METAL TOXICITIES AS MEASURED BY THE BIOCHEMICAL OXYGEN DEMAND TEST

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Ofelia Esguerra Vito

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by Ofelia Esguerra Vito

Earlier investigators have studied the toxic effects of industrial wastes on the microorganisms responsible for stabilization of organic matter in receiving waters. Although general trends were discovered, most of the reports produced different toxic limits. To provide more nearly uniform results, Hermann devised a procedure for determining the toxicities of various compounds in which the effects of environmental factors, such as temperature, pH, time of observation, food concentration, and number of organisms, were minimized. Hermann's test is closely allied to the standard biochemical oxygen demand test.

Based on methods developed by Hermann, toxicities of many of the metals used in stainless steels, including aluminum, barium, boron, chromium (III), cobalt, copper, iron (III), manganese, mercury (II), molybdenum, nickel, niobium (columbium), tantalum, titanium, tungsten, vanadium, zirconium, were determined. Because the standard five-day test for biochemical oxygen demand (BOD) is an integral part of the method used for setting up this toxicity index, the method possesses most of the advantages and disadvantages inherent in BOD determinations.

Toxicity indexes developed for these metals can be used to approximate the concentrations of metal wastes that can be

efficiently treated by aerobic biological processes. This technique will also enable investigators working in different locations to obtain comparable results, and will provide a basis for comparison of the toxicity of one substance against that of another.

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METAL TOXICITIES AS MEASURED BY THE BIOCHEMICAL OXYGEN DEMAND TEST

The operations of substantially all manufacturing industries result in the creation of waste products (12). These may be solid, liquid, or gaseous. Water-borne wastes, with a few exceptions where they are discharged into wells for underground disposal, eventually find their way into surface waters. Their effects, in lakes and streams, may be calamitous. Toxicity is one of the most important waste properties that may lead to trouble.

For proper control of the effects of mixed wastes discharged into receiving waters, some measure of toxicity of the various waste substances must be known. Literature surveys, however, report a wide variation in toxic limits. To establish more nearly uniform results, Hermann (14) developed a method for measuring the toxicity of chemicals to the microorganisms responsible for stabilization of organic matter in waste waters, taking into account different environmental factors that cause variation in the results.

To meet the requirements of simplicity, reproducibility, and applicability, Hermann's experimental development of a toxicity index was begun by selecting a suitable culture medium and test population. Toxicity tests on synthetic substrates were initiated to eliminate the variable strength factor caused by variations in the biochemical oxygen demand (BOD) of waste waters from different sources and even from a single source at different times. Basic information was presented by Hermann on the nature of response of organisms in the BOD test toward a number of substances known to be toxic. His concept is dependent on the idea that an increasing quantity of toxic material will cause a decreasing rate of oxygen utilization.

Based on the method devised by Hermann, the author has measured the toxicity of several of the alloying metals used in the manufacture of stainless steel. The toxicity indexes developed can be used to estimate the types and concentrations of waste metals that can be tolerated in biologically active streams or can be efficiently treated by aerobic biological processes. These indexes should enable investigators in different localities to obtain comparable results, and provide a basis for comparison of the toxicity of one substance against that of another.

REVIEW OF LITERATURE

Extensive research on the toxicity of industrial wastes began when a special subcommittee of the Federation of Sewage Works Associations Research Committee was created in 1948. Previous to the formation of this subcommittee, a few investigations on toxic effects had been conducted. The study of the effects of toxic materials on mesophilic digestion by Rudolfs (24) in 1937, and the study of the toxic effects of nickel by Weischmeyer and Chapman (36) in 1947 were noteworthy.

Toxicity Tests with Fish

Bio-assay methods for the evaluation of acute toxicity of industrial wastes to fish were reported in 1951 by the Committee on Research, Subcommittee on Toxicity, Section III, of the Federation of Sewage and Industrial Wastes Associations (5). At approximately the same time, a proposed toxicity test for industrial wastes to be discharged to marine waters was reported by Daugherty (6). The test was simple, inexpensive, and of short duration. Basic procedure and test animals were standardized, but not the constituents of the water or its temperature. The standard fish chosen was the Lagodon Rhomboides.

Freeman and Fowler (10) modified the method of culturing and testing with Daphnia magna to produce a more standardized procedure. This modification consisted of the substitution of standard reference water for the usual lake water previously employed. Equally satisfactory results were obtained by the new procedure.

Extensive experimental studies (32) on the Kama River in Russia, of the effects of dilute solutions of toxic substances on various types of river bottom animals, indicated that 0.01, 0.002, and 0.001 N solutions of potassium dichromate were lethal in 25, 47, and 67 minutes, respectively, to Daphnia pulex. Similar tests were conducted with lead nitrate, methyl violet, and other industrial wastes.

Cerkinsky (4) proposed a theoretical basis for the hygienic standardization of water courses receiving combinations of several harmful substances with similar noxious properties such as smell, taste, and toxicity. The values of the maximum permissible concentration of each substance should be reduced by a factor equal to the number of similar substances in the wastes, and the sum of the concentration of all such substances, expressed in percent of the maximum permissible concentration of each substance, should not exceed 100 percent.

There is now experimental confirmation, as reported by Shaw and Grushkin (29), of the hypothesis that metallic cations are toxic because they combine with the essential sulfhydryl group of key enzymes. Heavy metal ions affect a living cell by blocking the enzyme systems or by interfering with the essential cellular constituents required for metabolism of most oxidizing bacteria and some protozoa. Heavy metals combine with the -SH groups necessary for metabolism. It is assumed that the affinity of metal cations for sulfhydryl groups is an inverse linear function of the solubility of the corresponding metal sulfide. Metal ions that form the least soluble sulfides are the most toxic.

Heavy metals are also known to coagulate and precipitate proteins, many of which are denatured by this action. The presence of the peptide bond -CONH- in the protein molecule provides the mechanism by which metals denature the proteins.

Heavy metals vary markedly in toxicity. A logical explanation is that some of the heavy metals form tighter bonds with the enzymes and proteinaceous substances. This same reasoning also explains why some heavy metals, such as zinc and copper, form reversible systems.

Many advances in toxicity determinations of industrial wastes were made in 1953. Doudoroff and Katz (8) published a critical review of the literature on toxicity to fish of industrial wastes and their components. These authors reported that all metal cations apparently can be toxic to fish in rather dilute (less than 0.05M) physiologically unbalanced solutions of single metal salts. In solutions prepared with natural waters containing certain dissolved materials, and in other mixed salt solutions, the metals are often much less toxic than they are in simple solutions because of the antagonistic action of different cations.

On the other hand, some toxic metals such as zinc and copper are highly synergistic. When equally toxic solutions of salts of these metals are combined, the resulting mixture is much more rapidly fatal to fish than are the solutions of individual components. Cupric, mercuric, and silver salts have been found to be extremely toxic. Nickel and chromium have not been reported to be demonstrably toxic in any water in concentrations much less than 1 mg/l; and some comparable data reported that there is no pronounced difference between the toxicity of cobalt and manganese and that of nickel. Chromium (VI) was reported to be not as toxic as chromium (III) to fish.

Iron, in some waters, proved to be harmful to fish at concentrations as low as 0.1 to 1 mg/l. However, sufficient evidence of toxicity of small amounts of iron in solutions of the common ferric and ferrous salts with tolerable pH values has not been found. Some investigators reported much higher concentrations to be apparently harmless in various waters, or were unable to demonstrate toxicity of solutions that were not rendered excessively acidic through hydrolysis of salts. In these investigations, the toxicity of iron has not been adequately separated from the effect of low pH brought about by hydrolysis of the iron salts. La Roze (18) in 1955 attempted to overcome this difficulty by studies using iron lactate. He observed that anionic iron penetrates only after death of the fish and therefore has no toxic action. Death does not occur before two hours regardless of the concentration of iron. Studies of the toxicities of sodium citrate, mercuric chloride, and ethyl mercuric chloride to crustaceans were also made. Mixtures of copper salts and mercuric chloride showed more than additive toxic effects.

Fischer (9), in tests to determine the toxic effects of copper sulfate on daphnia, cylops, and chironomids (crustaceans), concluded that the former were most sensitive. Copper sulfate was more toxic at high temperatures; and equal concentrations differed in toxicity in different waters, being most toxic in distilled water, less in tap water, and still less in pond water. Lowering of pH increased the toxicity.

Measurement of toxicity of organic wastes to marine organisms was conducted by Hood, Duke, and Stevenson (15) in 1960. The authors concluded that experiments on the metabolic effects of wastes give more useful information on the tolerance of important ecological groups of the environment than can be obtained from acute toxicity of wastes to fish or to individual organisms. It is suggested that further work on metabolic effects of wastes, perhaps to include enzyme inhibition, will provide a better understanding of the effects of industrial effluents on the environment.

Toxicity Tests with Microorganisms

The variability in toxicity of copper salts for algae control was established in 1950. Scott (28) noted the effect of copper on the BOD of sewage as determined by the dilution method, and reported that copper, in as low a concentration as 0.1 mg/1 in an incubation sample, prevented the determination of the true

BOD. Chromates in amounts greater than 0.3 mg/l gave appreciably lower results than the true BOD.

The special subcommittee of the Federation of Sewage Works Associations Research Committee reported on the toxicity of mercuric chloride, chromic sulfate, and sodium chromate (22), in the dilution BOD test. The results of 58 determinations of toxicity of mercuric chloride indicated a slow increase in toxicity from 0.02 to 0.2 mg/l; beyond 0.2 mg/l, a sharp rise in toxicity; and at approximately 2 mg/l, complete bacteriostasis, indicated by zero BOD in the five-day period. From the fifteen determinations of toxicity of chromium, it was noted that chromium is much less toxic than mercury; in the dilution BOD test chromic ion is more toxic than chromate ion in the range from 1 to 10 mg/l.

The special subcommittee of the Federation of Sewage Works Associations Research Committee gave a second report (23) in 1956 on the toxicity of copper and zinc ions in the standard dilution BOD test. It was concluded from these findings that copper is more toxic than zinc in all concentrations from 0.1 to 10 mg/l; and that many factors influence the specific toxic effects of any given ion, including pH, time of observation, food concentration, and number of organisms. Sawyer, Frame, and Wold (26) have added temperature to these factors.

In 1957, toxicity studies on metal-finishing wastes were made by Sheets (30). In the tests carried out, a synthetic sewage was used in which 13 grams of lactose broth (Difco) was dissloved in one liter of water. Five milliliters of this solution was placed in a test tube, sterilized, and stored until used. In use, 5 ml of sterile broth was seeded with one milliliter of settled sewage and then diluted to 100 ml. The results of these tests using buffered and unbuffered solutions of toxic metals are shown in Table 1.

BOD determinations in wastes containing chelated copper or chromium were carried out by Morgan and Lackey in 1958 (20). It may be concluded from their experiments that wastes containing considerable copper, when entering a stream will tend to sterilize the stream until dilution or some type of ion exchange lowers the copper concentration sufficiently to allow biological action to proceed. From the results obtained, the toxicity of chromium is much greater than that of copper, in a ratio of approximately five to one. It has been postulated that this can be accounted for by the high valence state (hexa-) of the metal; however, this does not appear valid for mercury, which is more toxic than chromium. Other results show that chelation effectively reduces the toxicity of these metals.

Hermann Method

Hermann (14) in 1958 devised a procedure todetermine toxicity indexes for industrial wastes, based on the nature of response of organisms in the BOD dilution procedure toward substances of various toxicities. The concept is dependent on the idea that a toxic material will cause a decreasing quantity of oxygen utilization with increasing quantity of toxic material.

TABLE 1. Concentrations of Toxic Metals Required to Affect BOD Values by 50 Percent (TC_{50} point)

Based on Sheets (30)

Element	Compound Used	Buffered	TC_{50} Concentration of Element (mg/l)
Cr	CrO_3	Yes	4.0
Cr	CrO_3	No	0.9
Cr	K_2CrO_4	Yes	50.0
Cr	K_2CrO_4	No	10.5
Cr	$CrCl_3$	Yes	0.23
Ni	$NiSO_4 \cdot 6H_2O$	No	0.18
Ni	$NiSO_4 \cdot 6H_2O$	Yes	16
Ni	$NiCl_2 \cdot 6H_2O$	Yes	38
Ni	$Ni(NO_3)_2 \cdot 6H_2O$	Yes	64
B	H_3BO_3	Yes	480
Al	$Al_2(SO_4)_3$	Yes	18
Cu	$CuSO_4$	Yes	0.4

The points obtained by plotting oxygen utilization against concentration of toxic material, on a semi-logarithmic scale, produced a graph from which the 50 percent depletion of oxygen in relation to the control may be read. This value, called the TC_{50} level, is used as the toxicity index.

The TC_{50} value is the major characteristic of the toxicity curve. The choice of TC_{50} was based on the time-honored generalization that rate of biochemical oxidation of organic matter is a first order reaction of the type

$$\frac{-\,dc}{dt} = Kc$$

in which c is organic matter concentration, t is time, and K is a constant. The inhibition of oxygen utilization indicates retardation of the reaction rate. The biochemical half life of the decaying substance is related to the rate constant by the expression:

$$t_{1/2} = \frac{0.693}{K}$$

Designation of the toxic concentration at the 50 percent level provides a convenient reference point for making toxicity measurements as indicated by the empirical data.

Curves obtained by plotting toxicity data from various substances have been designated as toxigrams. Five different types of toxigrams were observed by Hermann. The characteristics of substances falling into each category are as follows:

Toxigram Type 1. Substances belonging to Type 1 may be classed as simple poisons. They exhibit some inhibitory effect at concentrations considerably smaller than the TC_{50}.

Toxigram Type 2. Compounds belonging to Type 2 manifest the peculiar characteristic of having practically no effect on the BOD test over a wide range of concentrations.

Toxigram Type 3. Substances of Type 3 affect the BOD determination because they are reducing agents.

Toxigram Type 4. Many organic compounds belong to Type 4. Each such compound first exerts an oxygen demand of its own; then as its concentration is increased, oxygen utilization is sharply inhibited. The compounds apparently serve as bacterial food unless used in a concentration high enough to cause toxic effects.

Toxigram Type 5. Of many substances tested for their toxicity index, one (acetaldehyde) reacted in a manner similar to Type 4 except that at concentrations higher than the toxic effect, the oxygen utilization curve rose again to approximately 100 percent. This peculiar effect was presumably due to exertion of a simple chemical oxygen demand.

Preparation of a toxicity index for a chemical compound therefore consists of three essential parts:

1. Testing by a standardized and reproducible analytical procedure, based on the BOD analysis;

2. Construction of a toxigram, or curve of oxygen utilization vs. concentration of the compound;

3. Designation, if possible, of the concentration that produces 50 percent inhibition of oxygen utilization as compared with unintoxicated blanks. This concentration is the TC_{50}.

Based on the method developed by Hermann, the author has measured the toxicities of several metals including many that are used in stainless steels. Among these have been aluminum, barium, boron, chromium (III), cobalt, copper, iron (III), manganese, mercury (II), molybdenum, nickel, niobium (columbium), tantalum, titanium, tungsten, vanadium, and zirconium. Because the standard five-day test for biochemical oxygen demand (BOD) is an integral part of the method used for setting up this toxicity index, the method possesses most of the advantages and the disadvantages inherent in BOD determinations.

EXPERIMENTAL PROCEDURES

The biochemical oxygen demand (BOD) of waters and waste waters is the quantity of dissolved oxygen, in mg/l, required during the stabilization of the decomposable organic matter by aerobic biochemical action. Determination of this quantity is accomplished by dilution of suitable portions of the sample with oxygen saturated water and measuring the dissolved oxygen in the mixture both immediately and after a period of incubation (usually five days) under favorable conditions for such action. The dissolved oxygen depletion during the incubation period serves as an indication of the activity of the organisms and of the organic matter concentration. A large oxygen depletion therefore indicates suitable conditions for aerobic biochemical action. Inhibited oxygen depletion, on the other hand, indicates the effect of interferences such as extreme pH, unfavorable temperature, or the presence of toxic substances such as heavy metal ions. It is the toxic effect of heavy metal ions that is given first consideration in these experiments.

In the determination of dissolved oxygen, the unmodified Winkler method (1) is usually employed. In cases where nitrites, ferric and ferrous iron, organic matter, sulfides, sulfites, polythionates, hypochlorites, suspended matter, or other oxidizing and reducing substances interfere with the Winkler method, a suitable modification should be used. The choice of the exact procedure will depend upon the nature of the sample and the interferences present.

The basic Winkler procedure is common to all modifications. This basic procedure entails oxidation, by the dissolved elemental oxygen, of manganous hydroxide under highly alkaline conditions. Upon subsequent acidification in the presence of an iodide, the manganic hydroxide dissolves and free iodine is liberated in an amount equivalent to the oxygen originally dissolved in the sample. The free iodine is titrated with a standard sodium thiosulfate solution, using starch as an internal indicator after most of the iodine had been reduced. The normality of the thiosulfate solution is adjusted so that 1 ml is equivalent to 1 mg/l of dissolved oxygen when 200 ml of the original sample is titrated.

Hermann's method employed the Alsterberg (azide) modification of the Winkler method. This modification is used for waters containing more than 0.1 mg/l of nitrite nitrogen and not more than 1 mg/l of ferrous iron. Other reducing or oxidizing materials should be absent. If 1 ml of fluoride solution is added before acidifying the sample, and if there is no delay in the titration, the method is also applicable in the presence of 100 to 200 mg/l of ferric iron. The toxicity test specifications developed by Hermann are shown in Table 2.

The author employed the method developed by Hermann to determine toxigrams for many of the alloying metals used in the production of stainless steels. In applying the toxicity test

TABLE 2. Toxicity Test Specifications

Incubation period	5 days±4 hours
Incubation temperature	20°C ± 0.5°C
Substrate: -	
Dextrose	4.0 mg/l
Peptone	4.0 mg/l
Settled sewage, aged 24 hours at 25°C ± 3°C	2.0 ml/l

in standard BOD dilution water.*

*Note: Standard BOD dilution water is prepared by adding 1 ml of each of the following chemicals to one liter of distilled water:

phosphate buffer solution	
magnesium sulfate solution	(22.5 grams/liter)
ferric chloride solution	(0.25 gram/liter)
calcium chloride solution	(27.5 grams/liter)

For further specifications, see Reference 1.

procedure, dissolved oxygen (DO) determinations were generally made by means of the Alsterberg (sodium azide) modification of the Winkler method. To facilitate measurement of quantities of toxic substances with minimum dilution effect, standard stock solutions were pipetted directly into the BOD bottles, solubility permitting.

In the case of difficultly soluble metals such as molybdenum, niobium (columbium), tantalum, titanium, tungsten, vanadium, and zirconium, special techniques were employed. The metals were dissolved, in very strong acids if necessary, and were pipetted idrectly to the BOD bottles. If precipitation occurred at that time, it was not a matter of concern. It is understood that such metals were present in the BOD test, but were wholly or partially in insoluble form.

Molybdic anhydride (MoO_3) was dissolved by warm concentrated ammonium hydroxide solution to obtain the ion Mo^6. The colorless concentrated standard solution was added directly to the BOD bottle in the proper amounts.

Treatment of metallic niobium (coumbium) with a mixture of hydrofluoric acid and nitric acid yielded a light yellow liquid (NbF_5) with a fine white precipitate. Metallic tantalum, when treated similarly, also yielded a light yellow liquid (TaF_5) containing a fine white precipitate.

Metallic titanium was dissolved by prolonged boiling in concentrated hydrochloric acid. A bright blue solution of the mixture was obtained.

Metallic tungsten was dissolved in a mixture of hydrofluoric acid and nitric acid in a polyethylene container. A light yellow concentrated solution of tungsten fluoride (WF_6) was obtained.

Ammonium metavanadate (NH_4VO_3) was dissolved in boiling water to produce a colorless solution.

An alloy of zirconium containing 37 percent zirconium, 50 percent silicon, 9.25 percent iron, and 0.5 percent carbon, was dissolved in a mixture of hydrofluoric acid and nitric acid, yielding a greenish yellow liquid (ZrF_4). There was some undissolved residue, but the whole mixture was used on the assumption that all of the zirconium in the alloy had dissolved.

By the standardized analytical procedure, initial dissolved oxygen tests were conducted for the BOD water containing the specified substrates. Similarly, five-day tests were conducted on the samples to which the toxic metal was added. These values were then subtracted from the initial dissolved oxygen to give the amount of oxygen utilized by the sample for the corresponding concentrations of the toxic metal. The ratio of the amount of oxygen utilized to the initial dissolved oxygen multiplied by 100 is termed the percentage oxygen utilization. The points obtained by plotting the oxygen utilization against the toxic material concentration on a semi-logarithmic scale produced a graph which is known as a toxigram. From the toxigram, the 50 percent depletion of oxygen in relation to the control could be read. The 50 percent depletion value was used as the toxicity index.

The same analytical procedure, except for some modifications for iron (III), and calculations were followed for each of the metals tested for toxicity. The data obtained were classified into two sets: the summer results and the fall results. Each point plotted for percent oxygen utilized was the average from two or more determinations. All these values, for both sets of data, were plotted in the toxigram. Curves were drawn through average oxygen utilization points at each concentration measured, but only if the data at that concentration were complete.

RESULTS

Twelve chemical compounds of reagent grade, 4 pure metals, and an alloy, representing in all 17 chemical elements, were tested for their individual toxicities at various ionic concentrations. The results for each are found in Tables 4 to 20 in the Appendix. Graphical representation of the data obtained for each substance is shown in Figures I to XVII. The toxicity index value is designated as Toxic Concentration$_{50}$ (TC$_{50}$). It is the concentration of the toxic substance in mg/l required to obtain 50 percent inhibition of oxygen utilization, using the available oxygen utilization of the incubated blank substrate as a base. Values for the toxicity indexes are shown in Table 3.

Two sets of data were obtained for the metals aluminum, barium, boron, chromium (III), cobalt, copper, manganese, mercury (II), molybdenum, nickel, titanium, and vanadium. The first set of data was obtained during the summer, and the second set during the fall. It was shown statistically that there is a significant difference between the two sets of data; the author attributes this difference to the fact that deviations or errors arising from analytical methods were not fully controlled and depended on the acquired skill and training of the analyst. The summer results therefore show the lack of experience that the author had in the earlier experiments. Careful examination of the fall results is indicative of the acquired skill, since the data obtained were consistent. The metals iron (III),

TABLE 3. Toxicity of Various Metals

Metal	Toxic Concentration TC_{50}, mg/1	Toxigram Type
Aluminum	10.1	1
Barium	>1000	2
Boron	>500	2
Chromium (III)	4.2	1
Cobalt	23	1
Copper	33	1
Iron (III)	12	1
Manganese	>500	2
Mercury (II)	0.26	1
Molybdenum	1.25	1
Nickel	9.6	1
Niobium (columbium)	0.107	1
Tantalum	0.125	1
Titanium	0.33	1
Tungsten	0.19	1
Vanadium	31	1
Zirconium	0.053	1

niobium (columbium), tantalum, tungsten, and zirconium were tested only during the fall.

Most of the metals tested, including aluminum, chromium (III), cobalt, copper, iron (III), mercury (II), molybdenum, niobium (columbium), tantalum, titanium, tungsten, vanadium, and zirconium, belong to Hermann's toxigram Type 1. These metals are classed as simple poisons. They exhibit some inhibitory effect at concentrations considerably less than the TC_{50}. From the toxigrams, it can be seen that for small concentrations, the toxic effect is not pronounced for the metals aluminum, chromium (III), cobalt, copper, iron (III), mercury (II), nickel, and vanadium. However, after a critical point is reached, the toxicity effect becomes very pronounced. For the metals molybdenum, niobium (columbium), tantalum, titanium, tungsten and zirconium, there is a steep sloping down of the toxigram. The author attributes this to a joint effect of the metal ion and the acid in solution.

In the case of the metals niobium (columbium), tantalum, titanium, tungsten, and zirconium, large concentrations of the metal interferred with the five-day BOD test by causing a rise in apparent oxygen utilization after the point of minimum oxygen utilization was reached. To prove that the metal ions interferred with the BOD test, samples containing the metals in large concentrations were tested for immediate oxygen demand. Apparent oxygen utilization increases similar to the five-day BOD test were observed. The author attributes this interference to lowering of the solution pH rather than to the metal ions, because the metals niobium, tantalum, tungsten, and zirconium were dissolved in a mixture of hydrofluoric acid and nitric acid, and titanium was dissolved in a concentrated solution of hydrochloric acid.

The metals barium, boron, and manganese are classed under Hermann's toxigram Type 2. Metals belonging to this type manifest a peculiar characteristic of having practically no effect on the BOD test over a wide range of concentrations. There is a gradual sloping down of the toxicity curve. There are no TC_{50} values for these 3 metals, since concentrations below 500 mg/1 have very little inhibitory effect on the BOD test. These metals do not appear as toxic as the others, either because they do not form tight bonds with the enzymes or proteinaceous substances, or because they are precipitated during the BOD test.

A comparison of the results obtained by the author with those obtained by Hermann showed nearly uniform results for the metals barium, boron, and mercury. There are discrepancies in the results obtained by Hermann, Sheets, and the author for the metals chromium, cobalt, and copper. The author suggests that further work be done on these metals, including an investigation of pH and chelation effects. Further work on molybdenum, niobium (columbium), tantalum, titanium, tungsten, vanadium, and zirconium with regard to pH control is also suggested.

CONCLUSIONS

1. The method developed by Hermann is limited by the disadvantages inherent in the biochemical oxygen demand (BOD) determinations, such as pH, temperature, and skill and training of the analyst.
2. Subject to these limitations, the technique provides a convenient means of measuring the toxicity to microorganisms of metal ions and other chemical materials. In order of increasing toxicity, the metals studied are as follows: Barium, boron, manganese, copper, vanadium, cobalt, iron (III), aluminum, nickel, chromium (III), molybdenum, titanium, mercury (II), tungsten, tantalum, niobium (columbium), and zirconium.
3. Most of the metals tested, including aluminum, chromium (III), cobalt, copper, iron (III), mercury (II), molybdenum, nickel, niobium (columbium), tantalum, titanium, tungsten, vanadium, and zirconium, belong to Hermann's toxigram Type 1. These metals are classed as simple poisons. They exhibit some inhibitory effect at concentrations considerably less than the TC_{50}. These metals form tight bonds with the enzymes.
4. The metals barium, boron, and manganese are classed under toxigram Type 2. Metals belonging to this type manifest a peculiar characteristic of having practically no effect on the BOD test over a wide range of concentration. These metals do not form tight bonds with the enzymes.

5. The toxicity indexes developed can be used to estimate types and concentrations of waste metals that can be tolerated in biologically active streams or can be efficiently treated by aerobic biological processes. These indexes should enable investigators in different localities to obtain comparable results, and provide a basis for comparison of the toxicity of one substance against that of another.

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APPENDIXES

TABLE 4. Toxicity of Aluminum

Aluminum added as aluminum sulfate [$Al_2(SO_4)_3$].

Run A September 1960 — 7.8 mg/l initial available oxygen
Run B September 1960 — 7.4 mg/l initial available oxygen

Aluminum Concentration mg/l	Oxygen Utilized in Test, mg/l		Percent of Available Oxygen Utilized		
	Run A	Run B	Run A	Run B	Aver.
0	6.4	4.4	82.0	59.5	70.8
1	5.7	4.2	73.0	56.8	64.9
5	--	3.2	--	43.3	--
10	3.2	2.6	41.0	35.1	38.1
25	2.6	1.3	33.3	17.5	25.4
50	1.8	0.9	23.1	12.1	17.6
75	1.4	1.0	17.9	13.5	15.7
100	1.3	1.0	16.6	13.5	15.1
150	1.2	1.3	15.4	17.6	16.5
250	0.7	1.5	8.9	20.3	14.6

TABLE 5. Toxicity of Barium

Barium added as barium chloride ($BaCl_2$).

Run A September 1960 8.1 mg/1 initial available oxygen
Run B October 1960 7.0 mg/1 initial available oxygen
Run C October 1960 6.3 mg/1 initial available oxygen

Barium Concentration mg/1	Oxygen Utilized in Test, mg/1			Percent of Available Oxygen Utilized			
	Run A	Run B	Run C	Run A	Run B	Run C	Aver.
0	5.5	6.0	5.0	68.0	85.7	79.5	77.7
1	5.2	6.2	--	64.2	88.5	--	--
5	5.2	5.9	--	64.2	84.3	--	--
10	4.8	5.9	4.6	59.3	84.3	73.0	72.2
50	4.5	5.7	4.1	55.5	81.5	65.0	67.3
100	4.4	5.3	4.1	54.3	75.7	65.0	65.0
250	4.1	5.1	--	50.6	73.0	--	--
500	3.9	4.3	3.3	48.2	61.5	52.5	54.0
750	3.8	4.1	3.2	47.0	58.6	50.8	52.1
1000	3.5	3.6	3.1	43.2	51.5	49.3	48.0
1500	--	2.9	--	--	41.5	--	--

TABLE 6. Toxicity of Boron

Boron added as boron oxide (B_2O_3).

Run A September 1960 7.6 mg/1 initial available oxygen
Run B October 1960 7.0 mg/1 initial available oxygen
Run C October 1960 6.3 mg/1 initial available oxygen

Boron Concentration mg/1	Oxygen Utilized in Test, mg/1			Percent of Available Oxygen Utilized			
	Run A	Run B	Run C	Run A	Run B	Run C	Aver.
0	4.9	6.0	5.0	64.5	85.7	79.5	76.6
1	4.9	5.5	--	64.5	78.5	--	--
10	5.0	5.8	5.1	66.8	82.8	81.0	75.9
50	4.4	5.2	4.2	58.0	74.3	66.8	66.4
100	4.0	4.9	4.1	52.7	70.0	65.0	62.6
250	3.8	--	3.6	50.0	--	57.0	--
500	3.8	4.1	2.8	50.0	58.6	44.5	51.0
700	--	3.7	--	--	52.8	--	--
750	3.2	--	1.8	42.1	--	28.6	--
900	--	2.6	--	--	37.1	--	--
1000	2.0	2.5	2.5	26.3	35.8	39.7	33.6

TABLE 7. Toxicity of Chromium (III)

Chromium added as chromium oxide (Cr_2O_3).

Run A	August	1960	7.3 mg/1 initial available oxygen
Run B	October	1960	7.8 mg/1 initial available oxygen

Chromium Concentration mg/1	Oxygen Utilized in Test, mg/1		Percent of Available Oxygen Utilized		
	Run A	Run B	Run A	Run B	Aver.
0	5.1	5.2	69.8	66.8	68.3
1	4.8	3.6	65.8	46.3	56.1
2.5	4.1	3.2	56.2	41.0	48.6
4	--	2.2	--	28.2	--
5	2.2	2.2	30.1	28.2	29.2
6	--	0.8	--	10.3	--
7.5	0.1	0.0	1.37	0.0	0.7
10	0.0	0.0	0.0	0.0	0.0
15	0.0	0.0	0.0	0.0	0.0
20	0.0	0.0	0.0	0.0	0.0

At concentrations >6 mg/1, the original yellow color of the chromium ion interfered with the BOD test.

TABLE 8. Toxicity of Cobalt

Cobalt added as cobalt nitrate [$Co(NO_3)_2$].

Run A September 1960 8.4 mg/1 initial available oxygen
Run B September 1960 8.0 mg/1 initial available oxygen
Run C October 1960 7.4 mg/1 initial available oxygen

Cobalt Concentration mg/1	Oxygen Utilized in Test, mg/1			Percent of Available Oxygen Utilized			
	Run A	Run B	Run C	Run A	Run B	Run C	Aver.
0	5.7	5.2	4.4	67.9	65.0	59.5	64.1
1	6.4	5.5	--	76.3	68.8	--	--
5	5.4	3.9	4.7	64.3	48.8	63.5	58.9
10	4.8	1.7	3.4	57.1	21.3	46.0	41.5
20	3.5	1.5	3.2	41.6	18.8	43.3	34.6
30	3.3	0.7	2.7	39.3	8.76	36.5	28.2
40	3.2	0.5	2.6	38.1	6.3	35.1	26.5
50	2.1	0.6	1.9	25.0	7.5	25.7	19.4
60	1.9	0.3	1.4	22.6	3.8	18.9	15.1
70	1.7	0.4	1.1	20.3	5.0	14.9	13.4
80	1.6	0.3	1.1	19.1	3.8	14.9	12.6
90	1.4	0.4	1.0	16.7	5.0	13.5	11.7
100	1.1	0.3	1.2	13.1	3.8	16.2	11.0
110	1.2	--	--	14.3	--	--	--
120	1.6	0.3	1.0	19.1	3.8	13.5	12.1

TABLE 9. Toxicity of Copper

Copper added as copper sulfate ($CuSO_4 \cdot 5H_2O$)

Run A September 1960 8.2 mg/1 initial available oxygen
Run B October 1960 7.2 mg/1 initial available oxygen

Copper Concentration mg/1	Oxygen Utilized in Test, mg/1		Percent of Available Oxygen Utilized		
	Run A	Run B	Run A	Run B	Aver.
0	6.1	4.9	74.5	68.1	71.3
1	4.8	3.7	58.5	51.4	55.0
1.5	4.5	3.8	54.9	52.8	53.9
2	4.5	3.8	54.9	52.8	53.9
3	3.6	3.4	44.0	47.3	--
10	--	3.8	--	52.8	--
15	3.9	4.0	47.6	55.6	--
17.5	4.6	3.7	56.1	51.4	53.8
20	4.2	3.9	51.2	54.2	52.7
22.5	3.8	3.8	46.3	52.8	49.6
25	3.5	3.8	42.7	52.8	47.8
30	3.1	3.0	37.8	41.7	39.8
40	1.9	2.2	23.2	30.6	26.9

TABLE 10. Toxicity of Iron (III)

Iron added as ferric chlorice ($FeCl_3$)

Run A October 1960 7.8 mg/1 initial available oxygen
Run B October 1960 7.8 mg/1 initial available oxygen

Iron Concentration mg/1	Oxygen Utilized in Test, mg/1		Percent of Available Oxygen Utilized		
	Run A	Run B	Run A	Run B	Aver.
0=	6.4	5.2	82.0	66.6	74.3
1	5.3	5.0	68.0	64.1	66.0
5	--	4.4	--	56.5	--
10	3.6	3.1	46.2	39.8	43.0
17.5	--	2.3	--	29.5	--
25.	0.7	1.9	9.0	24.4	16.7
40	--	1.0	--	12.8	--
50	0.6	--	7.7	--	--
60	--	0.6	--	7.7	--
75	0.2	1.0	2.6	12.8	7.7
100	0.3	--	3.9	--	--
125	0.3	--	3.9	--	--
150	0.2	--	2.6	--	--

A modification of the Alsterberg (sodium azide) method was used to test for the 5-day DO of the Fe (III) samples. Potassium fluoride was added to the samples before titration.

TABLE 11. Toxicity of Manganese

Manganese added as manganese sulfate ($MnSO_4 \cdot H_2O$).

Run A	September	1960	7.9 mg/l initial available oxygen
Run B	October	1960	7.4 mg/l initial available oxygen
Run C	November	1960	7.4 mg/l initial available oxygen

Manganese Concentration mg/l	Oxygen Utilized in Test, mg/l			Percent of Available Oxygen Utilized			
	Run A	Run B	Run C	Run A	Run B	Run C	Aver.
0	5.0	5.1	6.2	63.3	69.0	83.8	72.0
1	5.0	6.1	--	63.3	82.5	--	--
10	4.8	5.0	--	60.8	68.0	--	--
30	4.6	--	--	58.3	--	--	--
50	4.5	4.7	--	57.0	64.5	--	--
75	4.4	--	--	55.7	--	--	--
100	4.3	4.1	5.8	54.5	56.2	78.5	63.1
150	4.0	--	--	50.6	--	--	--
250	3.9	3.5	4.4	49.3	48.3	59.5	52.4
350	--	3.1	--	--	42.6	--	--
500	2.8	2.7	4.1	35.5	37.3	55.5	42.8
700	--	--	3.9	--	--	52.7	--
900	--	--	3.3	--	--	44.6	--
1000	--	--	3.5	--	--	47.3	--

TABLE 12. Toxicity of Mercury (II)

Mercury added as mercuric chloride ($HgCl_2$).

Run A	August	1960	8.1 mg/l initial available oxygen
Run B	August	1960	7.8 mg/l initial available oxygen
Run C	September	1960	7.0 mg/l initial available oxygen
Run D	October	1960	7.4 mg/l initial available oxygen

Mercury Concentration mg/l	Oxygen Utilized in Test, mg/l				Percent of Available Oxygen Utilized				
	Run A	Run B	Run C	Run D	Run A	Run B	Run C	Run D	Aver.
0	5.5	5.0	6.0	6.2	67.9	64.1	85.6	83.8	75.4
0.05	5.0	4.9	2.9	4.4	61.8	62.8	41.5	59.5	56.4
0.1	4.5	4.5	2.9	4.1	55.5	57.8	41.5	55.5	52.6
0.2	4.7	3.9	2.8	4.0	58.0	50.0	40.0	54.0	50.5
0.25	--	--	--	3.7	--	--	--	50.0	--
0.3	4.2	1.2	0.5	3.7	51.9	15.4	7.2	50.0	31.1
0.4	4.4	0.8	0.4	--	54.3	10.3	5.7	--	--
0.5	3.6	0.3	0.4	--	44.5	3.9	5.7	--	--
0.6	--	0.4	0.3	--	--	5.1	4.3	--	--
0.7	--	0.3	0.5	--	--	3.9	7.2	--	--
0.8	--	0.4	0.2	--	--	5.1	2.9	--	--
0.9	--	0.2	0.2	--	--	2.6	2.9	--	--
1.0	--	0.4	0.3	--	--	5.1	4.3	--	--
1.5	--	0.3	--	--	--	3.9	--	--	--
2.0	--	0.4	--	--	--	5.1	--	--	--

TABLE 13. Toxicity of Molybdenum

Molybdenum added as molybdic anhydride (MoO_3) dissolved in concentrated ammonium hydroxide solution

Run A August 1960 8.2 mg/1 initial available oxygen
Run B September 1960 7.9 mg/1 initial available oxygen
Run C October 1960 7.3 mg/1 initial available oxygen
Run D October 1960 6.9 mg/1 initial available oxygen

Molybdenum Concentration mg/1	Oxygen Utilized in Test, mg/1				Percent of Available Oxygen Utilized				
	Run A	Run B	Run C	Run D	Run A	Run B	Run C	Run D	Aver.
0	6.1	6.2	5.9	5.7	74.5	78.5	81.0	82.8	79.2
0.2	4.5	4.7	3.1	4.3	54.9	59.5	42.5	62.5	54.9
0.5	4.0	4.4	2.9	3.7	48.8	55.6	39.8	53.6	49.5
0.75	--	4.2	2.5	3.4	--	53.2	34.2	49.3	--
1	3.0	3.8	2.6	2.7	36.6	48.1	35.6	39.2	39.9
1.25	--	--	0.7	2.4	--	--	9.6	34.8	--
1.5	0.3	--	0.4	2.6	3.7	--	5.5	37.7	15.6
1.75	--	--	0.6	1.0	--	--	8.2	14.5	--
2	0.0	--	--	--	0.0	--	--	--	--

Upon the addition of $MnSO_4$ solution in the DO test, samples containing molybdenum concentrations of 1 mg/1 or more assumed a yellow coloration:

1 mg/1 = slightly colored
1.5 mg/1 = darker
2.0 mg/1 = yellow color.

Dilution of the concentrated solution produced a fine precipitate.

TABLE 14. Toxicity of Nickel

Nickel added as nickel nitrate $[Ni(NO_3)_2]$.

Run A	August	1960	8.1 mg/l initial available oxygen
Run B	October	1960	8.2 mg/l initial available oxygen
Run C	October	1960	7.8 mg/l initial available oxygen

Nickel Concentration mg/l	Oxygen Utilized in Test, mg/l			Percent of Available Oxygen Utilized			
	Run A	Run B	Run C	Run A	Run B	Run C	Aver.
0	5.5	5.2	5.0	68.0	63.5	64.0	65.2
0.1	5.6	6.1	5.1	69.1	74.5	65.5	69.7
0.5	5.5	4.1	4.8	68.0	50.0	61.5	60.1
1	5.2	4.4	4.8	64.2	53.6	61.5	59.8
5	4.1	3.4	4.1	50.1	41.5	52.6	48.0
7.5	--	--	3.2	--	--	41.0	--
10	1.5	1.5	--	18.5	18.3	--	--
15	--	--	3.8	--	--	48.8	--
17.5	0.9	0.7	2.9	11.1	8.6	37.2	19.0
20	--	--	2.7	--	--	34.7	--
25	0.9	0.2	1.0	11.1	2.5	12.9	8.8
50	0.7	0.4	--	8.7	4.9	--	--
75	0.9	0.2	1.0	11.1	2.4	12.9	8.8
100	2.0	0.1	0.3	24.7	1.2	3.9	9.9
110	--	--	0.4	--	--	5.1	--
120	--	--	0.4	--	--	5.1	--

TABLE 15. Toxicity of Niobium (Columbium)

Niobium added in nitric-hydrofluoric acid solution.

Run A October 1960 7.4 mg/l initial available oxygen
Run B November 1960 6.8 mg/l initial available oxygen

Niobium Concentration mg/l	Oxygen Utilized in Test, mg/l		Percent of Available Oxygen Utilized		
	Run A	Run B	Run A	Run B	Aver.
0	5.4	4.3	73.0	63.2	68.1
0.05	--	2.1	--	30.9	--
0.1	--	0.6	--	8.8	--
0.2	--	0.8	--	11.8	--
0.5	1.5	0.6	20.3	8.8	14.6
1	1.3	0.6	17.6	8.8	--
2	0.7	--	9.5	--	--
5	0.6	--	8.1	--	--
10	0.7	0.5	9.5	7.4	8.92
20	0.7	--	9.5	--	--
50	0.5	0.5	6.8	7.4	7.1
100	0.8	0.4	10.8	5.9	8.4
200	2.4	1.8	32.4	26.5	29.5
500	4.0	4.9	54.0	72.0	63.0
1000	4.2	--	56.8	--	--

TABLE 16. Toxicity of Tantalum

Tantalum added in nitric-hydrofluoric acid solution.

Run A	October	1960	7.8 mg/l initial available oxygen
Run B	November	1960	6.7 mg/l initial available oxygen
Run C	November	1960	6.2 mg/l initial available oxygen

Tantalum Concentration mg/l	Oxygen Utilized in Test, mg/l			Percent of Available Oxygen Utilized			
	Run A	Run B	Run C	Run A	Run B	Run C	Aver.
0	6.2	5.7	4.9	79.5	85.1	79.0	81.2
0.05	--	--	3.6	--	--	58.2	--
0.1	--	--	1.8	-	--	29.1	--
0.2	--	--	1.4	--	--	22.6	--
0.5	--	--	1.4	--	--	22.6	--
1	0.8	0.7	0.1	10.3	10.5	1.6	7.5
2	1.0	0.6	--	12.8	9.0	--	--
5	0.8	0.6	--	10.3	9.0	--	--
10	0.7	0.7	0.0	9.0	10.5	0.0	6.5
20	0.7	0.7	--	9.0	10.5	--	--
50	0.7	1.7	0.4	9.0	25.4	6.5	13.6
100	0.9	2.4	0.0	11.5	35.8	0.0	15.8
150	--	--	1.0	--	--	16.1	--
200	4.6	5.5	2.6	59.0	82.0	42.0	61.0
500	5.6	6.1	4.6	71.8	91.0	74.2	79.0
1000	5.6	--	4.3	71.8	--	69.5	--

TABLE 17. Toxicity of Titanium

Titanium added in concentrated hydrochloric acid solution.

Run A	August	1960	7.2 mg/1 initial available oxygen
Run B	October	1960	7.3 mg/1 initial available oxygen
Run C	November	1960	6.9 mg/1 initial available oxygen

Titanium Concentration mg/1	Oxygen Utilized in Test, mg/1			Percent of Available Oxygen Utilized			
	Run A	Run B	Run C	Run A	Run B	Run C	Aver.
0	4.9	5.9	5.7	68.2	80.8	82.8	77.3
0.2	3.4	3.2	2.4	47.3	43.8	34.8	42.0
0.5	3.3	--	--	45.8	--	--	--
1	2.7	1.6	1.9	37.6	21.9	27.6	29.0
1.5	2.1	--	--	29.2	--	--	--
2	2.0	1.1	1.5	27.8	15.1	21.8	21.6
5	2.2	2.0	1.6	30.6	27.4	23.2	27.1
10	4.1	1.8	1.9	57.0	24.7	27.6	36.4
15	--	1.1	--	--	15.1	--	--
20	7.2	1.1	2.6	100.0	15.1	37.7	50.9
50	--	2.5	6.9	100.0	34.3	100.0	--
100	--	-	6.9	100.0	--	100.0	--

Concentrations of the metal greater than 20 mg/1 caused interference with the BOD test.

TABLE 18. Toxicity of Tungsten

Tungsten added in nitric-hydrofluoric acid solution.

Run A October 1960 7.8 mg/l initial available oxygen
Run B November 1960 6.7 mg/l initial available oxygen
Run C November 1960 6.2 mg/l initial available oxygen

Tungsten Concentration mg/l	Oxygen Utilized in Test, mg/l			Percent of Available Oxygen Utilized			
	Run A	Run B	Run C	Run A	Run B	Run C	Aver.
0	6.2	5.7	4.9	79.5	85.0	79.0	81.2
0.05	--	--	2.2	--	--	35.5	--
0.1	--	--	2.5	--	--	40.3	--
0.2	1.3	--	1.6	16.7	--	25.8	--
0.5	0.0	--	2.2	14.1	--	35.5	--
1	0.8	0.5	2.1	10.3	7.5	33.9	17.3
2	0.9	1.1	--	11.6	16.4	--	--
5	0.8	0.5	0.1	10.3	7.5	1.6	6.4
10	0.9	0.6	0.0	11.6	9.0	0.0	6.9
20	0.9	0.4	--	11.6	6.0	--	--
50	0.9	0.6	0.1	11.6	9.0	1.6	7.4
100	0.8	0.7	0.0	10.3	10.5	0.0	6.9
200	1.1	1.1	0.2	14.1	16.4	3.2	11.2
300	--	--	2.2	--	--	35.5	--
500	5.2	4.9	4.1	66.6	73.2	66.1	68.6

Samples containing 500 mg/l were still cloudy and yellow after addition of H_2SO_4.

TABLE 19. Toxicity of Vanadium

Vanadium added as ammonium metavanadate (NH_4VO_3).

Run A	September	1960	8.2 mg/l initial available oxygen
Run B	September	1960	7.9 mg/l initial available oxygen

Vanadium Concentration mg/l	Oxygen Utilized in Test, mg/l		Percent of Available Oxygen Utilized		
	Run A	Run B	Run A	Run B	Aver.
0	5.2	5.2	63.4	65.8	64.6
0.1	5.3	4.7	64.7	59.5	62.1
0.5	4.7	4.6	51.4	58.3	57.9
1	4.5	4.4	54.9	55.8	55.4
5	4.0	4.4	48.8	55.8	52.3
10	3.7	4.0	45.2	50.7	48.0
25	--	3.2	--	40.5	--
35	--	3.7	--	47.0	--
50	1.8	2.4	22.0	30.4	26.2
100	1.4	2.4	17.1	30.4	23.8
125	--	1.0	--	12.7	--
150	--	0.3	--	3.8	--

The stock solution became more yellow the greater the amount of dilution water added.

Samples containing concentrations of the metal from 50 mg/l and up turned light green upon addition of $MnSO_4$.

TABLE 20. Toxicity of Zirconium

Zirconium added in nitric-hydrofluoric acid solution.

Run A	October	1960	7.4 mg/1 initial available oxygen
Run B	November	1960	6.2 mg/1 initial available oxygen
Run C	November	1960	6.8 mg/1 initial available oxygen

Zirconium Concentration mg/1	Oxygen Utilized in Test, mg/1			Percent of Available Oxygen Utilized			
	Run A	Run B	Run C	Run A	Run B	Run C	Aver.
0	5.4	4.9	4.3	73.0	79.0	63.2	71.4
0.05	--	1.9	1.3	--	30.7	19.1	--
0.1	--	2.3	0.5	--	37.1	7.4	--
0.2	1.1	0.1	0.5	14.9	1.6	7.4	8.0
0.5	0.5	0.0	0.3	6.8	0.0	4.4	3.7
1	0.6	0.0	0.3	8.1	0.0	4.4	4.2
2	0.6	--	--	8.1	--	--	--
5	0.5	--	--	6.8	--	--	--
10	0.6	0.0	0.3	8.1	0.0	4.4	4.2
20	0.6	0.0	0.3	8.1	0.0	4.4	4.2
50	2.8	1.7	2.6	37.9	27.4	38.3	34.5
100	4.4	4.1	4.8	59.5	66.2	70.5	65.5
200	5.1	4.4	5.5	69.0	71.0	80.5	73.5
500	2.4	2.5	4.8	32.5	40.3	70.5	47.8

Samples containing 200 and 500 mg/1 turned pale yellow upon addition of alkaline iodide. They turned turbid light yellow upon addition of H_2SO_4.

FIGURE I
TOXIGRAM FOR ALUMINUM

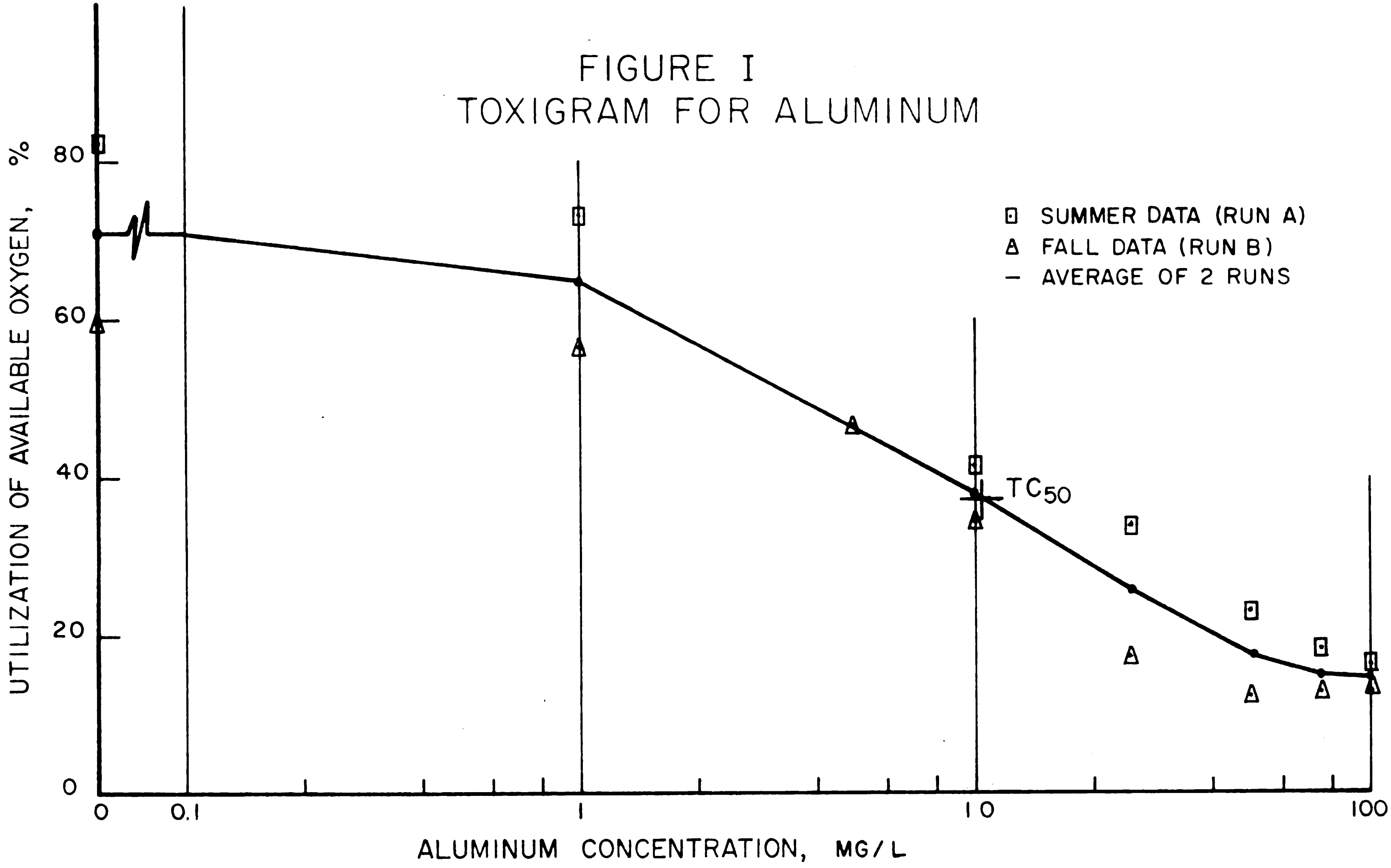


FIGURE II
TOXIGRAM FOR BARIUM

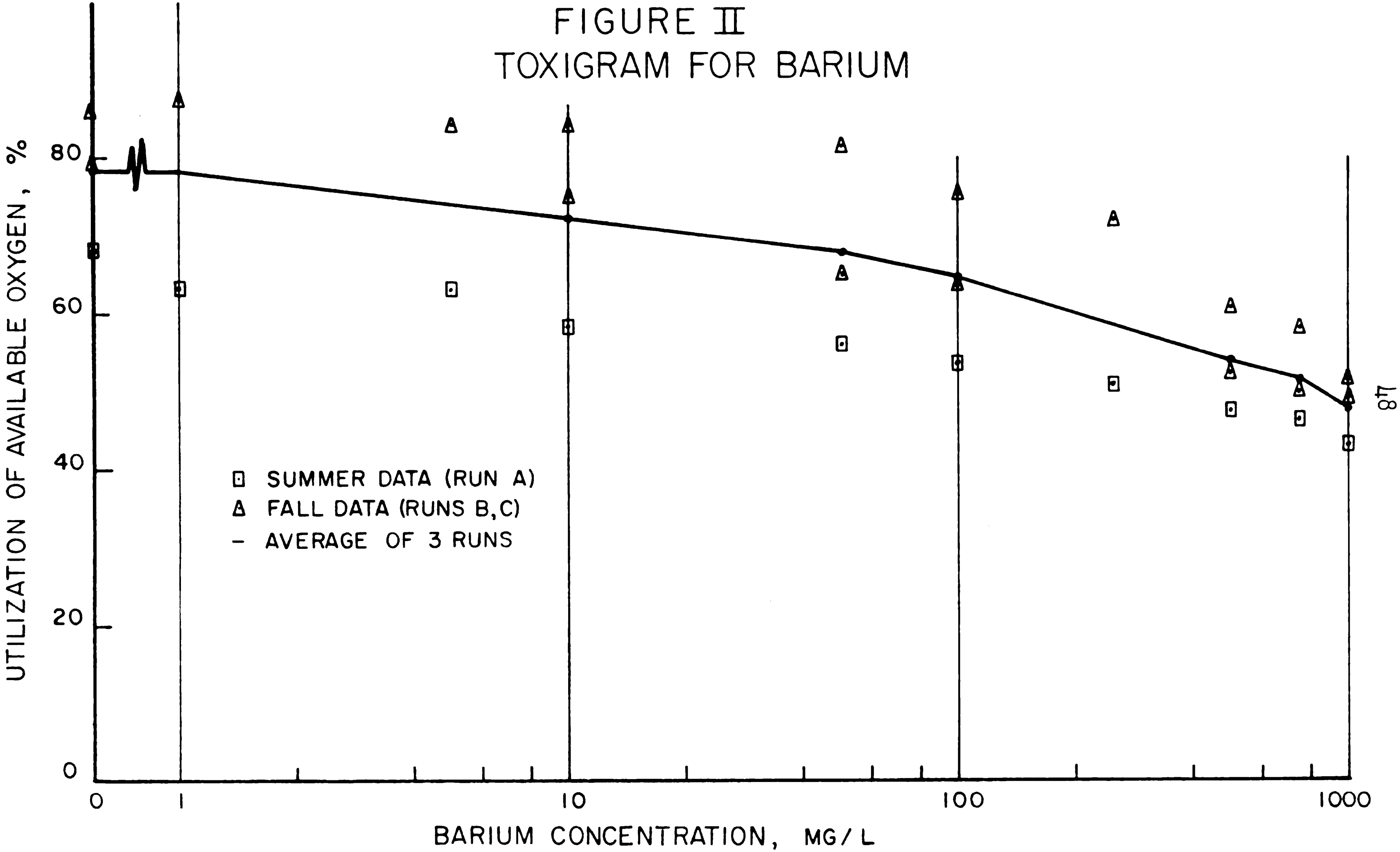


FIGURE III
TOXIGRAM FOR BORON

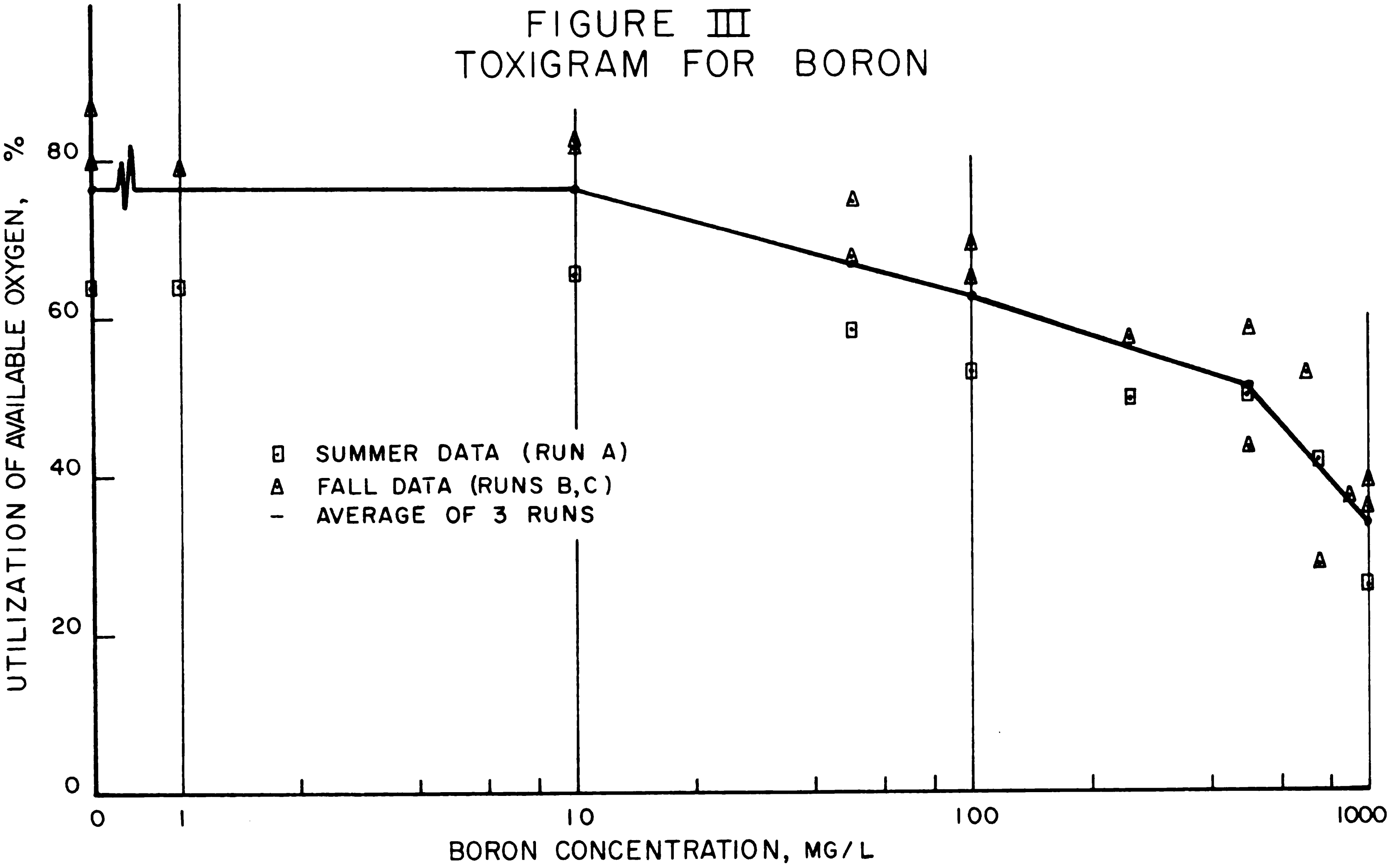
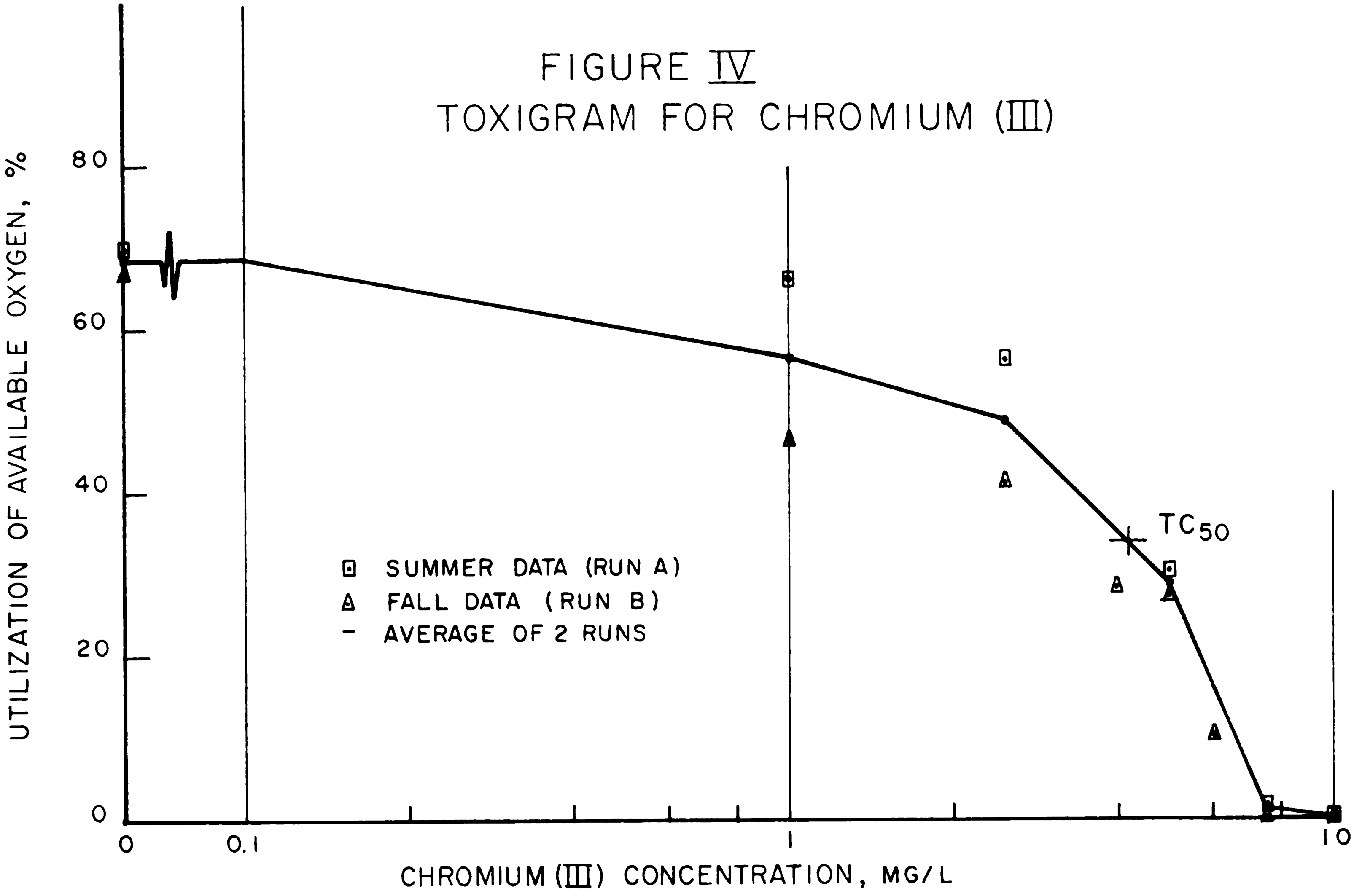


FIGURE IV
TOXIGRAM FOR CHROMIUM (III)



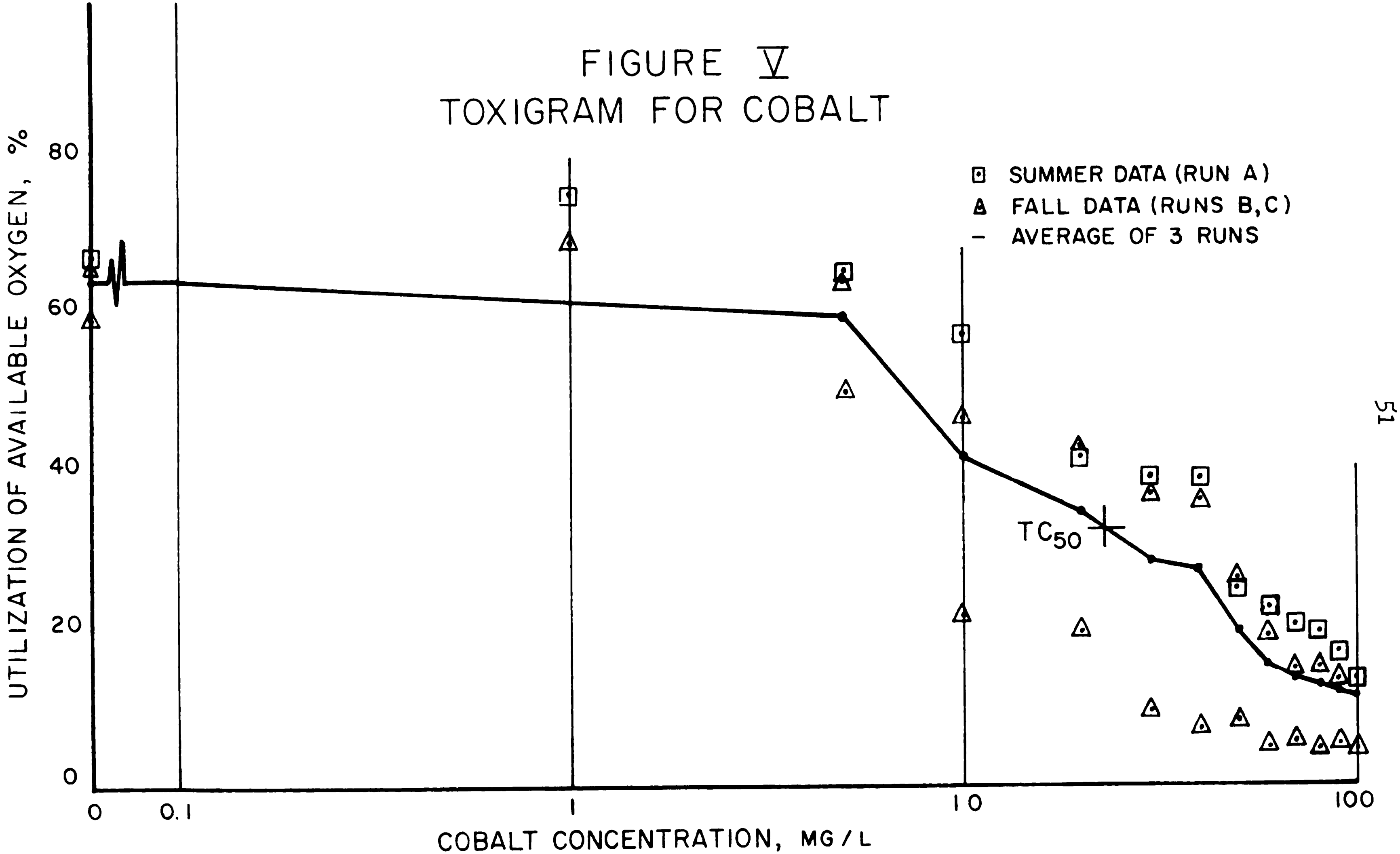


FIGURE V
TOXIGRAM FOR COBALT

FIGURE VI
TOXIGRAM FOR COPPER

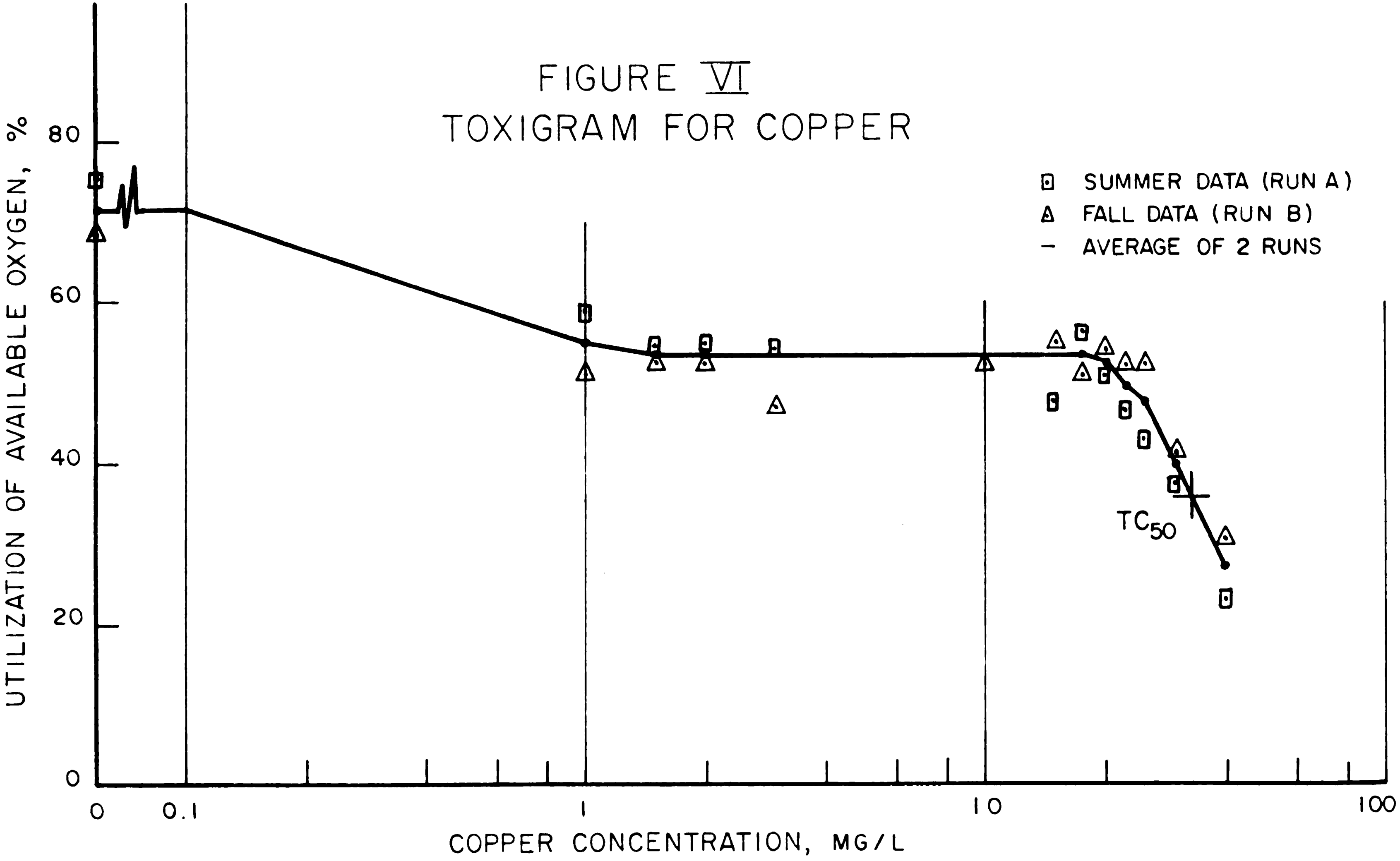


FIGURE VII
TOXIGRAM FOR IRON (III)

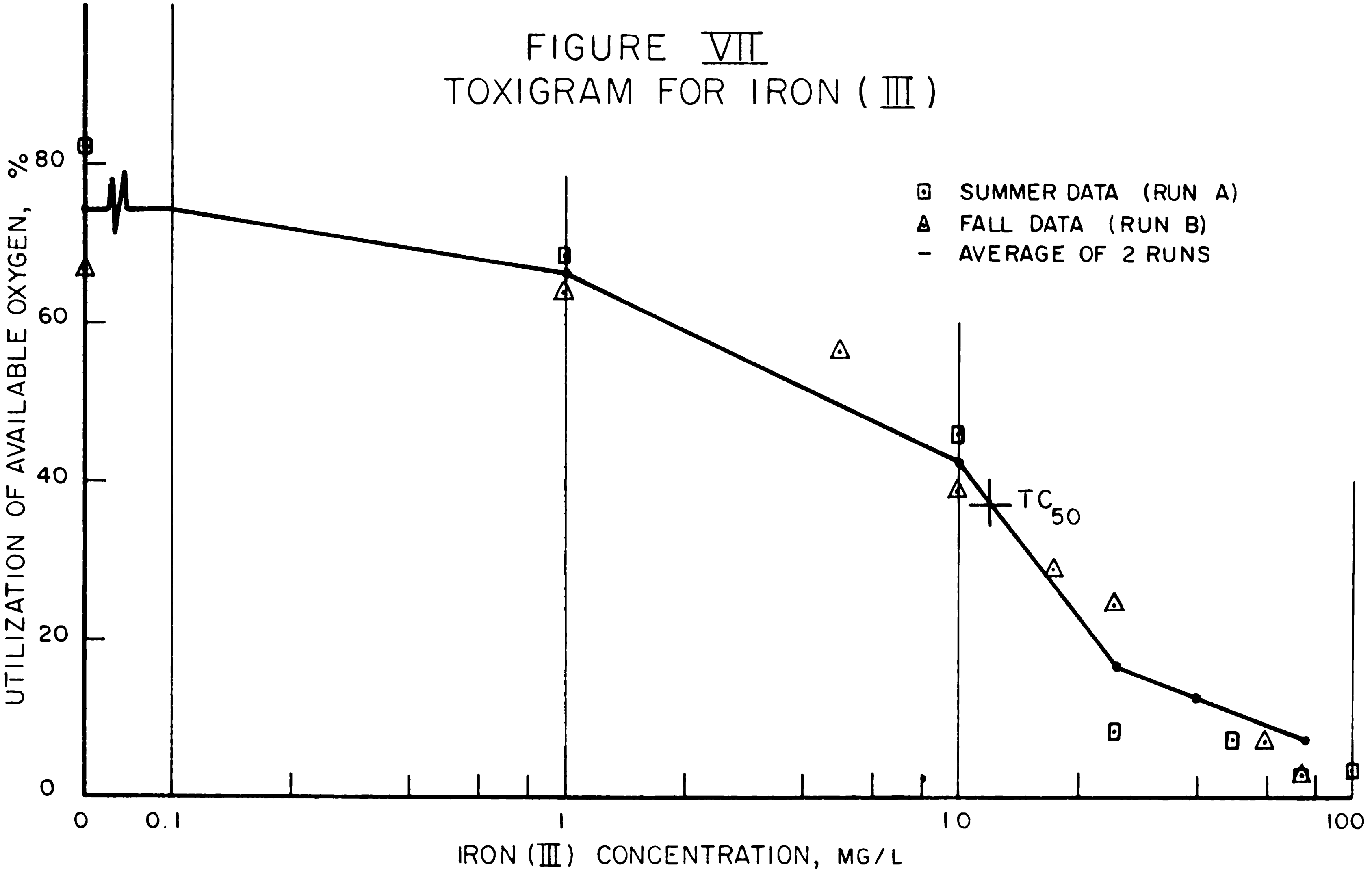


FIGURE VIII
TOXIGRAM FOR MANGANESE

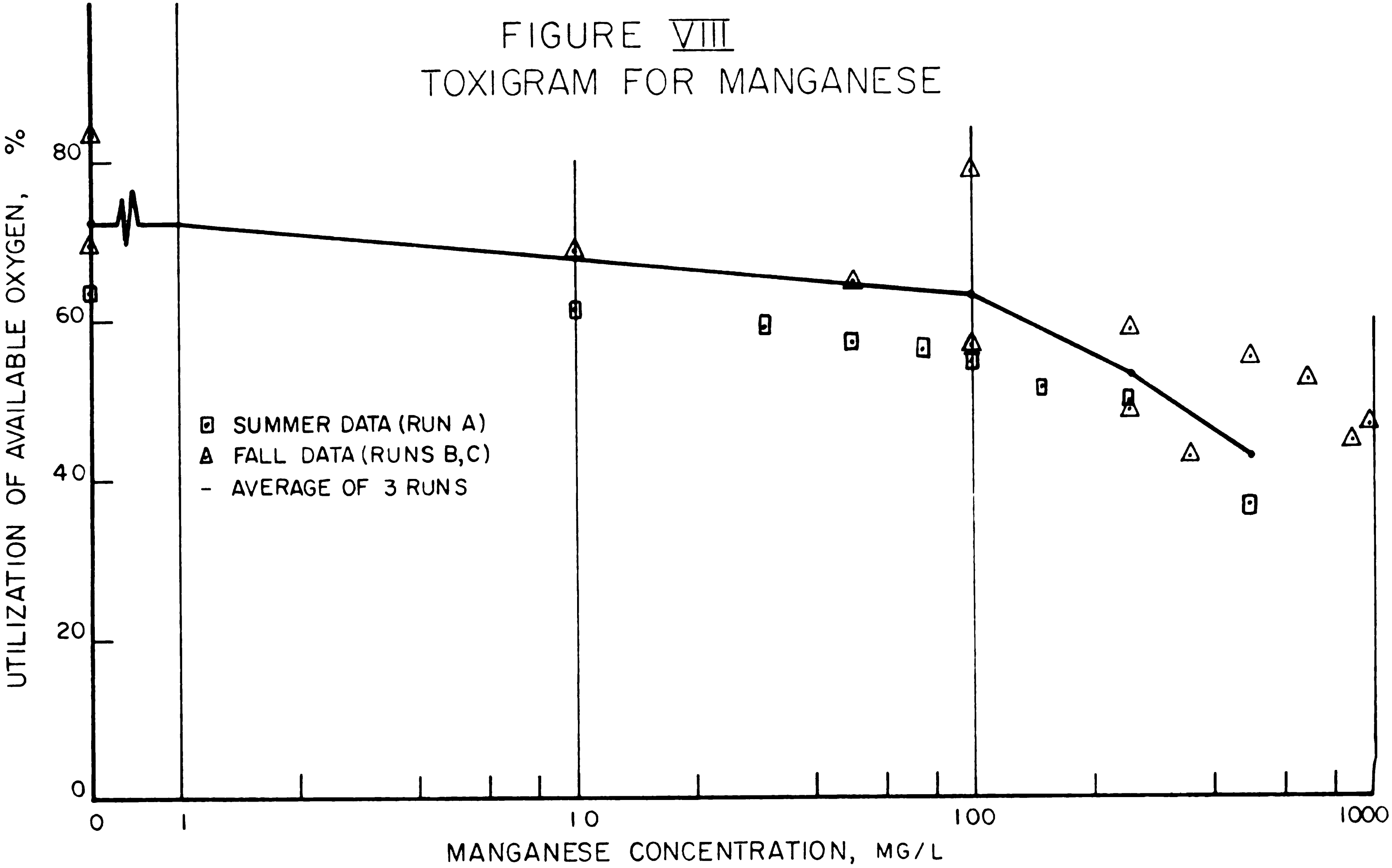


FIGURE IX
TOXIGRAM FOR MERCURY (II)

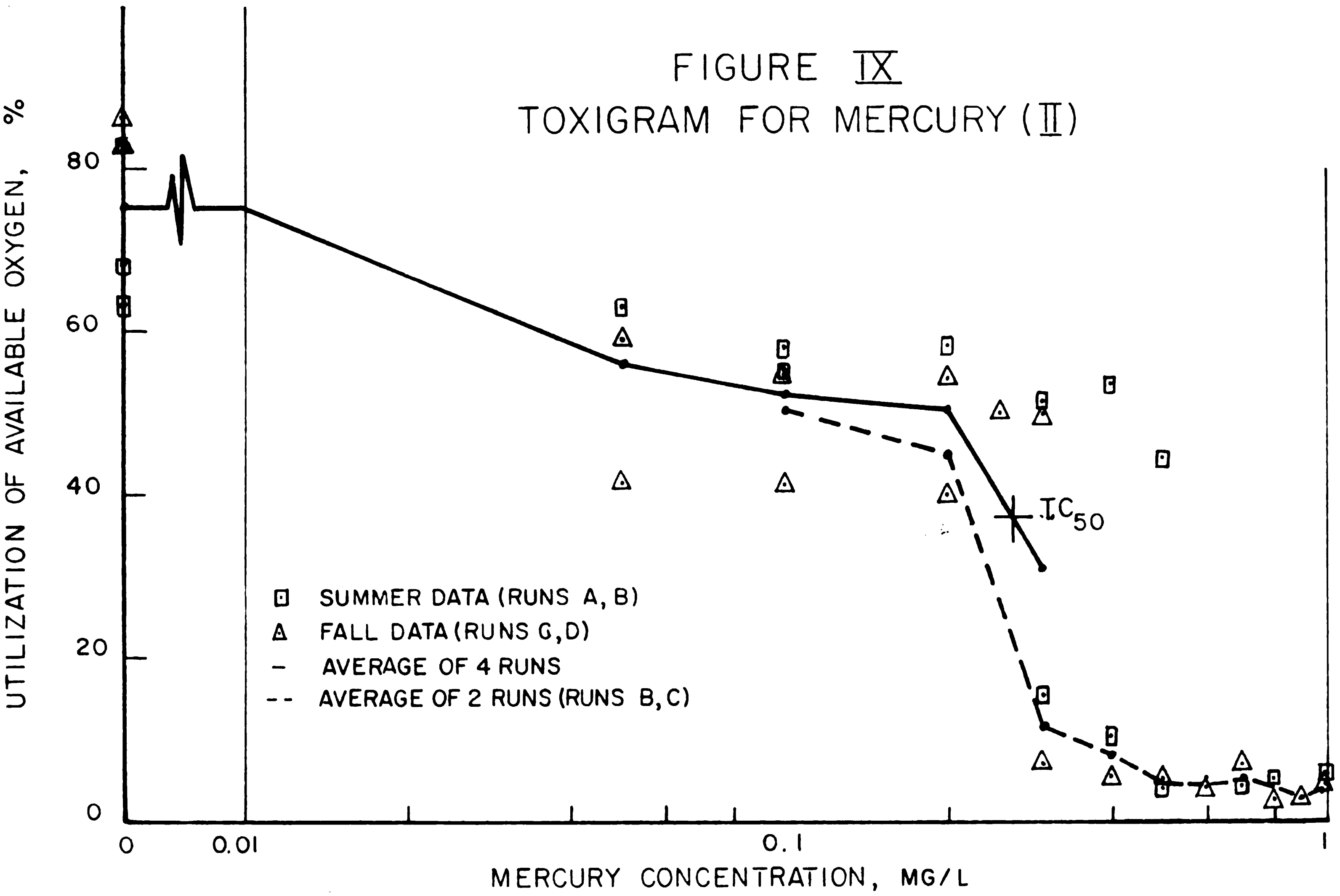


FIGURE X
TOXIGRAM FOR MOLYBDENUM

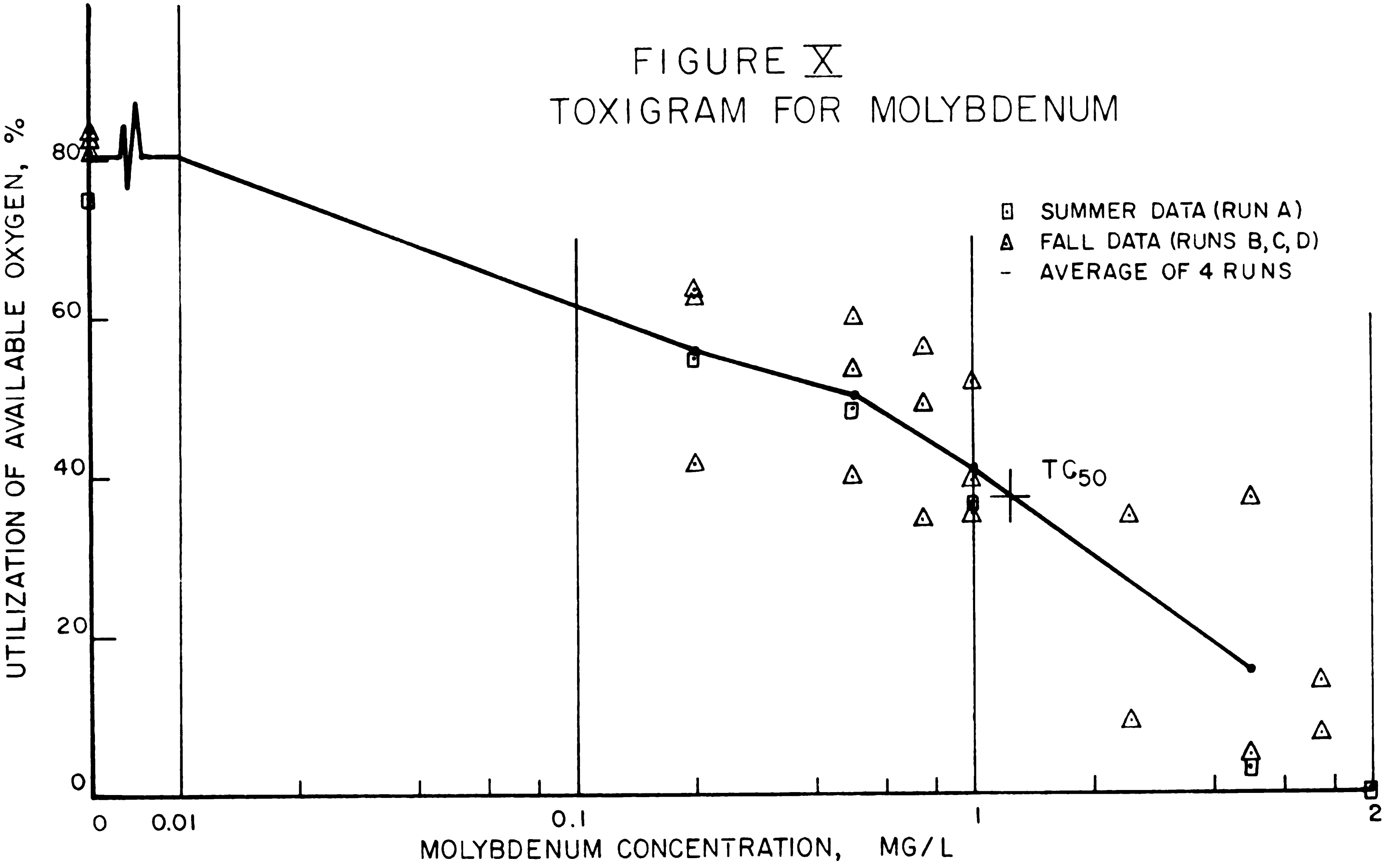
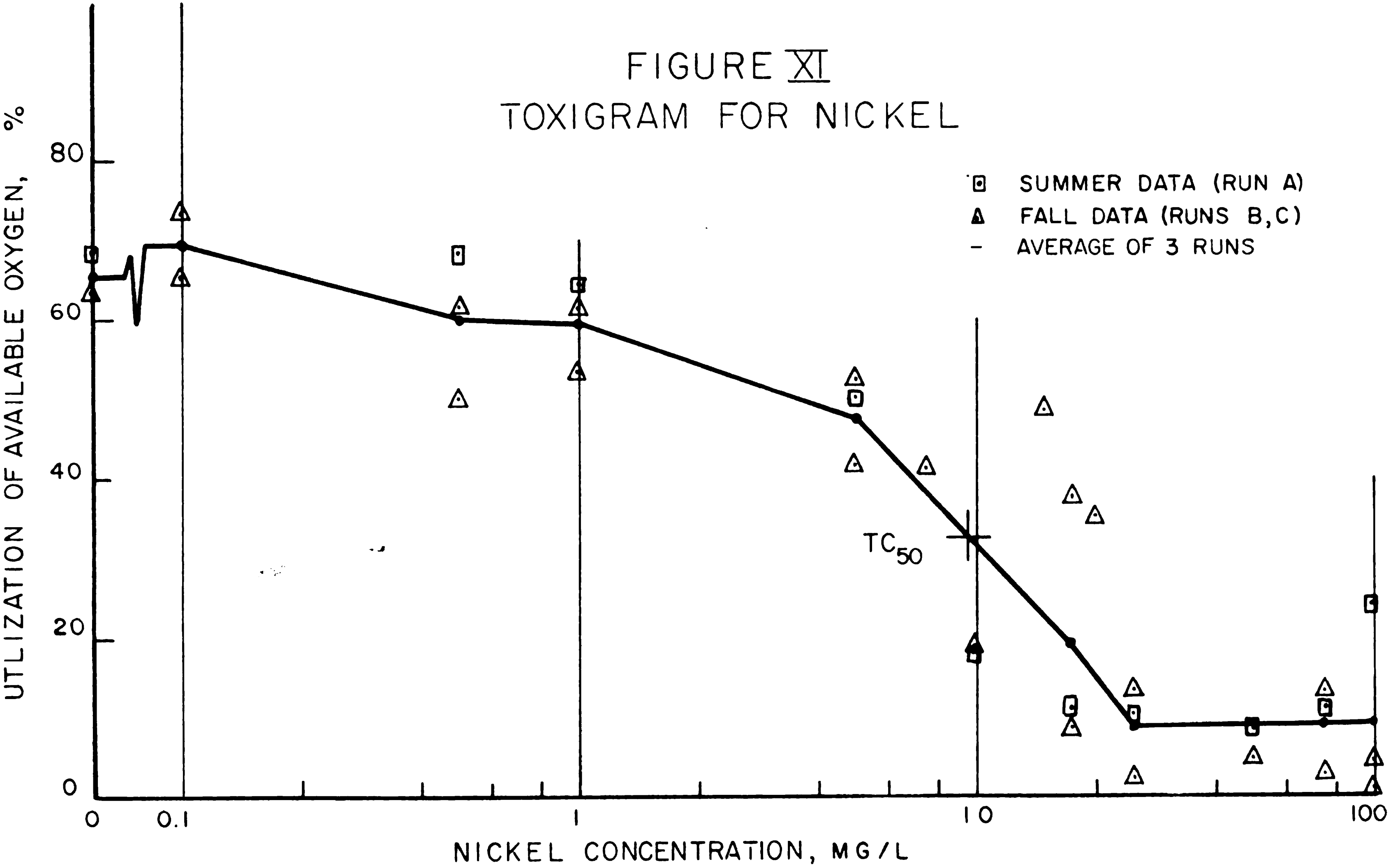


FIGURE XI

TOXIGRAM FOR NICKEL



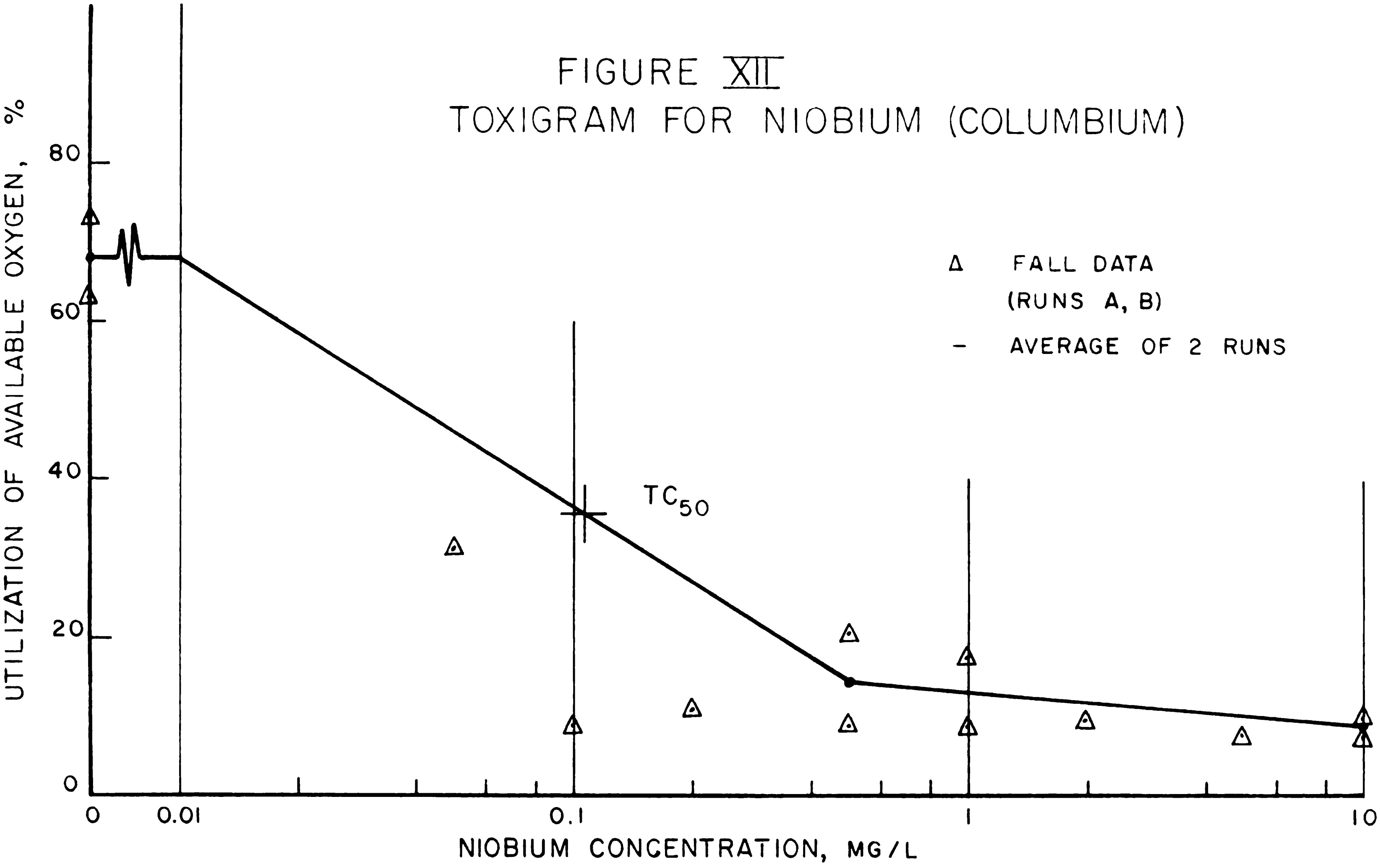


FIGURE XII
TOXIGRAM FOR NIOBIUM (COLUMBIUM)

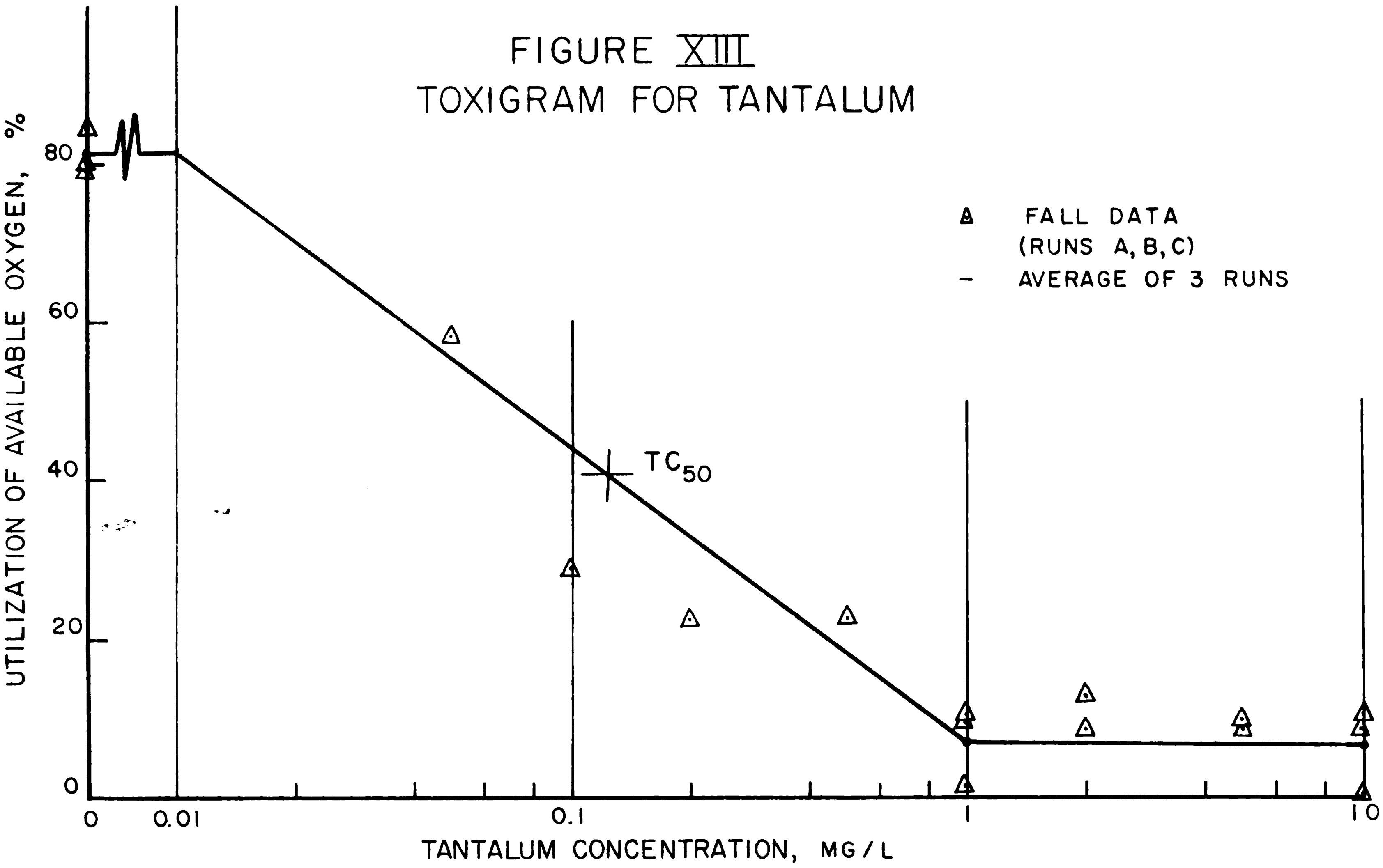
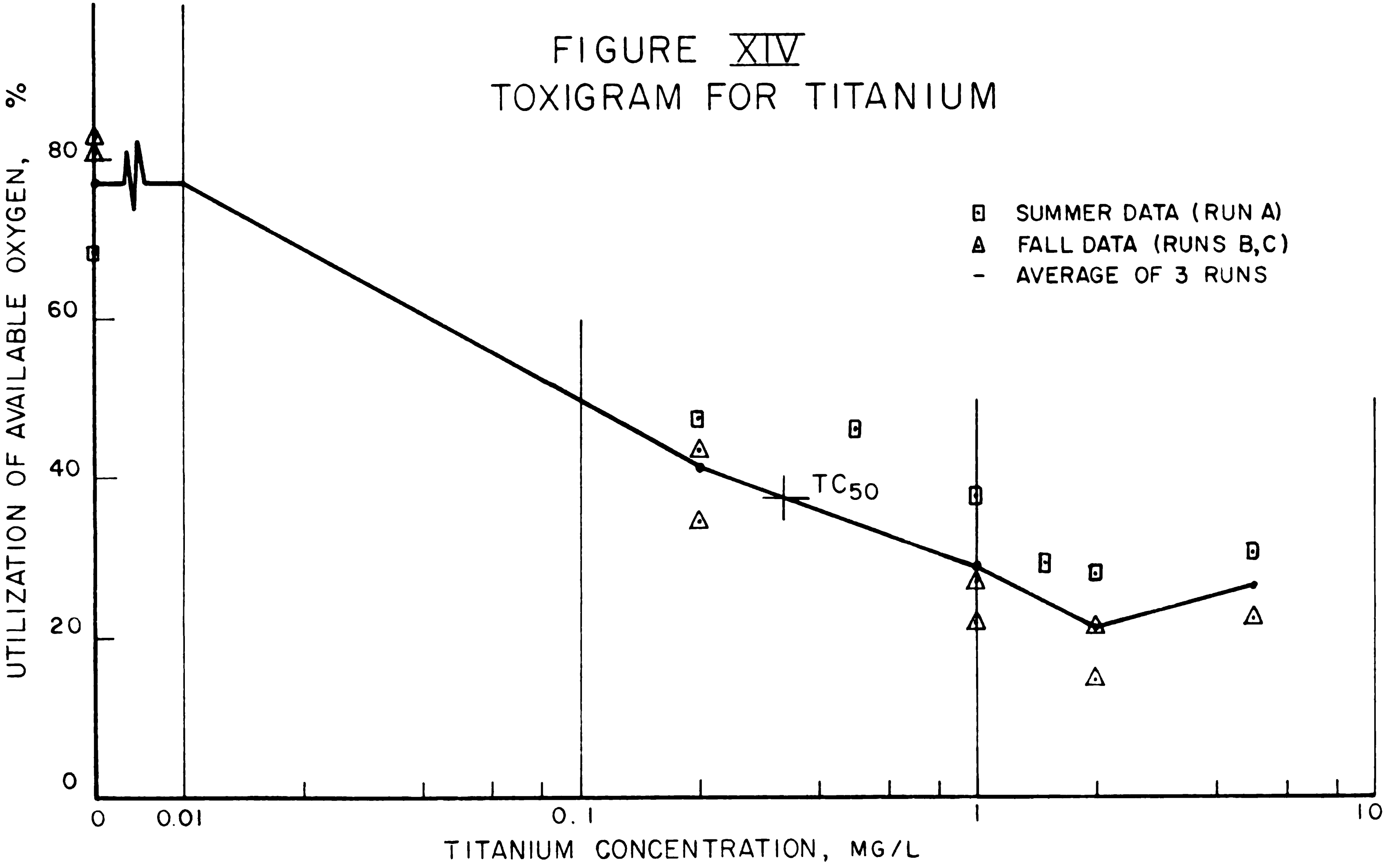


FIGURE XIII
TOXIGRAM FOR TANTALUM

FIGURE XIV
TOXIGRAM FOR TITANIUM



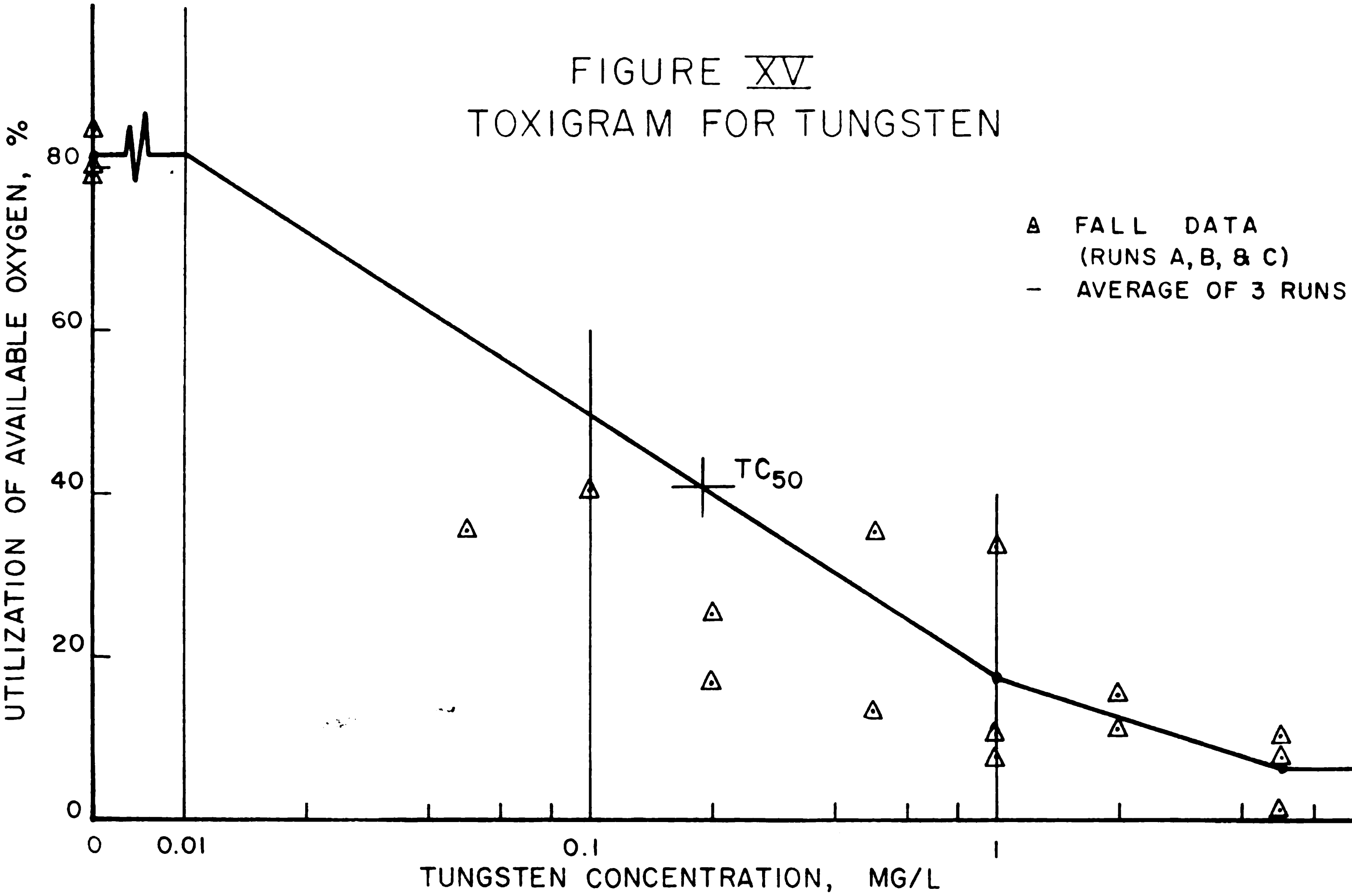


FIGURE XV
TOXIGRAM FOR TUNGSTEN

FIGURE XVI
TOXIGRAM FOR VANADIUM

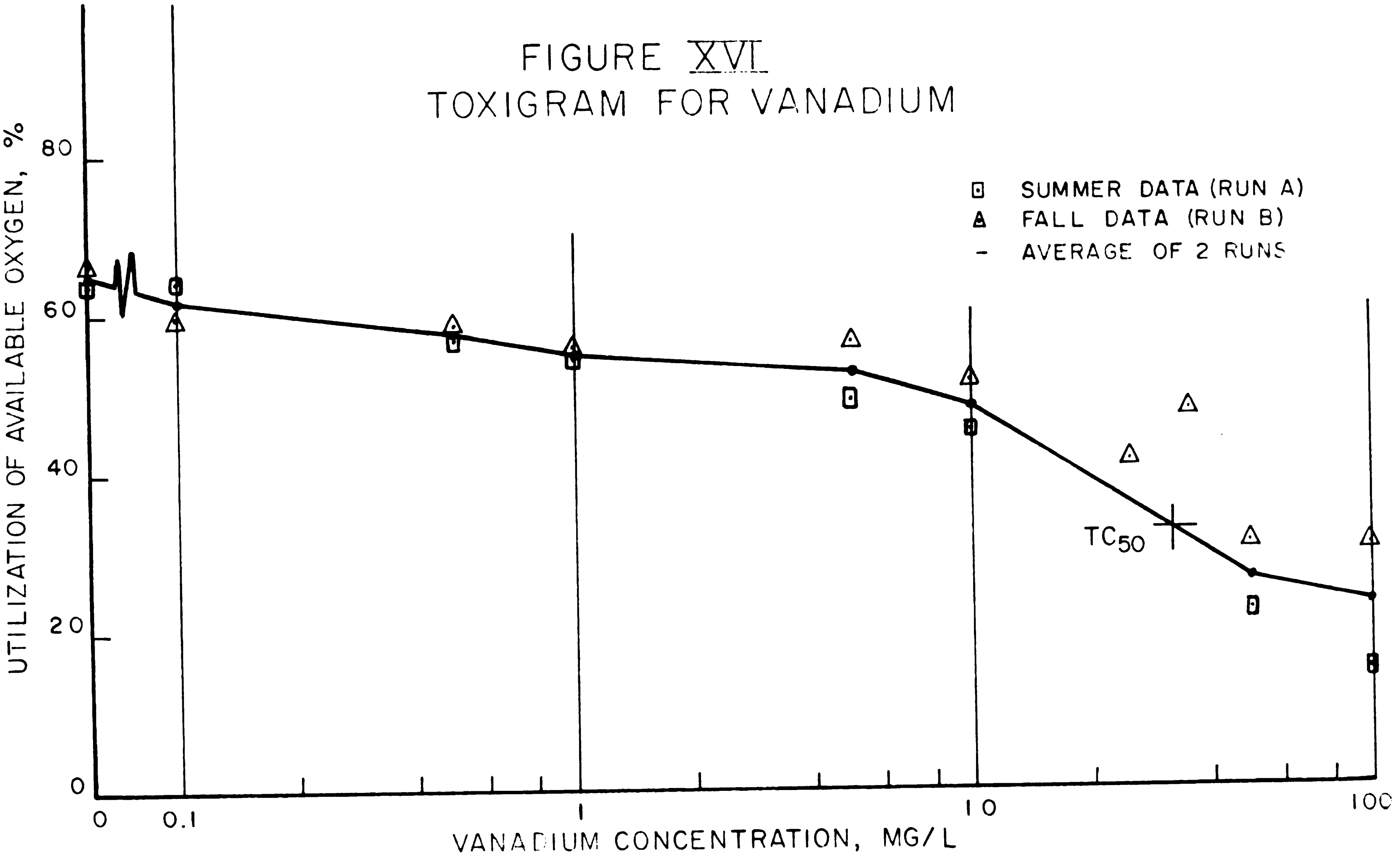


FIGURE XVII
TOXIGRAM FOR ZIRCONIUM