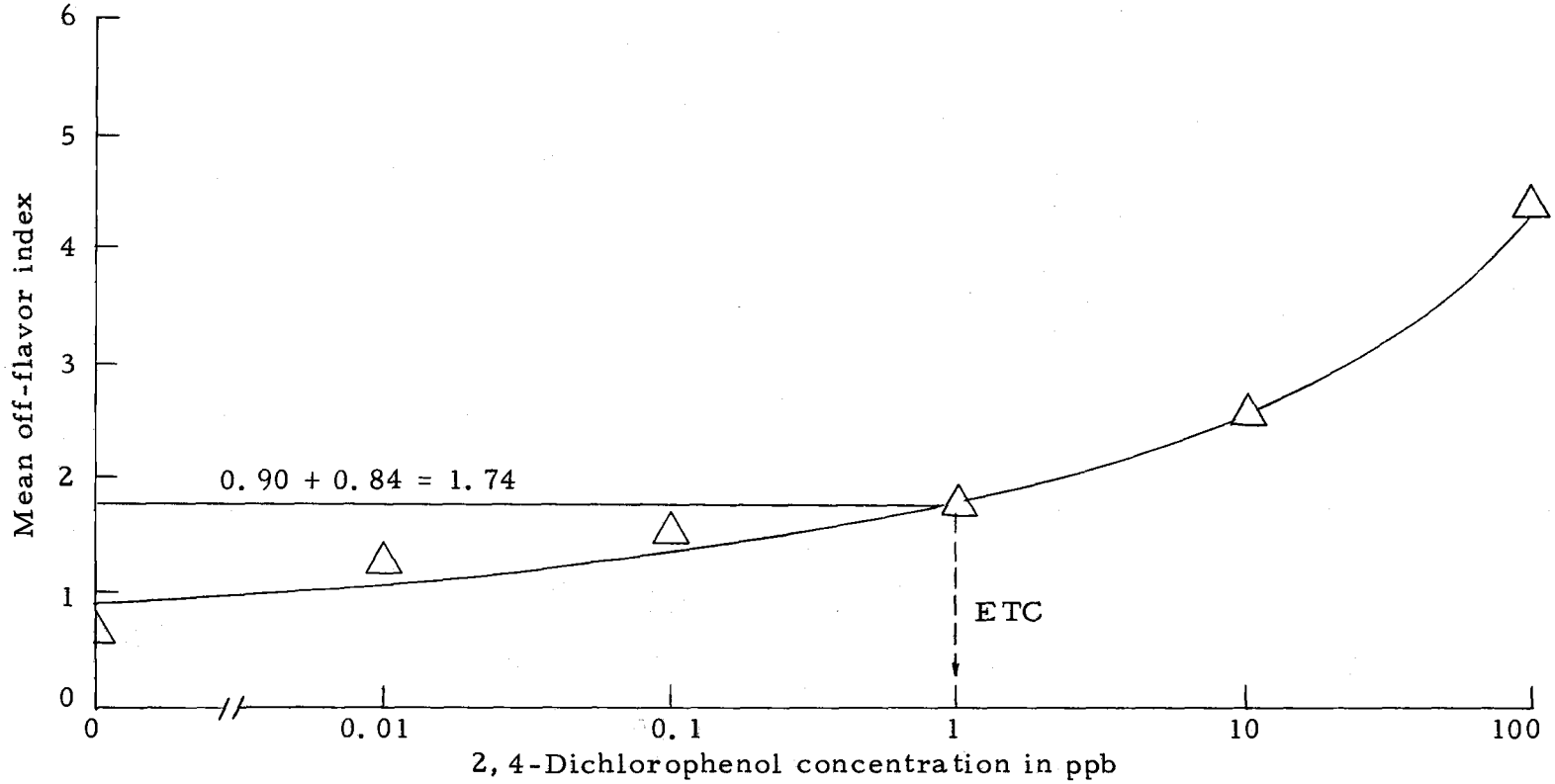


Figure 4. Relationship between 2, 4-dichlorophenol concentration and mean off-flavor index of rainbow trout exposed for 96 hr at 15 C.

across to the eye-fitted curve. At the point of intercept, the line was extended vertically to the abscissa in the manner shown in Figure 4 (dashed line). As may be seen in Figure 4, the ETC for 2, 4-DCP was determined to be 1 ppb.

In order to determine the rate of uptake or exposure duration required to produce maximum impairment of flavor, trout were exposed to 2, 4-DCP concentrations of 0. 1, 10, and 100 ppb for 0. 25, 1. 27, 6. 5, 33. 5, and 168 hr. The experimental conditions and results are presented in Table 1. The flavor of trout was unaffected by the length of exposure at 0. 1 and 10 ppb of 2, 4-DCP (Figure 5). Off-flavor was detected after exposure of only 0. 25 hr at the 100 ppb level, and maximum flavor-impairment was attained by the end of 1. 27 hr. Continued exposure beyond 1. 27 hr did not induce further change in the degree of flavor-impairment.

Trout were exposed to 2, 4-DCP concentrations of 1, 10, and 100 ppb for 24 hr, removed, and placed in chambers containing clean water where they were allowed to remain for 0. 25, 1. 27, 6. 5, 33. 5, and 168 hr. The experimental conditions and results of these tests are presented in Table 2. Samples of trout were not taken at clearing time zero and the values plotted on the Y-axis in Figure 6 were obtained from data presented in Figure 4.

The mean off-flavor index for trout exposed to 100 ppb of 2, 4-DCP and then held in fresh water decreased with time from an

Table 1. The experimental conditions and results of tests in which rainbow trout were exposed for various periods of time to 0.01, 10, and 100 ppb of 2,4-dichlorophenol at 15 C.

Experiment no. and panel size	Exposure concentration (ppb)	Exposure period (hr)	Mean off-flavor index (0-6)	LSD $_{.05}$ [1/]	Standard error of the mean	Fish per chamber	
						grams	number
U-1	0	0.25	1.58	n. s.	0.24	255	1
10 Judges	0.1	0.25	1.03		0.14	246	1
	0.1	1.27	1.28		0.18	440	2
	0.1	6.5	1.25		0.16	212	1
	0.1	33.5	1.53		0.27	453	2
	0.1	168	1.45		0.22	239	1
U-2	0	1.27	1.38	n. s.	0.19	393	2
10 Judges	10	0.25	2.20		0.22	193	1
	10	1.27	1.80		0.23	367	2
	10	6.5	1.95		0.17	213	1
	10	33.5	2.20		0.29	368	2
	10	168	1.85		0.21	188	1
U-3	0	168	1.33	0.62	0.22	222	1
10 Judges	100	0.25	2.65	*	0.21	192	1
	100	1.27	3.83	*	0.29	446	2
	100	6.5	3.93	*	0.18	179	1
	100	33.5	3.30	*	0.23	381	2
	100	168	3.50	*	0.28	183	1

[1/] The least significant difference at P = 0.05 based on a two-way analysis of variance. An asterisk (*) indicates a statistically significant change in flavor from that of the control sample.

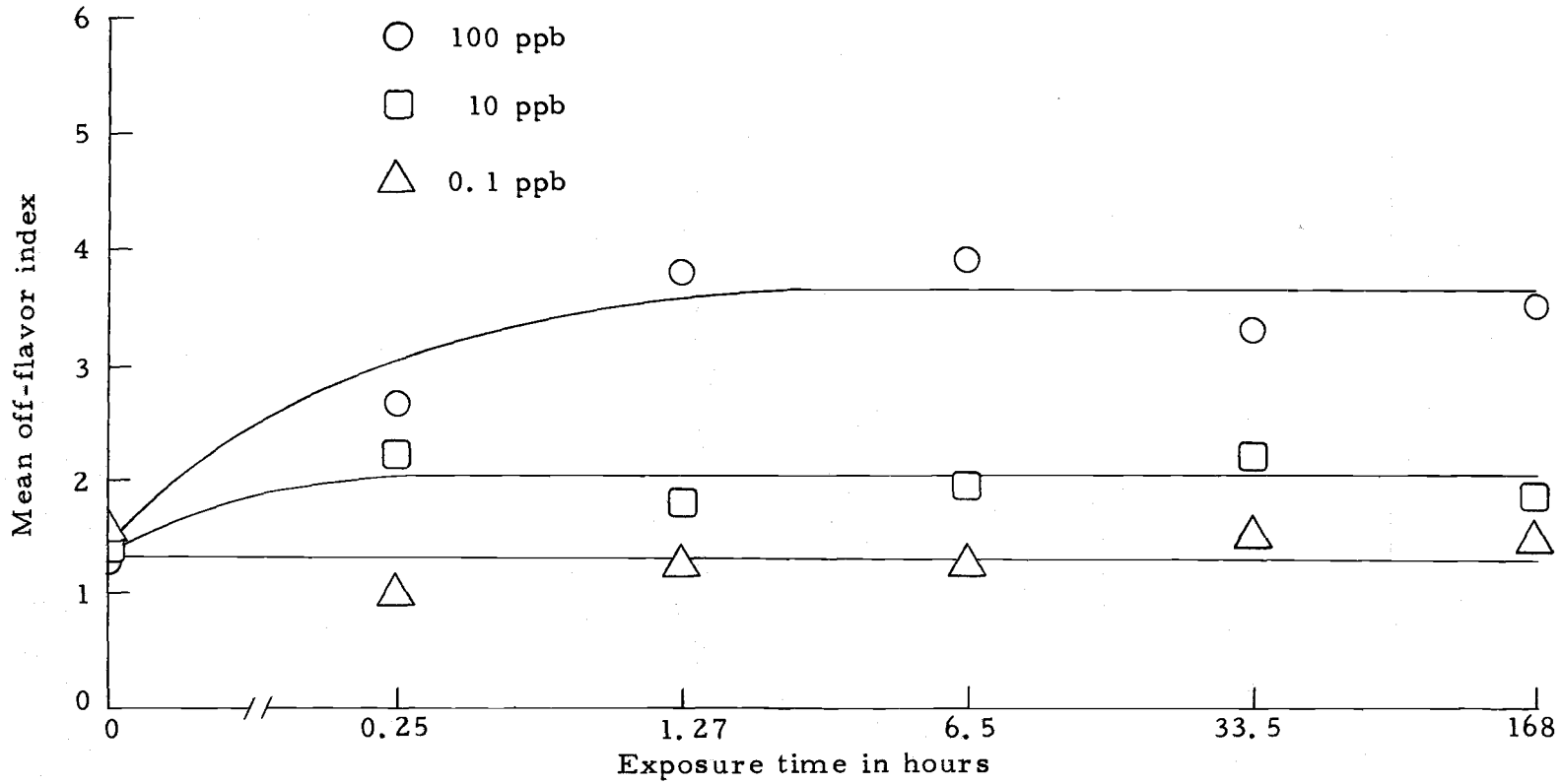


Figure 5. Relationship between mean off-flavor index of rainbow trout held at 0.1, 10, and 100 ppb of 2,4-DCP and the exposure duration in hr. The tests were conducted at 15 C.

Table 2. Experimental conditions and results of tests in which rainbow trout were exposed to 1, 10, and 100 ppb of 2, 4-dichlorophenol for 24 hr, removed, and placed in fresh water and held for various periods of time. The tests were conducted at 15 C.

Experiment no. and panel size	Exposure concentration (ppb)	Clearing time (hr)	Mean off-flavor index (0-6)	LSD $_{.05}$ [1/]	Standard error of the mean	Fish per chamber	
						grams	number
C-1	1	0.25	1.80	n. s.	0.40	137	1
10 Judges	1	1.27	1.55		0.25	166	1
	1	6.5	1.65		0.22	131	1
	1	33.5	1.20		0.23	155	1
	1	168	1.65		0.36	143	1
C-2	10	0.25	2.40	n. s.	0.43	165	1
10 Judges	10	1.27	2.15		0.37	176	1
	10	6.5	1.20		0.27	168	1
	10	33.5	1.40		0.36	206	1
	10	168	1.20		0.25	238	1
C-3	100	0.25	3.90	1.18	0.44	170	1
10 Judges	100	1.27	3.60		0.42	126	1
	100	6.5	2.40	*	0.56	154	1
	100	33.5	1.10	*	0.33	115	1
	100	168	1.70	*	0.34	148	1

[1/] The least significant difference at P = 0.05 based on a two-way analysis of variance. An asterisk (*) indicates a statistically significant reduction in off-flavor from the sample of trout that was exposed for 0.25 hr.

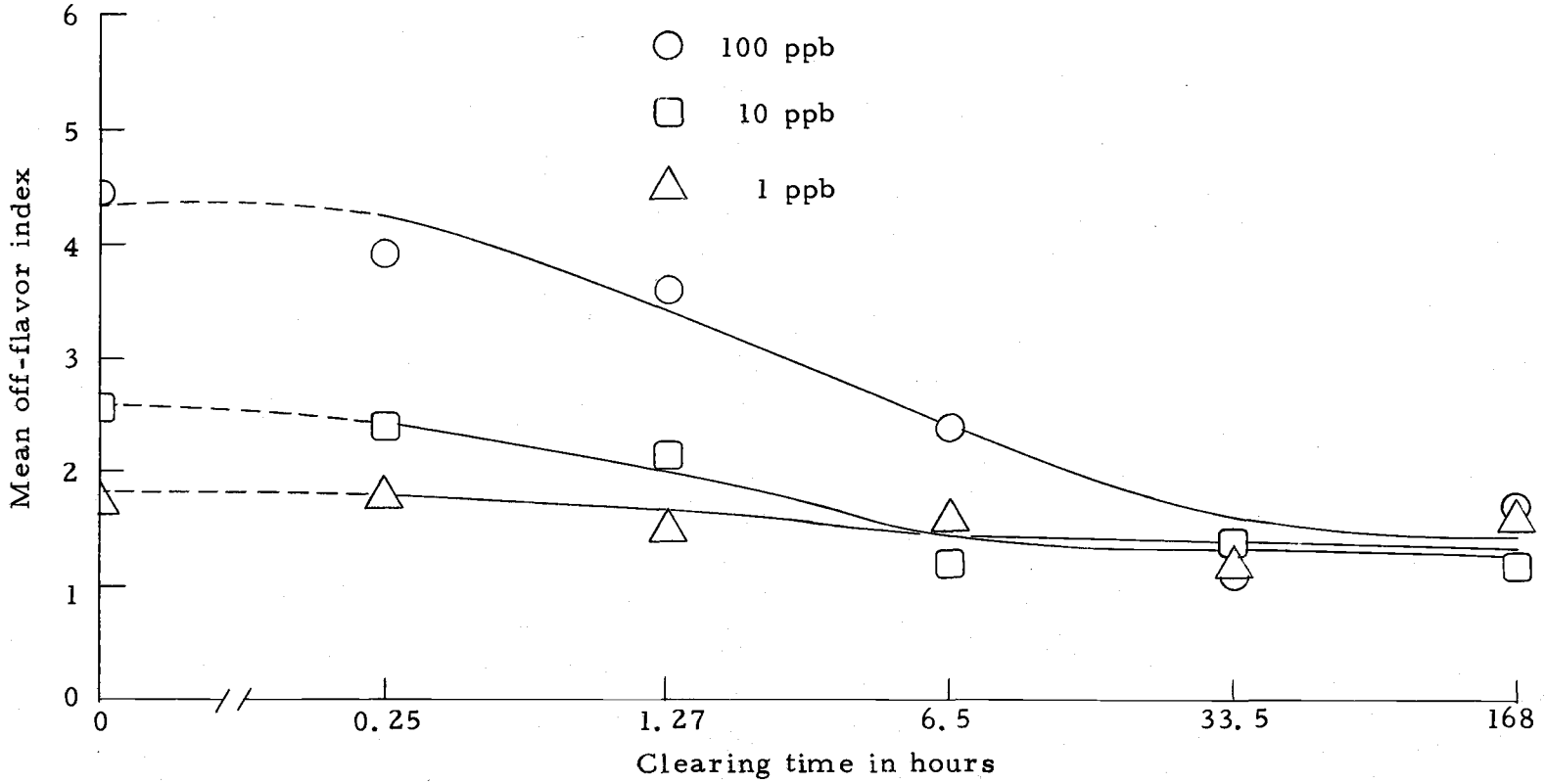


Figure 6. Relationship between mean off-flavor indices for rainbow trout and time held in uncontaminated water after 24 hr exposure to 1, 10, and 100 ppb of 2, 4-dichlorophenol. Off-flavor indices plotted on the ordinate were taken from mean indices presented in Figure 4.

initial 3.90 to 1.10 at the end of 33.5 hr. A decrease in the off-flavor index from 2.40 to 1.20 occurred in trout that had been exposed to 10 ppb of 2,4-DCP and maintained in fresh water for 168 hr; however, the decrease was not statistically significant. Mean off-flavor indices for trout remained nearly constant throughout the 168-hr clearing period after exposure to 1 ppb of 2,4-DCP.

In order to determine the quantity of trout that can be held in an exposure chamber without influencing the degree of flavor-impairment attained, different weights of trout were exposed to 10 and 100 ppb of 2,4-DCP for 96 hr at 15 C. The desired weight of fish per chamber was achieved by varying the number of fish exposed rather than the size of fish. Table 3 lists the exposure concentration, number and total weight of trout held at each concentration, the mean off-flavor index for each sample, and gives the results of the statistical analysis.

Although the mean off-flavor indices for trout exposed to 10 and 100 ppb varied from 0.70 to 1.85 and from 1.65 to 3.15 respectively, the differences were not significant at the 5 percent level. From the data, it appears that the exposure of 133 to 1418 g of trout per chamber does not effect the degree of off-flavor attained.

Rainbow trout were exposed to 10 and 100 ppb of 2,4-DCP at three pH levels (high, intermediate, and low) for 24, 48, and 96 hr at 15 C. The experimental conditions, statistical evaluation, and

Table 3. The experimental conditions and results of tests in which different weights of rainbow trout were exposed for 96 hr to 10 and 100 ppb of 2, 4-dichlorophenol at 15 C.

Experiment no. and panel size	Exposure concentration (ppb)	Mean off-flavor index (0-6)	Fish per chamber		LSD .05 [1/]	Standard error of the mean
			grams	number		
W-1	10	1.85	137	1	n. s.	0.42
10 Judges	10	0.75	462	3		0.28
	10	0.90	844	5		0.28
	10	0.70	1418	8		0.20
W-2	100	2.10	133	1	n. s.	0.34
10 Judges	100	3.15	402	3		0.33
	100	1.65	708	5		0.40
	100	2.40	1279	8		0.36

[1/] The least significant difference at P = 0.05 on a two-way analysis of variance.

results of these experiments are presented in Table 4. The results are shown graphically in Figure 7.

To achieve the desired low and high pH levels, it was necessary to adjust the natural pH of the well water (7.8 to 8.0). The low and high pH levels were achieved by the introduction of sulfuric acid and sodium hydroxide respectively into the reservoir portion of the water control boxes. Unconditioned well water was used for the intermediate pH level.

Increasing pH resulted in sharp decreases in mean off-flavor indices for trout held at 100 ppb of 2,4-DCP for 24 and 48 hr. In the 96-hr test (100 ppb), however, the relationship between pH and off-flavor was not well-defined. A significant decrease in off-flavor indices occurred with an increase in pH from 7.97 to 8.88; however, the mean off-flavor index for trout held at a pH of 6.11 was significantly lower than the index for trout held at the intermediate pH level. The off-flavor of trout exposed to 10 ppb of 2,4-DCP for 24, 48, and 96 hr appeared to decrease with increasing pH, although the differences were not statistically significant.

The flavor-imparting capacity of 2,4-DCP is apparently influenced by the pH of the water, at least at relatively high 2,4-DCP concentrations. At levels only slightly above the ETC, however, pH has little or no influence on the flavor-imparting capacity of the compound. Although no positive correlation could be made between

Table 4. Experimental conditions and results of tests in which rainbow trout were exposed to 10 and 100 ppb of 2, 4-dichlorophenol at high, intermediate, and low pH values for 24, 48, and 96 hr at 15 C.

Experiment no. and panel size	Exposure concentration (ppb)	Mean off-flavor index (0-6)	Mean pH	LSD .05 [1]	Standard error of the mean	Fish per chamber	
						grams	number
24-hr Exposure							
P-1	10	0.40	8.88		0.12	268	1
20 Judges		1.45	7.79	n. s.	0.39	331	1
		1.75	6.45		0.32	293	1
P-2	100	1.05	8.87	*	0.25	288	1
20 Judges		2.95	7.66	0.74	0.44	305	1
		3.75	6.26	*	0.40	303	1
48-hr Exposure							
P-3	10	1.00	8.90		0.25	342	1
20 Judges		0.50	8.01	n. s.	0.24	330	1
		2.05	6.39		0.46	305	1
P-4	100	1.40	8.88	*	0.26	261	1
20 Judges		3.40	7.83	0.92	0.54	324	1
		3.95	6.28	*	0.36	376	1
96-hr Exposure							
P-5	10	0.75	8.92		0.30	286	1
20 Judges		1.00	7.93	n. s.	0.22	408	1
		1.05	6.21		0.27	267	1
P-6	100	2.15	8.88	*	0.61	414	1
20 Judges		3.50	7.97	0.87	0.48	274	1
		2.60	6.11	*	0.45	387	1

[1] The least significant difference at P = 0.05 based on a two-way analysis of variance. An asterisk (*) indicates a statistically significant change in flavor from that of the fish held at intermediate pH levels.

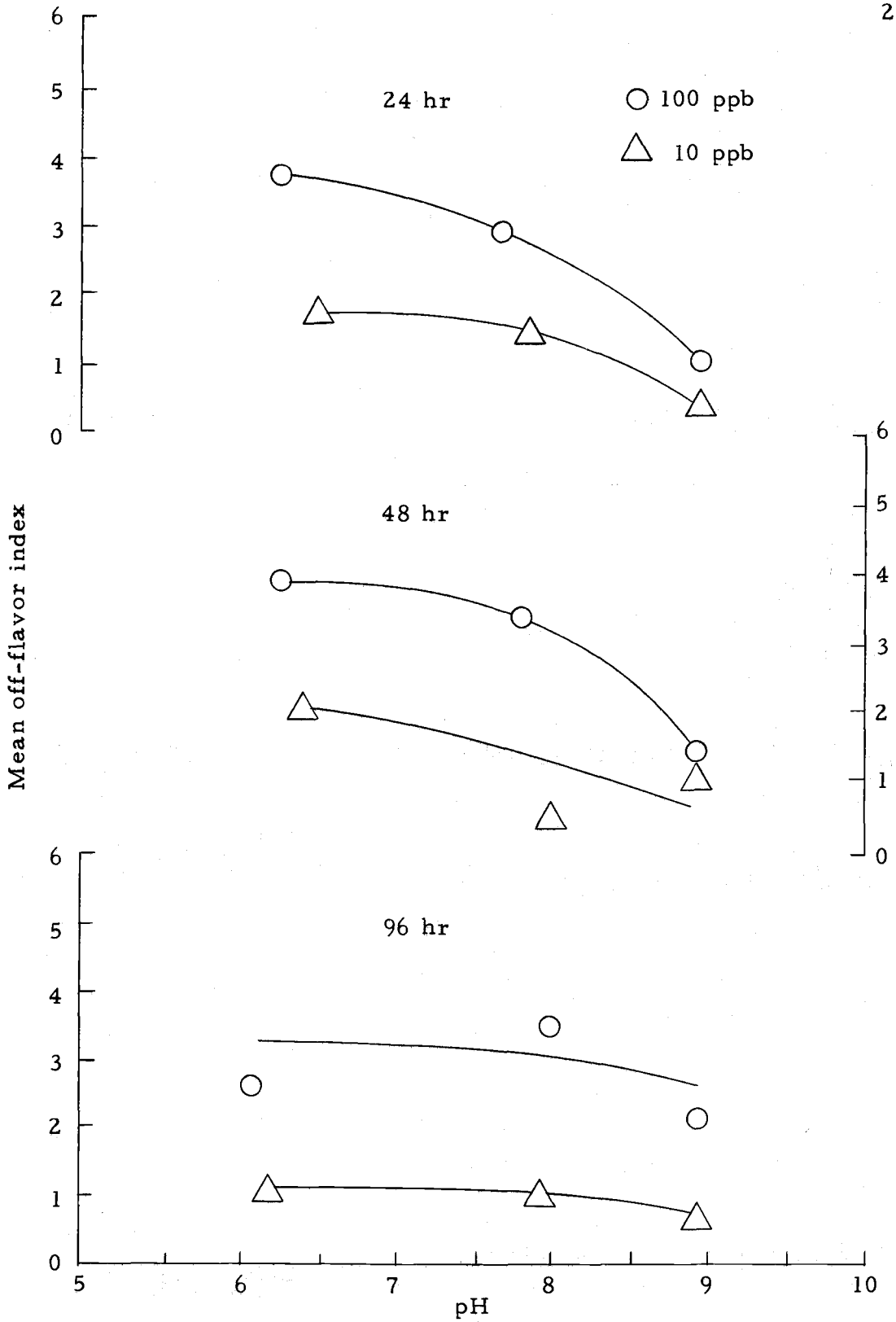


Figure 7. Relationship between pH of the water and mean off-flavor indices for rainbow trout exposed to 10 and 100 ppb of 2, 4-dichlorophenol for 24, 48, and 96 hr at 15 C.

mean off-flavor indices and length of exposure, it is interesting to note that the mean off-flavor indices for trout held in 100 ppb of 2, 4-DCP at the high and intermediate pH levels increased with increasing exposure duration (Table 4). This increase in off-flavor indices was not observed for trout held at the low or intermediate pH levels.

Trout were exposed to 1, 10, and 100 ppb of 2, 4-DCP at high, intermediate, and low dissolved oxygen concentrations for 48 hr at 15 C. Table 5 lists the exposure concentration, mean dissolved oxygen concentration, and mean off-flavor indices for each experiment. Dissolved oxygen concentrations as low as 3.8 mg/l caused no change in the mean off-flavor index for exposed trout. At each 2, 4-DCP concentration tested, the highest off-flavor index was noted at the intermediate oxygen level.

Largemouth bass and bluegill were exposed to 2, 4-DCP concentrations of 0.1, 1, 10, and 100 ppb for 48 hr at 20 C. In addition, bass were exposed to 0.01 ppb of 2, 4-DCP. The exposure concentrations, mean off-flavor indices, results of statistical analysis, and weight and number of fish exposed are listed in Table 6. A graphical presentation of the results is shown in Figure 8.

Significant off-flavors were detected only in the flesh of bass and bluegill exposed to the highest concentration of 2, 4-DCP tested, 100 ppb. Flavor-impairment was not detected in bulegill exposed to

Table 5. Results and experimental conditions of tests in which rainbow trout were held at various dissolved oxygen concentrations and exposed for 48 hr to 2, 4-dichlorophenol concentrations of 1, 10, and 100 ppb at 15 C.

Experiment no. and panel size	Exposure concentration (ppb)	Mean off-flavor index (0-6)	Mean dissolved oxygen (mg/l)	LSD .05 [1/]	Standard error of the mean	Fish per chamber	
						grams	number
D-1	1	0.55	4.1		0.25	374	2
10 Judges	1	1.00	6.5		0.30	311	2
	1	0.60	10.4	n. s.	0.16	316	2
D-2	10	1.85	3.9		0.39	351	2
10 Judges	10	2.35	6.9		0.42	355	2
	10	1.35	10.4	n. s.	0.25	381	2
D-3	100	3.60	3.9		0.54	341	2
10 Judges	100	3.80	6.2		0.33	398	2
	100	3.55	10.6	n. s.	0.50	379	2

[1/] The least significant difference at P = 0.05 based on a two-way analysis of variance.

Table 6. The experimental conditions and results of tests in which largemouth bass and bluegill were exposed to various concentrations of 2, 4-dichlorophenol for 48 hr at 20 C.

Fish species and panel size	Exposure concentration (ppb)	Mean off-flavor index (0-6)	$LSD_{.05}$ [1/]	Standard error of the mean	Fish per chamber	
					grams	number
Largemouth bass	0	0.40	0.88	0.19	802	2
10 Judges	0.01	1.55	*	0.29	546	2
	0.1	0.90		0.39	509	2
	1	2.10	*	0.35	477	2
	10	1.75	*	0.36	472	2
	100	3.75	*	0.32	520	2
Bluegill	0	0.55	0.71	0.24	446	6
10 Judges	0.1	0.50		0.21	478	6
	1	0.55		0.22	446	6
	10	1.00		0.34	401	6
	100	3.50	*	0.38	419	6

[1/] The least significant difference at $P = 0.05$ based on a two-way analysis of variance. An asterisk (*) indicates a statistically significant change in flavor from that of the control sample.

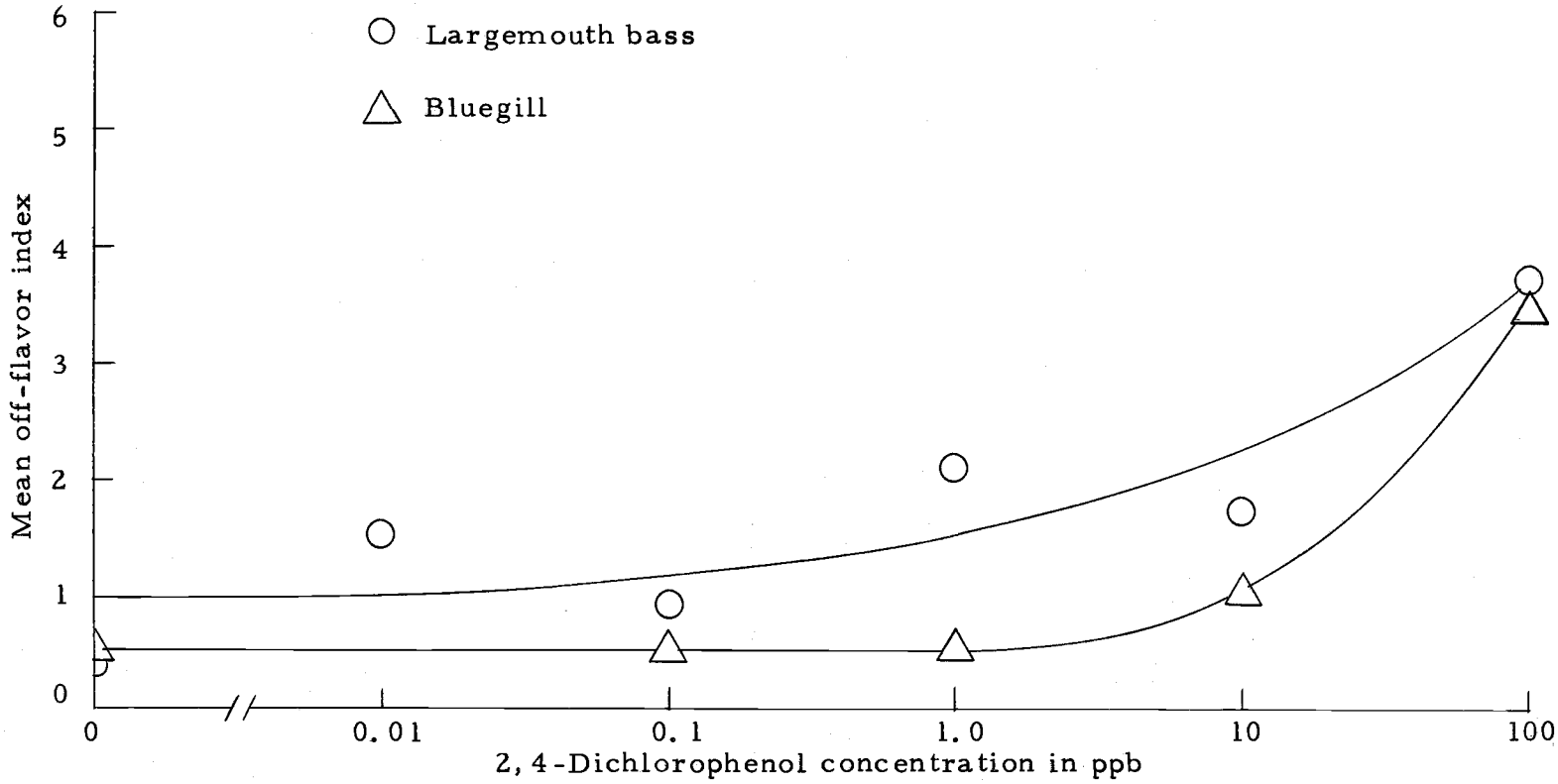


Figure 8. Relationship between 2, 4-dichlorophenol concentration and mean off-flavor indices for largemouth bass and bluegill exposed for 48 hr at 20 C.

0. 1 and 1 ppb of 2, 4-DCP, and only a slight impairment was found in bluegill exposed to 10 ppb. Although the mean off-flavor indices determined for bass were quite variable, there appeared to be some degradation of flavor at all 2, 4-DCP concentrations tested, except 0. 1 ppb. The ETC's for 2, 4-DCP using bass and bluegill as test fish were determined to be 4 and 14 ppb, respectively.

An experiment was conducted to determine how the addition of another flavor-producing compound, pyridine, in combination with 2, 4-DCP would affect the flavor of rainbow trout. Prior to conducting this experiment, however, it was necessary to determine the flavor-imparting capacity of pyridine.

Trout were exposed to a control and to pyridine concentrations of 0. 01, 0. 1, 1, 10, and 100 ppm for 96 hr at 15 C. The results of this test are shown in Figure 9. Flavor was not impaired in trout exposed to 0. 01, 0. 1, 1, or 10 ppm of pyridine; however, a substantial degree of off-flavor was apparent at 100 ppm (mean off-flavor index of 4. 20). The ETC for pyridine was 28 ppm.

Table 7 lists the experimental conditions and results of the test in which trout were exposed for 48 hr to 1, 10, and 100 ppb of 2, 4-DCP at a pyridine concentration of 100 ppm. The results are presented graphically in Figure 10. The combination of 100 ppm pyridine and 100 ppb of 2, 4-DCP resulted in a mean off-flavor index of 4. 00, a value that would be expected for trout exposed to either of the

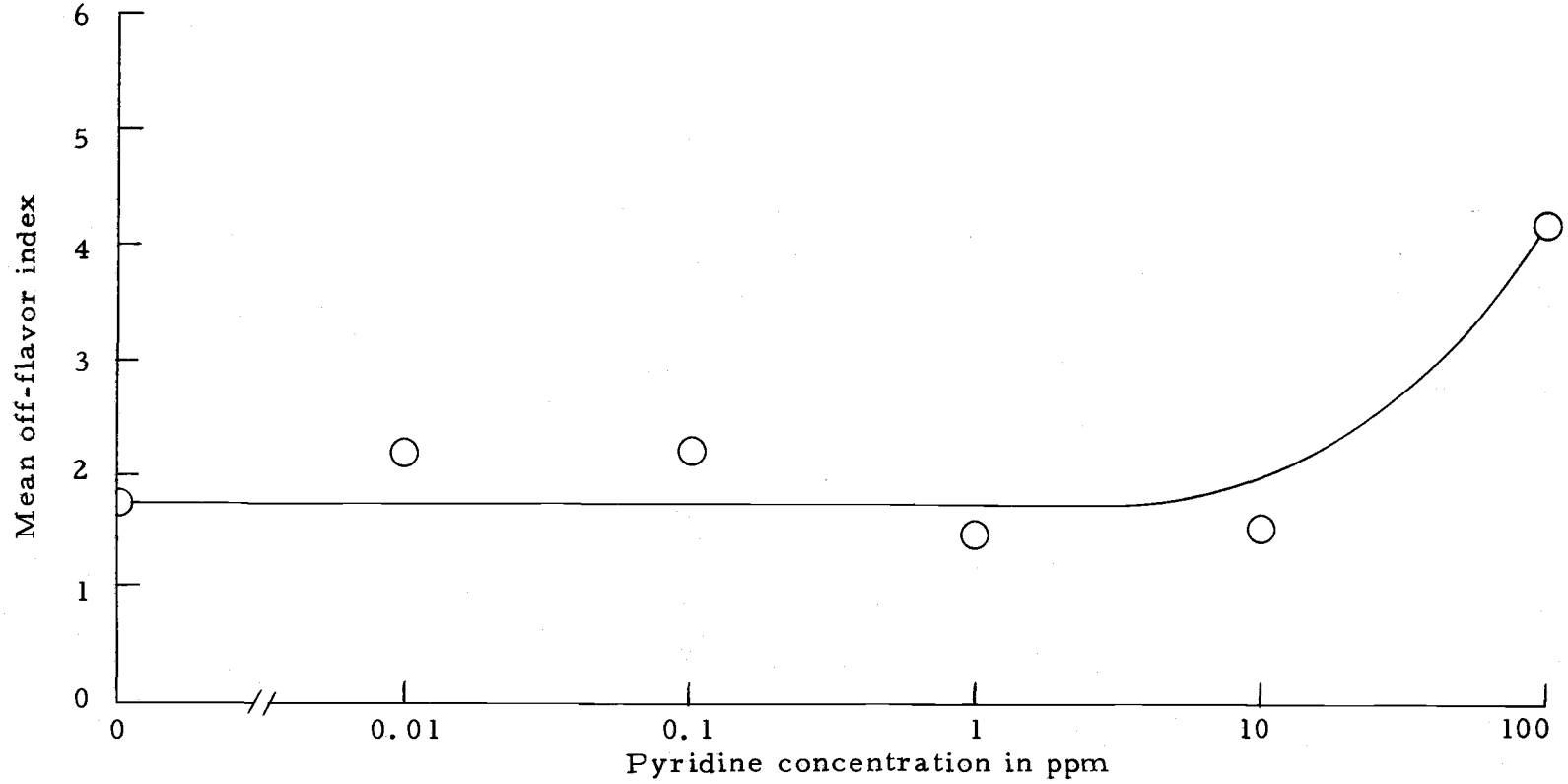


Figure 9. Relationship between concentration of pyridine and mean off-flavor indices for rainbow trout exposed for 96 hr at 15 C.

Table 7. Experimental conditions and results of tests in which rainbow trout were exposed for 48 hr to various concentrations of 2, 4-dichlorophenol plus 100 ppm of pyridine at 15 C.

Experiment no. and panel size	Exposure concentration		Mean off-flavor index (0-6)	$LSD_{.05}$ [1/]	Standard error of the mean	Fish per chamber	
	2, 4-DCP (ppb)	Pyridine (ppm)				grams	number
M-1 9 Judges	0	0	1.11	1.35	0.48	271	2
	1	100	2.33		0.63	314	2
	10	100	3.11	*	0.68	314	2
	100	100	4.00	*	0.54	267	2

[1/] The least significant difference at P = 0.05 based on a two-way analysis of variance. An asterisk (*) indicates a statistically significant difference in flavor from that of the control sample.

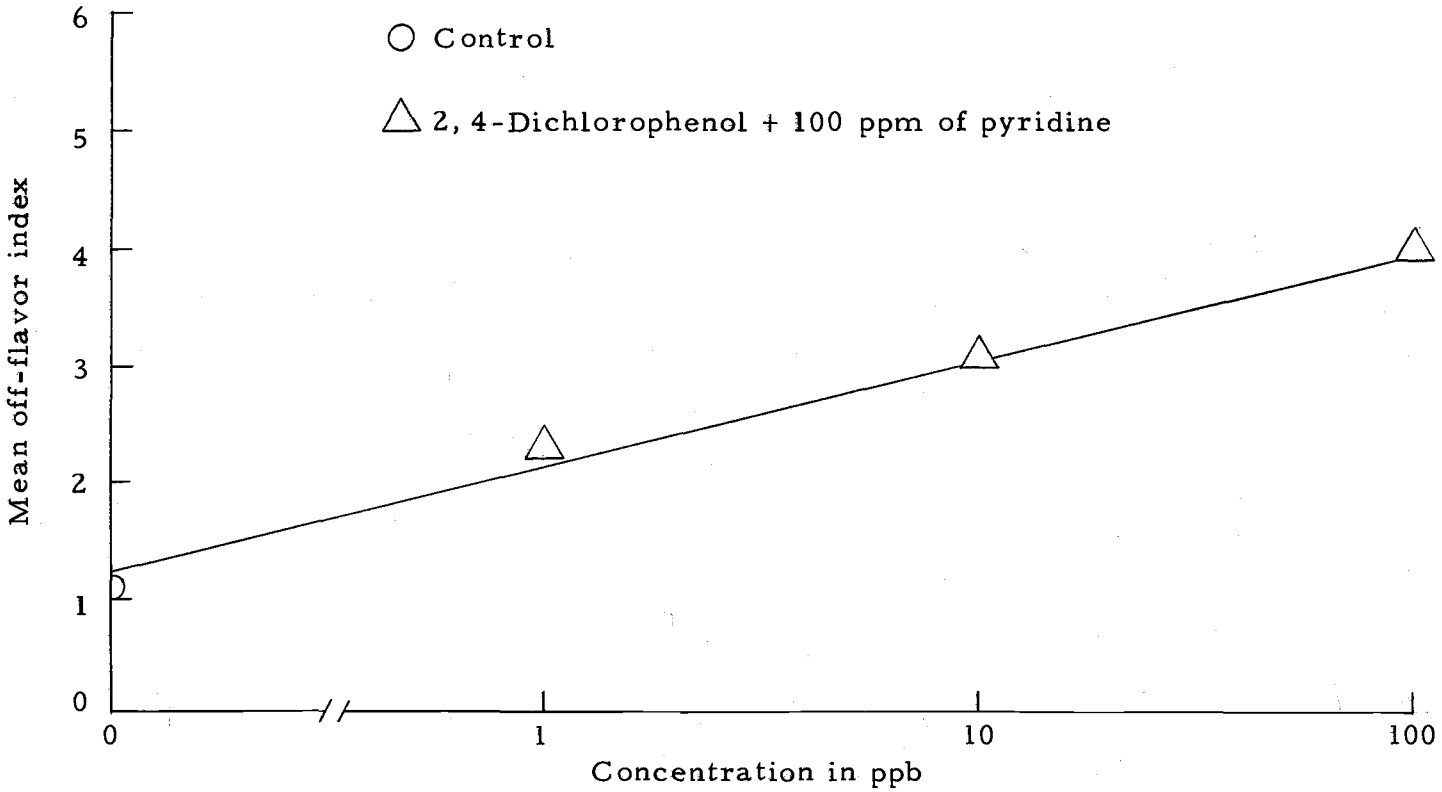


Figure 10. Relationship between exposure concentration and mean off-flavor indices for rainbow trout exposed to 2, 4-dichlorophenol and pyridine in combination. Trout were exposed for 48 hr at 15 C.

chemicals individually (see Tables 4 and 9). Mean off-flavor indices obtained for trout exposed to 1 and 10 ppb of 2,4-DCP, along with 100 ppm of pyridine, were in each case less than would be expected for trout exposed to 100 ppm of pyridine alone. They were, however, greater than the off-flavor indices determined in earlier tests for trout exposed to 1 and 10 ppb of 2,4-DCP (Figure 4).

DISCUSSION

Off-flavor indices resulting from organoleptic evaluation are not precise measurements but are only mean values derived from a range of judgement values. Not only do values reflect variability between test animals, but they also reflect the response of an individual panel member to the samples of fish flesh on a given day.

Although precautions were taken to insure continuity between flavor tests, there are factors which influence an individual's response to flavor stimuli that are difficult, if not impossible, to control. These factors include such things as what the panel member ate at his last meal, his physical health at the time, whether or not he smoked prior to a test, his attitude toward the test itself, and many others.

Organoleptic tests using 2, 4-DCP as the contaminant were not conducted as a continuous series, but rather were interspersed with many other tests using different organic compounds. This was done to reduce the possibility of bias among panelists. Panel members were never advised of the nature of the contaminant or the exposure concentrations used until after the flavor evaluation was completed. Because there was an apparent "order" to the samples in most tests (based on the intensity of off-flavor), panelists tended to order the samples in all tests, whether or not differences in the samples actually

existed. In those tests in which off-flavor differences were not discernible, "ordering" by each panel member was probably random, thereby producing insignificant differences in the mean index values for the samples.

In view of the sources of variability inherent in organoleptic testing, emphasis should then be given to differences in mean off-flavor indices that can be statistically compared, i. e., indices within a single experiment, and caution used when attempting to compare mean off-flavor indices between experiments.

There are four ways in which a fish may obtain a tainting substance from the water: 1) across the gill membrane, 2) across the gut membrane, 3) absorption through the skin, and 4) adsorption to the mucosa. Since test fish were never fed during an experiment and since freshwater fish ingest very little water, uptake of 2, 4-DCP across the gut membrane was probably negligible. Since the fish in this study were carefully washed prior to cooking and their skins removed before they were evaluated, the off-flavor indices yielded more than likely represent the uptake of 2, 4-DCP mainly across the gill membrane and to a lesser extent through the skin. After entering the fish through the gills, the contaminant can be easily and quickly transported throughout the body by the blood.

Schulze (1961) found that carp exposed for 4 days to 10 ppm of phenol concentrated the phenol in selected body tissues at levels

exceeding that of the exposure water. The greatest concentrations of phenol occurred in the liver (19 ppm), gills (17 ppm), and kidneys (12 ppm). The concentration of phenol found in the muscles of the carp was 10 ppm.

The difference between the rates of uptake and elimination of 2, 4-DCP determines the rate at which the chemical is accumulated in the tissues of the fish. The amount of chemical accumulated then determines the level of flavor-impairment at any given time after the initiation of the exposure period. The leveling-off of flavor-impairment curves with time may indicate that an equilibrium between the uptake and excretion rates was attained, or nearly attained, and that the rate of accumulation (net gain) of the contaminant by the fish, if not zero, was so low that it could not be detected by organoleptic means.

My data for trout exposed to 100 ppb of 2, 4-DCP suggest that initially the rate of uptake is much greater than the rate of loss, but that an equilibrium between the two rates was reached within 1.27 hr, the time maximum off-flavor was attained. After the equilibrium was achieved, continued exposure served only to maintain the high level of flavor-impairment. Trout exposed to 10 ppb of 2, 4-DCP appeared to reach maximum flavor-impairment in only 0.25 hr, after which no further increase in off-flavor occurred. The possibility of acquiring additional off-flavor after the first few hours of exposure seems remote, even if the trout were held for a substantially longer period of time.

Once attained, off-flavor may be lost by holding fish in water free of the contaminant for an appropriate period of time. Mann (1965) suggested at least three weeks in clean water were necessary to remove the disagreeable taste of phenol in eels. Korschgen et al. (1970) reported that the flavor of contaminated (tainted) carp failed to improve after 18 days retention in a clean-water pond. They also reported a study by Leslie E. Whitesel, in which she reported that catfish transferred from the Ohio River to clean water lost about one-half of their off-flavor in 7 days, and nearly all in 21 days. Shumway (1966) reported that some off-flavor still remained in contaminated coho salmon after as long as 72 hr of cleansing.

The findings reported herein show that the rate of loss of off-flavor is substantially less than the rate of uptake. No significant loss of off-flavor occurred until trout exposed to 100 ppb of 2, 4-DCP had been held in uncontaminated water for 6.5 hr, more than four times the time required to obtain maximum off-flavor. It cannot be said with certainty that all off-flavor had been eliminated at the end of 168 hr, the duration of the cleansing period.

The rapid rate of uptake and relatively slow clearing rate of 2, 4-DCP, and presumably most other organic compounds, is of great importance when considering tainting problems in migratory species of fish. The flesh of salmon or steelhead, for example, may be rendered inedible for some time after only a brief encounter with a

tainting substance. The effect on a migratory species would not be a localized problem, but could despoil a fishery for miles upstream from the source of contamination.

The ability of 2, 4-DCP to impart an off-flavor to the flesh of fish is dependent upon the pH of the exposure solution. Based on a pKa of 7. 75 (Hansch et al., 1964), at a pH of 6. 11 (the lowest pH tested) about 97. 5 percent of the 2, 4-DCP in solution would be available in molecular form, while at a pH of 8. 92 (the highest pH tested) only about 9 percent existed in molecular form (Albert, 1965). Because the phenate anion cannot readily cross cellular membranes, the off-flavor produced in test fish is caused by the molecular or non-ionic portion of the 2, 4-DCP in solution. The effect of pH on the flavor-imparting capacity of 2, 4-DCP is then similar to altering the concentration of the chemical available.

The result of increasing or reducing the pH of the exposure solution from a nominal value of about 7. 8 was to reduce or to increase respectively the degree of off-flavor acquired by test fish. Similar results have been obtained in other experiments conducted at Oregon State University dealing with the effect of pH on the flavor-imparting capacity of pyridine, an organic compound which reacts as a base. In experiments with pyridine, increased pH resulted in higher mean off-flavor indices for exposed trout.

Unfortunately, surface waters are seldom contaminated by a

single pollutant, but more often by mixtures of two or more pollutants which may act together to cause tainting of the flesh of exposed fish. Results of the experiment using 2, 4-DCP and pyridine in combination show that the flavor-imparting capacity of pyridine was reduced by the presence of 2, 4-DCP. Similar results were obtained in another study conducted at Oregon State University using a mixture of pyridine and p-chlorophenol. The off-flavor index of trout exposed to a combination of these two compounds was always lower than would have been expected had the fish been exposed to the same concentration of the chemical normally expected to cause the highest degree of off-flavor. In no case was the off-flavor index lower than that expected for trout exposed to the chemical expected to produce the lowest degree of off-flavor.

The above experiments involved two compounds of dissimilar type which in nearly all cases interacted to produce less off-flavor when combined than would have been caused by the compound producing the highest degree of off-flavor if used alone. Mixtures of compounds of similar nature may react altogether differently and produce additive flavor characteristics.

The purpose of the laboratory investigation reported here was to generate information that could be applied to natural systems. Consideration of the data obtained in this study shows that sublethal concentrations of water pollutants can have a deleterious effect on the

quality of fish. In addition to largemouth bass, bluegill, and rainbow trout, less economically important species of fish, and other aquatic organisms, may also be the recipients of the indiscriminate discharge of malodorous pollutants. While fish can acquire an off-flavor by consuming contaminated food (Mann, 1965), I know of no study that has been conducted that considered both contamination from the water and from the food.

The relationship between off-flavor and tissue concentration of contaminants in fish has not yet been explored and may be the key to a good understanding of the fish-tainting problem. The effect of mixtures of compounds on off-flavor is poorly understood and merits further investigation. Clearly, more laboratory studies encompassing the entire scope of the fish-tainting question are needed.

There are many ways to assess the impact of pollution on our fisheries resources, and certainly, off-flavor problems in fish have not been in the mainstream of biological investigation. Nevertheless, tainting of fish should not be overlooked when determining water quality standards that will protect the value of our important fisheries. I hope this study, and the others that were conducted concurrently at Oregon State University, will help bring more attention to the general problem, as well as generate additional research in this area.

LITERATURE CITED

Albert, A. 1965. Selective toxicity. John Wiley and Sons, Inc., New York, 394 pp.

Baker, R.A. 1961. Problems of tastes and odors. Journal of the Water Pollution Control Federation 33:1099-1106.

Bandt, H. J. 1946. Damage to the taste of fish. Beiträge zur Wasser-, Abwasser- und Fischereichemie aus dem Flusswasser Untersuchungsamt in Magdeburg 1:36-39.

Böetius, J. 1954. Foul taste of fish and oysters caused by chlorophenol. Meddelelser Fra Danmarks Fiskeri-Og Havundersgelser 1(4):1-7.

Chadwick, G. G., J. R. Palensky and D. L. Shumway. 1973. Continuous-flow dilution apparatus for toxicity studies. Proceedings 39th Industrial Waste Conference, Pacific Northwest Pollution Control Association. (In press)

English, J. N., E. W. Surber and G. N. McDermott. 1963. Pollutional effects of outboard motor exhaust - field studies. Journal of the Water Pollution Control Federation 35:1121-1132.

Fetterolf, C. M. 1964. Taste and odor problems in fish from Michigan waters. In: Proceedings of the 18th Industrial Waste Conference. Engineering Bulletin, Purdue University, (Engineering Extension Series No. 115), 48(3):174-182.

Hansch, C., K. Kiehs and G. Lawrence. 1965. The role of substituents in the hydrophobic bonding of phenols by serum and mitochondrial proteins. Journal of the American Chemical Society 89(6):5770-5773.

Hasselrot, T. B. 1965. Investigations with caged fish. Sartryck ur Vattenhygien 2:1-10.

Kinney, J. E. 1961. Fusing the phenol frenzy. In: Proceedings of the 15th Industrial Waste Conference. Engineering Bulletin, Purdue University, (Engineering Extension Series No. 106), 45(2):28-34.

Korschgen, B. M., R. E. Baldwin, and J. W. Robinson. 1970. Influence of environment on palatability of carp. Journal of the Water Pollution Control Federation 41(5):189-196.

Krishnaswami, S. K. and E. E. Kupchanko. 1969. Relationship between odour of petroleum refinery wastes and occurrence of "oily" taste-flavour in rainbow trout, Salmo gairdneri. Journal of the Water Pollution Control Federation 41(5):189-196.

Mann, H. 1962. Increase by detergents of effects on taste in fish. Fischwirt 12:237-240.

Mann, H. 1965. Effects on the flavour of fishes by oils and phenols. In: Pollution Marines par les Microorganismes et les Produits Petroliers: Symposium de la Commission Internationale pour l'Exploration Scientifique de la Mer Mediterranee, Monaco, 1964. Paris, 371-374.

Nitta, T., K. Arakawa, K. Okubo, T. Okubo, and K. Tabata. 1965. Studies on the problems of offensive odors in fish caused by wastes from petroleum industries. Tokai Regional Fisheries Research Laboratory. Bulletin 42:23-37. (Tokai-Ku Suisan Kenkyujo Hokoku)

Schaafsma, N. D. R. 1935. The action of chlorine on water containing phenols and the production of tastes and odors. Water 19:207.

Schulze, E. 1961. The influence of phenol-containing effluent on the taste of fish. Internationale Revue der gesamten Hydrobiologie 46(1):84-90.

Shumway, D. L. 1966. Effect of effluents on flavor of salmon flesh. Research Report, Agricultural Experiment Station, Oregon State University. 17 pp.

Shumway, D. L. and G. G. Chadwick. 1971. Influence of kraft mill effluent on the flavor of salmon flesh. Water Research 5:997-1003.

Tamura, T., Y. Itazawa, and Y. Morita. 1954. River water pollution by sulfate paper mill wastes. Bulletin of the Japanese Society of Scientific Fisheries 20:344-349.

APPENDICES

APPENDIX 1

GEOLOGICAL SURVEY ANALYSIS OF WELL WATER CONDUCTED FEBRUARY 21, 1966

Sample of water was collected after 9 months of steady pumping on well

Specific conductance (micromhos at 25°C)	259 ppm	pH	7.5
Dissolved solids (evaporated at 180°C)	165 ppm	Temperature (°C)	10
Hardness as $CaCO_3$	121 ppm	Color	0
Silica (SiO_2)	32 ppm	Sodium (Na)	8.5 ppm
Magnesium (Mg)	9.9 ppm	Nitrate (NO_3)	0.3 ppm
Potassium (K)	0.3 ppm	Fluoride (F)	0.1 ppm
Bicarbonate (HCO_3)	162 ppm	Chloride (Cl)	6.0 ppm
Carbonate (CO_3)	0	Sulfate (SO_4)	1.0 ppm
Iron (Fe)	0.08 ppm	Calcium (Ca)	32 ppm

APPENDIX 2

EVALUATION SHEET USED BY FLAVOR PANEL MEMBERS TO RATE SAMPLES OF FISH FLESH

Department of Food Science and Technology
Oregon State University

Name: _____

Date: _____