RESEARCH TECHNICAL COMPLETION REPORT

PROJECT A-013-IDA

A Lethal Index For Classifying Chemicals Which Affect Water Quality Of Aquatic Life

Project Investigator — Craig MacPhee

Research Assistant — Dennis Norman

Water Resources Research Institute
University of Idaho
Moscow, Idaho

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PRINCIPAL INVESTIGATOR - Craig MacPhee
Professor of Fishery Management
College of Forestry
University of Idaho
Moscow, Idaho

PERIOD OF PROJECT - June 1965 to September 1967

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ABSTRACT

Seventeen toxic chemicals were bioassayed to investigate the possibility of measurable physiological responses to death in northern squawfish. Lethal indices, dependent upon concentration and temperature for each chemical, in terms of time between loss of equilibrium and death, were established.

The physiological process of suffocation caused by the action of heavy metals and organic compounds of lactic acid, formaldehyde, and p-nitrophenol was identifiable and unaffected by concentration or temperature. The anesthetic effect of p-chlorophenol was discrete and also unaffected by concentration or temperature. The physiological actions of nitrites, other phenols, chlorinated hydrocarbons, and organophosphorus compounds were not discretely identifiable and the data indicate multiple physiological effects for these chemicals depending on concentration.

NORMAN, D. E.

A Lethal Index for Classifying Chemicals Which Affect Water Quality

Technical Completion Report Project

KEY WORDS: Bioassay indicators, water pollution, toxicity, fishkill, water quality

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INTRODUCTION

This project was initiated to study the tolerance of fish subjected to toxic compounds having known physiological modes of action. More specifically, the objectives of this study were: (1) to arrange common toxic chemicals which have known physiological affects on fish into broad physiological categories by calculating lethal indices, (2) to use these indices as standards or references to classify chemicals by physiological effect for which the lethal causes are not yet known, and (3) to investigate the effect of water temperature as a variable on the value of the lethal index.

The reasoning behind this study is that if fish, in the process of dying, exhibit a measurable characteristic resistance to certain chemicals with known physiological effects, then it is possible that the magnitude of this resistance could be used as a lethal index to separate groups of chemicals with different physiological roles. The measurable characteristic resistance used as the lethal index is the relationship, in terms of time, between loss of equilibrium and death

in fish exposed to lethal concentrations of toxic agents. Such an index, established by bioassay, may serve directly to ascertain the effect of a pollutant and indirectly to identify and determine the amount of a pollutant present. An index which reflects the nature, type and amount of pollution might serve as a tool in water quality control.

Knowledge of the physiological reactions of fish to certain toxicants has been useful in the past. Hazeltine (1963), used a specific reaction between an organic phosphorous compound and a bluegill sunfish to protect wildlife during the control of the Clear Lake gnat in California. Other workers have successfully used physiological responses to assess concentration levels of toxicants (Sun, 1952; Bushland, 1951; and Fleming, 1951). Davidow (1954 and 1955), used a physiological response in goldfish to separate a series of chlorinated hydrocarbons because biological identification was more accurate and less expensive than analytical laboratory methods.

Water quality as affected by industrial wastes and pesticides is a major national problem. This is especially true for downstream agricultural and recreational areas. The problem has become increasingly complex and the hazards progressively great with the rapid commercial development and expanded use of pesticides.

The development of new pesticides has advanced so rapidly that evaluations of side effects have not kept pace. These side effects are often catastrophic to aquatic life. Furthermore, it is difficult to identify and evaluate concentration levels of these pollutants once they have been diluted and moved downstream.

The problems concerning water pollution can be enlightened by a better understanding of the biochemical systems of fish which lie in the primary line of response to toxic materials. In assessing the effect of a pollutant, the mechanism involved in the mode of entry and transport to the site of toxic action needs to be evaluated before the ultimate effect of the toxic substance can be assessed.

FACILITIES

Fish assay facilities.

Fish assay facilities are housed in the Small Animal Laboratory Building, University of Idaho. A 4,567 gallon glass-lined water storage tank, buried adjacent to the building, supplies water to the assay laboratory. Non-toxic water is delivered and circulated through the laboratory through polyvinyl chloride plumbing with fiberglass lined or stainless stell fittings.

Fish holding space was provided by four 270-gallon stainless steel vats. These refrigerated vats could maintain water temperatures between room temperature and 32 F.

Assay facilities consisted of four tables, each containing 20 aquaria in a circulated water bath. Each assay table was equipped with a heating element in addition to refrigeration coils so that temperatures above room temperature could be maintained.

Test aquaria were plastic waste baskets 11.5 inches in diameter, 11.5 inches high and with a capacity of 14 liters. During tests each

aquarium was lined with a disposable polyethylene poultry bag to prevent chemical contamination and fitted with a clear plexiglass cover to prevent fish escapement. Oil free air was supplied to each aquarium, by a special air compressor, through a single stone air-breaker.

Water supply.

All assays were conducted in water transported by tank truck from Rochat Creek, a small tributary of the St. Joe River, near St. Maries, Idaho. The test water was uncontaminated, clear and of a quality suitable for trout. Rochat Creek water has a pH of 7.2, methyl orange alkalinity of 2 ppm, total hardness of 4 ppm and a conductivity less than 0.1 millimhos/cm on August 19, 1965 (MacPhee, 1966). Photovolt meter, Model 115 pH readings were taken on water delivered to the laboratory during the summer and fall of 1966: June 25, pH 7.2; August 8, pH 7.0; and October 30, pH 6.7.

No attempt was made to standardize a synthetic test water because of the difficulty in modifying the various properties of a water supply. Antagonistic or synergistic interaction between toxic substances and dissolved Rochat Creek salts was probably at a minimum considering the conductivity reported by MacPhee.

Test solutions.

A total of 17 toxic chemicals were tested during the period June 14, 1966 to November 25, 1966. A test solution consisted of a quantity of chemical by weight dissolved in 10 liters of water. The concentrations of the toxic solutions in ppm were multiples or fractions of the

ratio one milligram chemical/liter of water is equivalent to one part per million. The concentrations of hydrated salts were calculated on the basis of their hydrated form.

To obtain the desired concentrations, calculated amounts of chemical were weighed on a Mettler H15 single pan electronic balance and added directly into the water. Test solutions of low concentration were prepared by making a concentrated stock solution and diluting portions of it as required.

All chemicals were soluable in either water or acetone. Portions of concentrated aqueous solutions of chemical were added to the 10 liters of test water with a hypodermic syringe and needle. No adjustment in the volume of test water was made for these small additions because the 10-liter test volume varied ± 20 milliliters.

Below is a resume of the chemicals used in the tests.

Chemical	Formula	Grade*	Supplier	Code
Cadmium chloride	$CdCl_2$	Purest	J.T. Baker	1212
p-Chlorophenol	ClC_6H_4OH	Purest	Eastman	366
Chromium trioxide	CrO_3	Purest	Mallinckrodt	2576
Cobalt chloride	$CoCl_2.6H_2O$	Purest	Allied	1594
Dieldrin	Insecticide	Unknown	Hammond Bay	
Formaldehyde	HCHO	Practical	Eastman	P450
Lactic acid	$CH_3CHOHCOOH$	Purest	J.T. Baker	0194
Malathion	Insecticide	Technical	Am. Cyanamid	W-50-107-3
Mercuric chloride	$HgCl_2$	Purest	J.T. Baker	2594
Nickle chloride	$NiCl_2.6H_2O$	Purest	J.T. Baker	2768

Chemical	Formula	Grade*	Supplier	Code
o-Nitrophenol	$NO_2C_6H_4OH$	Practical	Eastman	P191
p-Nitrophenol	$NO_2C_6H_4OH$	Purest	Eastman	192
Pentachlorophenol	C_6Cl_5OH	Technical	Eastman	3462
Potassium cyanide	KCN	Purest	J.T. Baker	3080
Sodium nitrite	$NaNO_2$	Purest	J.T. Baker	3780
Zinc chloride	$ZnCl_2$	Purest	J.T. Baker	4321
Zinc sulfate	$ZnSO_4.7H_2O$	Purest	J.T. Baker	4382

*Reagent, as given in supply catalog.

Test fish.

Fingerling northern squawfish, Ptychocheilus oregonensis Richardson, were used for assays because this species is abundant near the Moscow area and is used in other research being conducted on the Moscow campus. The northern squawfish is rated as being intermediate to moderately sensitive to toxic materials (Hart, 1945) and adequately insensitive to handling and laboratory conditions.

Test fish were seined from four local streams with a 50-foot fine meshed, nylon beach seine. Over eighty per cent of the fish used in the assays were taken from the St. Maries River, a tributary of the St. Joe River, near St. Maries, Idaho. These fish had an average fork length of 52 millimeters. Larger fish, fork length averaging 67 millimeters but comprizing less than 10 per cent of the test fish used, were collected in Rochat Creek. The remaining small portion of fish were collected in a side channel of the Clearwater River near Spalding, Idaho and in the

Potlatch River near Kendrick, Idaho. The Clearwater squawfish averaged 45 millimeters fork length and the Potlatch fish averaged 49 millimeters. The Potlatch River is a tributary of the Clearwater River and collecting in these areas was conducted in the spring and fall of the year when fishing conditions in the St. Maries area were not optimal.

Captured fish were transported to the laboratory in large plastic garbage cans. Fish arriving at the campus were immediately transferred to the holding vats where they were acclimated and conditioned to assay facilities for two days prior to testing. The fish were transferred to the assay aquaria about 24 hours before the chemical was added. The fish were never fed.

ASSAY PROCEDURES

An experimental unit.

Each assay aquarium contained five fish in 10 liters of test solution. Total weight of the fish in each aquarium was about 8.5 grams, or more than one liter of test solution per gram of fish.

Continuous observation of the fish was initiated upon addition of the chemical and maintained until the last fish in an aquarium died. The times at loss of equilibrium and death were recorded to the nearest tenth of an hour. Loss of equilibrium was the state at which a fish could no longer maintain itself in a normal upright position. No opercular movement was considered to be a state of death. Fish from different sources were never mixed and the fork length of the largest fish in an aquarium was rarely more than 1.25 times the fork length of

the smallest fish.

More than one fish in an aquarium could exhibit loss of equilibrium at the same time and individual fish dying early in an experiment were not necessarily the same individuals that lost equilibrium early. Attempts to identify individual fish by fin clipping increased incidental mortality due to the rigors of handling. As a result, the five loss of equilibrium and death times were arithmetically averaged and converted to logrithmic values to obtain linear relationships.

The distribution of losses of equilibrium and deaths in an experimental unit (5 fish in one aquarium) were not always normal. Some unit deaths were skewed toward early or late mortalities while others were normally distributed. In most cases skewed distributions were caused by one abherant fish.

The possibility of some skewed distributions influencing the average values of loss of equilibrium and death, thus necessitating conversion of all individual recordings of time into logrithmic values, was checked. Groups of fish exposed to different concentrations of chemical produced a series of loss of equilibrium and death times. Averages of these groups were calculated on the basis of the average logrithmic value and the logarithm of the arithmetic average. Deviations of both averages from the over all means were compared and found to be non-significant at the 0.01 level.

Concentration as a variable.

Toxicity of the chemicals was established by preliminary assays. Since time was a variable in developing the index, toxicity of a chemical

was denoted by the concentration which killed all of the test fish within a specified time (LD_{100}).

A concentration interval for each chemical was established which would allow test fish to live for a period of 2 to 20 hours at 50 F. The size of an interval determined how many different concentrations would be tested. Assays were repeated with 10 chemicals so that 4 groups of 5 fish, or 20 fish, were killed at each concentration. Tests with six chemicals showing less variability were repeated three times, killing 15 fish at each concentration. One chemical was only repeated once at each concentration. All tests were conducted at 50 F.

Temperature as a variable.

Eight chemicals were tested using temperature as a variable. The concentration, previously established, which allowed the test fish to live for approximately eight hours at 50 F were used with a series of temperatures ranging between 40 and 75 F, in five degree intervals. As data for 50 F tests was already obtained, this temperature was excluded from the series. The tests were repeated twice and killed a total of 15 fish at each temperature.

Establishing the index.

The lethal index for this study was the quotient of the time until loss of equilibrium divided by the time until death. This ratio has the limits of being greater than zero but less than one. Different index values for the variables of concentration, temperature and time were established by calculating the regressions of concentration and temperature

on time. From these regressions the index value at any specific concentration, temperature or time could be calculated; however, such index values are theoretical because they are derived from lines of best fit.

Most curvilinear relationships between concentration and temperature on time were transformed into linear ones for obtaining lines of best fit by transforming time, concentration or temperature into logarithmic values. In some instances, where log transformation alone would not suffice, linear relationships were obtained by applying correction factors (Burdict, 1957) at the threshold levels of concentration or time.

RESULTS

Concentration as a variable.

The toxicities of the chemicals used varied greatly and required a wide range of concentrations (Figure 1) to produce a series of deaths which approached the specified time interval of 2 to 20 hours. Data for the upper and lower limits of this time interval were not obtained for all chemicals. At threshold levels, small reductions in concentration for most chemicals resulted in greatly extended survival times. No attempt was made to extrapolate beyond the limits of the data obtained.

Solutions of cadmium chloride, cobalt chloride, dieldrin, mercuric chloride, sodium nitrite, zinc chloride, and zinc sulfate did not produce deaths at two hours. Exploratory assays, using concentrations several times greater than the highest concentration shown in Figure 1 for these chemicals, still failed to result in deaths within two hours. The elapsed time until death at these higher exploratory concentrations became constant

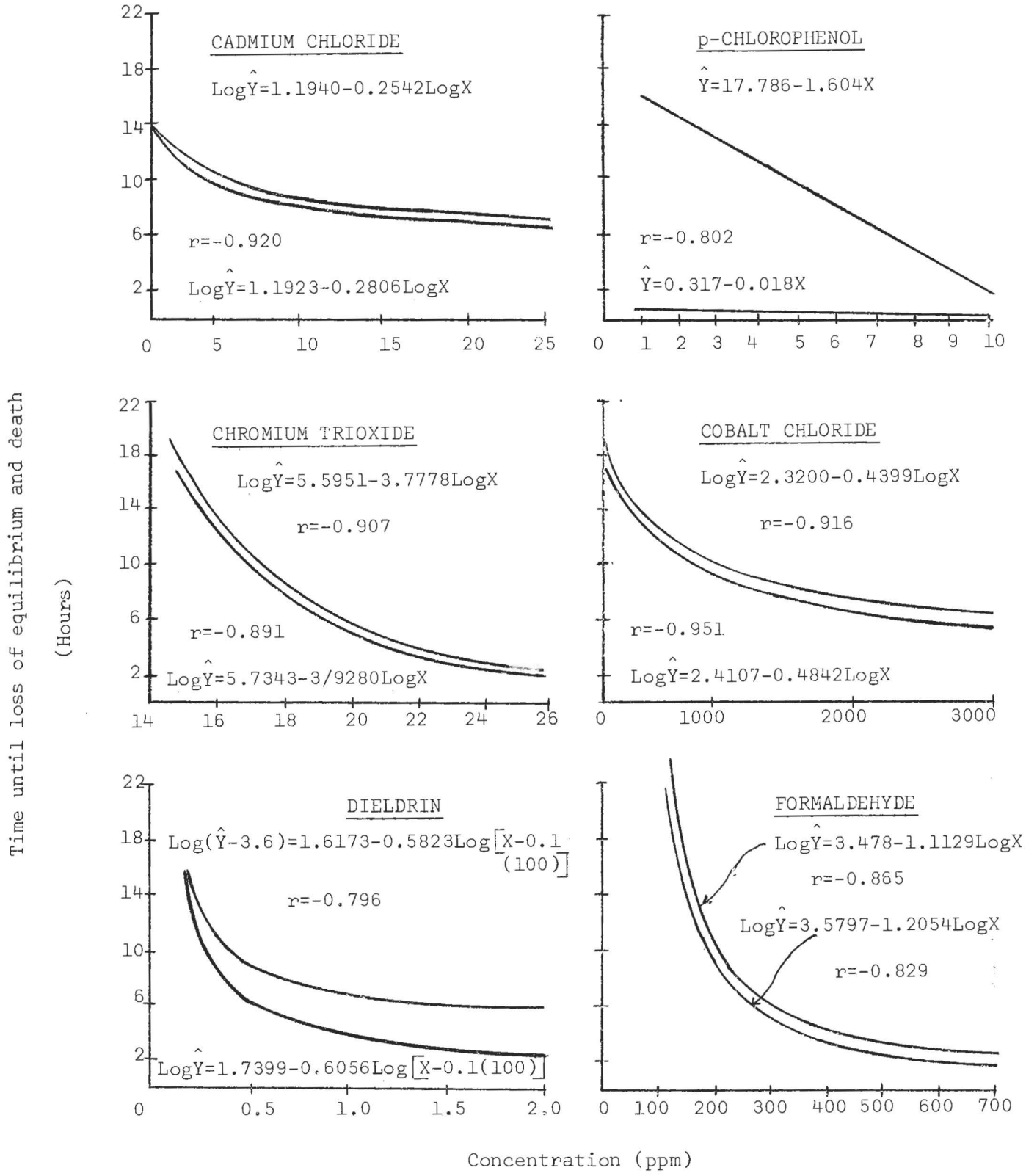
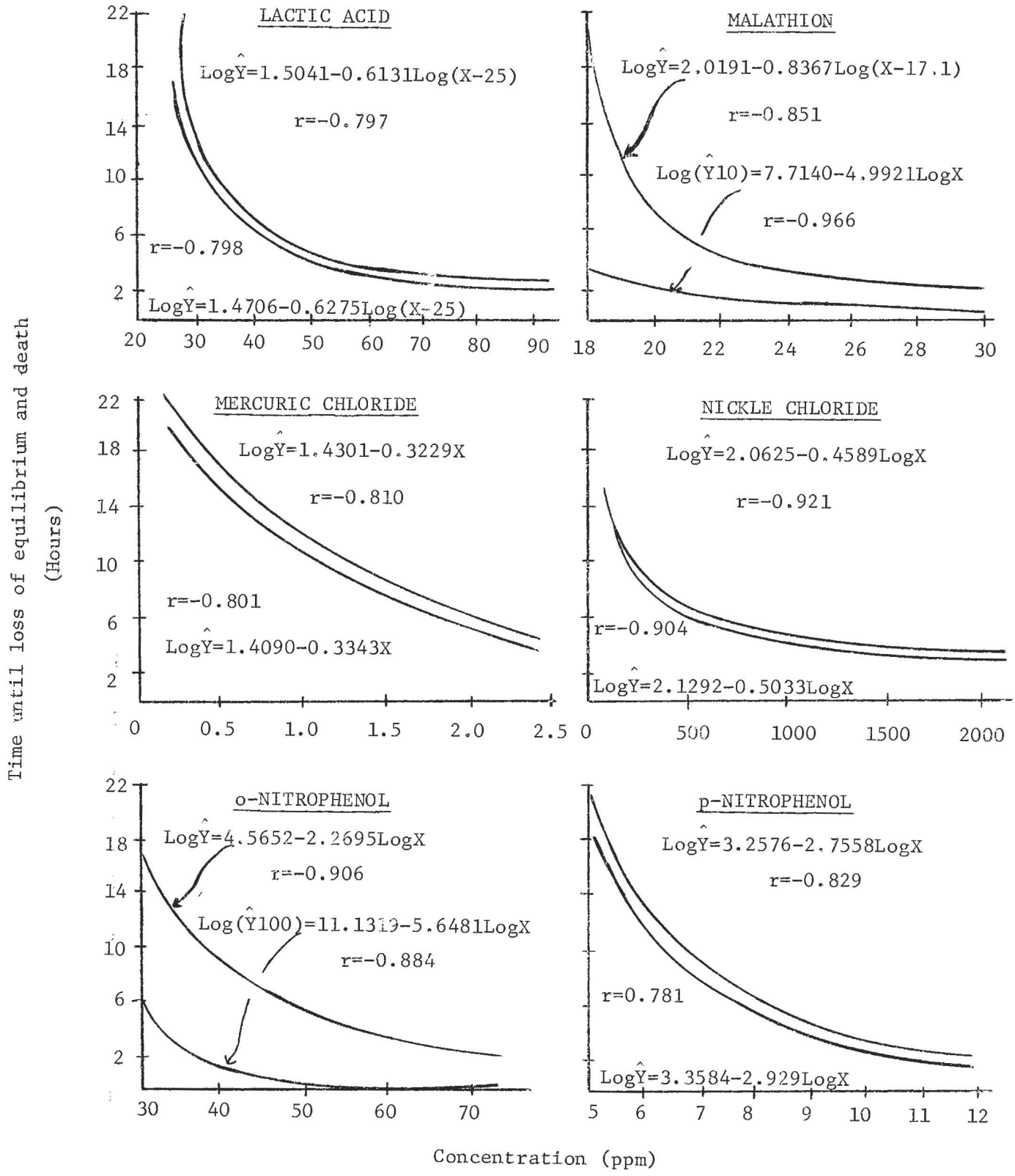


Figure 1.--Loss of equilibrium (lower line) and death (upper line) responses in northern squawfish. Temperature held constant at 50 F.



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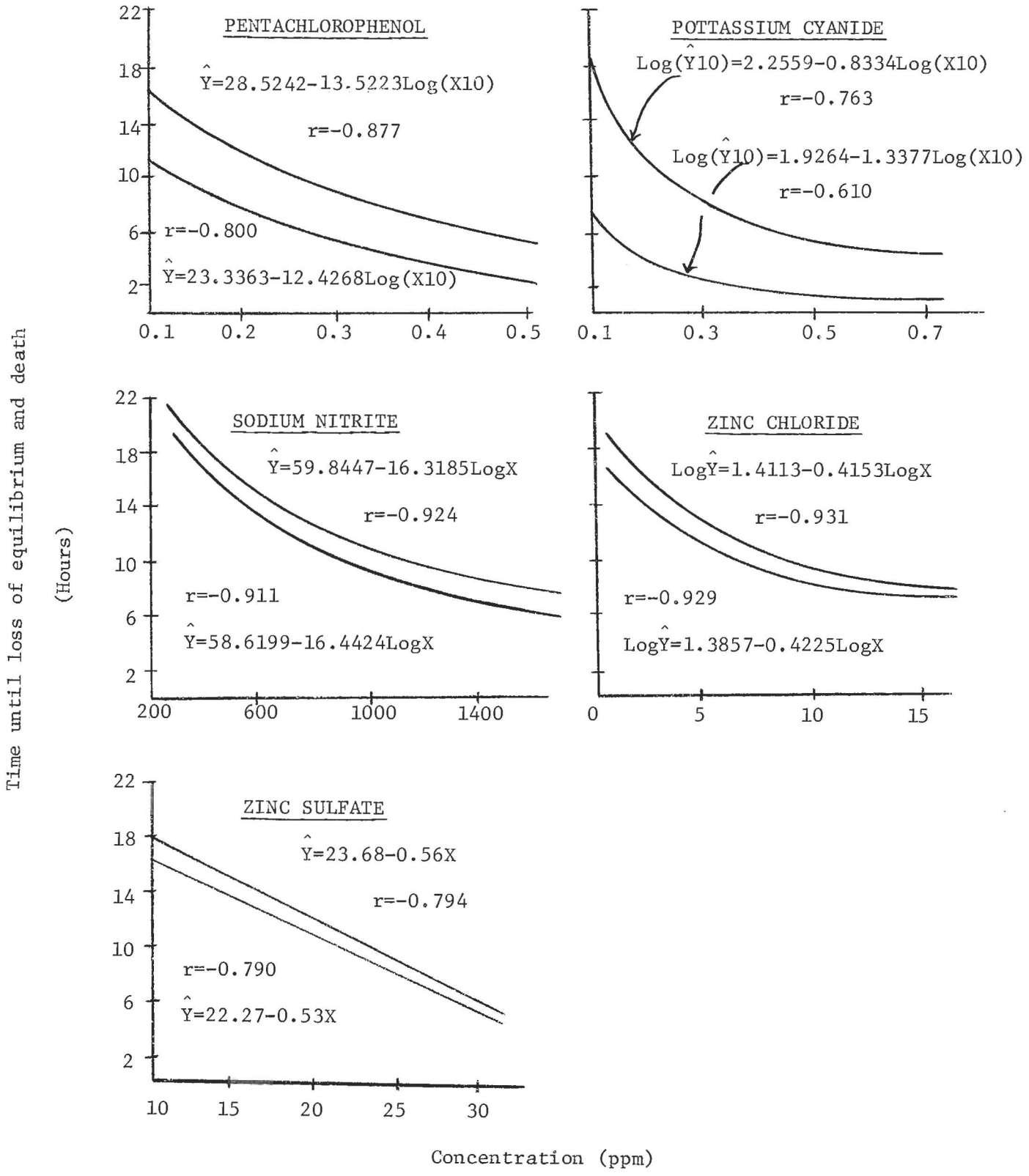

Figure 1.--continued

due to a time requirement, independent of concentration, needed for the physiological reaction to produce death.

The correlation coefficents for concentration and time were always negative and highly significant (Figure 1). From the regression of time on concentration any elapsed time at loss of equilibrium can be related to a corresponding time until death.

The loss of equilibrium/death ratio (lethal index) when plotted over a series of times resulted in near linear relationships for most chemicals (Figure 2). Potassium cyanide, o-nitrophenol, and malathion exhibited curvilinear relationships which tended to straighten with longer survival periods.

The value of the lethal index varied with survival time. Since data were not always obtained for assays of short and long duration, comparisons of the lethal index ranges for survival periods were made (Table 1). The average index value for the period of 6 to 16 hours was calculated based on the six 2-hour index values for this 10-hour period (Table 1).

Temperature as a variable.

Fish were tested with eight chemicals in assays using temperature as a variable (Figure 3). All of the fish, except those exposed to solutions of cadmium chloride, died within two hours at the higher temperatures of 70 or 75 F. At the lowest temperature of 40 F only fish exposed to chromium trioxide survived much longer than 14 hours. Fish exposed to pentachlorophenol did not survive longer than 10 hours.

Fish exposed to temperature gradients produced equilibrium/death

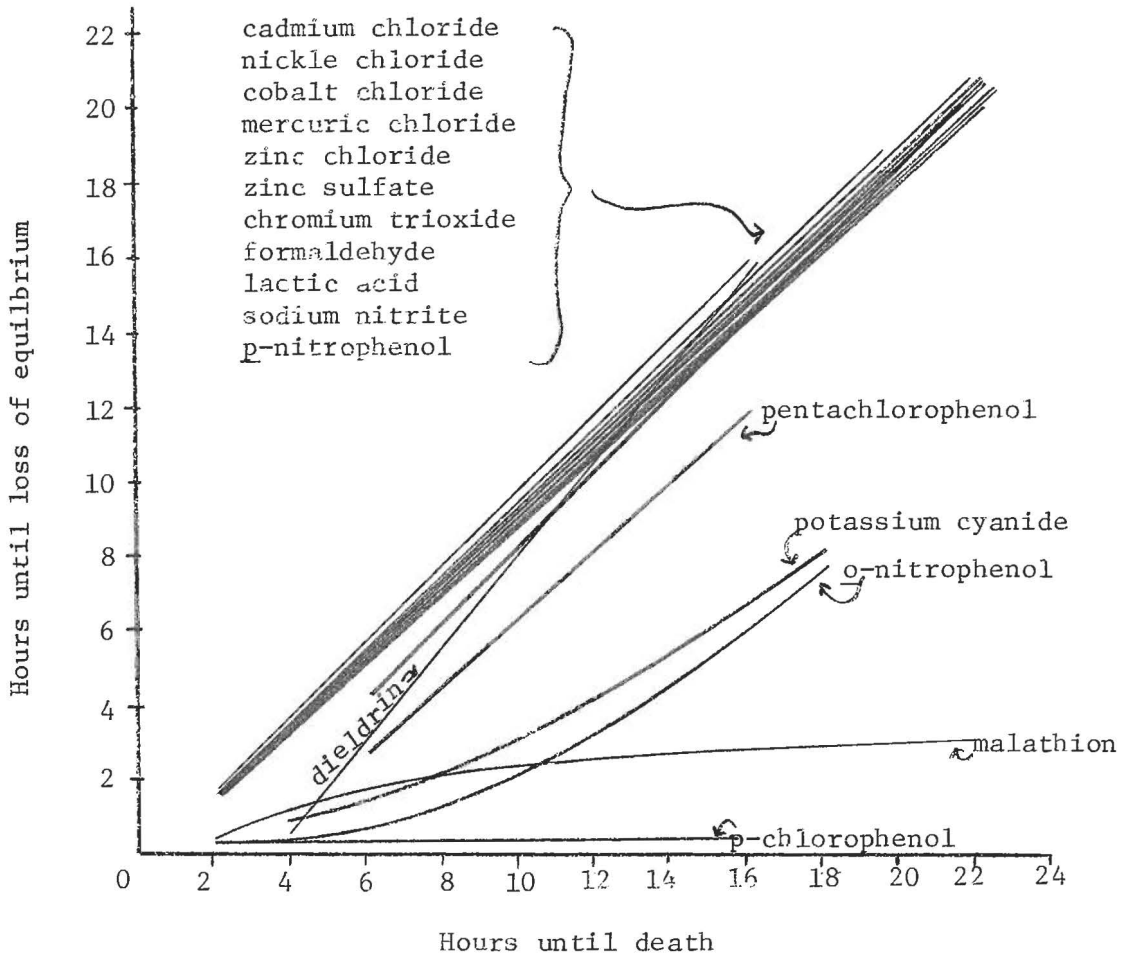

Figure 2.--Loss of equilibrium/death relationship (lethal index) for northern squawfish. Concentration variable, temperature maintained at 50 F.

Table 1.--Lethal indices of northern squawfish produced by a concentration variable at 50 F.

Chemical	Hours until death											6-16 hour average
	2	4	6	8	10	12	14	16	18	20	22	
Cadmium chloride			0.902	0.929	0.950	0.969	0.984	0.996				0.955
p-Chlorophenol	0.070	0.040	0.032	0.026	0.023	0.021	0.019	0.019				0.023
Chromium trioxide	0.845	0.872	0.887	0.896	0.905	0.911	0.916	0.922	0.926	0.929		0.906
Cobalt chloride			0.862	0.887	0.907	0.925	0.939	0.951	0.963	0.972		0.912
Dieldrin			0.475	0.667	0.788	0.871	0.932	0.979				0.785
Formaldehyde	0.760	0.805	0.833	0.854	0.870	0.882	0.894	0.904	0.913	0.921	0.929	0.873
Lactic acid	0.865	0.882	0.890	0.896	0.901	0.905	0.909	0.911	0.913	0.915	0.917	0.902
Malathion			0.270	0.247	0.232	0.209	0.189	0.175	0.160	0.148	0.139	0.220
Mercuric chloride		0.887	0.903	0.914	0.921	0.924	0.930	0.935	0.940	0.942	0.947	0.921
Nickle chloride	0.790	0.842	0.877	0.900	0.920	0.937	0.951	0.962				0.924
o-Nitrophenol	0.015	0.047	0.085	0.130	0.182	0.238	0.300	0.366	0.436			0.217
p-Nitrophenol	0.755	0.797	0.822	0.837	0.854	0.865	0.876	0.884	0.892	0.899	0.907	0.856
Pentachlorophenol			0.440	0.559	0.631	0.680	0.714	0.739				0.627
Potassium cyanide	0.174	0.187	0.240	0.275	0.328	0.366	0.402	0.436	0.459			0.341
Sodium nitrite			0.694	0.798	0.840	0.867	0.888	0.902	0.914	0.923	0.931	0.831
Zinc chloride			0.912	0.924	0.927	0.928	0.933	0.935	0.937	0.938		0.926
Zinc sulfate			0.920	0.929	0.934	0.935	0.936	0.936				0.932

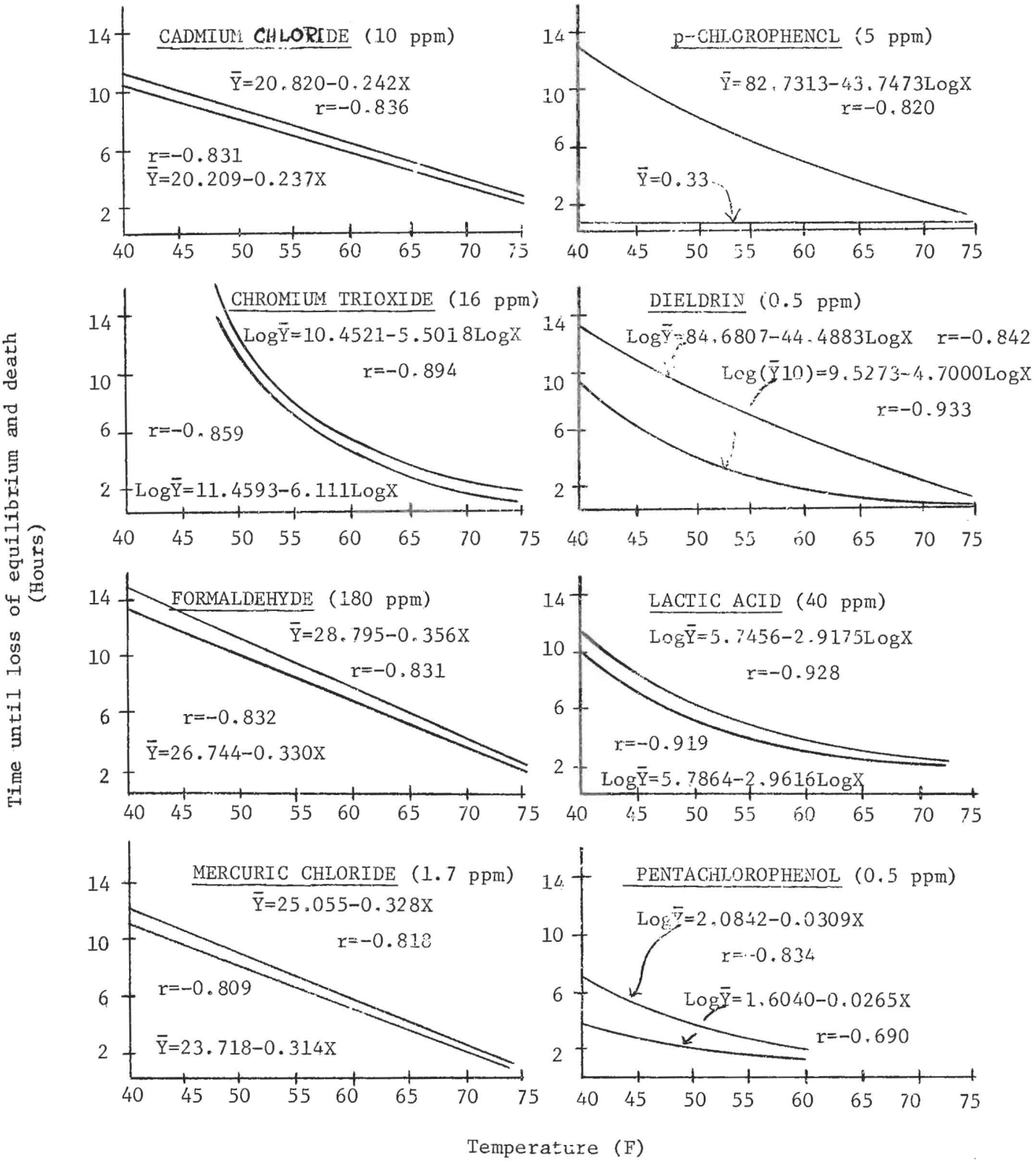

Figure 3.--Loss of equilibrium (lower line) and death (upper line) responses in northern squawfish. Temperature variable, concentrations held constant.

relationships similar to those produced by concentration gradients (Figure 4). The average index value for the 10-hour survival period between 4 and 14 hours was used to compare the ranges of the indices produced by the temperature variable (Table 2). Except for formaldehyde the ranges of these indices were contained in or overlapped the indices produced by the concentration gradient.

PHYSIOLOGICAL ACTION OF CHEMICALS TESTED

The chemicals assayed can be classified into eight major groups: Inorganic salts of heavy metals, nitrites and cyanides; organic ketones, aldehydes, phenols, chlorinated hydrocarbons, and organophosphorus compounds. Only the heavy metals and phenols were represented by more than one chemical in this study. The physiological modes of action for most of the chemicals tested are well documented.

Heavy metals.

The lethal action of the soluble salts of the heavy metals on fish is attributed chiefly to a disruption in the exchange of gases across the gill membranes. Death is a result of suffocation. Several theories have been advanced concerning the exact mode of action on gills at lethal concentrations: Intense mucus formation accompanied by precipitation of the metal ion in this mucus which covers the gill lamella and prevents exchange of gases, cytological damage to the gill lamella, and coagulation of protoplasm in the gills and body after absorption of

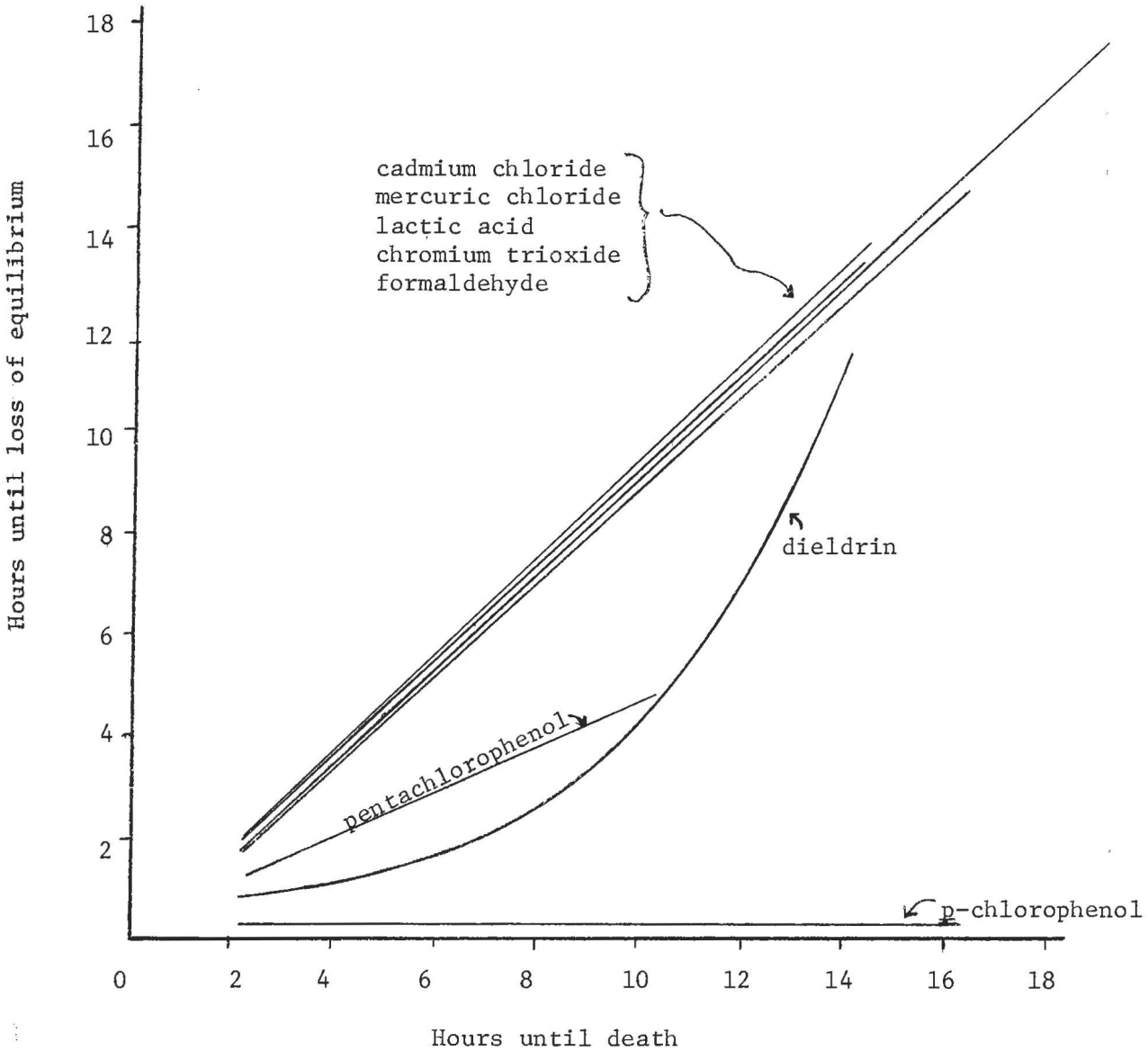

Figure 4.--Loss of equilibrium/death relationship (lethal index) for northern squawfish. Temperature variable, concentration constant.

Table 2.--Lethal indices of northern squawfish produced by a temperature variable; concentration held constant.

Chemical	Hours until death											4-14 hour average
	2	4	6	8	10	12	14	16	18	20	22	
Cadmium chloride		0.945	0.955	0.961	0.964	0.966	0.968					0.959
p-Chlorophenol	0.165	0.082	0.055	0.041	0.033	0.027	0.024					0.044
Chromium trioxide	0.765	0.825	0.863	0.891	0.913	0.932	0.948	0.962	0.974	0.986	0.996	0.895
Dieldrin		0.252	0.273	0.334	0.430	0.588	0.821					0.450
Formaldehyde	0.950	0.940	0.935	0.934	0.932	0.931	0.931	0.930				0.934
Mercuric chloride	0.825	0.890	0.913	0.924	0.930	0.935	0.939					0.922
Lactic acid	0.910	0.917	0.925	0.929	0.931	0.934	0.936					0.929
Pentachlorophenol	0.595	0.537	0.508	0.487	0.472							0.501*

* 4-10 hour average

the metal ion. No conclusive evidence has been presented to support the theory of coagulation of protoplasm (Skidmore, 1964). Carpenter (1927 and 1930) does not believe that the metal ions penetrate the body of freshwater fish. Schweiger (1957) injected carp with solutions of heavy metals without mortality occuring, which suggested to him that the metals are not general internal poisons but that they act specifically on the gills of fish.

Zinc chloride, zinc sulfate, mercuric chloride, and chromium trioxide definitely have distructive affects on the epithelium of the gill membranes. Herbert (1964), Lloyd (1960) and Carpenter (1927) agree that the zinc ion, whether in the chloride or sulfate form, acts to disrupt the gill tissue. Sometimes, depending on the concentration of the ion, mucus formation can accompany this disruption. Wood (1960) describes the disruption as a swelling of the lamella to a non-functional state.

Mercuric chloride has a corrosive and astringent affect on the gill membranes (Jones, 1947).

Chromium trioxide is a strong oxidant which causes severe burns and ulcers. This chemical was noted in the laboratory to cause extreme swelling of the gill filaments to a point of completely filling and expanding the opercular cavity and distending the branchiostegals. Little mucus formation was noted, but some precipitation of the chemical appeared to occur over the general body surface as evidenced by the fish assuming the color of the toxic solution, a pale yellow.

The remaining heavy metals tested, cadmium chloride, cobalt chloride and nickle chloride, caused suffocation by precipitation of

mucus on the gills, thereby interfering with the intake of oxygen (Carpenter, 1927; Westfall, 1945; and Dourodoroff, 1953). Intense mucus formation was noted on fish exposed to these chemicals.

Sodium nitrite.

At the high concentrations used, this chemical also causes death by suffocation. However, the chemical does not interfer with the normal function of the gills. In excessive doses sodium nitrite converts hemoglobin to methemoglobin; in lesser, non-toxic doses the chemical relaxes the smooth muscle of the finer blood vessels (Lewis, 1963; and Albert, 1960). Death is a result of cyanosis caused by a fall in blood pressure and slow capillary action, and functional anemia caused by the alteration of hemoglobin. In no way does sodium nitrite affect the nervous system (Lewis, 1963).

Potassium cyanide.

The cyanides act to bind the iron group in cytochrome oxidase, thus preventing transfer of electrons to oxygen and thereby interfering with tissue respiration (Giese, 1963; and Hart, 1945). Lack of oxygen affects the brain first followed by paralysis of the remainder of the central nervous system. Death is due to respiratory arrest (Gleason, 1963).

Lactic acid.

The lethal action of lactic acid is similar to the heavy metals that cause suffocation by precipitating and coagulating mucus on the

gills. Death by large doses is caused by coagulation film anoxia; however, smaller doses may have systemic affects on fish (Westfall, 1945 and Ellis, 1937).

Formaldehyde.

This aldehyde is a powerful corrosive and general protoplasmic poison which acts as a protein precipitant because of its high chemical reactivity with amino groups (Goodman, 1941). Formaldehyde resembles the inorganic acids in corrosive damage (Gleason, 1963). Discoloration of the gills and heavy mucus secretions were noted in fish exposed to formaldehyde.

Phenols.

The phenols are generally described as corrosive protoplasmic or narcotic poisons. The simple aromatic hydrocarbons used in this study have different physiological affects and rates of action depending upon the presence of halogen or nitro substitution on the ring structure and the position of these additions on the ring. Any substitution on the ring structure increases the toxicity of the chemical and halogen substitution is more toxic than nitro substitution. Toxicity also increases with the number of substitutions. The para-phenolic site has a higher biological reactivity than the meta or ortho site and exerts a strong electron releasing effect which weakens acids and thus increases the numbers of neutral molecules (Lewis, 1963 and Albert, 1960). The neutral molecule of the weakly acetic and highly lipid soluble phenols easily penetrate cell membranes which hinder

the passage of ions, such as the nerve tissue of the central nervous system.

Para-chlorophenol with a narcotic chloride ion on a highly reactive site acts as an anesthetic. Rapid loss of equilibrium is caused by general nerve relaxation and death is probably due to decreased oxygen uptake caused by reduced metabolism.

Pentachlorophenol increases the metabolic rate and blood pressure which causes rupture of the finer blood vessels and capillaries (Goodnight, 1942). Bleeding about the pectoral region was noted on test fish in this study.

Para-nitrophenol and ortho-nitrophenol are corrosive and protoplasmic poisons which cause inflammation and necrosis of the gill membranes (Lewis, 1963 and Vishnivetskii, 1963). Havelka (1957) reported no gill disruption of fish exposed to nitrophenol compounds. He attributed death to nerve poisoning which resulted in respiratory failure and suffocation. Fish exposed to para-nitrophenol in this study had no visible gill disorders but did have heavy mucus formation on much of the body. Death was probably caused by the mucus interfering with the uptake of oxygen. Fish exposed to ortho-nitrophenol showed no signs of tissue disruption but did exhibit the stages of imperfect consciousness, excitement, and anaesthesia , indicating that the compound affects the nervous system and death is probably due to respiratory arrest.

The results of the assays agree with the published literature concerning the relationship between molecular structure and toxicity of the phenols tested. Referring to the concentration range (Figure 1) used in assaying the four phenolic compounds, ortho-nitro (30 to 70 ppm)

was the least toxic, while para-nitro (6 to 12 ppm) was less toxic than the para-chloro (1 to 10 ppm) substitution. The multiple substituted pentachloro compound (0.10 to 0.50 ppm) was the most toxic.

Malathion.

The organophosphorus insecticide, malathion, affects nervous transmission by inhibiting cholinesterase, an enzyme responsible for the hydrolysis of acetylcholine. As a result, excessive concentrations of acetylcholine are maintained and neuromuscular transmission is arrested. The reaction site is the brain (Weiss, 1965 and 1961). The transfer of malathion from the water, across the gills and directly to the brain is the shortest route and can account for the rapid loss of equilibrium. No other toxic action is known (Golz, 1960).

Dieldrin.

The action of the chlorinated hydrocarbons and dieldrin in particular is not certain. In general, both metabolism and nervous transmission are affected (Albert, 1960; Holden, 1965; Weiss, 1965 and Ferguson, 1964). Dieldrin could be a psychomotor drug that stimulates nervous convulsion and causes death by exhaustion, which is similar to DDT, another chlorinated hydrocarbon. Test fish in this study were observed to have an increased rate of ventilation and a high state of excitability.

DISCUSSION

Cataloging chemicals.

The chemicals tested which cause death through suffocation produce discrete and identifiable lethal indices which are not greatly affected by concentration or temperature. Death occurs a short time after loss of equilibrium which results in a high index value. Identification of the toxic agent through inspection of the indices is possible. High index values ranging from 0.950 to 0.900 are characteristic of the heavy metals, most of which cause suffocation by damaging gill tissue. Index values of 0.900 to 0.850 are typical of the organic compounds, lactic acid, formaldehyde, and p-nitrophenol, that cause suffocation by mucus precipitation. However, some of the heavy metalic compounds also cause suffocation by the precipitation of mucus; therefore, the lethal index cannot be used in all instances to define the specific physiological role of tissue disruption or mucus precipitation. Where mucus precipitation is involved the organic compounds tested always produced a lower average index value than the metalic compounds when using concentration as a variable. The higher lethal index values of the metalic compounds are probably attributable to the combined physiological effects of tissue disruption and mucus precipitation; while the organic compounds contribute only to mucus precipitation.

Sodium nitrite, another chemical which causes suffocation, has on the average, lower lethal index values than the other suffocating chemicals. Sodium nitrite causes systemic disorders and the lethal indices are influenced by concentration. At high concentrations,

allowing test fish to live six or eight hours, sodium nitrite produces discrete lethal indices of low value ranging between 0.694 and 0.798. However, at low concentrations the index values approach 0.900 which is similar to some of the other suffocating chemicals tested. The range of the lethal indices produced by sodium nitrite is best explained by the combined effects of cyanosis and anemia at high concentrations. Both physiological reactions experienced at the same time could quickly deprive the tissues of adequate oxygen and cause early loss of equilibrium. Death would be a function of time and activity. At low concentrations only cyanosis would occur which produces an index value similar to the mucus precipitating chemicals.

Low lethal index values are indicative of an anesthetic effect. Para-chlorophenol was the only true anesthetic compound tested and this aromatic hydrocarbon produced index values of 0.700 or lower. Concentration, like in the suffocating chemicals, did not greatly affect the index values.

Malathion developes indices that are restricted between the values of 0.139 and 0.270, which is a considerable range. However, the lower index values are associated with both high and low chemical concentrations. No other chemical tested exhibited this characteristic because none of the other chemicals had such a defined threshold level. At high concentrations test fish lost equilibrium early due to shear availability of the chemical in the water and death followed after a reaction period. At low concentrations the test fish lost equilibrium after a slightly longer exposure period, but the reaction period resulting in death was delayed. At a low enough concentration of malathion

test fish were able to regain equilibrium and survive for days, indicating that the fish were able, at least in part, to regenerate cholinesterase.

The above mentioned chemicals, the metals, p-nitrophenol, lactic acid, formaldehyde, sodium nitrite, p-chlorophenol, and malathion have in common fairly stable lethal index values regardless of concentration. A narrow range of index values for any one chemical at various concentrations would suggest that the chemical is disturbing only one physiological entity. Lethal indices of different values for different chemicals would suggest separate physiological entites. However, the remaining chemicals tested, dieldrin, pentachlorophenol, o-nitrophenol and potassium cyanide have extremely wide index ranges which are dependent upon concentration.

Dieldrin, for instance, has a lethal index range from 0.475 to 0,979 which includes the lethal indices of all the suffocating chemicals and overlaps the index range of pentachlorophenol, 0.440 to 0.739. Pentachlorophenol, however, only shares common index values with dieldrin and sodium nitrite. The physiological role of dieldrin, although uncertain, is attributed to nervous transmission and increased metabolism. Pentachlorophenol also increases metabolism and has the additional systemic affect of increasing blood pressure. Dieldrin and pentachlorophenol, therefore, share a common physiological effect and common lethal index values at high concentrations. At lower concentrations the index values of dieldrin exceed 0.900, which suggests a physiological role similar to the heavy metals. Pentachlorophenol does not produce index values which approach those of the suffocating metals and therefore probably does not affect the exchange of gases across the gill membranes.

Potassium cyanide and o-nitrophenol have similar index values

which are discrete from the other chemicals tested, except malathion, when extremely high concentrations are ignored. The ultimate cause of death by these two chemicals is respiratory arrest. The cyanide compound affects an oxidase system which paralyses the central nervous system. The nitrophenolic compound also affects the central nervous system and at high concentrations acts as an anesthetic. The indices of both chemicals are influenced by concentration, however, both chemicals are influenced in a similar manner.

The lethal index values of potassium cyanide and o-nitrophenol which range from 0.015 to 0.459 include the entire index range of malathion, 0.139 to 0.270. Malathion produces neuromuscular arrest and death is actually due to respiratory failure. Therefore, malathion, o-nitrophenol and potassium cyanide have a common physiological affect and also related lethal indices.

Based on data obtained through this study, suffocation through the combined affects of tissue disruption and mucus precipitation, notable in the heavy metals, is characterized by lethal index values of 0.950 to 0.900 when the effects of shock and trauma are avoided by allowing test fish to live at least six hours. Since concentration does not seem to influence the index value, laboratory bioassay to identify heavy metals or to establish that death occured by suffocation would be a simple and rapid procedure requiring little time and few test fish.

The physiological role of suffocation through mucus precipitation is characterized by lethal index values approaching 0.900 to 0.850. The identification of this physiological effect would be easier if test fish were not allowed to live longer than 16 or 18 hours. Again,

concentration has little influence on the index value and the results of several bioassays could be averaged. Average index values lower than 0.900 could indicate the absence of heavy metals.

Lethal index values in the area of 0.700 to 0.800 could indicate systemic disorders. Sodium nitrite and pentachlorophenol both affect blood pressure and both chemicals share common index values in this range. Pentachlorophenol also has common index values with dieldrin in the range of about 0.450 to 0.600 or 0.700. Pentachlorophenol also affects metabolism as does dieldrin. The data, however, are not conclusive.

Similarly, potassium cyanide, o-nitrophenol, and malathion share wide and overlapping index values ranging from about 0.100 to 0.450. All three chemicals cause death by respiratory arrest, but the physiological modes of action that cause the arrest differ. None of the other chemicals tested produced index values in this range so respiratory arrest might be identified by low index values; however, because of the possible wide range involved and the fact that o-nitrophenol developes indices similar to p-chlorophenol at high concentrations, assay procedures would be more complicated.

It is probable that o-nitrophenol, pentachlorophenol, sodium nitrite, dieldrin and possibly potassium cyanide each can exhibit different physiological effects depending on concentration. The significance of the indices developed by these chemicals is therefore low until other chemicals having only one physiological effect concerning metabolism, neuromuscular arrest, and circulatory disorders are tested.

Classifying chemicals.

In the course of this study chemicals were purchased and preliminary

assays were performed to determine toxicities. A time factor prevented complete assay of some of these chemicals, but preliminary assay data at 50 F are available.

Lead nitrate at varying concentrations which allowed 45 test fish to live from 7 to 16 hours produced an average index value of 0.940. Lead acetate, killing 55 fish in 6 to 16 hours, produced an average index value of 0.949. Another suffocant, 3-trifluoromethyl-4-nitrophenol (TFM), produced an average index value of 0.924 while allowing 12 fish to live about two hours. These average lethal index values are similar to the known suffocants tested.

Sulfuric acid, a chemical known to cause mucus precipitation and closely related to lactic acid and formaldehyde in its mode of action (Westfall, 1945), produced an average lethal index of 0.874 while killing 20 fish in 4 to 11 hours. The average index value of lactic acid for a comparable 4 to 10 hour survival period in this was 0.890. Formaldehyde produced an average index value of 0.840 for the same period.

Ortho-chlorophenol and quinaline, both anesthetics, exhibited average index values of 0.005 and 0.082 respectively, while killing 25 fish. A 16-hour bioassay using five fish and trichloromethylpropanol, an unknown in this study, resulted in a lethal index value of 0.049, indicating an anesthetic effect.

Two other chlorinated hydrocarbons, chlordane and endrine were also assayed using about 140 fish which resulted in index values ranging from 0.117 to 0.900. Chlordane, with index values ranging between 0.621 and 0.900, more closely resembled dieldrin than did endrine which produced index values of 0.117 to 0.690. The chlorinated hydrocarbons

do not seem to follow any particular pattern concerning lethal indices.

Effect of water temperature on the index.

Dieldrin was the only chemical tested that greatly changed its index value with temperature. The indices of the suffocating chemicals remained fairly stable with temperature variation. The range of the indices for the suffocating chemicals are approximately equal for the variables of concentration and temperature. However, the separation of mucus precipitation and tissue disruption which was evident with concentration as a variable was lost when using temperature as a variable. This loss of separation of these two physiological effects could cast doubt on the validity of the presence of a separation with concentration.

The lethal index values of lead nitrate and lead acetate, when using the temperature variable, were 0.938 and 0.939, respectively. Once again illustrating the tendency of the heavy metals to produce stable index values regardless of concentration or temperature.

The indices of pentachlorophenol and p-chlorophenol remained stable and equaled the index values derived by the concentration variable; however, the range of the index values resulting from the temperature variable for pentachlorophenol approximated the lower range of the index values derived by the concentration variable. More interesting is the fact that the concentration used while assaying the temperature series for pentachlorophenol produced indices similar to the index produced by that concentration in the concentration series. This occurence supports the suggestion that pentachlorophenol has different physiological roles at different concentrations.

The limited assays with o-chlorophenol using temperature as a variable resulted in an average index value of 0.018. The anesthetic chemicals also show a tendency to produce stable indices regardless of concentration or temperature.

SUMMARY

1. A lethal index, the quotient of the time until loss of equilibrium by fish divided by the time until death, was utilized to identify certain toxicants. The index has the limits of being greater than zero and less than one.
2. The objectives were (1) to arrange common toxic chemicals which have known physiological affects on fish into broad physiological categories by calculating lethal indices, (2) to use these indices as standards or references to classify chemicals by physiological effect for which the lethal causes are not yet known, and (3) to investigate the effect of water temperature as a variable on the value of the lethal index.
3. Index values varying between 0.950 to 0.900 are characteristic of the heavy metals which cause death through suffocation. Index values between 0.900 to 0.850 are characteristic of organic compounds (lactic acid, formaldehyde, and p-nitrophenol) which also cause death through suffocation. The physiological roles of tissue disruption, generally associated with the organic compounds, are not in all instances separable; however, the occurrence of an index value less than 0.900 could indicate that heavy metals were not the cause of death.
4. Concentration has little affect on the lethal index value of chemicals causing death by suffocation.
5. Para-chlorophenol was the only anesthetic compound tested which produced discrete, low index values that were not affected by concentration.

6. Pentachlorophenol, sodium nitrite, dieldrin, potassium cyanide, o-nitrophenol, and malathion produced nondiscrete, wide-range indices dependent upon concentration. Most of these chemicals have multiple physiological affects which depend on concentration. In all situations where the lethal indices of one chemical overlapped the lethal indices of another chemical a common physiological effect was shared.
7. Limited assays with additional chemicals of known physiological action or related to the test chemicals produced indices which fit well into the catagories established by the test chemicals. Lead nitrate, lead acetate, and TFM developed indices within the 0.900 to 0.950 range. Sulfuric acid produced an average index of 0.874. Quinaline, trichloromethylpropanol, and o-chlorophenol all produced indices of less than 0.100. The chlorinated hydrocarbons, chlorodane, endrine, and dieldrin do not seem to follow any particular pattern concerning lethal indices.
8. The index value of dieldrin was the only value which greatly changed with temperature.
9. Providing that bioassays could be performed in time, this research suggests that the lethal index could be adapted at least in some specific instances for the fast detection of certain groups of pollutants which are poisonous to fish. The quick identification of suspect chemicals might permit remedial action to be taken before damage to a fishery resource could become widespread. Its use as a resource tool, however, would have to await the development of field techniques that are practical and the organization of "trouble-shooting" teams in resource agencies which are geared to recognize and combat effects of aquatic pollution.

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