A STUDY OF SOME TOXIC COMPONENTS IN OIL REFINERY EFFLUENTS

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CHAPTER I

INTRODUCTION

Ammonia, sulfide, and phenol are fish toxicants found in petroleum refinery effluents. Concentrations of these materials in effluents vary from refinery to refinery and from time to time at a single refinery. Other toxic materials may be present in the effluents. Liquid wastes generally contain more than one toxic component. Little is known of the combined properties of toxic materials found in refinery and other industrial wastes. Several workers including Doudoroff (1956), Ellis (1937), Lloyd (1961), Merkens, et al. (1957), Trama (1955), Turnbull, et al. (1954), and Wallen (1957) have studied the toxicity of single pure compounds. However, toxicants when mixed may not act independently, and little is known of the toxic properties of such mixtures. In combination, antagonistic or synergistic actions between toxic components of wastes will be of an additive nature. Information on such effects would be useful in developing criteria for determining admissible concentrations to be discharged into public waterways and in making decisions as to the type and degree of treatment to be attained.

This study has three parts. Bioassays were performed on refinery effluents in which the concentration of ammonia, sulfide, and phenol, and chemical oxygen demand were known. In the second portion a statistical model for predicting fish survival was determined from the toxicity of prepared solutions of the three toxicants. Hydrogen ion concentration is known to affect toxicity of ammonia and sulfide, and this effect was also

considered. Equations developed from the statistical model were used to determine toxic relationships of the components. In the third portion, the statistical model was applied to the information gathered from bioassays of refinery effluents. The feasibility of developing equations for estimating the survival of fish from known chemical composition, and the possible presence of unknown toxic factors were considered.

CHAPTER II

REVIEW OF LITERATURE

Industrial and Oil Refinery Wastes

Literature concerning industrial wastes and their toxic properties is voluminous. Notable reviews of the literature have been made by Doudoroff, et al (1950), and Beak (1958). Ingram, et al (1955) published a useful bibliography of publications relating to the undesirable effects of various materials upon aquatic life.

Brant and Wallen (1954) listed many chemicals found in oil refinery waste water. They stated that these chemicals may have adverse effects, or in some cases may be beneficial. They felt that if toxic components could be rated on a comparative basis, singly or in combination, the waste could receive only a partial chemical analysis for adequate regulation of waste disposal. Hubault (1957) studied the change in toxicity with temperature change. He pointed out that though chemical analysis will remain the basis for the study of water pollution, it will be necessary to have the analysis controlled and verified by the biological method.

Burroughs, et al. (1958) reviewed past and present problems in the treatment of oil refinery wastes. They noted that with larger amounts of high sulfur content crude oil being processed, and with higher quality product demands there has been an increase in the sources and types of wastes. Turnbull, et al. (1954) studied the toxicity of waste water from specific areas within a refinery and of pure compounds found in the wastes. They discussed problems encountered in toxicity bioassay of petroleum

refinery effluents and described reactions of fish to certain pure compounds. The aim of the study was to determine permissible concentrations of toxic materials so that chemical tests could be used for control purposes. Dorris, et al. (1959) studied the toxic characteristics of catalytic cracker effluents, cooling tower blowdown, and final effluents. The catalytic cracker effluents had high concentrations of ammonia, sulfide, and phenolic compounds. The cooling tower blowdown contained chromate, ammonia, and chlorine. The final effluents consisted of process effluents gathered into a single stream, and had not been subjected to biological treatment. Most of the effluents were found to be toxic. Cooling tower effluent was toxic if the water had been treated with chlorine. The results of the study indicated that toxicity was an important effect of oil refinery effluents on receiving streams.

Ludzack, et al. (1956, 1957, 1958) have studied the persistence of oily wastes and their effects on streams. They found oily sludge deposits to be one of the more harmful aspects of oil refinery wastes. Ruchhoft (1953) pointed out that phenolic wastes and hydrocarbon or petroleum wastes are among the principal taste and odor contributing wastes.

Tarzwell (1957) discussed some of the problems in developing water quality criteria in relation to concentration of toxicants. He expressed the belief that each toxic waste should be considered only in relation to the receiving stream. Tarzwell (1958) discussed biological studies as a method of evaluating the self-purification of a stream. Wastes are usually classified according to their origin. Tarzwell (1958) classified wastes according to their effects on the receiving water and its biota. He noted that practically all wastes would fall into one or more of the following groups: (1) inert inorganic and organic materials; (2) putrescible wastes; (3) toxic wastes; (4) wastes of significant heat content; (5) radioactive

wastes; and (6) wastes that taint fish flesh. Henderson, et al. (1957) noted that complex wastes when mixed in an effluent may produce an entirely different toxicity from that of pure chemicals, and that most of the information available was concerned with toxicity of pure chemicals to a particular fish.

Bioassay Technique

Use of the bioassay technique for testing toxic properties of industrial effluents has been discussed by a number of authors. Belding (1927) considered some of the factors that affect bioassay results and reactions of fish to certain toxicants. Tarzwell, et al. (1958) described uses of the toxicity bioassay for studying industrial wastes. Hart, et al. (1945) and Doudoroff, et al. (1951) characterized the procedure for conducting acute toxicity bioassays. This procedure became the basis for the American Society for Testing Materials (1959) standard method for evaluating acute toxicity of industrial waste water to fresh-water fishes (ASTM Designation: D 1345). Anderson (1953) proposed a toxicity test for industrial wastes using standard species of fishes. Freeman (1953) proposed a standardized method for determining toxicity of pure compounds to fish. This method utilized a standard dilution water and standard handling practices for test organisms.

Results of bioassays have been reported in various ways. The median tolerance limit (TL_m) technique of utilizing bioassay data is described by Doudoroff, et al. (1951). This is a graphical method of estimating the concentration of toxicant that would be required to kill just 50% of the test organisms. The number of hours required to produce the TL_m is also reported. For example, a median tolerance limit determined by a 24-hour test is reported as the TL_m^{24}. Gillette, et al (1952) appraised a

chemical waste by reporting the range in concentration below which all fish lived and above which all fish died. Ellis (1937) reported the lowest concentration in which fish died. Herbert (1952) and other English workers used the "reciprocal method", based on the concept that survival time is inversely proportional to a power of the concentration. An equation was used to predict the time of death at low toxicant concentrations. Henderson (1957) discussed the possibility of developing application factors based on bioassay results to estimate the required dilution for safe disposal.

Specific Toxicants

Ammonia is found in many industrial effluents, and numerous studies of its toxic properties have been made. The undissociated molecule of NH_4OH, or the free un-ionized ammonia dissolved in water ($NH_3 \cdot H_2O$), is the toxic material. Wuhrmann, et al. (1947) discussed the factor and showed that the hydrogen ion concentration of a solution is a major factor in determining the toxicity of ammonia. The greater the pH the greater the concentration of undissociated NH_4OH. Herbert (1955) noted that a fall in pH value decreased the toxicity of ammonia because more ammonium ions are formed which are less toxic than ammonia molecules. Ellis (1937) reported that toxicity of ammonium compounds increased 200% or more between pH 7.4 and 8.0. Grindley (1946) found rainbow trout to be affected more rapidly in a solution of ammonium chloride prepared with alkaline water than in a solution prepared with distilled water. Alabaster and Herbert (1954) found that concentrations of carbon dioxide, up to 30 mg/l, reduced the toxicity of ammonia. It was concluded that this reduction was due to the lowered pH with the addition of carbon dioxide. At concentrations above 30 mg/l the carbon dioxide was toxic. No interaction was

evident between ammonia and carbon dioxide toxicity. Downing and Merkens (1955) and Merkens and Downing (1957) found a significant interaction between concentrations of ammonia and dissolved oxygen. At a constant concentration of un-ionized ammonia survival time increased as oxygen tension increased. The effect of oxygen tension on survival time was greatest in lower concentrations of un-ionized ammonia. Lloyd (1961) related toxicity of ammonia to bicarbonate alkalinity, pH, temperature, free carbon dioxide and dissolved oxygen, and devised a graphical method of predicting toxicity when these factors were known. Turnbull, et al (1954) found the TL_m^{24} concentration of ammonia to be 8.2 mg/l expressed as nitrogen.

Jones (in Klein, 1957) presumed that ammonia acts as a true internal poison on fish, entering the body via the gills and circulating in the blood stream, since its toxicity seems to be strictly correlated with the permeability of the gills for the toxic molecules. In man, ammonia is said to destroy blood corpuscles and the blood does not coagulate normally. It is possible that fish are affected in a similar way. Jones (1948) found fish to be repelled by 0.04 N and 0.01 N solutions. Turnbull, et al. (1954) found that fish were blinded and that the gill region became covered with a gray mucus sheath in an ammonium hydroxide solution.

When sulfides are hydrolyzed toxic hydrogen sulfide is formed. The H_2S eventually escapes into the atmosphere or is gradually oxidized, yielding colloidal sulfur. This oxidation occurs more rapidly in lower H_2S concentrations. Longwell and Pentenlow (1935) found that survival time of fish was increased fifteen-fold by raising the pH from 7.5 to 9.0. No measurable variation in toxicity was observed between pH 6.9 to 7.6. According to Doudoroff and Katz (1950), Dodero (1924, 1926) found that Na_2S was more toxic in distilled water than in hard water, but that

Kreitmann (1929) found the opposite to be true. Turnbull, et al (1954) found the TL_m^{24} concentration of sulfide to be 25 mg/l.

Belding (1927) stated that H_2S produces respiratory paralysis, but that fish can recover in fresh water. Jones (in Klein, 1957) stated that sulfides inhibit oxygen utilization. The undissociated molecule of H_2S appeared to penetrate living tissue more rapidly than sulfide ions. The rate of opercular movement falls with oxygen consumption and fish exhibit a remarkable power of recovery if removed from the solution before they cease breathing. Turnbull, et al. (1954) found it difficult to obtain reproducible results because of sulfide loss. They found fish to be relatively inactive near the bottom of the jar, and after a period of time to lose their equilibrium.

Phenols are important aromatic compounds and toxicants. There are numerous monohydric and polyhydric phenols. Trama (1955) conducted toxicity bioassays on phenol solutions and concluded that measurements should be based on 48-hour rather than 24-hour tests. He found that a 12 mg/l solution of phenol lost 1-2 mg/l phenol in 24 hours, and at the end of 72 hours all the phenol had disappeared. The instability was attributed to oxidation by dissolved oxygen or to bacterial decomposition. McKinney, et al. (1956) showed that phenol may serve as a bacterial food without serious toxic effect. Belding (1927) reported that phenol has an irritating action, but produced no evidence of oxygen hunger. Jones (in Klein, 1957) stated that phenol has an irritating action on mucous membranes and appears to have some direct effect on the nervous system. When fish were placed in a phenol solution they quickly lost their balance and fell over on their sides. They lived for sometime in a helpless condition, breathing feebly and irregularly, and recoiling violently if touched. Even in very dilute solutions swimming was not co-ordinated. Jones (1951) found that the

minnow Phoxinus phoxinus (L.) had little capacity for avoiding phenol, with little power to discriminate between phenol and water. Turnbull, et al. (1954) reported that the fish moved about violently immediately after being placed in concentrations between 13 and 23 mg/l phenol. The TL_m^{24} concentration was 19 mg/l phenol.

Toxic characteristics of high and low hydrogen-ion concentrations have been studied by numerous workers. Doudoroff and Katz (1950) reviewed this work and concluded that pH values between 5.0 and 9.0 are not lethal for most fully developed fresh-water fishes. Extreme pH values below 4.0 and above 10.0 may be tolerated indefinitely by resistant species. Ellis (1937) reported the general pH range of unpolluted water to be pH 6.7 to 8.6. Trama (1954) found pH tolerance of the bluegill to be 4.0 to 10.35. Jones (1948) found that fish responded indifferently to hydrogen-ion concentrations within the range pH 5.8 to 11.2. Ellis (1937) showed that toxicity of many acids is due to their anions or undissociated molecules and that hydrogen ions may be relatively unimportant. Jones (in Klein, 1957) stated that higher hydrogen ion concentrations (i.e., lower pH values) apparently produce a coagulation of gill secretions and asphyxia, or may exert an astringent or corrosive effect upon gill tissues with a similar result. Sodium hydroxide and other strong alkalis probably produce asphyxiation by coagulation of gill secretions. Turnbull, et al. (1954) observed that the bodies of fish become covered with a gray mucus-like sheath, and that the eyes become the first organs visibly affected, when placed in a sodium hydroxide solution.

CHAPTER III

METHODS

General Procedures

Bioassay methods used for this study were essentially those suggested by Hart, et al. (1945) and Doudoroff, et al. (1951). The methods are useful in obtaining information on the relative acute toxicity of the material tested. Acute toxicity is expressed as a median tolerance limit (TL_m). Methods of chemical analysis of test materials were basically those described in "Standard Methods" (1960).

Handling of Fish

Test fish utilized were fathead minnows (Pimephales promelas Raf.). Doudoroff (1956) found that these fish remained in good condition in the laboratory for many months. They are thought to be moderately sensitive to toxicants as compared with other fishes (Hart, et al.1945). Douglas and Irwin (1962) compared the resistance of P. promelas and 15 other species of fishes to oil refinery effluents. Four significantly different populations existed among the 16 species studied. P. promelas was in three of the populations, one species, the guppy, Lebistes reticulatus (Peters) was significantly more resistant, and none of the remaining were significantly less resistant.

Minnows were raised in ponds located near Stillwater, Oklahoma, and were collected by seining. They were held in the laboratory in porcelain-lined tanks of approximately 50 gallons capacity. They were fed a mixture

of ground chicken feed and powdered egg. All fish used for testing mixtures of pure compounds were acclimated in dechlorinated tap water for ten days or more. On a few occasions fish used for bioassays of oil refinery effluents were acclimated for only three days. Fish used in the tests were 3 to 4.5 cm long, in good physical condition, and came from ponds within a single watershed. During the summer months a small number of fish were infected with tail-rot. Satisfactory treatment was accomplished by adding terramycin and acroflavin to the holding water (Irwin, 1959). Fish collected during fall, winter and spring usually needed no treatment. Normally less than one percent of the fish died as a result of holding.

Toxicity Bioassays of Refinery Effluents

Members of the Oklahoma Oil Refiners Waste Control Council sent one or more samples of their waste water to Oklahoma State University for toxicity bioassay each month. Chemical analyses of samples were made by refinery personnel. Concentrations of ammonia-nitrogen, sulfide, phenol, chemical oxygen demand, and alkalinity, and pH value were measured. Samples were collected in five-gallon polyethylene bottles and delivered to the Stillwater laboratory within 24 hours. Different concentrations of waste water/dilution water were made and survival of fish in each determined.

Dechlorinated tap water was used as dilution water. This water had a relatively constant chemical composition. Three-gallon polyethylene buckets were used for test containers. Ratio of waste water to dilution water in test solutions was based on a logarithmic scale suggested by Hart, et al. (1945). Ten liters of solution were made up in each test container. Two test solutions were used at each concentration. Five

fish were placed in each container, so that each concentration was tested with ten fish.

Each test was conducted over a 48-hour period. Initial and final pH values were recorded for each test solution. A Beckman "180 Pocket pH Meter" was used to measure pH values. Test solutions that maintained greater than 1.0 mg/l dissolved oxygen were considered satisfactory (Dorris, et al. 1959). Whitworth, et al. (1961) found that P. promelas survived at an oxygen tension of 1.0 mg/l. Oxygen was bubbled slowly through test solutions having low oxygen concentrations. Test solutions having an initial dissolved oxygen concentration (DO) of less than 1.0 mg/l were treated with oxygen before fish were placed in the test solution. Dissolved oxygen measurements were made using the "Short" Theriault modification of the Winkler method. Bioassays were conducted in a constant temperature room, and temperatures of test solutions were held within a range of 20 C to 24 C. Surviving fish were counted at 24 and 48 hours. Dead fish were removed when found, or at the time of counting.

Toxicity Bioassays of Mixtures of Pure Compounds

Ammonium hydroxide, sodium sulfide, and phenol were used in preparing solutions to study toxic properties of pure compounds and mixtures of pure chemicals. Appropriate amounts of the materials were added to 80 liters of dilution water to produce a stock solution containing desired proportions of the toxicants. A chemical analysis was made of each stock solution. Bioassays were run in 5-gallon glass containers. Ten liters of test solution were used. Tests were conducted at two pH levels. Adjustments in pH were made by adding small amounts of hydrochloric acid or sodium hydroxide until the desired level was reached. Two test solutions were set up for each concentration and 10 fish were placed in each

container. Tests were conducted for 24 hours and survival counts were made at 12 and 24 hours. The pH levels were checked and adjusted at the 12-hour check period. In other respects toxicity bioassays of mixtures of pure compounds were the same as with refinery effluents.

Chemical Analysis

Chemical analyses of refinery effluents and prepared toxic solutions were based on methods described in "Standard Methods" (1960). In some cases refinery personnel modified the methods or developed new ones to fit their particular problems. In the analysis of prepared solutions of pure compounds ammonia-nitrogen and phenol were determined using the "Direct Nesslerization Method" and "Aminoantipyrine Method" respectively. A Bausch and Lomb "Spectronic 20" colorimeter was used to measure color intensities, at a wavelength of 510 mμ. Sulfide was measured by the "Titrimetric Method".

Statistical Analysis

The statistical design was selected in consultation with staff of the Oklahoma State University Statistical Laboratory. The method of analysis is known as the "Multiple Linear Regression" method and is discussed in Cochran and Cox (1957). An IBM 650 digital computer was utilized in the analysis.

CHAPTER IV

CHARACTERISTICS OF REFINERY OPERATIONS AND EFFLUENTS

A high degree of variability was observed in chemical and toxic characteristics of the effluents considered. Variability was pronounced between different refineries. Effluents from a single refinery varied considerably in some cases. Variability might arise from complexity of operations, type of crude oil being refined, refining processes characteristic of each refinery, and degree and type of waste treatment.

Effluents consisted of combined wastes from all portions of a refinery. Since it was necessary that test material be toxic to fish, sampling stations were chosen that had previously produced toxic samples. During the course of the study some refineries introduced treatment practices which improved the waste at sampling stations. In some refineries two sampling stations were chosen to measure effectiveness of treatment methods. Effluents described in this study do not necessarily represent waste water outfalls to receiving streams. A brief description of refinery operations and waste treatment methods at the different refineries is given in the following paragraphs. The letters used to identify refineries correspond to the letters used to identify effluents in Tables I through V.

Refinery Operations

Refining processes at refinery A included crude distillation, vacuum distillation, catalytic cracking, catalytic reforming, hydrogen fluoride

alkylation, and propane deasphalting. Waste water passed through a series of oil settling ponds, a spray system and a series of oxidation ponds.

Processes at refinery B included crude distillation with light naptha specialties, vacuum distillation, catalytic cracking, and polymerization. Waste treatment facilities included an American Petroleum Institute (API) oil separator, a series of holding ponds, aeration tower, and a series of holding ponds with aeration weirs separating the final series of ponds.

Refinery C had crude distillation, vacuum distillation, catalytic cracking, polymerization, HF alkylation, and asphalt facilities. Waste water passed through an oil separator, aerator and a holding pond system.

Refining processes at refinery D included crude distillation, vacuum distillation, catalytic cracking, catalytic reforming, phosphoric acid polymerization, HF alkylation, desalting, cleaners solvent manufacture, phenol and cresol treatment of lube stocks, and a heptane stripper with ammonia refrigeration. All waste water flowed through an API type oil separator to a holding pond. It was then pumped to a pit, mixed with lime slurry, and settled in pits. After recarbonation by an underwater burner the waste passed through a series of oxidation ponds where additional aeration was introduced by diffusing compressed air.

Processes at refinery E included crude distillation, vacuum distillation, catalytic and thermal cracking, catalytic reforming, HF alkylation, lubricating oil heating and dewaxing, and delayed coking. Waste treatment included segregation and separate disposal of caustics and other chemicals harmful to the quality of effluent water, segregation of sour water streams with steam stripping and bio-oxidation prior to entering refinery effluent, neutralization of hydrogen fluoride sludge with lime, oil separation in traps and ponds, and final treatment of composite effluent in a series of oxidation ponds.

Processing at refinery F consisted of crude distillation, vacuum distillation, catalytic cracking, catalytic reforming, and oil blending. Waste treatment processes included an API oil separator, foul water stripping, reuse of process water in desalter, flue gas neutralization of low cresylic-acids-content caustic solutions, disposal by sale of high cresylic-acids-content caustic solutions, an oxidation pond, and burning and burial of solids.

Major operations at refinery G included crude distillation, vacuum distillation, catalytic cracking, catalytic reforming, catalytic polymerization, HF alkylation, and delayed coking. Waste treatment included an API oil separator, lime treatment, aeration by spraying, and a series of oxidation ponds.

Basic refining processes at refinery H were crude distillation, vacuum distillation, catalytic cracking, catalytic polymerization, propane deasphalting, and phenol treating. Waste water passed through an oil separator before discharge.

Refining processes at refinery J included crude distillation, vacuum distillation, catalytic cracking, catalytic reforming, and polymerization. Waste treatment included oil separation, impounding of spent caustic solutions, reclamation of boiler feed water, stripping and neutralization of process water containing high concentrations of sulfur compounds, and an oxidation pond.

Operations at refinery K were crude distillation, vacuum distillation, catalytic cracking, polymerization, and asphalt blending. Waste treatment practices included steam stripping of process water containing high concentrations of sulfur and ammonia, reuse of this water for desalting crude oil, followed by mixing with high pH boiler blowdown water under high pressure, releasing water to atmospheric pressure produced steam flashing

and release of much ammonia, cooling this water and mixing with water from oil trap, and passage of water through air flotation and activated sludge units.

At refinery L the refining processes included crude distillation, vacuum distillation, catalytic and thermal cracking, catalytic reforming, polymerization, sulfuric acid alkylation, isomerization, and gasoline treating. Waste treatment included oil separation, stripping of slurry waters, aeration, and final settling in oxidation ponds.

Refinery Effluents

Range, mean, and median of chemical and toxic characteristics of effluents of the refineries are shown in Tables I through V. Number of determinations used to obtain each set of data is also given. Median values are used for comparative purposes in the following discussion, and an attempt is made to point out possible reasons for differences in toxicity.

Effluent G-1 in Table I was the most toxic effluent considered in this study. Median concentrations of ammonia, phenol, sulfide, chemical oxygen demand (COD), and phenolphthalein alkalinity were greater than in any other effluent studied. Ammonia concentration was about one-third greater than the next highest concentration (G-2), phenol was almost twice as great as the next highest concentration (F), and sulfide concentration was more than three times greater than any other sulfide concentration except in effluent C.

Effluents J-1, J-2, and F (Table II) had median TL_m^{48} values of 11.8% to 13.5%. Effluents J-1 and J-2 were similar. Differences in median concentrations of ammonia and phenol in effluents J-1 and J-2 were probably of little importance. Median sulfide concentration of 7 mg/l in

TABLE I

CHARACTERISTICS OF REFINERY EFFLUENTS

Effluent		pH	NH_3-N mg/l	Phenol mg/l	Sulfide mg/l	C.O.D. mg/l	Alkalinity ($CaCO_3$) mg/l		TL_m^{48} %
							P	Total	
G-1	R	8.7 - 9.6	68 -- 377	11 -- 78	0 -- 339	520-3500	0 - 550	224--1100	1.8 - 46
	$\bar{X}$	9.0	163	34	76	1214	242	514	8.3
	M	9.2	126	27	23	882	201	385	5.6
	N	15	15	15	15	15	8	8	23

R. Range

$\bar{X}$. Mean

M. Median

N. Number of Determinations

TABLE II

CHARACTERISTICS OF REFINERY EFFLUENTS

Effluent		pH	NH_3-N mg/l	Phenol mg/l	Sulfide mg/l	C.O.D. mg/l	Alkalinity ($CaCO_3$) mg/l		TL_m^{48} %
							P	Total	
J-1	R	8.0--11.1	46 - 200	0 - 12	0 - 40	24 - 486	84 - 556	284 - 800	1.8 - 51
	$\bar{X}$	8.8	80	4	12	233	186	492	19.3
	M	8.9	70	2	7	218	141	460	11.8
	N	24	21	20	24	24	11	11	24
J-2	R	8.1--10.9	32 - 400	0 - 9	0 - 31	30 - 418	0 - 450	250 - 680	4.8 - 65
	$\bar{X}$	8.9	85	3	10	176	170	448	19.2
	M	8.9	68	3	3	134	149	468	13.5
	N	23	20	25	23	23	10	10	27
F	R	8.2--10.6	1 - 110	0 - 111	0 - 8	158 - 997	32 - 180	90 - 855	4.2 - 84
	$\bar{X}$	9.2	38	22	0	348	131	353	20.2
	M	9.2	36	15	0	295	130	235	13.3
	N	29	29	29	27	29	13	13	28

R. Range M. Median

$\bar{X}$. Mean N. Number of Determinations

effluent J-1 compared to 3 mg/l in effluent J-2 might have been the cause of the slightly greater toxicity of J-1. In effluent F median concentration of ammonia was approximately half that of effluents J-1 and J-2, but median phenol concentration in effluent F was approximately five times greater than in effluent J-2. Combined effect of ammonia and phenol might have been responsible for the relatively high toxicity (low TL_m^{48}) of effluent F.

Median TL_m^{48} values of effluents L, C, and G-2 (Table III) ranged from 21.0% to 32.0%. Effluent L had the same median ammonia concentration as effluent J-1, but the higher TL_m^{48} of L might have resulted from lower pH and sulfide values. Effluent C had an ammonia concentration about half that of effluent L, but the high pH of 9.4 and sulfide concentration of 18 mg/l probably tended to hold the TL_m^{48} at a lower level. Median values of ammonia and pH of effluent G-2 illustrate the expected effect of low pH on a high ammonia level. Ammonia concentration was 86.0 mg/l and the resulting high degree of ionization at pH 7.6 might have produced the TL_m^{48} of 32%.

Effluents D, A-1, and H (Table IV) had median TL_m^{48} values of 41.3% to 42.0%. Effluents D and G-2 had similar pH, phenol, and sulfide values. However, ammonia concentration of effluent D was much lower than effluent G-2, and this may explain the higher TL_m^{48} value of effluent D. Effluent A-1 had lower ammonia and phenol concentrations than effluent H, but the pH 8.9 of the effluent A-1 compared to pH 8.0 of effluent H was possibly the reason for TL_m^{48} being the same in both effluents.

Five effluents, K, E-1, E-2, A-2, and B (Table V) had median TL_m^{48} values greater than 100, that is, a 100% concentration of the waste would not kill 50% or more of the test fish. This should be interpreted as no measurable TL_m^{48}. Although toxicity was sometimes evident, less than 50%

TABLE III

CHARACTERISTICS OF REFINERY EFFLUENTS

Effluent		pH	NH_3-N mg/l	Phenol mg/l	Sulfide mg/l	C.O.D. mg/l	Alkalinity ($CaCO_3$) mg/l		TL_m^{48} %
							P	Total	
L	R	6.2 - 10.1	8 - 355	1 - 44	0 - 40	12 - 555	0 - 389	105 - 483	4.0 GT 100
	$\bar{X}$	8.5	91	7	4	192	134	234	26.6
	M	8.7	70	5	1	180	127	242	21.0
	N	28	28	28	28	28	13	13	28
C	R	7.2 - 11.0	6 - 70	0 - 0	0 - 170	234 - 3680	0 - 134	132 - 220	4.0 GT 100
	$\bar{X}$	9.2	36	0	38	765	46	171	35.1
	M	9.4	40	0	18	454	34	156	24.0
	N	22	22	21	21	20	12	12	28
G-2	R	7.0 - 8.5	27 - 135	0 - 60	0 - 19	270 - 974	0 - 62	130 - 292	13.0 -- 56
	$\bar{X}$	7.7	80	10	4	569	7	185	29.7
	M	7.6	86	4	2	533	0	180	32.0
	N	15	15	15	15	15	9	9	22

R. Range M. Median

$\bar{X}$. Mean N. Number of Determinations

TABLE IV

CHARACTERISTICS OF REFINERY EFFLUENTS

Effluent		pH	NH_3-N mg/l	Phenol mg/l	Sulfide mg/l	C.O.D. mg/l	Alkalinity ($CaCO_3$) mg/l		TL_m^{48} %
							P	Total	
D	R	6.8 - 8.2	5 - 80	0 - 37	0 - 13	98 - 502	0 - 0	150 - 400	13.0 GT 100
	$\bar{X}$	7.4	35	8	3	329	0	280	42.2
	M	7.4	32	4	2	341	0	300	41.3
	N	25	24	22	23	22	12	12	28
A-1	R	8.3 - 9.5	12 - 53	0 - 100	0 - 2	119 - 688	18 - 84	95 - 208	20.0 GT 100
	$\bar{X}$	8.9	23	10	0	242	30	165	50.4
	M	8.9	22	2	0	197	48	182	42.0
	N	27	29	27	29	28	20	14	29
H	R	6.8 - 9.5	7 - 51	0 - 271	0 - 25	42 - 1250	0 - 50	142 - 360	7.3 GT 100
	$\bar{X}$	7.9	28	40	2	359	28	278	42.8
	M	8.0	28	10	0	247	40	299	42.0
	N	18	20	20	20	20	6	6	30

R. Range M. Median

$\bar{X}$. Mean N. Number of Determinations

of the test fish died. In most cases no fish were killed in the solutions. Median ammonia concentration was less than 20 mg/l and median values of phenol and sulfide concentrations were relatively low in all effluents.

Effluent K is of interest because of the extreme TL_m^{48} range. Concentrations of ammonia, phenol, and sulfide were below the level normally considered unsafe for fish with the exception of one instance when the sulfide concentration reached 2.24 mg/l. The pH remained below 9.0 and had a median value of 7.5. Relatively low median COD value of 96.0 mg/l indicates a low concentration of oxidizable material. On some occasions the TL_m^{48} was very low, but the source of toxicity was not evident since concentrations of ammonia, phenol, and sulfide were at a low level. This one case indicates the existence of toxicants in the effluent that were not studied.

Effluents E-1, E-2, and A-2 were toxic 10% to 30% of the time, apparently because of occasional high ammonia concentrations. Ammonia concentration of effluent B consistently remained below 4 mg/l. Occasional toxicity of effluent B might have resulted from occasional high concentrations of phenol and sulfide.

Chemical oxygen demand (COD) determinations were made on all effluents. Greatest COD was found in effluent G-1, which was most toxic. Least COD was found in effluent K, which usually had a low toxicity. However, COD values between these extremes were variable and no direct relationship between COD and toxicity was distinguishable.

Prather (1959) found that refinery waste approached chemical stabilization when the ratio of phenolphthalein to total alkalinity approached zero. He also found that TL_m^{48} increased from 15.5% to 78% when the alkalinity ratio approached zero. Effluents K, E-1, E-2, A-2, and B had alkalinity ratios of less than 0.1 and TL_m^{48} values of greater than 100%, but

TABLE V

CHARACTERISTICS OF REFINERY EFFLUENTS

Effluent		pH	NH_3-N mg/l	Phenol mg/l	Sulfide mg/l	C.O.D. mg/l	Alkalinity ($CaCO_3$) mg/l		TL_m^{48} %
							P	Total	
K	R	6.8 - 817	0 - 6	0 - 0	0 - 2	0 - 258	0 - 0	20 - 88	1.8 GT 100
	$\bar{X}$	7.6	0	0	0	81	0	54	73.5
	M	7.5	1	0	0	96	0	56	GT 100.0
	N	24	23	24	24	24	13	13	26
E-1	R	6.8 - 8.6	7 - 55	0 - 4	0 - 2	100 - 324	0 - 22	96 - 134	63.0 GT 100
	$\bar{X}$	7.7	22	2	0	285	5	118	95.6
	M	7.6	18	1	0	177	5	118	GT 100.0
	N	27	27	27	27	27	12	12	29
E-2	R	6.8 - 8.3	5 - 51	0 - 2	0 - 1	95 - 268	0 - 12	106 - 140	80.0 GT 100
	$\bar{X}$	7.4	19	0	0	176	1	125	99.1
	M	7.4	13	0	0	165	0	126	GT 100.0
	N	27	27	27	27	27	11	12	28

R. Range M. Median

$\bar{X}$. Mean N. Number of Determinations

TABLE V (Continued)

Effluent		pH	NH_3-N mg/l	Phenol mg/l	Sulfide mg/l	C.O.D. mg/l	Alkalinity ($CaCO_3$) mg/l		TL_m^{48} %
							P	Total	
A-2	R	7.9 - 9.7	2 - 35	0 - 7	0 - 0	93 - 682	0 - 35	60 - 156	84.0 GT 100
	$\bar{X}$	8.7	13	0	0	187	11	109	99.0
	M	8.7	12	2	0	141	10	117	GT 100.0
	N	28	29	29	28	29	15	14	30
B	R	7.0 - 10.0	0 - 4	0 - 100	0 - 4	140 - 1000	0 - 40	75 - 240	32.0 GT 100
	$\bar{X}$	8.2	0	4	1	292	7	134	92.3
	M	8.0	0	0	1	233	0	110	GT 100.0
	N	28	25	25	26	22	13	13	28

R. Range

$\bar{X}$. Mean

M. Median

N. Number of Determinations

effluents G-2 and D, which also had alkalinity ratios less than 0.1, had median TL_m^{48} values of 32% and 41.3% respectively. A low alkalinity ratio did not necessarily accompany a relatively non-toxic waste.

CHAPTER V

RESULTS

Relationship Between Median Tolerance Limits and Concentrations of Effluent Components

Relationship between median tolerance limits (TL_m^{48}) and single factors measured in refinery effluents is shown in Figs. 1 to 4. Factors considered were ammonia, sulfide, phenol, and chemical oxygen demand. Results from four hundred and thirty-three bioassay tests of refinery effluents were used. Concentrations of effluent components were plotted against TL_m^{48} determined for each test. Boundary lines were drawn so that nearly all data points were enclosed beneath the lines. The lines do not necessarily represent maximum TL_m^{48} that may be attained at corresponding concentration, but are drawn only to enclose a large majority of observed data points and to point out the general trend of increased toxicity with increased component concentration. Though such a boundary undoubtedly exists it may not be in fact a straight line as is shown in Figs. 1 to 4. Since any factor may occur at very low concentration in a waste where other factors occur at high concentration, it is not uncommon for an effluent to be quite toxic even though the concentration of a particular component is of low value. A true boundary would be expected to become asymptotic to the abscissa. For this reason boundary lines were not estimated for values less than $TL_m^{48} = 10\%$. Values not included beneath the lines were assumed to be in error due to unknown causes. Over 95% of the points are beneath the assumed boundary lines.

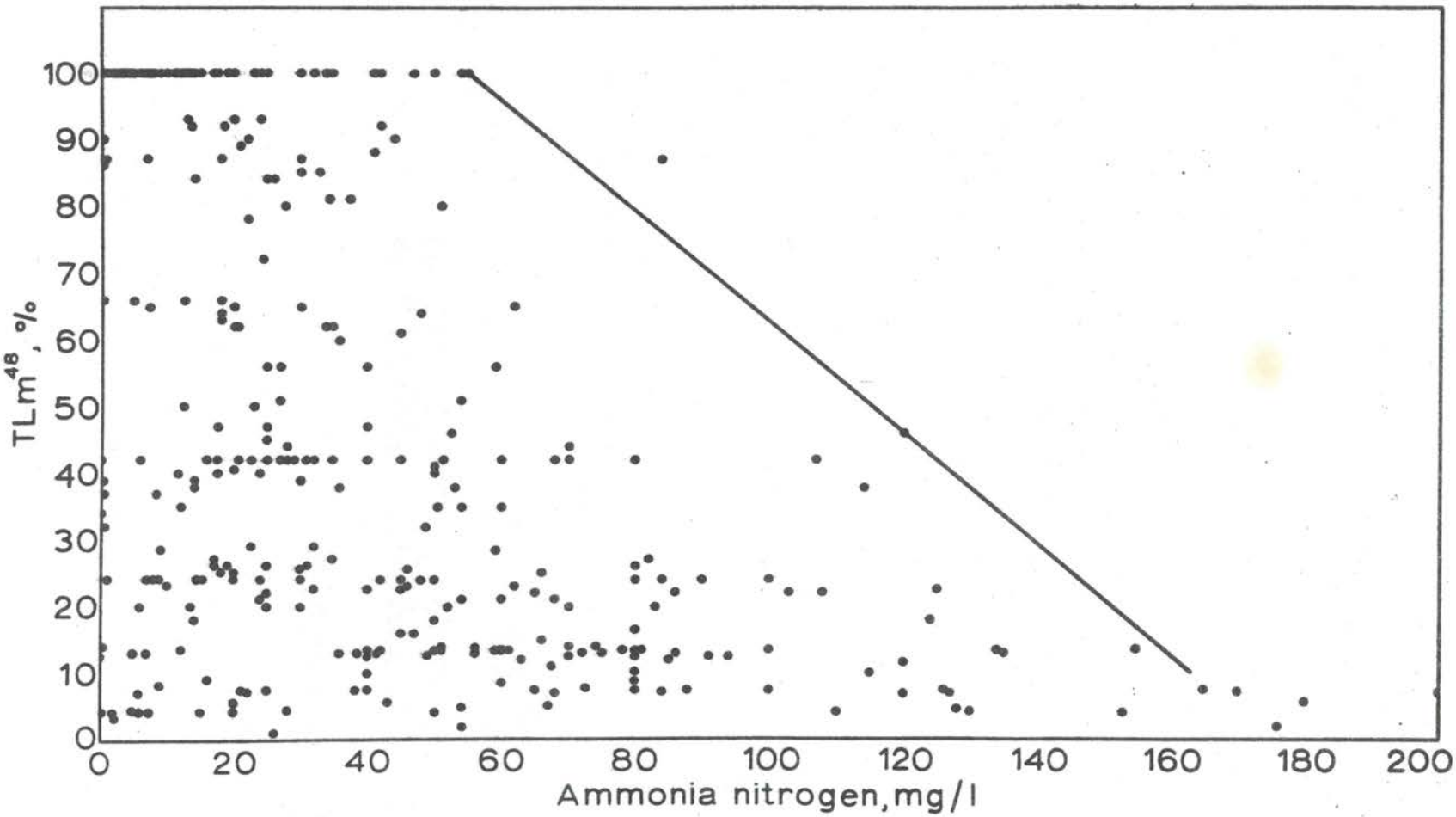

Fig. 1. Relationship of ammonia concentration in refinery effluents to toxicity.

Relationship of ammonia-nitrogen concentration, hereafter referred to as ammonia concentration, and TL_m^{48} is shown in Fig. 1. Concentration of ammonia at any TL_m^{48} in a solution may be obtained by multiplying the concentration by the corresponding TL_m^{48} percentage and dividing by 100. Maximum concentrations can therefore be estimated from the boundary line. For example: at TL_m^{48} = 100%, NH_3 = 60 mg/l; at TL_m^{48} = 10%, NH_3 = 16 mg/l. Overall maximum concentration estimated from the line is 64 mg/l at TL_m^{48} = 72%.

The boundary of sulfide concentration and TL_m^{48} is shown in Fig. 2. Maximum sulfide concentrations present in TL_m^{48} solutions vary from 4 mg/l at TL_m^{48} = 100%, to 30 mg/l at TL_m^{48} = 58%, to 10 mg/l at TL_m^{48} = 10%.

Phenol and TL_m^{48} relationship is shown in Fig. 3. Maximum phenol concentrations estimated for TL_m^{48} solutions vary from 8 mg/l at TL_m^{48} = 100%, to 29 mg/l at TL_m^{48} = 57%, to 10 mg/l at TL_m^{48} = 10%.

Chemical oxygen demand (COD) is not a measure of any particular toxicant but represents the concentration of oxidizable matter in an effluent. Oxidizable matter in a refinery effluent is largely of an organic nature. Some of these organic compounds may be toxic to fish. Fig. 4 illustrates the relationship between toxicity and observed COD values. Fifty percent or more of test specimens at times survived COD values up to 1710 mg/l. Beyond this there was a definite reduction in the observed TL_m^{48} values. Upper COD concentrations at TL_m^{48} vary from 1710 mg/l at TL_m^{48} = 100%, to 235 mg/l at TL_m^{48} = 10%.

Results of Bioassays Using Mixtures of Pure Compounds

Data from bioassays of mixtures of pure compounds were used to estimate TL_m^{24} concentrations, and results are shown in Table VI. Results from tests on single compounds and from mixtures at various ratios are

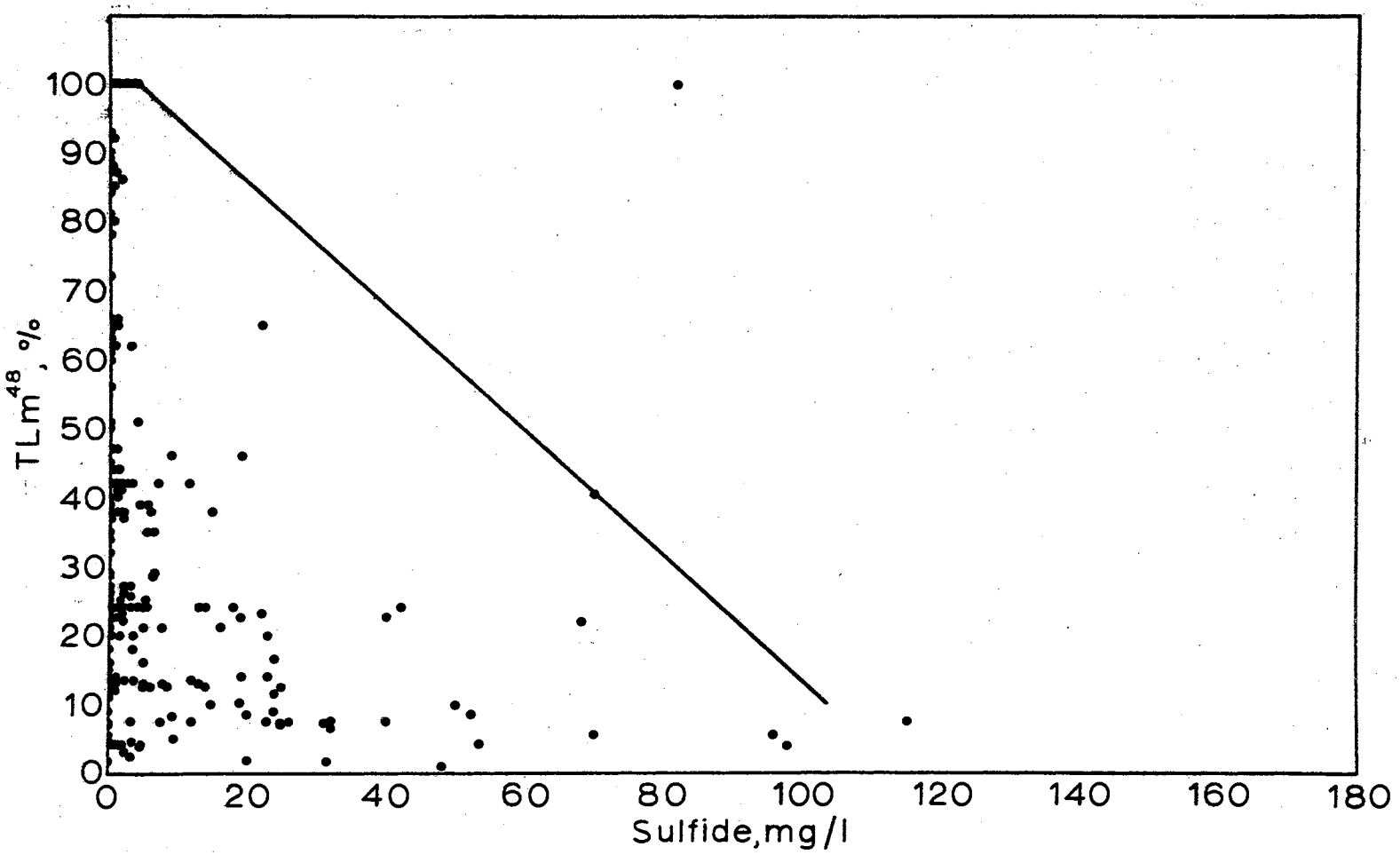

Fig. 2. Relationship of sulfide concentration in refinery effluents to toxicity.

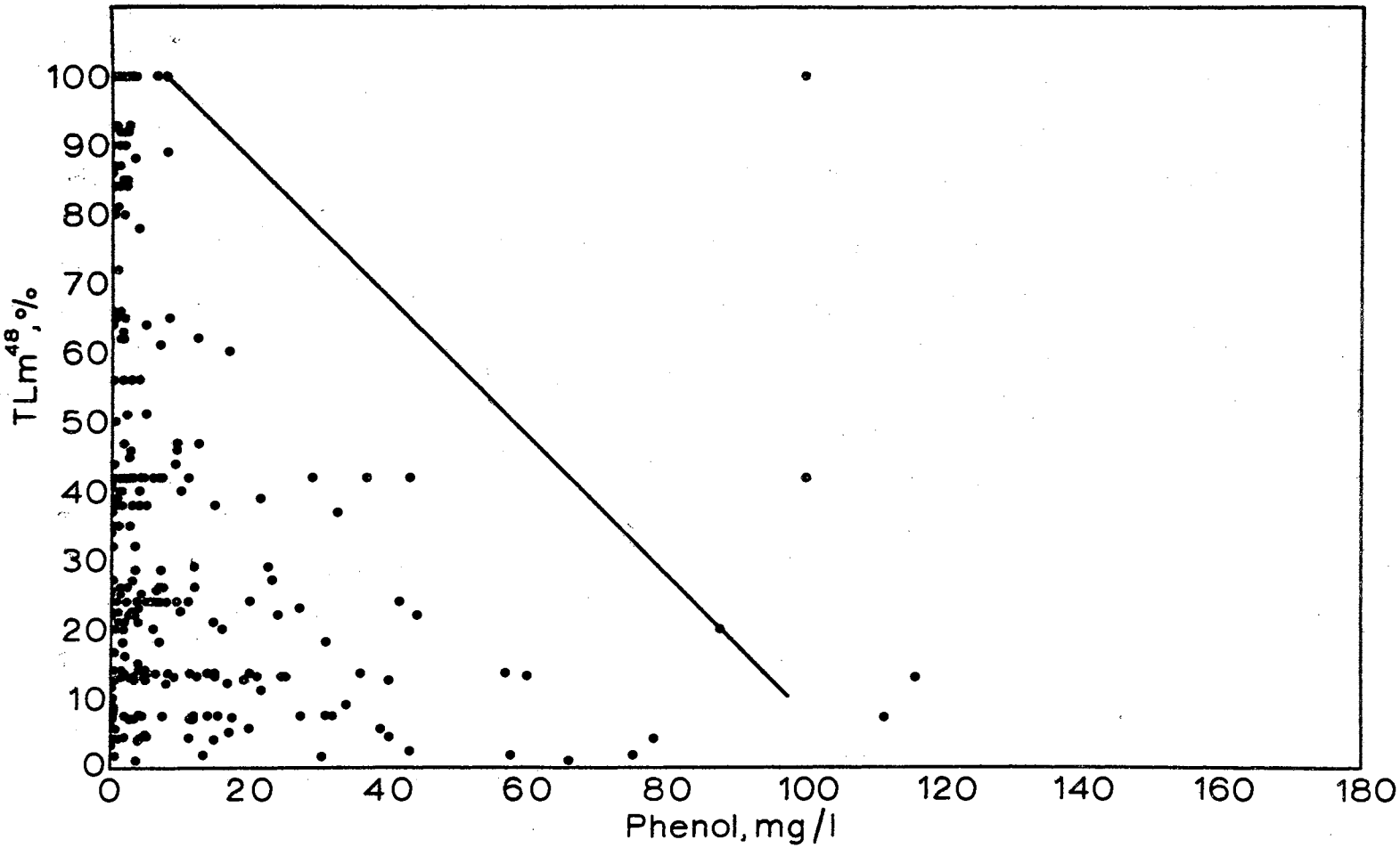

Fig. 3. Relationship of phenol concentration in refinery effluents to toxicity.

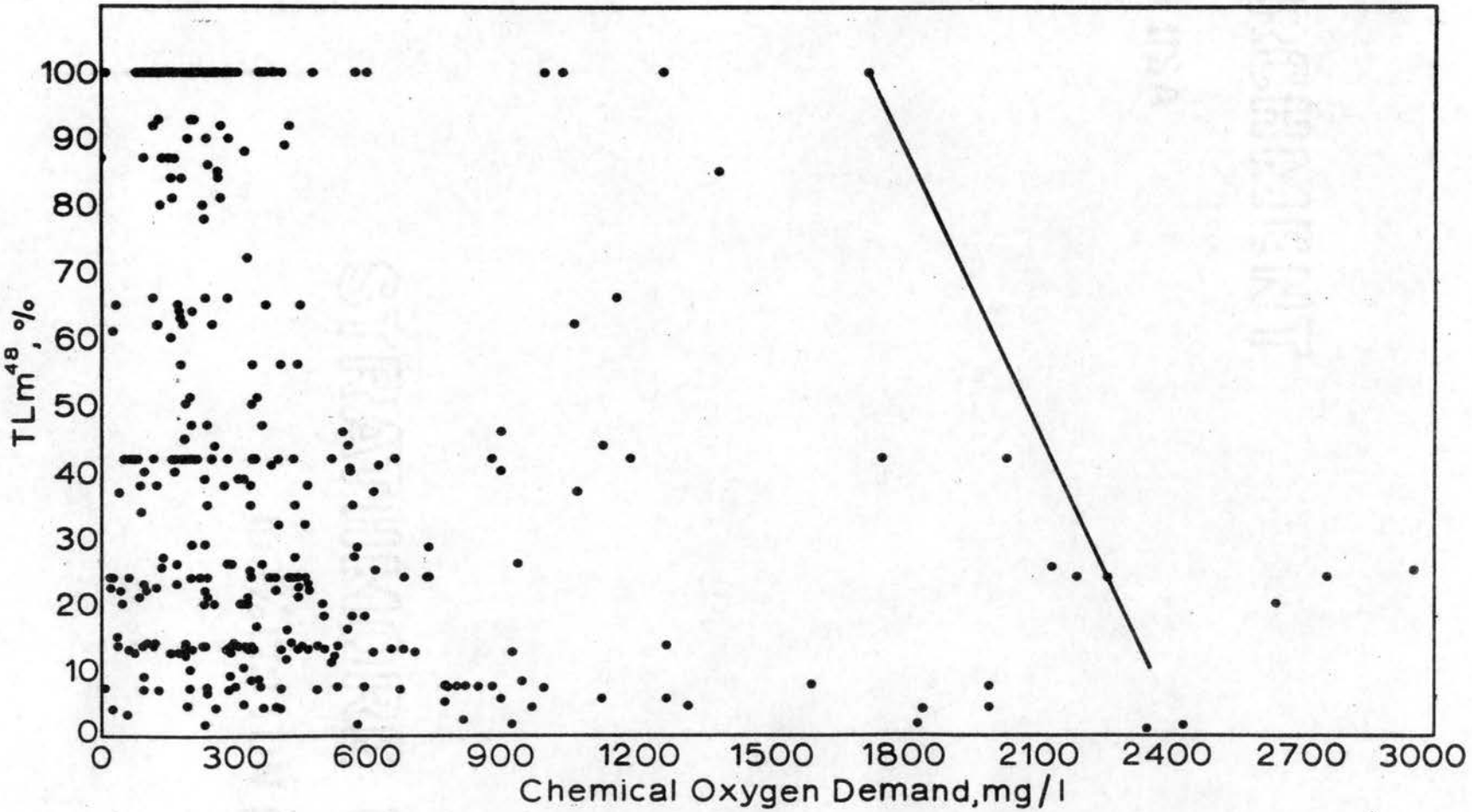

Fig. 4. Relationship of chemical oxygen demand of refinery effluents to toxicity.

arrayed in columns 1 through 11. Concentration of each toxicant at two pH levels is indicated under each column heading. For example, TL_m^{24} concentrations of ammonia mixed with sulfide in a 5:1 ratio is shown in column four to be 9.75 mg/l at pH 8.0, and 8.5 mg/l at pH 8.5.

Examination of data in Table VI suggest a number of tentative conclusions. Ammonia generally is more toxic at higher pH values, and may be more toxic in mixtures than as a single toxicant. Under similar test conditions Lloyd (1961) observed death in 50% of test specimens with ammonia-nitrogen concentrations of 14.5 and 23.9 mg/l at pH 8.2 and 7.8 respectively. Toxicity of sulfide is not materially affected by mixing with other toxicants. Sulfide generally is more toxic at lower pH level, and appeared to act independently of other materials used.

When present in high ammonia to phenol ratios (column 6 and 10) phenol may slightly reduce toxicity of ammonia. However, at a low ammonia to phenol ratio (columns 7 and 11), phenol increases toxicity of ammonia. These results were obtained at both higher and lower pH values. Phenol appears to be more toxic when other toxicants are present. Results from phenol tests were highly variable. Turnbull, et al. (1954) found no difference between 24- and 48-hour TL_m values for ammonia, sulfide, and phenol. Trama (1955) stated that toxicity measurements of phenol should be based on 48-hour tests. Variation in phenol test results in the present study may have been caused by using only a 24-hour test period. It was not uncommon for surviving fish in test solutions containing phenol to suffer loss of equilibrium.

Development of Prediction Equations

Data were taken from series of bioassay tests at two pH levels on each of three toxicants, on three combinations of two toxicants, and on

TABLE VI

ESTIMATED CONCENTRATIONS OF TOXICANTS TO PRODUCE 50% SURVIVAL OF TEST FISH (TL_m^{24})

Column No.		1	2	3	4	5	6	7	8	9	10	11
Toxicant Mixtures		N	S	PhOH	N:S	N:S	N:PhOH	N:PhOH	S:PhOH	S:PhOH	N:S:PhOH	N:S:PhOH
Mising Ratio					5:1	1:1	5:1	1:2	1:1	1:10	10:1:2	5:1:10
Toxicants & pH												
N mg/l	pH 8.0	17.00			9.75	1.90*	22.50*	8.50			17.00	7.50
	pH 8.5	6.60			8.50	3.40	9.50	5.10			9.50	5.25
S mg/l	pH 8.0		1.60		1.95	1.90*			1.90	1.70	1.70	1.50
	pH 8.5		4.70		1.70	3.40			5.30*	2.90	0.95	1.05
PhOH mg/l	pH 8.0			50.00			4.50*	17.00	1.90	17.00	3.40	15.00
	pH 8.5			52.00			1.90	10.20	5.30*	29.00	1.90	10.50

* TL_m estimated by extrapolation

N. Ammonia-nitrogen

S. Sulfide

PhOH. Phenol

combinations of all three toxicants to develop equations for prediction of survival. Percent survival of test organisms, concentrations of ammonia-nitrogen, sulfide, and phenol, and pH value were recorded for each bioassay test. A total of 212 tests was made using 2,420 fish.

It was assumed that a mathematical relationship existed between survival of fish and chemical composition of the test solution, and that this relationship resulted from accumulated actions of toxic materials. A mathematical model that best represented relationships between factors studied had to be selected. The ideal model would be the equation of a surface representing the relationship of survival to factors of toxicity. The better the choice of model, the better the model could be made to fit the collected data. After a number of trials the model chosen for the analysis was

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \beta_5 x_5 + \beta_6 x_6 + \beta_7 x_7 + \varepsilon$$

where

y = percent survival of fish,

x_1 = ammonia-nitrogen concentration in mg/l,

x_2 = sulfide concentration in mg/l,

x_3 = phenol concentration in mg/l,

x_4 = pH,

$x_5 = (x_1)(x_4)$,

$x_6 = (x_2)(x_4)$,

$x_7 = (x_1)(x_3)$,

ε = random error.

b_0 is the estimate of β_0, the constant regression coefficient of the equation which determines evaluation of the regression surface, $b_1, b_2, \ldots, b_7$ are estimates of $\beta_1, \beta_2, \ldots, \beta_7$, the regression coefficients for respective x-values which fix orientation of the

regression surface.

This equation represents a multiple linear regression of y on the x variables. A least squares method of analysis was used to estimate β values. Terms in the regression equation were chosen on the basis of what was known of the relationship of a particular toxicant to survival. Terms used in the model were simple expressions of concentration. Where interaction between toxicants was expected a cross product term such as (x_1x_4) was included in the equation. Interaction is defined (Steel, et al. 1960) as a measure of the departure of simple effects from a model based on main effects only. When interaction occurs between x_1 and x_2 the rate of change of response to various values of x_1 at one level of x_2 would not be the same at another level of x_2.

Analysis provided various types of information. The estimated β values provide fit of the model to the date. The coefficient of multiple correlation (R^2) provided a means of judging the choice of model. An R^2 value of 1 would indicate perfect correlation between the model and the data. If R^2 could be shown to be significantly greater than zero, it was indicated that the model was accounting for some of the effect of chemicals on survival (Anderson, 1958). A standard deviation, or standard error of the estimate, was determined from the analysis. Acceptance or rejection of an equation depended primarily on the coefficient of multiple correlation and the standard deviation. Standard deviation obtained with the equation ought to approximate deviations observed in duplicate test situations.

With b values established, survival may be estimated when concentration of chemicals are given. Values of b_0, b_1, ..., b_7 are given in Table VII. Choice of b values to be used is dependent upon which set of conditions are known. For example, if only ammonia concentration is

known, equation (1) would be used:

$$\hat{y} = 836.56 + 79.83X_1 - 84.02X - 10.60X_1X_4.$$

If both ammonia and sulfide concentrations are known, equation (4) would be used:

$$\hat{y} = 191.44 + 166.81X_1 - 616.28X_2 + 51.62X_4 - 21.19X_1X_4 + 67.36X_2X_4.$$

TL_m^{24} concentrations from Table VI, which were determined graphically, were substituted into equations given in Table VII to predict percent survival. Results are shown in Table VIII. In general, predicted values for conditions at pH 8.5 are nearer the expected 50% than those from pH 8.0. Out of 22 predicted values 15 are greater than 50% and seven predictions are low.

Goodness of fit of regression, or the fraction of variation in survival of fish which is accounted for by the model is measured by R^2 values shown in Table VII. Smaller values of R^2 may result from random variation or other independent variables not considered in the regression model. Bioassay results for phenol were somewhat variable and this could account in part for the small R^2 value of .37. All R^2 values in Table VII except for equation (3) were found to be significantly greater than zero. This indicates that the equations generally provide a significant means to predict survival of fish. The larger the R^2 value the more closely the data fit the equation.

Data from all series of tests were analyzed in an effort to obtain a general equation applicable for all situations. The resulting R^2 value of 0.44, while significantly greater than zero, is not as large as might be desired. The large number of zero concentration values introduced into the analysis from those series of tests that did not contain one or more toxicants may account for part of the lack of fit. Another possibility is that the combined toxic action is so different when any one or

TABLE VII

MULTIPLE REGRESSION ANALYSIS DATA

Toxicants and Equation No.		Regression Coefficient								Multiple Correlation Coefficient	Standard Deviation
		b_0	b_1	b_2	b_3	b_4	b_5	b_6	b_7	R^2	
N	(1)	836.56	79.83			-84.02	-10.60			0.72*	28.2
S	(2)	-1,050.52		- 45.52		142.91		2.57		0.70*	25.2
PhOH	(3)	143.17			- 1.34	- .66				0.37	17.2
N:S	(4)	- 191.44	166.81	-616.28		51.62	-21.19	67.36		0.79*	22.2
N:PhOH	(5)	232.87	177.45		-12.12	1.56	-23.04		.44	0.83*	19.8
S:PhOH	(6)	657.01		-905.48	- 2.43	-56.67		104.27		0.81*	15.4
N:S:PhOH	(7)	656.78	139.08	-447.53	- 4.24	-51.34	-18.14	50.11	-.16	0.82*	19.2
N:S:PhOH (all data)	(8)	- 189.60	113.02	- 49.66	- 1.24	38.77	-14.45	3.67	-.06	0.44*	31.1

* R Significantly greater than zero

TABLE VIII

PREDICTED PERCENT SURVIVAL OF TEST FISH

Equation		1	2	3	4	4	5	5	6	6	7	7
Column No. From Table VI		1	2	3	4	5	6	7	8	9	10	11
Predicted Percent	pH 8.0	79.9	52.82	70.89	44.17	65.31	80.78	44.50	63.53	41.10	40.41	49.19
Survival	pH 8.5	54.6	52.94	67.88	59.91	53.45	56.34	51.61	60.76	49.21	45.39	65.05

two toxic materials is added to another that a general equation cannot be applicable in the absence of any x-factor.

Standard deviation values shown in Table VII are expressed as percent survival of test fish. Since most of the values are of the order of 20 percent, the equation may be expected to provide a prediction within approximately four fish out of twenty 68% of the time. This is in agreement with the standard deviation of $\pm$ 20 percent that would be expected when 20 test organisms per test are used (Snedecor, 1956).

An attempt to estimate permissible levels of the factors was made by substituting approximate x values into equations given in Table VII. Estimated values are shown in Tables IX, X, and XI. Survival of 50% was assumed so that concentrations estimated by using the equations could be compared with values shown in Table VI obtained by the graphical method of estimating TL_m. The equations should not be used to predict an unknown x-factor when survival and other x-factors are known since that factor actually may not be present.

Ammonia-nitrogen concentrations shown in Table IX were obtained by substituting values of sulfide and phenol from Table VI and assuming y of 50%. Substituted values are noted in parentheses. The regression equation will not estimate ammonia-nitrogen levels in the same mixing ratios that were used for testing, but will estimate the permissible concentration of ammonia-nitrogen that would allow 50% of the fish to survive at the substituted sulfide and phenol levels.

Example:

Sulfide = 1.95 mg/l (Table VI)

pH = 8.0

Equation (4):

TABLE IX

CALCULATED PERMISSIBLE TL_m^{24} CONCENTRATIONS OF AMMONIA-NITROGEN

Column		1	2	3	4	5	6	7
Toxicant Mixtures		N	N:S	N:S	N:PhOH	N:PhOH	N:S:PhOH	N:S:PhOH
Original Mixing Ratio			5:1	1:1	5:1	1:2	10:1:2	5:1:10
Toxicants & pH								
N mg/l	pH 8.0	23.02	7.60	7.60	28.80	17.52	15.52	7.40
	pH 8.5	7.05	9.25	3.66	9.86	5.22	9.20	6.15
S mg/l	pH 8.0		(1.95)	(1.90)			(1.70)	(1.50)
	pH 8.5		(1.70)	(3.40)			(.95)	(1.05)
PhOh mg/l	pH 8.0				(4.50)	(17.00)	(3.40)	(15.00)
	pH 8.5				(1.90)	(10.20)	(1.90)	(10.50)

Values in parenthesis from Table VI

N. Ammonia-nitrogen

S. Sulfide

PhOH. Phenol

TABLE X

CALCULATED PERMISSIBLE TL_m^{24} CONCENTRATIONS OF SULFIDE

Column		1	2	3	4	5	6	7
Toxicant Mixtures		S	N:S	N:S	S:PhOH	S:PhOH	N:S:PhOH	N:S:PhOH
Original Mixing Ratios			5:1	1:1	1:1	1:10	10:1:2	5:1:10
Toxicants & pH								
N mg/l	pH 8.0		(9.75)	(1.95)			(17.00)	(7.50)
	pH 8.5		(8.50)	(3.40)			(9.50)	(5.25)
S mg/l	pH 8.0	1.71	1.88	2.15	2.10	1.58	1.49	1.48
	pH 8.5	4.82	1.95	3.49	5.86	2.86	0.74	1.75
PhOH mg/l	pH 8.0				(1.90)	(17.00)	(3.40)	(15.00)
	pH 8.5				(5.30)	(29.00)	(1.90)	(10.50)

Values in parenthesis from Table VI

N. Ammonia-nitrogen

S. Sulfide

PhOH. Phenol

$$y = -191.44 + 166.81x_1 - 616.28x_2 + 51.62x_4 - 21.19x_1x_4 + 67.36x_2x_4$$

$$50 = -191.44 + (166.81)x_1 - (616.28)(1.95) + (51.62)(8.0) - (21.19)(x_1)(8.0) + (67.36)(1.95)(8.0)$$

$$x_1 = \frac{50 + 191.44 + (616.29)(1.95) - (51.62)(8.0) - (67.36)(1.95)(8.0)}{166.81 - (21.19)(8.0)}$$

$$x_1 = \frac{20.59}{2.71} = 7.60 \text{ mg/l}$$

This value of x_1 is shown in column 2 of Table IX. Values estimated for ammonia and sulfide in Table IX and X are comparable to those obtained under test condition s as shown in Table VI. Concentrations estimated for phenol in Table XI differ from results in Table VI as would be expected from lack of fit of equation (3), and variation experienced with bioassays. The regression equation for ammonia and sulfide mixtures indicates that sulfide is the primary contributor of toxicity, and as concentration of one factor increases the tolerable concentration of the other decreases. Rate of increase or decrease in concentration is dependent upon pH. The equation has a fair fit and estimated concentrations are in accordance with test results shown in Table VI.

When ammonia concentration shown in Table XI at pH 8.0 was reduced from 22.5 mg/l to 8.5 mg/l, estimated phenol concentration decreased slightly from 18.38 mg/l to 16.34 mg/l. When ammonia concentration at pH 8.5 was reduced from 9.5 mg/l to 5.1 mg/l, estimated phenol concentration increased from 2.7 mg/l to 10.36 mg/l. This indicates that range of tolerable phenol concentrations is much more limited at higher pH values when ammonia is present. If a combined toxic effect is produced by the two toxicants it is possibly a function of concentrations of un-ionized ammonia and phenol.

Results in Tables X and XI using the sulfide and phenol regression equation indicate that sulfide contributes the major portion of toxicity,

TABLE XI

CALCULATED PERMISSIBLE TL_m^{24} CONCENTRATIONS OF PHENOL

Column		1	2	3	4	5	6	7
Toxicant Mixtures		PhOH	N:PhOH	N:PhOH	S:PhOH	S:PhOH	N:S:PhOH	N:S:PhOH
Original Mixing Ratio			5:1	1:2	1:1	1:10	10:1:2	5:1:10
Toxicants & pH								
N mg/l	pH 8.0		(22.50)	(8.50)			(17.00)	(7.50)
	pH 8.5		(9.50)	(5.10)			(9.50)	(5.25)
S mg/l	pH 8.0				(1.90)	(1.70)	(1.70)	(1.50)
	pH 8.5				(5.30)	(2.90)	(0.95)	(1.05)
PhOH mg/l	pH 8.0	65.59	18.38	16.34	7.46	13.34	2.02	14.85
	pH 8.5	65.34	2.70	10.36	9.72	28.67	1.10	13.46

Values in parenthesis from Table VI

N. Ammonia-nitrogen

S. Sulfide

PhOh. Phenol

TABLE XII

VARIATION IN SURVIVAL COMPARED WITH HOLDING TIME AND CAPTURE GROUP

Test #	Capture Group	Holding time (days)	Survival %
1	1	24	35
2	1	28	95
3	2	21	90
4	2	22	95
5	Test not completed		
6	2	42	95
7	2	44	45
8	3	14	85
9	3	17	90
10	3	19	100
11	3	27	95
12	4	16	95
13	4	18	65
14	4	21	85
15	5	12	90
16	5	14	90
17	5	23	100
18	6	12	95
19	6	15	95
20	Test not completed		
21	6	21	95
22	7	11	100
23	7	16	90

but that at higher sulfide levels allowable phenol concentrations are reduced. Estimated values are comparable to those in Table VI.

The general regression equation (8) does a fair job of estimating the concentration that will produce a 50% survival. This is illustrated by comparing values in Tables IX, X, and XI with those in Table VI.

Holding Time and Quality of Test Organisms

Tests were run concurrently with bioassays of prepared solutions to determine effect of holding time of fish on reaction of fish to toxic solutions. A solution containing 9.24 mg/l ammonia, 0.68 mg/l sulfide, and 12.6 mg/l phenol at pH 8.0 was used for each test. These tests made it possible to compare fish captured at different times, to compare fish held for various periods of time prior to exposure to toxic mixtures, and to determine consistency of quality of fish used to compare effects of ammonia, sulfide, and phenol in prepared solutions. Results are shown in Table XII.

Weiss, et al. (1957) found that resistance of fish increased as holding time increased from one to thirteen days, but that fish held more than thirty days tended to lose resistance. Herbert, et al. (1952) studied factors associated with varying resistance of trout to cyanide. They found that resistance of individual trout was mainly due to inherent properties. Fish for tests in the present study were held at least ten days before being used as test animals.

Fish for all tests could not be captured at one time and capture groups were compared for possible differences. Holding time varied from 11 to 44 days and fish were captured on seven different occasions. Survivals of 35% and 45% in the first and seventh test were less than normal.

TABLE XIII

CHEMICAL ANALYSIS OF STOCK SOLUTIONS

Test	Prepared Toxic Solutions						Holding Time Solution					
	Calculated			Analysis			Calculated			Analysis		
	NH_3-N	S	PhOH	NH_3-N	S	PhOH	NH_3-N	S	PhOH	NH_3-N	S	PhOH
1	200	0	0	200	0	0	22	1.63	30	20	T	23.8
2	0	0	80	0	0	62	22	1.63	30	24	3.84	20.8
3	0	20	0	0	19.44	0	22	1.63	30		NS	
4	0	20	0	0	18.5	0	22	1.63	30	17	2.8	26.0
5	100	0	0	75	0	0	22	1.63	30	14	0.5	17.5
6	Not completed									Not completed		
7	0	0	100	0	0	92	22	1.63	30	21.7	1.2	NS
8	50	0	100	48	0	NS	22	1.63	30	26.7	1.0	NS
9	50	0	100	80	0	98	22	1.63	30	21.0	2.3	20.5
10	0	10	0	0	6.7	0	22	1.63	30	21.9	NS	34.0
11	50	10	0	36	10.2	0	22	1.63	30	16.0	1.8	37.0
12	50	10	0	44	10	0	22	1.63	30	17.0	1.9	34.7

T: Trace

NS: No Sample

TABLE XIII (Continued)

Test	Prepared Toxic Solutions						Holding Time Solution					
	Calculated			Analysis			Calculated			Analysis		
	NH_3-N	S	PhOH	NH_3-N	S	PhOH	NH_3-N	S	PhOH	NH_3-N	S	PhOH
13	0	10	100	0	6.9	100	22	1.63	30	17.0	1.9	35.5
14	50	0	100	40	0	100	22	1.63	30		NS	
15	50	10	100	12	1.08	98	22	1.63	30	8.0	0	37.5
16	50	10	0	18	7.1	0	22	1.63	30	10.5	0.5	30.0
17	50	0	10	38	0	17	22	1.63	30	12.5	0.1	39.0
18	0	10	100	0	8.5	96	22	1.63	30	12.6	0.58	34.0
19	10	10	0	13.6	8.67	0	22	1.63	30	25.0	1.11	28.7
20	50	10	100	34.0	7.95	100	22	1.63	30	17.1	1.08	29.5
21	0	10	10	0	4.57	12.5	22	1.63	30	21.0	2.09	36.0
22	50	5	10	41.5	3.2	13.3	22	1.63	30	17.6	0.28	36.5
23	50	5	10	40.6	2.06	15.8	22	1.63	30	15.0	T	34.4

T: Trace

NS: No Sample

Equation (7) (Table VII) was used to estimate percent survival in test solutions, and a value of 86% was determined. Mean percent survival for 21 completed tests was 87%. Median percent survival was 95%. Two tests were not completed because of known errors. Neither holding time nor capture group could be shown to have a significant effect on response of test animals. Quality of fish used throughout the study was relatively constant.

Stock solutions containing toxicants were prepared and diluted to obtain a range of test concentrations. Chemical analyses were made on stock solutions used for tests. Results of analyses of prepared toxic solutions and holding time are shown in Table XIII, and varied from expected values in some cases. Samples were fixed for chemical analysis immediately after bioassays were started. Samples were stored in a refrigerator and at times were stored more than a week before analysis. Storage time might have affected some results. A 0.5 mg/l deviation from the prescribed ammonia-nitrogen concentration would normally be expected. The Nessler's reagent was found to be bad after test number 18 and this might account for the low values for ammonia observed from test 15 through 18. A normal deviation of 0.8 mg/l sulfide could be expected. This deviation was exceeded in about half the tests. Variation normally associated with the phenol test cannot be determined due to the numerous phenolic compounds that are detected by the test.

Interrelationships of Toxic Components of Oil Refining Effluents

Effect on toxicity resulting from changes in the four X-factors is shown in Fig. 5 through Fig. 12. Range of X values used in determining equations shown in Table VII is given in Table XIV. Equations would be

TABLE XIV

RANGE AND MEAN OF DATA USED TO DEVELOP REGRESSION EQUATIONS

Equation		1	2	3	4	5	6	7	8
y Percent Survival	R	0 -100	0 -100	35 -100	0 -100	0 -100	5 -100	0 -100	0 -100
	$\bar{X}$	38.9	40.4	73.9	45.5	47.6	71.9	60.9	54.7
X_1 NH_3-N mg/l	R	3.8- 36.0	0 - 0	0 - 0	1.4- 12	3.8- 25	0 - 0	3.8- 21	0 - 36
	$\bar{X}$	15.3			7.8	9.7		9.8	7.2
X_2 S mg/l	R	0 - 0	0.8- 7.4	0 - 0	1.4- 3.8	0 - 0	1.0- 5.6	0.7- 2.1	0 - 7.4
	$\bar{X}$		3.9		2.1		2.3	1.0	1.3
X_3 PhOH mg/l	R	0 - 0	0 - 0	33.6- 60.0	0 - 0	1.5- 24	1.5- 32	1.5- 18	0 - 60
	$\bar{X}$			47.5		11.5	14.0	10.4	9.8
X_4 pH	R	8.0- 8.5	8.0- 8.5	8.0- 8.5	8.0- 8.5	8.0- 8.5	8.0- 8.5	8.0- 8.5	8.0- 8.5
	$\bar{X}$	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2

R. Range

S. Sulfide

$\bar{X}$. Mean

NH_3-N. Ammonia

PhOH. Phenol

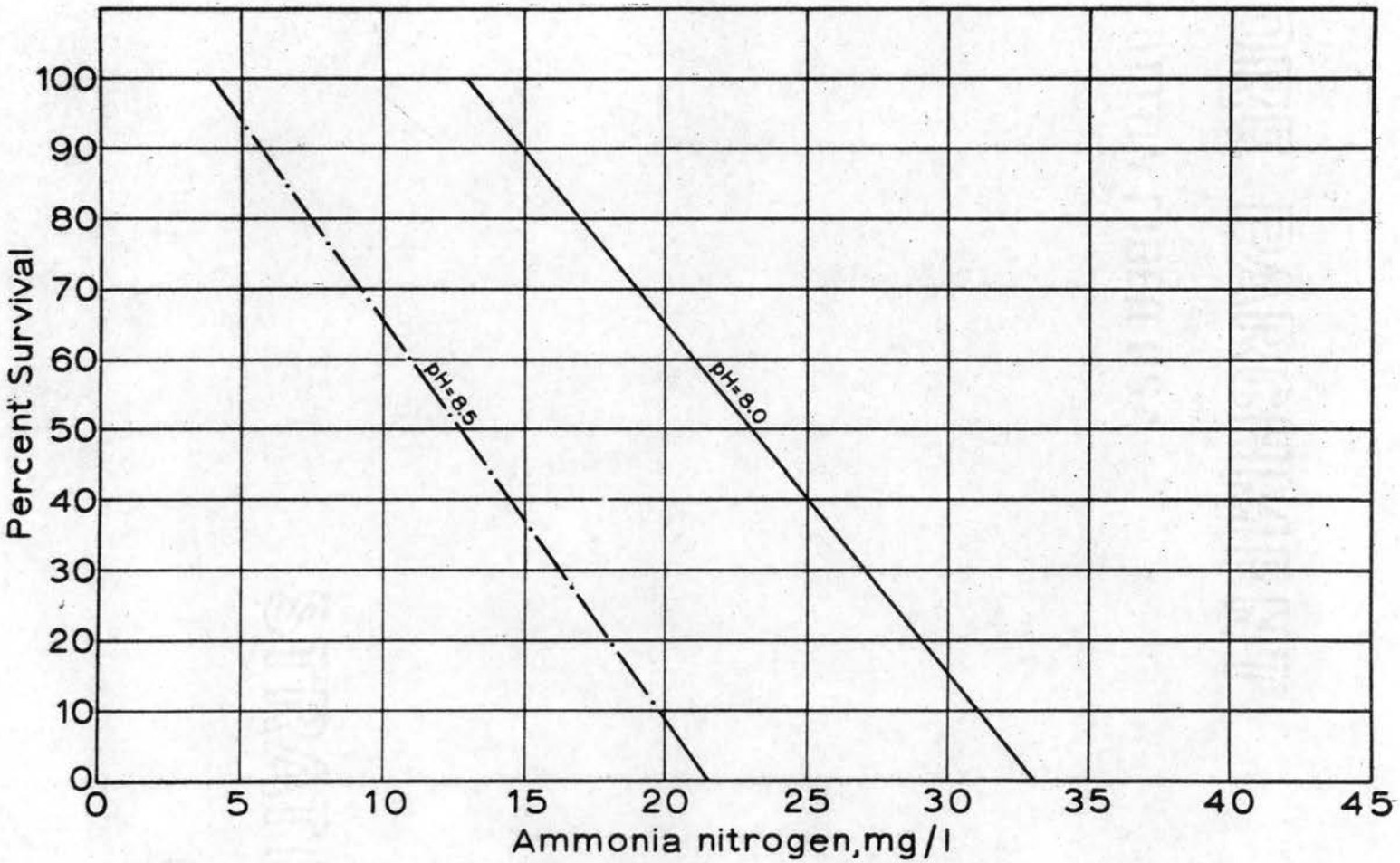

Fig. 5. Interrelationship of ammonia and pH to toxicity.

more precise when used with X values that are near mean X values of the original data used to estimate the regression equation (Steel, et al. 1960). A regression equation should not be used with levels of X beyond the range of values used to estimate the equation. Mean X and y values of the original data are also given in Table XIV.

Predicted survival of fish exposed to different concentrations of ammonia is shown in Fig. 5. Equation (1) (Table VII) and pH values of 8.0 and 8.5 were used to determine the lines. When interaction is present, a plot of this type would produce non-parallel lines. Although an interaction term was included in the equation, for purposes of predicting survival, the lines are essentially parallel. Increase in pH increased toxicity of ammonia. At a given ammonia concentration survival at pH 8.5 was approximately 50 percentage points less than at 8.0.

Survival of fish estimated by using equation (2) was plotted against sulfide concentration in Fig. 6. As with ammonia, lines are approximately parallel and, although interaction of sulfide concentration and pH was considered in the model, it did not seem to be of practical importance for this situation. Reduction of pH increased toxicity of sulfide compounds. At a given sulfide concentration survival was approximately 75 percentage points more at pH 8.5 than at pH 8.0.

Equation (3) was used to determine toxic action of phenol concentrations (Fig. 7). There was little or no relationship between toxicity of phenol and pH level. Phenol would be less than 10% ionized within the pH range used to estimate equation (3). At higher pH levels phenol would be more highly ionized. If phenol molecules are more toxic than ions, as in the case of ammonium hydroxide and hydrogen sulfide, it is possible that phenol would become less toxic at higher pH values and somewhat more toxic at lower pH values. At higher pH values the compound which produces the higher pH might become increasingly more toxic.

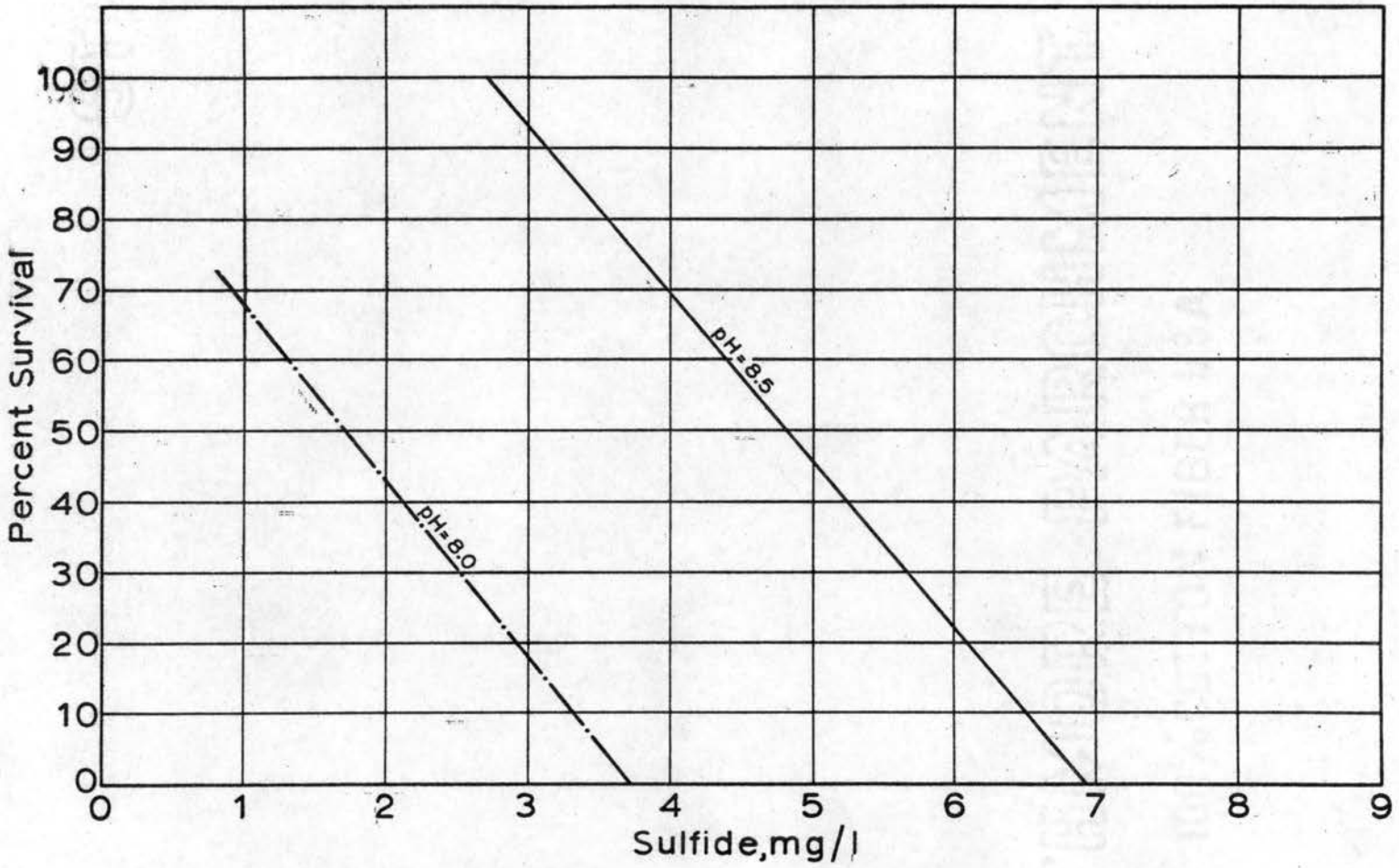

Fig. 6. Interrelationship of sulfide and pH to toxicity.

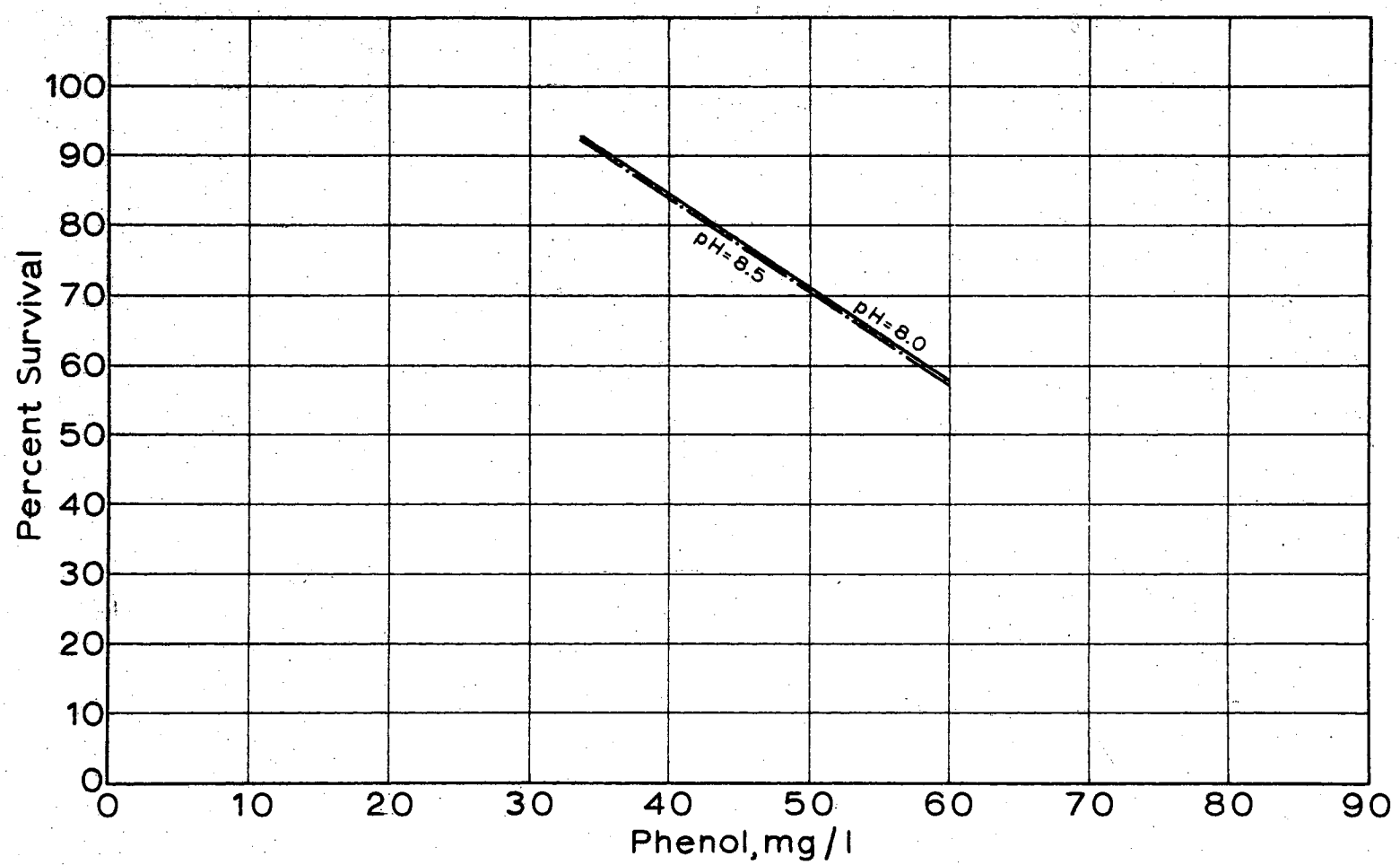

Fig. 7. Interrelationship of phenol and pH to toxicity.

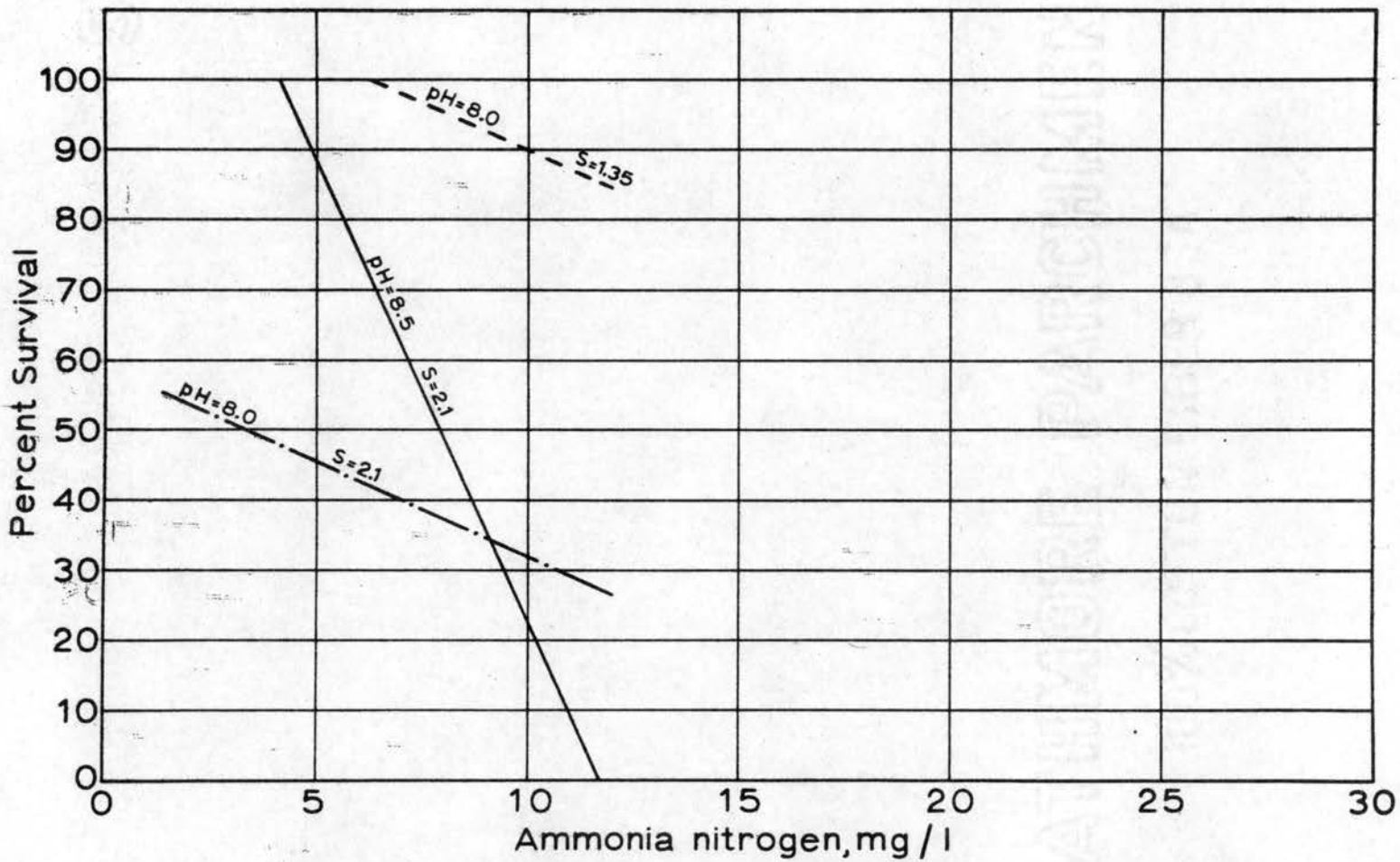

Fig. 8. Interrelationship of ammonia, sulfide, and pH to toxicity.

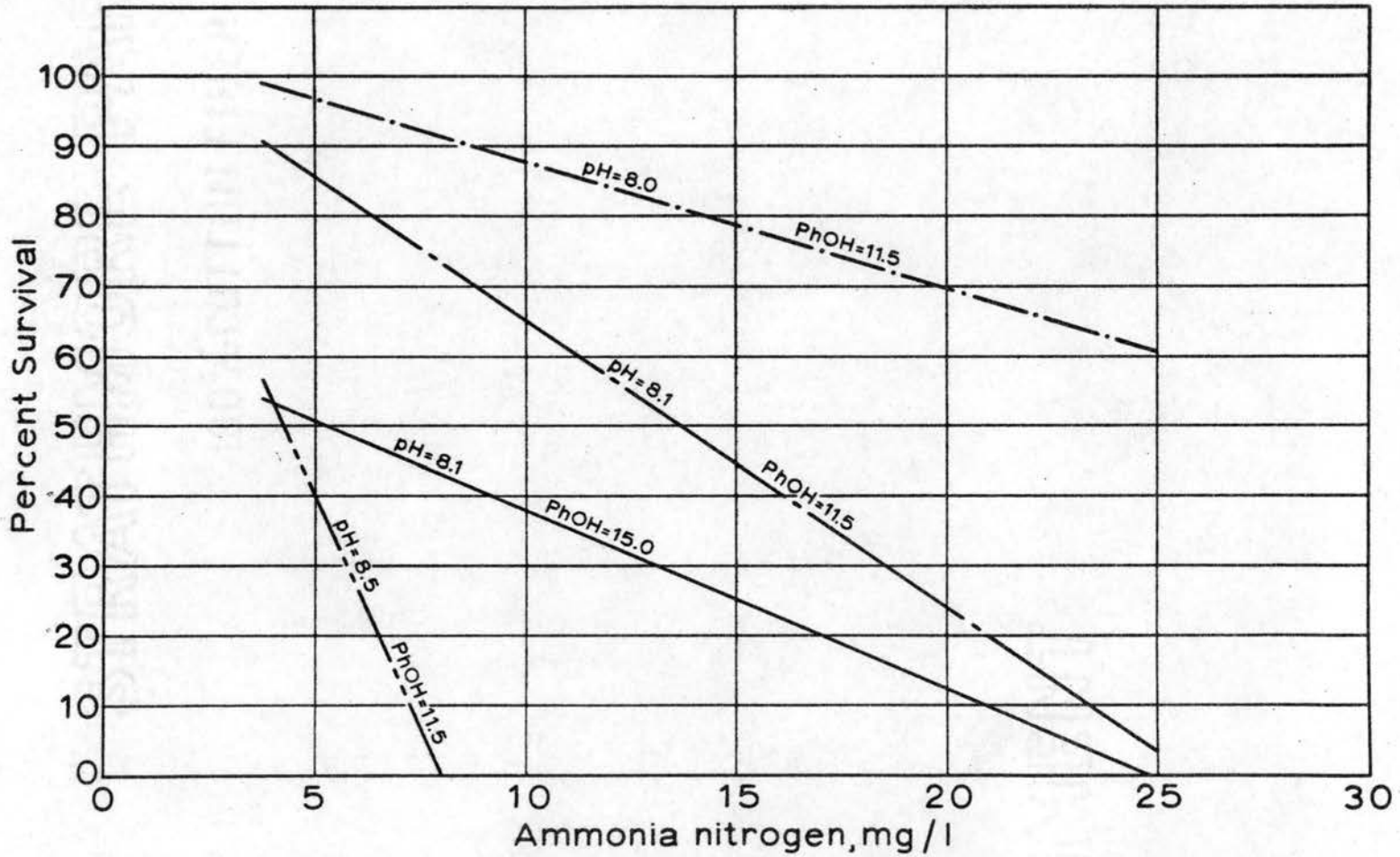

Fig. 9. Interrelationship of ammonia, phenol, and pH to toxicity.

Equation (4) was developed to predict toxicity of solutions containing ammonia and sulfide in absence of phenol and to determine the combined toxic effect of the two materials (Fig. 8). At constant sulfide concentration, change in pH resulted in a change in slope. The interaction between ammonia and pH in the presence of sulfide is illustrated by these lines. At pH 8.5 an increase from 5 to 10 mg/l of ammonia would result in death for approximately 6 out of 10 fish, but a corresponding increase of ammonia at pH 8.0 would result in death of approximately 1 out of 10 fish. Interaction also exists between pH and sulfide. If ammonia concentration were held constant at the value of the intersection of the pH 8.0 and 8.5 lines and sulfide were allowed to vary, the two lines would intersect at a sulfide concentration of 2.1 mg/l. Increase in either ammonia or sulfide would cause a decrease in survival of fish.

The procedure used to determine equation (5) was designed to evaluate toxic conditions resulting from mixtures of ammonia and phenol at the two pH levels (Fig. 9). With phenol concentration held constant at 11.5 mg/l the change in slope between pH 8.0, 8.1, and 8.5 lines illustrates interaction between ammonia and pH when phenol was present. The pH 8.0 line in Fig. 9 indicates greater toxicity at low concentrations and lesser toxicity at higher concentrations than does the pH 8.0 line in Fig. 5. By changing pH to 8.1 a line was obtained indicating conditions that are more compatible with results shown in Fig. 5. Use of equation (5) within pH range of 8.1 to 8.5 resulted in values compatible with results from equation one. Use of equation (5) with pH values between 8.0 and 8.1 produced results that are not realistic, especially at higher ammonia-nitrogen concentrations.

When pH was held constant and phenol was increased from 11.5 to 15 mg/l there was an increase in toxicity and a change in slope, illustrating

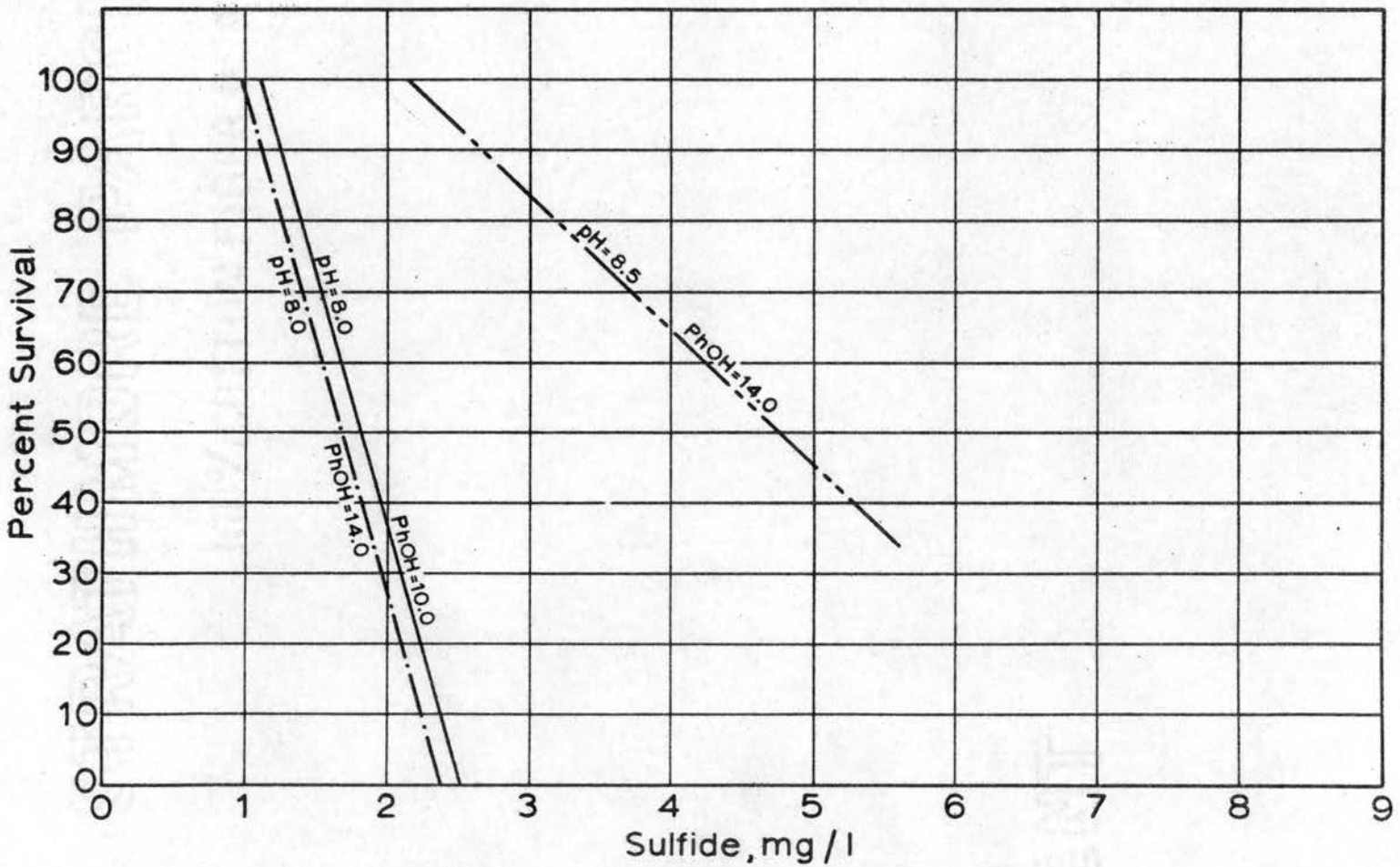

Fig. 10. Interrelationship of sulfide, phenol, and pH to toxicity.

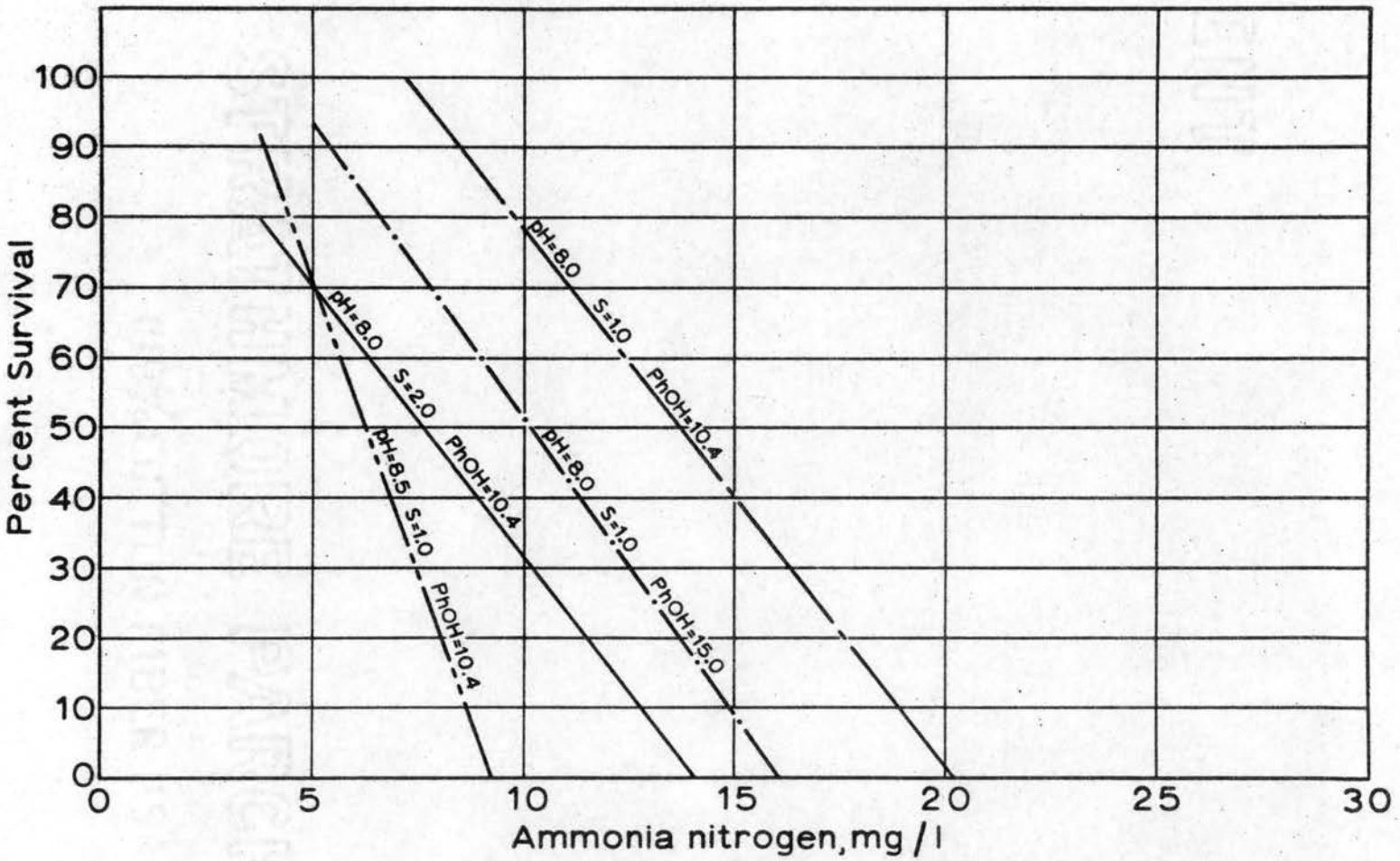

Fig. 11. Interrelationship of ammonia, sulfide, phenol, and pH to toxicity.

an interaction between phenol and ammonia. Covergence of pH 8.1 lines at greater ammonia concentrations indicated that phenol exerted a stronger influence on toxicity when ammonia concentrations were low, and that as ammonia increased, toxicity of the solution became more independent of phenol and more dependent on ammonia alone.

Results of equation (6) for sulfide and phenol are shown in Fig. 10. When phenol concentration was held constant there was a change in slope resulting from change in pH. This represents interaction between sulfide and pH in the presence of phenol. A given increase in sulfide concentration at pH 8.0 would result in a greater kill than the same increase at pH 8.5. Slope and location of the pH 8.5 line were very similar to the pH 8.5 line in Fig. 6. It appears that any combined toxic action between sulfide and phenol was of greater importance at the lower pH. When pH was held steady and phenol content was reduced, toxicity was only slightly reduced. Increase or decrease in either sulfide or phenol resulted in a corresponding change in toxicity.

Equation (7) was determined to account for conditions where ammonia, sulfide, and phenol were present in the test solution (Fig. 11). Interaction between pH and ammonia, pH and sulfide, and ammonia and phenol were included in the model. Response was greater for a given change in ammonia concentration at pH 8.5 then at pH 8.0. An increase in concentration of any of the three toxicants produced a more toxic solution.

Data used to determine equations (1) through (7) were lumped to determine equation (8) (Fig. 12). The purpose was to develop a general equation that could be used where one, two, or three of the toxicants were present. There was a large mass of data where one or more X-factors were absent and recorded as zero. The large number of zero data may account in part for the low coefficient of regression found in this equation.

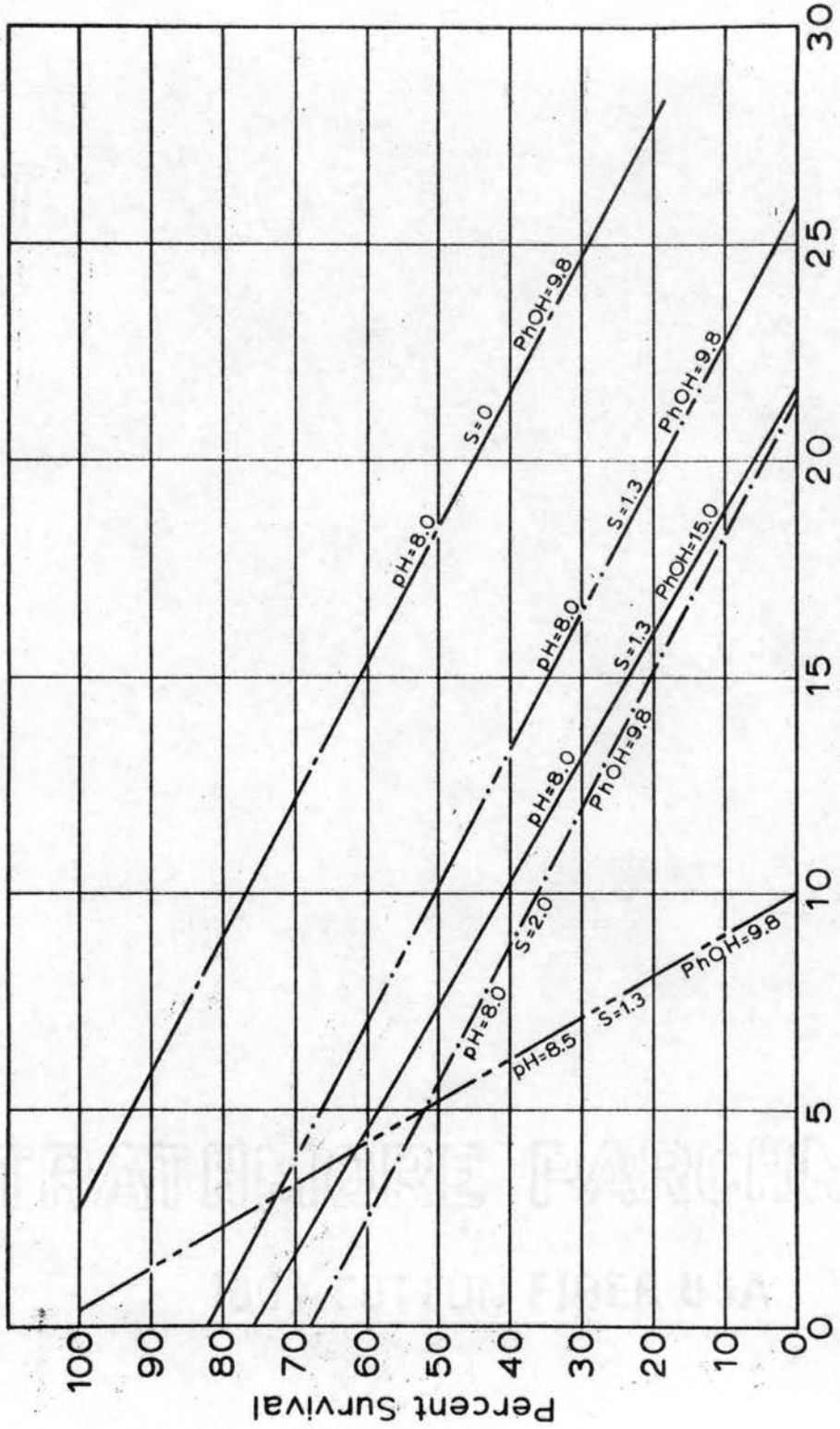

Fig. 12. Interrelationship of ammonia, sulfide, phenol, and pH to toxicity.

Interaction terms were again included in the model. Increased phenol concentrations exerted a greater effect at higher ammonia concentrations. An increase in ammonia concentration resulted in a greater response at pH 8.5 than at pH 8.0. Increased concentration in any of the toxicants resulted in greater toxicity.

Statistical Analysis of Refinery Effluent Data

The model that proved to fit data from prepared solutions best was used as a basis for a model to analyze refinery effluent data. A new term which was the cross product of pH, ammonia, and phenol values was introduced into the model, because it was felt that this interaction might occur. Addition of a term does not impair precision of the final equation. The resulting model was

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \beta_5 x_5 + \beta_6 x_6 + \beta_7 x_7 + \beta_8 x_8 + \varepsilon$$

Terms are identical to those in the model used in analysis of prepared solutions, except for the new term, $\beta_8 x_8$, where $x_8 = x_1 x_3 x_4$.

If ammonia, sulfide, phenol, and associated pH were essentially the only toxicants present in refinery effluents, the model would be expected to fit refinery effluent data as it fit data from prepared solutions. If other toxicants were present in appreciable quantities, the model would not fit. Results of the analysis are shown in Table XV. Multiple correlation coefficients (R^2) ranged from 0.15 to 0.65, and generally were smaller than those determined for prepared toxic solutions (Table VII). Standard deviations were somewhat higher than those found for prepared solutions. Each equation shown in Table XV was developed from data collected from effluents of one refinery and therefore is applicable only to that refinery. So much variation occurred among refineries that no attempt was made to determine a general equation for all refineries. The model seemed

TABLE XV

RESULTS FROM THE STATISTICAL ANALYSIS OF TOXIC REFINERY EFFLUENTS

Regression Coefficient	Refinery				
	A	B	C	D	E
b_0	-371.43	102.34	119.48	189.420	123.825
b_1	46.89	6.14	- 3.75	- 2.200	- 6.191
b_2	482.86	175.73	3.24	27.380	66.598
b_3	0.87	25.62	8,398,681,000.00	5.420	- 8.362
b_4	51.61	- 4.87	- 5.50	- 18.440	- 5.038
b_5	- 5.54	- 0.47	0.30	0.360	0.819
b_6	-59.13	-22.49	- 0.40	- 3.980	- 6.201
b_7	- 0.20	- 6.19	-2,470,199,300.00	- 1.730	- 1.242
b_8	0.01	0.01	- 235.82	0.190	0.111
R^2(%)	25	64*	19	25	57*
Standard Deviation	23.9	19.2	31.0	25.3	19.6

* Statistically significant

TABLE XV (Continued)

Regression Coeffieient	Refinery					
	F	G	H	J	K	L
b_0	538.823	226.313	241.120	79.650	- 311.290	48.20
b_1	- 81.030	- 8.232	5.560	8.120	193.75	12.39
b_2	70,642.820	5.476	-83.780	0.160	75,196.38	93.59
b_3	3.695	3.679	0.570	10.850	1,049.59	- 6.32
b_4	- 57.626	- 20.010	-24.700	-3.080	43.45	- 3.08
b_5	9.695	0.968	- 0.420	-0.920	- 19.57	- 1.58
b_6	- 8,407.926	- 0.572	9.200	0.120	- 9,230.36	-10.89
b_7	- 2.655	0.083	- 7.490	-0.110	5,167.28	- 3.57
b_8	0.301	- 0.020	0.860	-0.080	- 711.00	0.48
R^2(%)	26	19	24	15	65	20
Standard Deviation	24.0	22.6	24.6	28.2	28.7	21.9

to fit data from refineries B and E, as R^2 values for these two equations were the only ones significantly greater than zero. Effluents from refineries B and E were usually of low toxicity (Table V) with effluent B sometimes having phenol and sulfide in toxic concentrations and effluent E sometimes having ammonia and sulfide in toxic concentrations. There is a general though not consistent trend for low R^2 values to be associated with very toxic effluents and higher R^2 values to be associated with effluents of lesser toxicity. Low R^2 values obtained for the other refineries indicate that the model is not applicable. Extreme b values, such as those in equations C, F, and K, further indicate that the model is not applicable to the data. The common residual of approximately 25% survival in the standard deviation may be attributed to factors not included in the model.

Ten fish were used at each test concentration and with this number of test organisms a standard deviation of $\pm$ 31% survival could be expected (Snedecor, 1956). The equations can therefore be expected to predict survival as well as the experiment was designed to accomplish. A greater precision would be desired, but to obtain much greater precision would have required very large numbers of test organisms. Supply of fish for this type of operation was difficult, and to increase the number for each test would have been infeasible.

CHAPTER VI

SUMMARY AND CONCLUSIONS

1. In general, effluents containing higher concentrations of ammonia, phenol, and sulfide were more toxic than those with lower concentrations. This indicates that these materials are important toxicants in refinery effluents and as their concentrations decrease effluents become less toxic. However, as these materials are removed, other toxicants may likewise be reduced. One effluent was occasionally toxic even though the substances studied were at what would normally be considered low concentration. This indicates that in some cases substances other than ammonia, phenol, and sulfide may produce toxic effects. Under present waste treatment practices phenol and sulfide concentrations are reduced more readily than ammonia. No consistent relationship could be demonstrated between toxicity and chemical oxygen demand or alkalinity.

2. A great deal of variability between concentration of a component and related toxicity was observed. Much of this variability was due to presence of more than one toxicant in an effluent. Maximum concentrations of each component at measured median tolerance limits were estimated; ammonia 16-64 mg/l, sulfide 4-30 mg/l, phenol 8-29 mg/l, and COD 235-1725 mg/l.

3. Bioassays were made with prepared solutions of ammonia, phenol, and sulfide. Solutions were made containing single toxicants, pairs of toxicants, and all three toxicants at two pH levels. Data were analyzed and fitted to a statistical model to determine equations that might be

used to estimate survival of fish when concentrations of toxicants are known. Seven of eight equations had multiple correlation coefficients (R^2) significantly greater than zero, indicating that the model chosen could be used. There was large variation in data obtained from testing phenol and the equation based on this toxicant was not shown to be significant. Standard deviation of predicted survivals based on the equations ranged from 15.4 to 31.1% with a mean of 22.3%. Equations could be expected to predict survival within approximately 20 percent sixty-eight percent of the time. This standard deviation is within the range of deviations observed in duplicate tests.

4. Bioassay data were used to estimate concentration of toxicants that would produce a 50% survival (TL_m^{24}). These concentrations were used in the equations to find how near the estimates would be to 50% survival. Of 22 test situations, predicted survival ranged from 40.44 to 80.78% with a mean of 56.8%, and 14 predictions were within 10% of the expected 50% value. This method of analysis provided a comparison between estimating equations and the graphical method of determining TL_m concentrations.

5. Estimated concentration of toxicants in mixtures that produced a 50% survival and prediction equations were used to estimate the concentration of a toxicant when levels of other toxicants and survival were known. When compared with expected values, 10 estimates were more than 1 mg/l greater, 28 were within $\pm$ 1 mg/l, and 4 were more than 1 mg/l less. There was a tendency for the high estimates to be further from the expected values than the low estimates.

6. Tests were made of response of fish after holding for various periods of time. No significant difference could be shown for holding times ranging from 11 to 44 days. Fish were captured at seven different

times during the period of testing and no significant difference could be detected in the response of capture groups.

7. Interrelationships between toxicity and the different factors were studied. Where ammonia was the only toxicant present, increase in pH resulted in reduced survival of test organisms. If sulfide were the only toxicant, reduction in pH resulted in lower survival. Hydrogen-ion concentration could not be shown to affect toxicity when phenol was the only toxicant present.

If both ammonia and sulfide were present, with sulfide held constant, increase in pH produced a greater death rate for a given increase in ammonia concentration. This shows interaction between ammonia and pH when sulfide is present. Increase or reduction in sulfide concentration produced a corresponding change in toxicity.

Where only ammonia and phenol were present, interaction was observed between pH and ammonia, and between ammonia and phenol. At pH 8.5, presence of phenol greatly reduced the ammonia concentration that fish could withstand.

There was interaction between pH and sulfide when phenol was present. At constant phenol concentration, death rate was greater at pH 8.0 than at pH 8.5.

With all three toxicants present interaction occurred between pH and ammonia, pH and sulfide, and ammonia and phenol. Interaction between ammonia and pH appeared to be most important.

8. The statistical model which best fitted data from bioassays of prepared solutions was the basis for the model used to analyze data from refinery effluents. Multiple correlation coefficients (R^2) were generally low, and only two were significantly greater than zero. It may be concluded that the model did not adequately fit the majority of the data.

The factors measured had a greater effect on toxicity in some effluents than in others. In such cases the model accounted for a greater part of the variation. The model did not account for a significant amount of variation in most effluents. In these cases, factors not included in the study must have affected toxicity of the effluents.

9. Standard deviations, or standard errors of the estimate, ranged from 19.2 to 31.0%. A 95% confidence interval of $\pm$ 31% could be expected to occur when 10 fish are used in a test sample. Precision in the analysis could be improved if a larger number of fish were used in a test sample.

10. Present day methods for studying toxicants are commonly applicable primarily to single components. The experimental design used in this study permits consideration of a number of environmental factors and development of mathematical statements of relationships apparently existing among the factors. Equations were developed which can be used to predict response to various levels of factors and to examine interrelationships among factors. The equations gave fair results with prepared solutions of toxicants that showed the relationship between concentration of chemicals and toxicity of the chemical to a given species of fish. Equations also provided a good means of gaining information about the combined activity of toxicants occurring as mixtures. Future studies might well include a wider range of toxicants and a more detailed study of the combined effect of toxicants. This might make it possible to develop new terms for the model. Addition of information to the model might provide a better fit for refinery effluents. Precision of equations could be improved by increasing the number of animals per test or by increasing the number of replications.

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