1976

Part I. Thermodynamic acidities of substituted phenylacetylenes. Part II. Mercury accumulation in trout of Southern Missouri

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Department:

PART I: THERMODYNAMIC ACIDITIES OF SUBSTITUTED PHENYLACETYLENES

PART II: MERCURY ACCUMULATION IN TROUT OF SOUTHERN MISSOURI

BY

ERIC THOMAS LLOYD, 1946-

A THESIS

Presented to the Faculty of the Graduate School of the

UNIVERSITY OF MISSOURI-ROLLA

In Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE IN CHEMISTRY

1976

Approved by

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Franklin B. Pauls

This thesis is dedicated to my wife and our parents.

PUBLICATION THESIS OPTION

This thesis consists of two manuscripts prepared for publication. Pages 1-28 have been prepared in the style utilized by the Journal of Organic Chemistry and will be presented for publication in that journal. Pages 29-60 have been prepared in the style utilized by Environmental Research and will be presented for publication in that journal.

ABSTRACT

PART I: THERMODYNAMIC ACIDITIES OF SUBSTITUTED PHENYLACETYLENES

The thermodynamic acidities (pKa) of phenylacetylene and several para substituted phenylacetylenes in methanol were determined at 25°C. These acidity constants were determined using a quenching technique which involves converting the phenylacetylenic anion, present at equilibrium as sodium phenylacetylide, to the tritium labelled carbon acid. By liquid scintillation counting techniques, the concentration of the anion was determined and the pKa of the phenylacetylene compound calculated. The resulting pKa's are: p-nitrophenylacetylene, 17.98, p-bromophenylacetylene, 18.10, p-fluorophenylacetylene, 18.14, phenylacetylene, 18.50, and p-methylphenylacetylene, 18.60. The effect of a substituent in the para position on the relative acidities is consistent with inductive effect predictions. A Hammett sigma rho plot of the observed pKa's produces a straight line with a slope equal to 0.85 and the coefficient of correlation is 0.988 using a least squares fit.

PART II: MERCURY ACCUMULATION IN TROUT OF SOUTHERN MISSOURI

A study of mercury accumulation in trout taken from the trout parks and streams of southern Missouri is presented. Mercury in trout is determined by digestion in nitric acid, sulfuric acid, and potassium permanganate, followed by reduction and aeration for measurement by flameless atomic absorption. The mercury accumulation

in trout collected and analyzed in this project ranged from 0.1 to 0.3 ppm mercury (μg mercury/g of tissue). Previous analysis of trout collected in the mid to late 1950's from the same areas indicated mercury accumulations of approximately 3 ppm. Over the past 25 years, there has been a substantial decrease in mercury found in trout of southern Missouri as a result of cleaner streams.

ACKNOWLEDGEMENTS

The author deeply appreciates the guidance, support, and friendship shown by Dr. James O. Stoffer during the course of this research. The author would like to extend his sincere appreciation to the members of his advisory committee, Dr. H. O. McDonald and Dr. F. B. Pauls.

For providing the financial support while at the University of Missouri-Rolla, I wish to thank Dr. William H. Webb and the Department of Chemistry for the Teaching Assistantships. I also thank Mr. George E. Smith, Director of the Water Resources Research Center, Columbia, Missouri and the United States Department of the Interior for the Research Support.

To my wife, Pam, I am deeply indebted and wish to thank her for her love, companionship, and understanding. With her support, we have accomplished our goal of making this thesis a reality.

I wish to extend a special thanks to my parents, Irene C. and the late Emerson T. Lloyd for the love, encouragement and support they have most unselfishly given throughout my lifetime.

Finally, the author wishes to thank Lynn Flaim for typing this thesis and John Land for the drawings presented within.

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PART I: THERMODYNAMIC ACIDITIES OF SUBSTITUTED PHENYLACETYLENES

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ABSTRACT

The thermodynamic acidities (pKa) of phenylacetylene and several para substituted phenylacetylenes in methanol were determined at 25°C. These acidity constants were determined using a quenching technique which involves converting the acetylenic anion, present at equilibrium as sodium phenylacetylide, to the tritium labelled carbon acid. By liquid scintillation counting techniques, the concentration of the anion was determined and the pKa of the acetylene compound calculated. The resulting pKa's are: p-nitrophenylacetylene, 17.98, p-bromophenylacetylene, 18.10, p-fluorophenylacetylene, 18.14, phenylacetylene, 18.50, and p-methylphenylacetylene, 18.60. The effect of a substituent in the para position on the relative acidities is consistent with inductive effect predictions. A Hammett sigma rho plot of the observed pKa's produces a straight line with a slope equal to 0.85 and the coefficient of correlation is 0.988 using a least squares fit.

INTRODUCTION

The interaction of acids and bases is probably one of the oldest studied types of chemical reactions. Problems related to establishing a universal definition for an acid and a base dates to the work of Gay-Lussac,[1,2] Arrhenius,[3] Bronsted and Lowry,[4,5] and Lewis.[6] The work in this publication makes use of Bronsted-Lowry definition of acids and bases. The general equation which describes the actual transfer of a proton from an acid to a base in solution is:

$$A\text{-}H + B^- \rightleftarrows A^- + B\text{-}H \qquad (1)$$

Compounds which react with bases forming anions by the cleavage of a carbon-hydrogen bond are known as carbon acids, and the anions are referred to as carbanions.[7] It is true that many such carbon acids possess only slight acidic properties. The pKa's may range from 10 to 30 units. However, changes in solvent, temperature, base, and structure should have an effect on the acidities of carbon acids.

Several methods for determining thermodynamic acidity constants have been reported by Breslow,[8] Bordwell,[9,10] Ritchie,[11] and Streitwieser.[12] These and other methods are described in reviews by Jones,[7] Cookson,[13] and in Cram's[14] book. This paper will deal with a technique capable of measuring acidities of carbon acids whose pKa's fall between 17 and 30.

The pKa of phenylacetylene in methanol at 25°C was reported to be 18.50.[15] The acidity constant was calculated by obtaining the anion concentration at equilibrium using a quenching technique with isotopic labelling and liquid scintillation counting techniques. The equilibrium quench runs were made by allowing the phenylacetylene-methoxide solution to reach equilibrium in methanol at 25°C. After equilibrium was established, the solution was quenched by dropping the solution into tritiated benzene sulfonic acid in methanol. After necessary treatment of the samples, aliquots of the phenylacetylene solution were transferred into vials containing scintillation fluid and were counted in a Nuclear Chicago Model 2 Liquid Scintillation Spectrometer. Since the protium to tritium ratio in the quenched solution is known, the concentration of the phenylacetylene anion at equilibrium can be determined by liquid scintillation counting techniques. The sensitivity[16] of the liquid scintillation spectrometer is illustrated by the fact it is able to detect tritium concentrations on the order of 10^{-15}M.

The technique is a method for measuring anion concentrations and one would expect the method to be able to detect changes in acidity as the result of modifying the structure of a parent compound. Acidity measurements have been made on phenylacetylene and four para substituted phenylacetylenes ($-NO_2$, -F, -Br, -Me), and is the purpose of this publication.

In this study the only modification was in the structure of the parent carbon acid. Methanol was selected as the solvent in the analysis because it dissolves most organic compounds, it is similar to water in many respects, it is easy to obtain in pure form, and the preparation, handling, and measurement of the concentration of the conjugate base (MeO^-) is relatively easy.

The method used to measure the pKa of the carbon acids involves establishing the following equilibrium,

$$RH + {}^-OMe \rightleftarrows R^- + MeOH \qquad (2)$$

The equilibrium expression for equation (2) would be: (assuming dilute solution; activity = concentration)

$$K_{eq} = \frac{[MeOH]\,[R^-]}{[RH]\,[{}^-OMe]} \qquad (3)$$

The equation represented in equation (2) can be expressed as the sum of two hypothetical equations, (4) and (5):

$$RH \rightleftarrows R^- + H^+ \qquad (4)$$

$$H^+ + {}^-OMe \rightleftarrows MeOH \qquad (5)$$

The expression for K_{eq} for equations (4) and (5) would be:

$$K'_{eq} = \frac{[R^-]\,[H^+]}{[RH]} = K_a^{RH} \qquad (6)$$

$$K''_{eq} = \frac{[MeOH]}{[MeO^-]\,[H^+]} = 1/K_a^{MeOH} \qquad (7)$$

The expression for the sum of equations (4) and (5) would be:

$$K_{eq} = \frac{K_a^{RH}}{K_a^{MeOH}} \qquad (8)$$

Equating equations (3) and (8) with algebraic rearrangement yields:

$$K_a^{RH} = \frac{K_a^{MeOH} \cdot [MeOH]\,[R^-]}{[RH]\,[MeO^-]} \qquad (9)$$

By definition $K_i^{MeOH} = K_a^{MeOH} \cdot [MeOH]$, therefore equation (9) reduces to:

$$K_a^{RH} = \frac{K_i^{MeOH} \cdot [R^-]}{[RH]\,[MeO^-]} \qquad (10)$$

The parameters necessary to calculate the Ka (or pKa) of our carbon acid are reduced to the equilibrium concentration of the acid (RH), its conjugate base (R^-), base concentration (MeO^-), and K_i^{MeOH}. The first three we can measure and the latter is equal[17] to $1.87X10^{-17}$.

RESULTS AND DISCUSSION

The results of this study are summarized in Table I.

The concentrations of the phenylacetylene anion on the order of 10^{-4}M when the initial phenylacetylene concentrations were 10^{-2}M indicates significant tritium incorporation above background (blank) in the quenching process. The DPM's (disintegrations per minute) for the blanks usually were less than 150, while the DPM's for the samples were in excess of 1000 to 1500 and larger.

One possible source of a systematic error in the generation of this experimental data is the reactivity of the hydrocarbon. Since triple bonds (alkynes) are susceptible to nucleophilic attack,[18] it might be expected that bases catalyze addition of methanol to the phenylacetylenes. Vinyl ethers and acetals may be produced by this reaction.[19] If hydrocarbon reactivity is a fact here, the concentrations of the acetylenic anion at equilibrium (measured by tritium incorporation) will be lower than it really is. Thus the observed pKa would be higher, indicating the compound to be a weaker acid.

Experiments were performed to determine the specific magnitude of this systematic error. Runs TL 11 and TL 12, involving the simultaneous analysis of the phenylacetylene, the nitro- and the fluoro- substituted phenylacetylene as a group, had 12 and 6 hour equilibration times respectively for the phenylacetylene-methoxide solution. Earlier analysis had equilibration times of approximately

TABLE I

Thermodynamic acidities of Phenylacetylenes

Run	Sample	$[MeO^-]$	[RH]	$[R^-]$	K_i^{MeOH}	Ka	pKa	Average[b] for compound
TL1	p-Br-C_6H_4-C≡CH	$6.46X10^{-2}$	$3.84X10^{-2}$	$1.08X10^{-4}$	$1.87X10^{-17}$	$8.14X10^{-19}$	18.09	
TL2		$6.46X10^{-2}$	$4.51X10^{-2}$	$1.27X10^{-4}$	$1.87X10^{-17}$	$8.15X10^{-19}$	18.09	18.10
TL9		$6.50X10^{-2}$	$4.35X10^{-2}$	$1.09X10^{-4}$	$1.87X10^{-17}$	$7.21X10^{-19}$	18.14	
TL9	p-H-C_6H_4-C≡CH	$6.50X10^{-2}$	$1.88X10^{-1}$	$2.38X10^{-4}$	$1.87X10^{-17}$	$3.64X10^{-19}$	18.44	
TL10		$6.50X10^{-2}$	$7.73X10^{-2}$	$7.23X10^{-5}$	$1.87X10^{-17}$	$2.69X10^{-19}$	18.57	18.50
TL7	p-Me-C_6H_4-C≡CH	$6.50X10^{-2}$	$8.97X10^{-2}$	$6.67X10^{-5}$	$1.87X10^{-17}$	$2.14X10^{-19}$	18.67	
TL9		$6.50X10^{-2}$	$7.92X10^{-2}$	$8.51X10^{-5}$	$1.87X10^{-17}$	$3.09X10^{-19}$	18.51	18.60
TL11	p-O_2N-C_6H_4-C≡CH	$6.56X10^{-2}$	$1.65X10^{-2}$	$6.19X10^{-5}$	$1.87X10^{-17}$	$1.07X10^{-18}$	17.97[a]	
TL12		$6.56X10^{-2}$	$2.96X10^{-2}$	$1.09X10^{-4}$	$1.87X10^{-17}$	$1.05X10^{-18}$	17.98[a]	17.98
TL11	p-F-C_6H_4-C≡CH	$6.56X10^{-2}$	$3.03X10^{-2}$	$7.53X10^{-5}$	$1.87X10^{-17}$	$7.08X10^{-19}$	18.15[a]	
TL12		$6.56X10^{-2}$	$7.49X10^{-2}$	$1.95X10^{-4}$	$1.87X10^{-17}$	$7.42X10^{-19}$	18.13[a]	18.14

[a] Values adjusted to phenylacetylene value of 18.50

[b] Weighted average based on total number of data points

2 hours. The results of the phynylacetylene of analysis TL 11 and TL 12 indicated a slightly higher pKa than in previous analysis; however, the pKa differences between the hydrogen compound and the nitro- and the fluoro- compounds in both analyses were consistent. Consistent and reproducible values were obtained with short equilibration times. Once the magnitude of these systematic errors had been determined, the experimental results were corrected by the appropriate amounts as represented by the values for p-nitrophenylacetylene and p-fluorophenylacetylene.

The values reported for each phenylacetylene compound are averaged values from two or more sets of multiple determinations. Each set was composed of from five to nine determinations. Occasionally, one or two experimental values would vary considerably from the rest for no apparent reason. Data of this nature were judged discordant and rejected if the deviation of the suspected value from the mean (omitting the doubtful value) is at least four times the average deviation of the retained values.[20]

From Table I the calculated pKa in methanol of p-nitrophenylacetylene is 17.98, p-bromophenylcaetylene, 18.10, p-fluorophenylacetylene, 18.14, phenylacetylene, 18.50, and p-methylphenylacetylene, 18.60.

The rapidly increasing volume of data on equilibria and rates of orgnaic reactions has resulted in the development of quantitative correlations between structure and reactivity. One of the best known

and most widely acceptable correlations was described by Hammett.[21] He found that plotting the logarithm of the rate (k) constant or equilibrium (K) constant for one reaction vs. the ΔpKa of the corresponding benzoic acids resulted in essentially a straight line for a number of reactions of meta- and para-substituted benzene derivatives. The difference in acid strengths of the benzoic acids is referred to as the substituent constant, σ. This constant is a measure of the electron-donating or electron-withdrawing power of a substituent. When the logarithm of the rate or equilibrium constant is plotted against the substituent constant, the slope, ρ, is the reaction constant and is a measure of the sensitivity of the equilibrium constant to substituent modifications.

Effects of structure on the acidic strength of a series of benzene derivatives may be approximated as the sum of inductive, resonance, and steric involvements. In general, substituents in the para position have very little contributions from steric effects. Thus σ_p values can be represented as the sum of inductive and resonance effects. Taft et al.[22] proposed that these values could be divided into inductive and resonance contributions, σ_I representing the inductive portion and σ_R representing the resonance portion. These σ values are related through the equation:

$$\sigma_p = \sigma_I + \sigma_R.$$

Data from this study were plotted in typical Hammett fashion against σ_p,[23] σ_I,[22] and σ_R.[22] Figure 1 is the plot of $\log(K_{eq}/K^{\circ}_{eq})$ vs. σ_I. A least square fit of the data indicates a positive slope

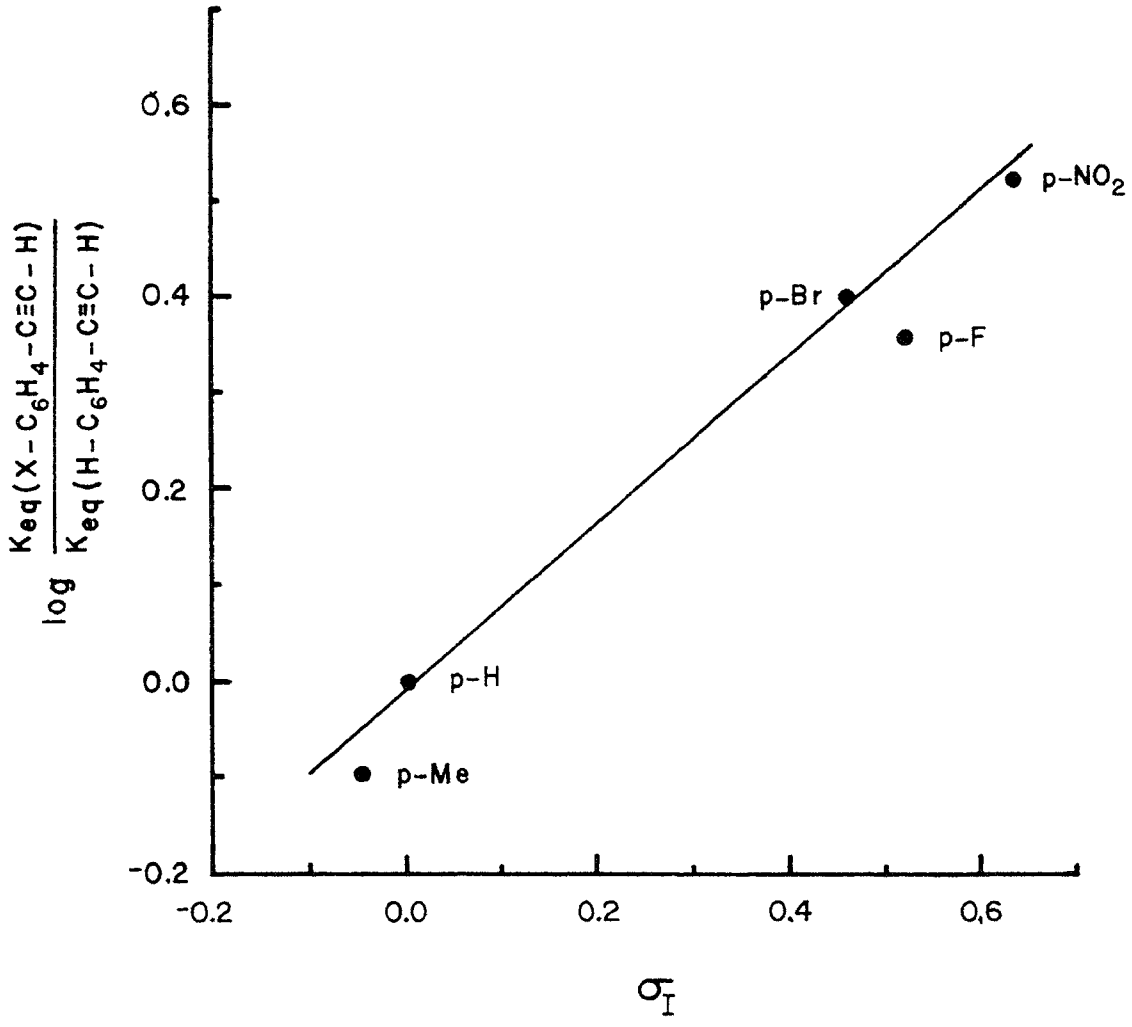

Figure I. A Hammett plot of pKa's for Phenylacetylenes

of 0.85 with a coefficient of correlation of 0.988. Figure 2 is the Hammett plot of σ_p and of σ_R. The results do not correlate well, showing more scatter and deviation than normally found in such straightforward plots. The correlation in Figure 1 indicates that modifications in structure changes the acidity of the phenylacetylenes through an inductive effect. In general, electron-donating groups, such as methyl, reduce acidity and electron-withdrawing groups, such as nitro, bromo, and fluoro increase acidity. The data collected in this study indicate such a trend.

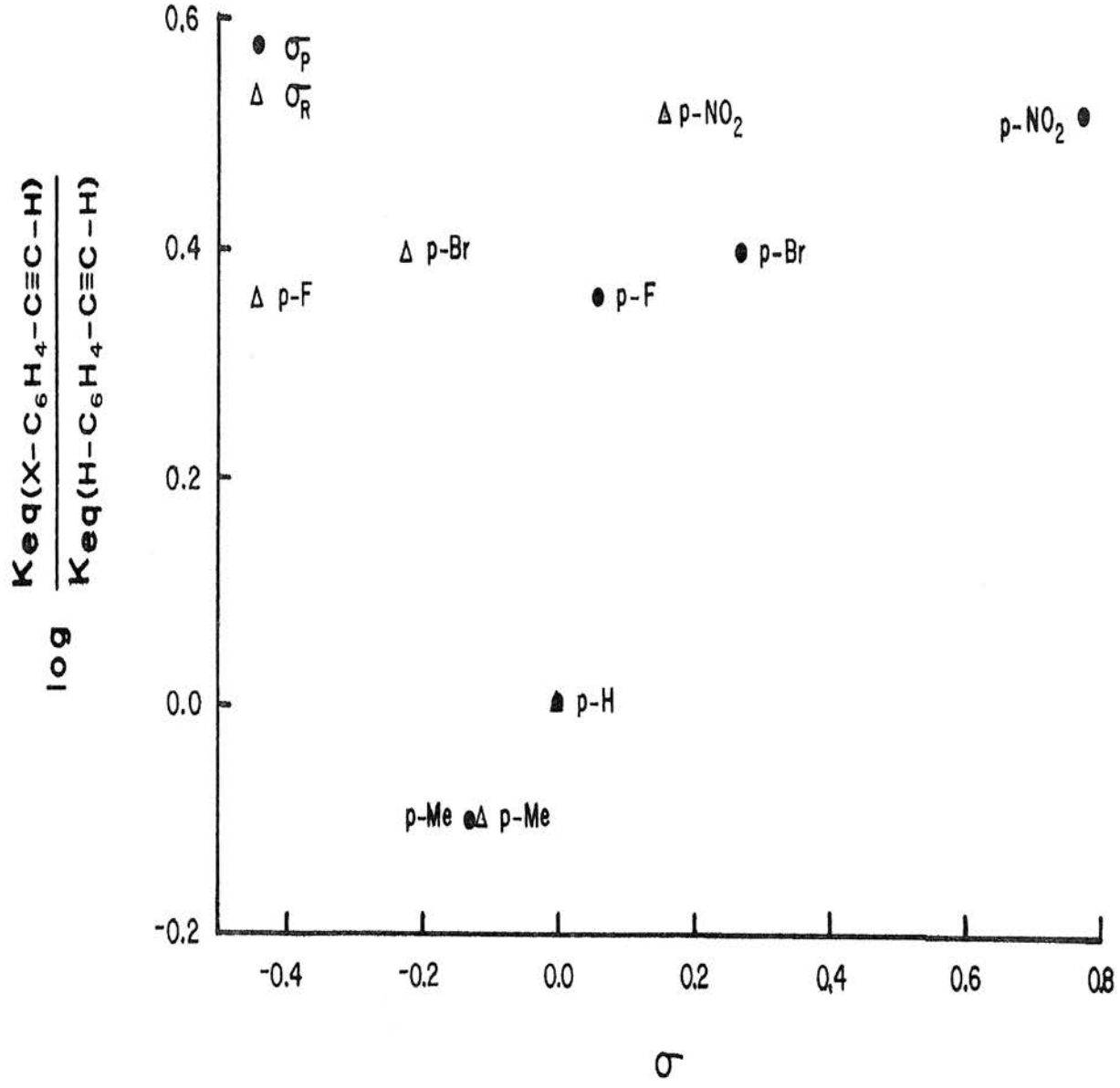

Figure 2. A Hammett plot of σ_P^- and σ_R^- for Phenylacetylenes

CONCLUSION

The author feels that the quench method used to obtain equilibrium phenylacetylenic anion concentrations are valid and reproducible. The calculated acidity constants for the respective phenylacetylene compounds generally encompass a range of ±0.15 pka units from the mean value. The results for the p-nitro- and p-fluoro-phenylacetylenic compounds are the averaged value of 12 individual equilibrium quench reactions, the parent compound (phenylacetylene) is an average of 13 individual reactions, the p-methyl compound is an average of 14 reactions, while the p-bromophenylacetylene is an average of 22 individual reactions.

The author feels the interpretation of the results of the study are valid. Since the quenching method can distinguish acid strengths as a function of substituents, the method is an effective tool for measuring acidity constants. The compounds studied with electron-withdrawing substituents ($-NO_2$, -F, and -Br), as expected, are more acidic than the parent compound (-H). This trend is due to the increased stability of the anion. The compound studied with an electron-donating group (-Me), as expected is less acidic than phenylacetylene. The correlations in this study, of acid strengths as effected by para substitution, are indicative that the equilibrium phenylacetylenic concentrations were truly

being measured. Therefore, the technique is valid for calculating the thermodynamic acidity constants.

EXPERIMENTAL SECTION

CHEMICALS

All melting points and boiling points are uncorrected. A Perkin-Elmer 137 NaCl Prism Infrared Spectrometer or a Perkin-Elmer 337 Grating Infrared Spectrometer were used to obtain all the ir spectra in this study. NMR spectra were obtained on a Varian EM 360 Spectrometer using Me_4Si (δ=0) as an internal standard. GC analysis was performed on a Varian Aerograph 920 equipped with a Varian G 1000 recorder using a 5 ft X 0.25 in Carbowax 20M column and showed only one peak for each compound.

Phenylacetylene

Phenylacetylene was obtained from Eastman Kodak Company. The compound was purified by distillation under aspirator pressure through a spinning band column, bp 44-45°C, lit[24] bp 142-144°C/ atmospheric pressure. The ir[25] and NMR[26] spectra were the same as those published.

p-Bromophenylacetylene

p-Bromophenylacetylene was prepared in our laboratory following the method of Dufraisse and Dequesnes,[27] by refluxing a mixture of p-bromoacetophenone and phosphorus pentachloride (slight excess). The product is obtained by dehydrohalogenation of the monochloro-ethylene and dichloroethane product mixture with alcoholic KOH

followed by extraction with ether. The ether solution is dried over potassium carbonate. The ether is removed and the product distilled under reduced pressure, and collected as a solid in the condenser. Recrystallization from ethanol gave colorless crystals, mp 65.0°C, lit[28] mp 64-65°C. The absorbance peaks on the ir spectrum[29] of this compound were the same as those published. The NMR was consistent with expectations.

p-Methylphenylacetylene

p-Methylphenylacetylene was prepared in our laboratory following the method of Smith and Hoehn.[30] p-Methylacetophenone was slowly added to phosphorus pentachloride (slight excess) and allowed to stand at room temperature for twelve hours. The monochloroethylene and the dichloroethane products are dehydrohalogenated with alcoholic KOH and the product extracted with ether. The ether solution is dried over potassium hydroxide. The ether is removed and the product distilled under aspirator pressure, bp 67-68°C, lit[28] bp 79-82°C/31-33mm. The ir and NMR are consistent with expected spectra.

p-Fluorophenylacetylene

p-Fluorophenylacetylene was prepared following the adapted procedure for the p-methylphenylacetylene synthesis. The starting material, p-fluoroacetophenone, is a liquid as is the starting material in the p-methyl synthesis, therefore, the same synthetic procedure was followed to give the title compound, bp 77-78°C. The ir and NMR were as expected.

p-Nitrophenylacetylene

p-Nitrophenylacetylene was prepared in our laboratory following the procedure of Drewsen.[31] The ethyl ester of p-nitrocinnamic acid was treated with bromine in carbon disulfide under reflux. The dibromo compound was dehydrohalogenated and saponified with alcoholic KOH yielding the potassium salt of p-nitrophenylpropynoic acid. The acid is precipitated with aqueous $NaHSO_4$. Following decarboxylation in boiling water, the p-nitrophenylacetylene steam distills and collects in the condenser. The product is recrystallized from ethanol, mp 150.0-150.6°C, lit[31] 152.0°C. The ir spectrum[32] was identical to published spectrum. The NMR was as expected.

Anhydrous Methanol

Methanol was obtained from Fisher Scientific Company. Approximately one liter of this methanol was allowed to react with 5 grams of magnesium turnings (a few crystals of iodine were added to initiate the reaction).[33] The methanol was then distilled from the turnings and stoppered in a glass-stoppered bottle wrapped with Parafilm. A Karl Fischer analysis of the redistilled methanol could not detect any water. The limits of this Karl Fischer apparatus was 0.03% water with one drop of the reagent. In all cases the titration reached its end point with one drop of Karl Fischer reagent.

SPECIAL SOLUTIONS

Scintillation Solution

The scintillation solution used for all non-aqueous tritium analyses was prepared according to the procedure of Holtz.[34] The

solution was prepared by dissolving 4.3 g of 2,5-diphenyloxazole (PPO, Packard Instrument Company, Inc., scintillation grade, fluoro. max. at 3650 Å) and 0.26 g of 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP, Packard Instrument Company, Inc., scintillation grade, fluoro. max. 4200 Å) in 950 ml scintanalyzed toluene (Fisher Scientific Company). This solution was allowed to stand overnight before use.

An emulsifying scintillation solution (Insta-Gel, Packard Instrument Company, Inc.) was used for aqueous tritium analyses, such as urine samples. Urine samples were counted periodically to monitor body incorporation.

Standard Sodium Methoxide Solution

Crude, fresh cut sodium was first cleaned in reagent grade methanol. The cleaned sodium (approximately 7.0 grams) was then placed in a 500 ml volumetric flask and diluted to volume with redistilled anhydrous methanol. This solution was stored in a teflon stoppered volumetric flask wrapped with parafilm.

A standard solution of potassium hydrogen phthalate was prepared according to standard procedures.[17] The standardization of the sodium methoxide-methanol was performed on four aliquots of the methoxide solution against the standard potassium hydrogen phthalate solutions, using phenophthalein as an indicator.

Tritiated Water

Tritium, in the form of tritium enriched water, was obtained and stored as described by Strait[35] and Filger[16].

Benzenesulfonic Acid Stock Solution

The benzenesulfonic acid stock solution was prepared by weighing 200.6030 grams of molten benzenesulfonic acid, $C_6H_5SO_3H \cdot H_2O$ (Eastman Kodak Company), in a 500 ml volumetric flask which was then filled to the mark at 25°C with anhydrous methanol.

EQUILIBRIUM QUENCH METHOD

All weighings were made on a Mettler H20 Electronic balance and were to within 0.01 mg. All temperature equilibrating in a Haake FK2 Constant Temperature Bath maintained at 25°C.

Preparation of Phenylacetylene-Methoxide Solution

The differential weight technique was used to measure the amount of the phenylacetylene compound in these analysis (approximately 0.3000 g in a 25 ml volumetric flask). In the case of liquid phenylacetylene compounds, they were syringed into the flask. After pipetting 3 ml of the standardized sodium methoxide solution into the flask, it was reweighed and filled to the mark with anhydrous methanol at 25°C. The flask was kept in the constant temperature bath at 25°C and allowed to come to thermoequilibrium and weighed for the final time, then was returned to the constant temperature bath until it was quenched.

Preparation of Tritiated Benzenesulfonic Acid Solution

A weighed quantity (differential weight technique) of the benzenesulfonic acid stock solution was transferred to a 50 ml Erlenmeyer flask and capillaries of tritium enriched water were crushed with a glass stirring rod below the level of the solution. A rubber septum over the mouth of the flask prevented concentration changes resulting from evaporation. Transfers were made with a syringe.

Quenching Procedure

The reaction vessels for the quench reactions were 20 ml vials fitted with pressure sealing screw caps. A small piece of iron nail sealed in glass was added to each vial as a stirring bar to facilitate mixing during the quenching process.

The experimental set up for the equilibrium quench runs involved a jacketed 10 ml pipette made by sealing the pipette inside a glass cylinder with two side arms. This type of set up permitted the temperature of the solution inside the pipette to be controlled. A pro-pipette bulb with a screw clamp was attached to the pipette. The screw clamp permitted control of the flow rate of the phenylacetylene methoxide solution from the pipette. The constant temperature media was allowed to circulate around the pipette at the desired temperature to maintain the established equilibrium of the phenylacetylene-methoxide solution as it was drawn into the pipette.

The quenching procedure involves syringing one ml of tritiated benzenesulfonic acid into preweighed vials. After reweighing,

each vial was placed on a magnetic stirrer and about one ml of the equilibrated phenylacetylene-methoxide solution was slowly dropped into them from the pipette. After this quenching, the vials and contents were weighed for the final time.

For each analysis, several blanks were used. The blanks were prepared by adding non-tritiated benzenesulfonic acid to each vial, followed by the phenylacetylene-methoxide solution, and tritiated benzenesulfonic acid. The blanks were used to show that little exchange takes place in acidic media conditions. From this point, the treatment of the blanks was identical to that of the samples.

After the quenching process, the phenylacetylene compound is extracted from its methanol solution with toluene. Ten ml of scintanalyzed toluene was pipetted into a 125 ml separatory funnel equipped with Teflon stopcock. Fifteen ml deionized water was then added. The contents of the equilibrium quench reaction (including stirring bar) were quantitatively transferred to the separatory funnel from the reaction vessel by washing with 5 portions (1 ml each) of anhydrous methanol.

After vigorous shaking, this first washing was allowed to stand a minimum of five hours. The water layer was drained off and replaced by 15 ml of deionized water. The second wasing stood for one hour. The washing process was continued through six washings, the last four at thirty minute intervals. Once free of tritiated methanol and benzenesulfonic acid impurities, the acetylene-toluene

layer was dried over calcium chloride. Then 1 ml aliquots from each sample (including blanks) were transferred to individual precounted scintillation vials for tritium analysis containing 15 ml of scintillation fluid or Insta-Gel.

In order to determine if six aqueous washings were sufficient to remove the radioactive impurities from the toluene extract layer, the washings were analyzed for tritium activity. The results of analysis on the water layer of washings six, seven, and nine indicated the activity of the aqueous layer decreased to background levels after six washings. Therefore, six washings were sufficient to remove any radioactive impurities from the toluene layer.

LIQUID SCINTILLATION COUNTING

A Nuclear Chicago Mark II liquid scintillation counter[36] was used in this study.

All samples were placed in the instrument's refrigeration unit for fifteen minutes, allowing them to come to equilibration. Each scintillation vial was counted for four minutes and the external standard was counted for 0.4 minutes. All data was automatically printed by a teletype.

A series of six quench standards are counted and their C/A ratio is calculated. A standard quench curve is obtained by plotting counting efficiency versus C/A ratio. By knowing the sample's C/A ratio, its counting efficiency can be obtained from the standard quench curve. The disintegrations per minute (DPM) are needed in

order to calculate an acetylenic anion concentration. The DPM's are obtained by dividing the CPM's (counts per minute) read directly from the teletype by the counting efficiency obtained from the standard quench curve.

TRITIUM ACTIVITY OF BENZENESULFONIC ACID SOLUTION

The amount of tritium present in the benzenesulfonic acid solution used in each analysis was determined by a dilution process. A portion of the tritiated benzenesulfonic acid solution (approximately 5 μl) was introduced into a Hamilton 10 μl syringe and weighed. The contents of the syringe were transferred into a 25 ml volumetric flask already containing some anhydrous methanol. The syringe reweighed and using the differential method, the amount of benzenesulfonic acid can be accurately measured. By accurately knowing the density of the benzenesulfonic acid, the volume of the solution added to each volumetric can be determined. The volumetric flask was filled to the mark with anhydrous methanol at 25°C and shaken to insure adequate mixing.

One ml of the above diluted tritiated benzenesulfonic acid solution was diluted to 25 ml in a volumetric flask at 25°C with anhydrous methanol and shaken to provide sufficient mixing. One ml of this tritiated benzenesulfonic acid solution was transferred into a precounted vial containing 15 ml scintillation solution and counted for tritium activity by liquid scintillation techniques. The absolute activity of the tritiated benzenesulfonic acid used in the

analysis was then calculated using dilution factors. Once the DPM's of the benzenesulfonic acid were determined, the number of tritiums were obtained by multiplying the DPM's by 9.73×10^6 ^3_1H/DPM.[37]

ACKNOWLEDGEMENTS

The author wishes to express his sincere appreciation and gratitude to Dr. James O. Stoffer whose advice and friendship has guided this investigation.

Because of their considerable influence to the development of and application for the quenching method used in this study, I wish to express my appreciation to Dr. Dennis L. Filger and Dr. Darrell R. Strait.

I also want to thank Claude Crain for the synthesis of the p-nitro-, p-bromo-, and the p-methylphenylacetylene used in this study.

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PART II: MERCURY ACCUMULATION IN TROUT OF SOUTHERN MISSOURI

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ABSTRACT

A study of mercury accumulation in trout taken from the trout parks and streams of southern Missouri is presented. Mercury in trout is determined by digestion in nitric acid, sulfuric acid, and potassium permanganate, followed by reduction and aeration for measurement by flameless atomic absorption. The mercury accumulation in trout collected and analyzed in this project ranged from 0.1 to 0.3 ppm mercury (μg mercury/g of tissue). Previous analysis of trout collected in the mid to late 1950's from the same areas indicated mercury accumulations of approximately 3 ppm. Over the past 25 years, there has been a substantial decrease in mercury found in trout of southern Missouri as a result of cleaner streams.

INTRODUCTION

Because of the recent awareness of mercury as a widespread environmental hazard, there have been a large number of papers published that involve the determination of mercury concentrations. The Japanese and Swedish problems are described in numerous reports from those countries. Papers by Irukayama (1967) and Westöö and Rydalv (1969) may be cited as examples of such documentation. Related discoveries in Canada and the United States have been reported by Kleinert and Degurse (1973), Dunlap (1971) and Wallace *et al.* (1971).

There are numerous references in the literature involving mercury accumulation in aquatic animals. Papers by Koirtyohann (1974) and Renken (1975) refer to accumulation of mercury in bass, Koirtyohann (1974) reports mercury in channel catfish, Kivalo *et al.* (1974) in pike, Uthe *et al.* (1973) and Bertrand (1974) in trout, and Kivalo *et al.* (1974) and Uthe *et al.* (1970) in walleye. Not only has accumulation been reported in fish, but also in shellfish. A report by Vermeer (1973) indicated accumulation in crayfish, also Kopler (1974) reported mercury accumulation in oysters.

Trout fishing is a sport enjoyed by many people in Missouri. There are four trout parks in the state which are stocked daily and open for fishing from March 1 through October 31. To illustrate the magnitude of interest in trout fishing in Missouri, Table 1 gives the total number of trout tags sold in the four conservation managed

TABLE 1

Total number of Trout tags sold in parks of Missouri[a]

Trout Park	1973	1974	1975
Bennett Spring	153,282	142,123	140,208
Montauk	82,562	76,782	91,897
Roaring River	74,638	68,649	76,210
Maramec Spring	46,084	47,486	58,513
TOTAL	356,566	335,040	366,828

[a]Jerrilyn Snodgrass, Missouri Department of Conservation, Fisheries Division, Jefferson City, Missouri. Personal Communication.

trout parks in the state. Each tag is not a different person, since many anglers buy several tags. However this still represents more than a third of a million angler-trips to the clear water spring branches of Missouri in each of the last three years. In addition to these four parks, there are several bodies of water in Missouri that support (with additional stocking) a trout population. These include Lake Taneycomo, Mill Creek, Little Piney River, Big Piney River, Current River, Eleven Point River, and Meramec River.

The recent reported disclosures of the presence of mercury pollution in lakes receiving industrial drainage and the high levels of mercury found in fish from such lakes has prompted the development of a simple, rapid, sensitive, and accurate method for determining mercury accumulation in aquatic life.

Many methods of analysis have been used for quantitative mercury determinations. All the methods typically involve preliminary treatment to release the mercury from the sample matrix, concentration of the mercury, and measurement. Reimer *et al.* (1973) report that prior to 1950 the wet methods, such as gravimetric, volumetric, polaragraphic, and colorimetric, were equal if not superior to instrumental methods. Since the middle of the 1950's, methods of trace metal analysis have advanced greatly with the continual refinement of modern technology. Not only are detection limits now orders of magnitude lower than in the past, but the origins of previously unexplained pollution problems are coming to

light. This is the result of the introduction of instrumental techniques as atomic absorption, neutron activation, x-ray fluorescence and mass spectroscopy, and electrometric techniques.

The development and application of the flameless atomic absorption technique to mercury analysis has greatly enhanced the accumulation of knowledge regarding mercury as an environmental problem. Flameless atomic absorption spectroscopy is presently one of the most popular microanalytical techniques for mercury determinations. The technique is in principle simple and sensitive (part per billion range), but in practice the accurate determination of mercury in natural samples is difficult.

The method of Jeffus *et al.* (1970) is used to free the mercury from the sample by the digestion in a mixture of nitric acid and sulfuric acid. The chemistry involved in this project is based on the method developed by Hatch and Ott (1968). The mercury in the sample is totally oxidized to mercury(II) with potassium permanganate in acidic media (nitric acid-sulfuric acid). Hydroxylamine hydrochloride is added to reduce excess permanganate. Stannous chloride is then added to reduce the mercuric ions to elemental mercury.

The elemental mercury is vaporized and circulated by an aerator system. Measurement is made with a flameless atomic absorption spectrophotometer. The 2537 Å mercury line emitted by a mercury lamp is absorbed by the mercury vapor in the flow-through absorption cell (15.5 cm). The change in transmittance is detected by the

phototube. The amount of absorption is related to the concentration of mercury vapor in the absorption cell which is directly related to the concentration of mercury in the sample.

Two large trout, one ten pounds and the other eight pounds, were taken from the Little Piney River during the period 1946-1950. After being stored in a deep freeze since then, they were analyzed in 1973 by the flameless atomic absorption technique. The results of this analysis by Bertrand (1974) indicated mercury concentrations of approximately 3 ppm. Two trout, considerably smaller, were taken from the same area in 1973 and analyzed using the same procedure. These fish contained less than 0.2 ppm mercury. Stocking procedures had been in effect in the Little Piney River between 1950 and 1973. Also, one trout taken from Mill Creek, an area where recent stocking had not taken place, was analyzed and found to contain 0.3 ppm mercury.

In addition to monitoring the levels of mercury in trout taken from streams in southern Missouri, this project had as its objectives:

1) To determine whether mercury levels of fish in the Little Piney River and Mill Creek watershed area have changed substantially over the past 25 to 30 years.
2) To study long-term accumulation of mercury by aquatic animals.
3) To compare mercury levels in the liver with mercury levels found in the flesh.
4) To make a correlation of mercury accumulation with size and/or age of the trout.

The investigator feels that knowledge concerning the possible causes for and the effects of the numerous releases of mercury into the environment is definitely incomplete. It is hoped that the data collected and conclusions drawn may be used to give insight into mercury pollution problems of the area, as well as lending itself to satisfying a fisherman questioning the quality of his catch.

MATERIALS AND METHODS

CHEMICALS

In this section, the chemicals used in the analysis of fish for mercury are described as to source, purity, concentrations used, and particular use in the analysis.

a) Mercuric chloride was obtained from Fisher Scientific Company as Certified A. C. S. This was used to prepare a standard solution of 1.000 mg mercury/ml in 5% HNO_3 (vol/vol).

b) Hydroxylamine hydrochloride was obtained from Fisher Scientific Company as Certified A. C. S. A solution of 10% (wt/wt) in 5% HNO_3 (vol/vol) was used to reduce the excess permanganate.

c) Potassium permanganate was obtained from Fisher Scientific Company as Certified A. C. S. A solution of 5% (wt/wt) in deionized water was used to assure complete oxidation of mercury to mercury(II).

d) Stannous chloride was obtained from Mallinckrodt as Analytical Reagent. A solution of 5% (wt/wt) in 5% H_2SO_4 (vol/vol) was used to reduce mercury(II) to elemental mercury.

e) Sulfuric acid was obtained from Fisher Scientific Company as Certified A. C. S. A solution of 50% (vol/vol) with deionized water was used in the digestion process. A solution of 5% (vol/vol)

with deionized water was used to dissolve the stannous chloride.

f) Nitric acid was obtained from Fisher Scientific Company as Certified A. C. S. A solution of 35% (vol/vol) with deionized water was used in the digestion process. A solution of 5% (vol/vol) with deionized water was used to dilute the standard mercury solution and to dissolve the hydroxylamine hydrochloride.

g) Tin metal (granular, 20 mesh) was obtained from General Chemical Division of Allied Chemical and Dye Corporation as Reagent Grade. Stannous chloride solution was stored over metallic tin in order to keep the tin in its lower oxidation state, tin(II).

h) Magnesium perchlorate (anhydrous) was obtained from City Chemical Corporation as Purified. It was used as a drying agent in the closed aeration system.

i) Amberlite (ion exchange resin) MB-1 was obtained from Mallinckrodt as Analytical Reagent. It was used to deionize all distilled water used in the analysis.

CLEANING GLASSWARE

Mercury contamination of glassware is an important consideration at the levels of mercury involved in this study as reported by

Bertrand (1974). The methods used to clean our glassware were strenuous but effective. It is briefly listed as follows:

1) Wash glassware with hydroxylamine hydrochloride solution and rinse with hot tap water.
2) Wash with hot soapy water and rinse with hot tap water.
3) Wash with concentrated HNO_3 and rinse with distilled water.
4) Soak overnight in an alcoholic KOH bath.
5) Rinse with hot tap water followed by rinsing with distilled water and dry.

SAMPLE COLLECTION

All fish studied were taken by sport fishing methods. The species included rainbow trout (*salmo gairdneri irideus*) and brown trout (*salmo trutta morpha fario*). Effort was made to include a range of sizes in those fish collected from the same area. The fish were stored frozen until analysis. There was no attempt to determine the age of the fish or to differentiate according to sex.

WHERE COLLECTED

Numerous trout samples were taken from Lake Taneycomo because there was no danger of depleting the trout in that area. Trout from Lake Taneycomo were used to develop and test our method of analysis. By studying Taneycomo trout, one could adequately study any variation in mercury accumulation as a function of size or seasonal variations.

Trout were also collected from the four trout park areas of the state: Bennett Spring, Montauk State Park, Roaring River, and Maramec Spring. Samples collected from these areas were used to accumulate data on mercury contamination as well as monitor the quality of fish from these locations.

Trout samples were collected from areas where occasional stocking is used to help maintain a native trout population, such as Little Piney River and Mill Creek. In the case of Mill Creek, stocking practices do not exist. Care was taken not to collect more samples than was necessary from these areas. This was done so the natural abundance of fish would not be depleted. Trout are known to reproduce naturally in these areas. The sample of trout collected were native trout born in these streams. Fig. 1 is a map of Missouri indicating areas where trout were collected.

PREPARATION OF STOCK MERCURY SOLUTION

A 1.000 mg mercury/ml stock solution was prepared by weighing 0.6768 grams of mercuric chloride into a 500 ml volumetric flask and diluting to volume with 5% HNO_3 (vol/vol). The stock solution was stored in a plastic bottle. Thorpe (1971) reported this standard solution to be stable for six months when stored in plastic bottles.

PREPARATION OF MERCURY STANDARDS

The stock mercury solution was diluted to a final concentration of 0.4 µg mercury/ml using 5% HNO_3 as the diluent. When making dilutions of mercury standards, the aliquot to be diluted was always added to the volumetric flask already containing part of the diluting

Fig. 1. Areas where Trout samples were collected

acid. This prevents adsorption of small amounts of mercury on the glass surface which can be critical when working with trace analysis. The 0.4 µg/ml solutions are only stable for a day, therefore they must be prepared daily.

The mercury working standard solutions were prepared containing 0.4, 0.8, 1.2, 1.6, and 2.0 µg mercury/100 ml solution in 5% HNO_3 by aliquoting appropriate amounts of 0.4 µg mercury/ml solutions. To these standard solutions, 1 ml $KMnO_4$ (2 grams/liter solution) was added. After diluting to final volume, the solution was transferred to a BOD (biological oxygen demand) bottle. The volumetric flask was then washed with three 15 ml portions of deionized water, the washings transferred to the BOD bottles. The standards were now ready for the reduction-aeration procedure. Three blanks were prepared identical to the standards but the standard mercury solution was omitted.

SAMPLE PREPARATION

The fillet (or liver) was removed from each fish, cut into small pieces and manually mixed to assure representative sampling.

About 1 gram of the fish was carefully placed in a 250 ml round bottom flask, fitted with a ground glass joint. The exact weight of the sample (±0.01 gram) was determined by weighing the flask before and after addition of the sample.

To the sample were added: four glass boiling beads (precleaned in concentrated HNO_3), 5 ml of 50% H_2SO_4 and 5 ml of 35% HNO_3. These reagents were added to dissolve the total sample as well as destroy

all mercury compounds and complexes. The entire sample must be covered by the acid mixture. The flask was immediately connected to an Allihn reflux condenser and was refluxed for one hour.

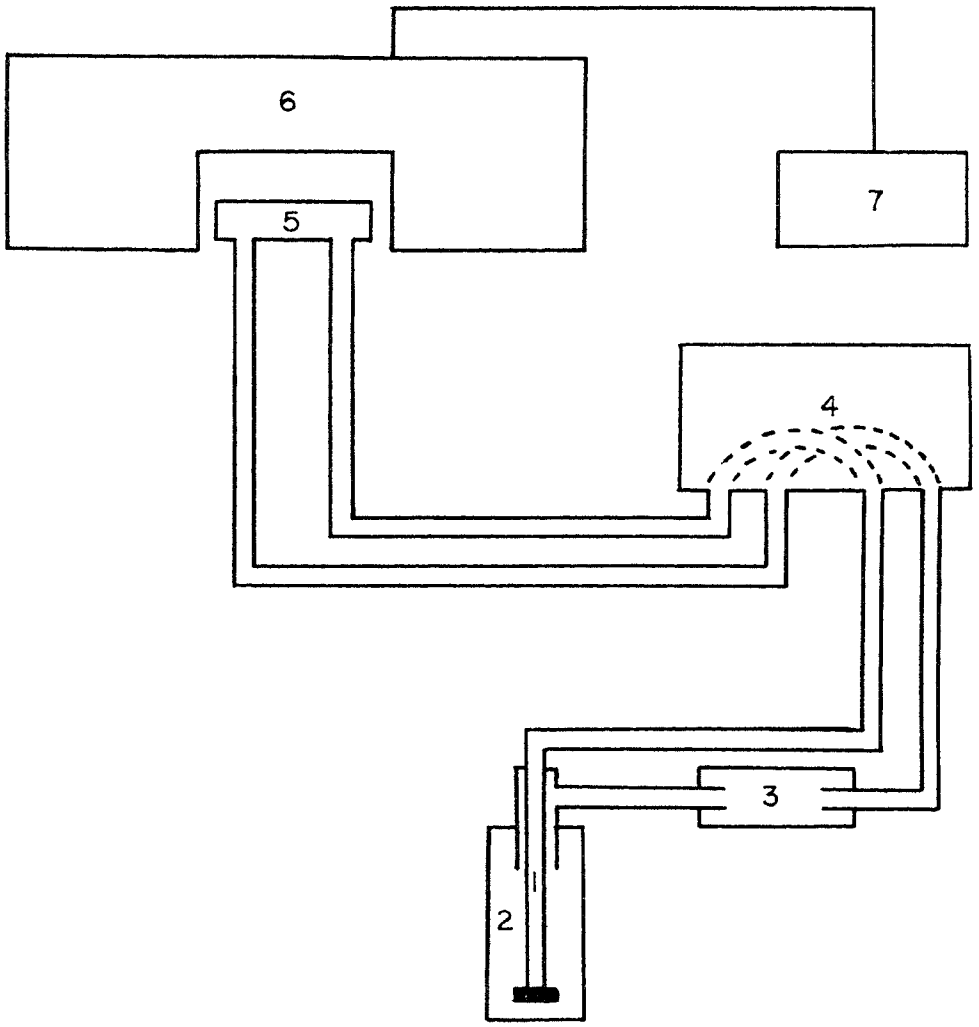
After refluxing was completed, the solutions were cooled and the entire system washed with two 15 ml portions of deionized water. Contents of the round bottom flask were quantitatively transferred to a 100 ml volumetric flask. The flask was washed with two 15 ml portions of deionized water and the washings transferred to the volumetric flask. Ten ml $KMnO_4$ solution (5% wt/wt) was added to each sample to assure complete oxidation of mercury to mercury(II). The samples were diluted to volume with deionized water and transferred to BOD bottles. After three 15 ml washings of the flask and transfer of washings to BOD bottles, the samples were ready for the reduction-aeration step. Three blanks were prepared identical to samples without addition of fish tissue.

REDUCTION-AERATION

The method described in this section assumes the investigator is completely familiar with the operation of the Perkin-Elmer Atomic Absorption Spectrophotometer 303 and has completed all preliminary adjustments so that the instrument is ready to operate. A schematic of the apparatus and instrumentation used in the reduction-aeration step is shown in Fig. 2.

The procedure involves:

1) syringe 10 ml of hydroxylamine hydrochloride solution into the BOD bottle already containing the sample,



1 Aeration Adaptor
2 Biological Oxygen Demand Bottle
3 Drying Tube
4 Air Pump
5 Absorption Cell
6 Perkin-Elmer 303 Atomic Absorption Spectrophotometer
7 DCR-1 Digital Concentration Readout

Fig. 2. Schematic of Reduction-Aeration Apparatus

2) replace the specially designed aerator making sure a good seal is achieved,
3) aerate the system for about ten seconds,
4) turn pump off, remove aerator, and syringe 10 ml stannous chloride solution,
5) replace aerator, turn on pump, and record maximum absorbance,
6) purge the circulating system by placing aerator on clean surface with the pump on.

The procedure is repeated for all standards, blanks, and samples.

INSTRUMENTATION

Measurement of mercury was made using a Perkin-Elmer Atomic Absorption Spectrophotometer 303. The spectrophotometer is equipped with a Perkin-Elmer Intensitron (hallow cathode) Lamp and a Perkin-Elmer Digital Concentration Readout (DCR 1) voltmeter.

The instrument settings are:

Lamp Current	6 ma
Wavelength	2537 Å
Slit	5
Scale	X1
Range	UV
Meter Response	2
Filter	OUT
DCR 1	
Noise Suppression	1

DCR 1 (cont'd.)

Mode	ABSORBANCE
Average	4X
Concentration	0000

The analysis utilizes the flameless atomic absorption technique, using the Perkin-Elmer Mercury Analysis System (303-0830) as the circulating system, pumping at a constant flow rate of 3 liters/minute.

CALCULATION

A working curve was obtained by plotting absorbance vs µg mercury. Since the volumes for the samples and the standards are the same, µg mercury may be plotted rather than a concentration expression. Fig. 3 is a typical working curve. The working range for mercury is linear up to concentrations of about 300 µg/ml in aqueous solution.

The quantity of mercury was read directly from the working curve. Ppm mercury is then calculated by dividing µg mercury by sample weight.

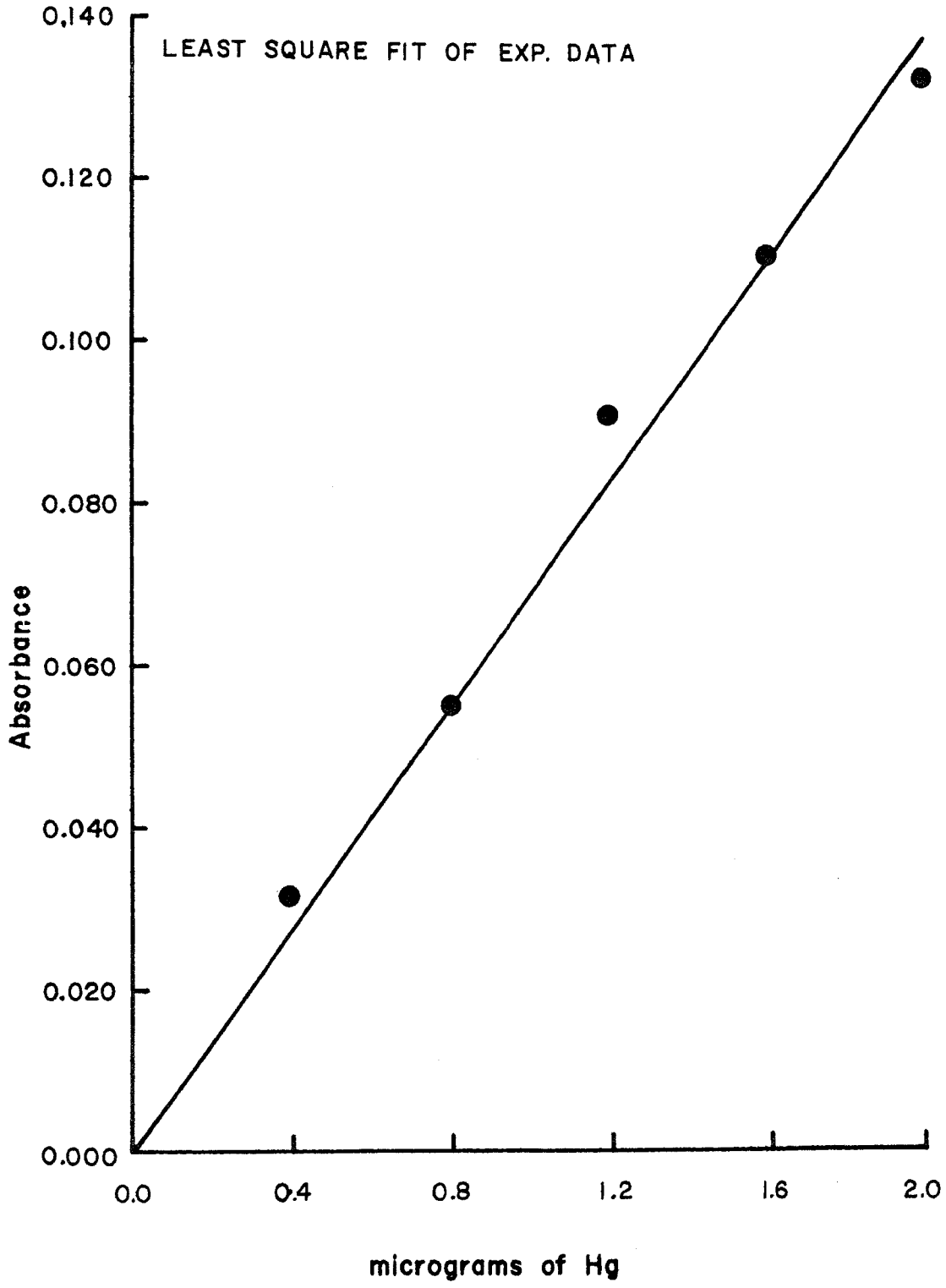


Fig. 3. A typical working standard curve

RESULTS AND DISCUSSION

The results of this study are presented in Table 2 and summarized in Figures 4 and 5.

There are some literature references indicating a direct correlation of mercury accumulation with size. Kleinert and Degurse (1973) report that larger fish of the same species often contain higher concentrations of mercury than do smaller fish taken from the same waters. Evidence for this correlation has been reported for Swedish pike (Environmental Research, 1971) and again by Koirtyohann (1974) for largemouth bass. Figure 4 correlates mercury accumulation with size from the data collected in this study. The results of this study indicate a relatively constant level of mercury in the fish with no variation according to size.

Various species of fish tend to accumulate mercury to different degrees. For example, Swedish pike tend to have one third to one half more mercury than other species of fish taken from the Swedish lakes. There may be a more significant explanation for low mercury levels than just lack of accumulation on the part of trout. From an environmental point of view, the most sought after explanation would be a substantial decrease of mercury in the streams. This could be a very acceptable explanation in light of the fact that fish taken from the Little Piney River during the period 1946-1950 contained approximately 3 ppm mercury, while those collected and analyzed in this project ranged from 0.1 to 0.3 ppm mercury. It can

TABLE 2

Analytical results for Mercury content in Trout of southern Missouri

Size	Source	Date of Catch	Hg (ppm)[a]
130 g	Mill Creek	3/23/75	0.21 (1)[b]
450 g		7/29/75	0.13 (1)
140 g	Little Piney River	10/22/75	0.20 (1)
			0.22 (2)
125 g		11/19/75	0.18 (1)
460 g	Big Piney River	8/15/75	0.49 (1)
650 g	Maramec Spring	10/3/74	0.29 (1)
890 g		10/26/74	0.19 (1)
460 g		2/25/75	0.28 (1)
380 g		3/4/75	0.10 (1)
1800 g		10/25/75	0.13 (1)
240 g	Bennett Spring	2/20/75	0.13 (1)
270 g		2/20/75	0.11 (1)
2160 g		8/6/75	0.40 (1)
			0.31 (2)
340 g		10/30/75	0.15 (1)
240 g	Roaring River	10/23/75	0.13 (1)
			0.23 (2)
310 g		10/23/75	0.08 (1)
			0.12 (2)
310 g	Montauk	2/26/75	0.16 (1)
340 g		2/26/75	0.23 (1)
300 g		3/1/75	0.13 (1)
1800 g		8/31/75	0.20 (1)[c]
460 g	Lake Taneycomo	12/15/74	0.18 (1)
410 g		1/8/75	0.25 (1)
360 g		1/25/75	0.24 (1)
370 g		5/10/75	0.32 (1)
2380 g		5/10/75	0.22 (1)
1590 g		7/2/75	0.09 (1)
1800 g		7/2/75	0.10 (1)
1360 g		10/30/75	0.09 (1)
3630 g		10/30/75	0.20 (1)
2140 g	Springfield, MO	2/25/75	0.00 (1)
300 g	Basalt, CO	6/19/75	0.22 (1)

[a]Standard deviation was ±0.025 ppm at the 0.1 ppm level and ±0.038 ppm at the 0.2 ppm level.

[b]Numbers in parenthesis indicate type of tissue analyzed: (1) flesh and (2) liver.

[c]Brown trout

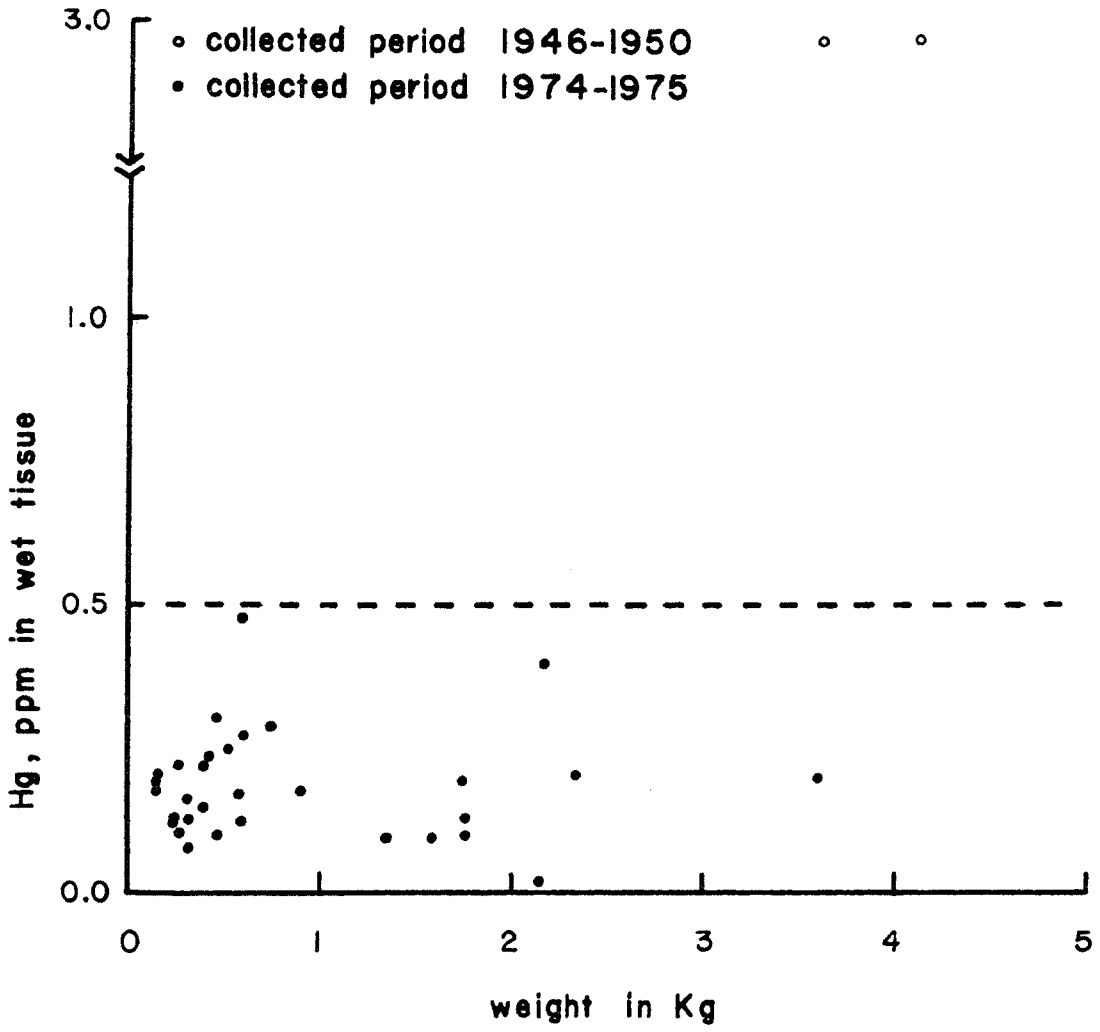


Fig. 4. Mercury content of Trout in southern Missouri

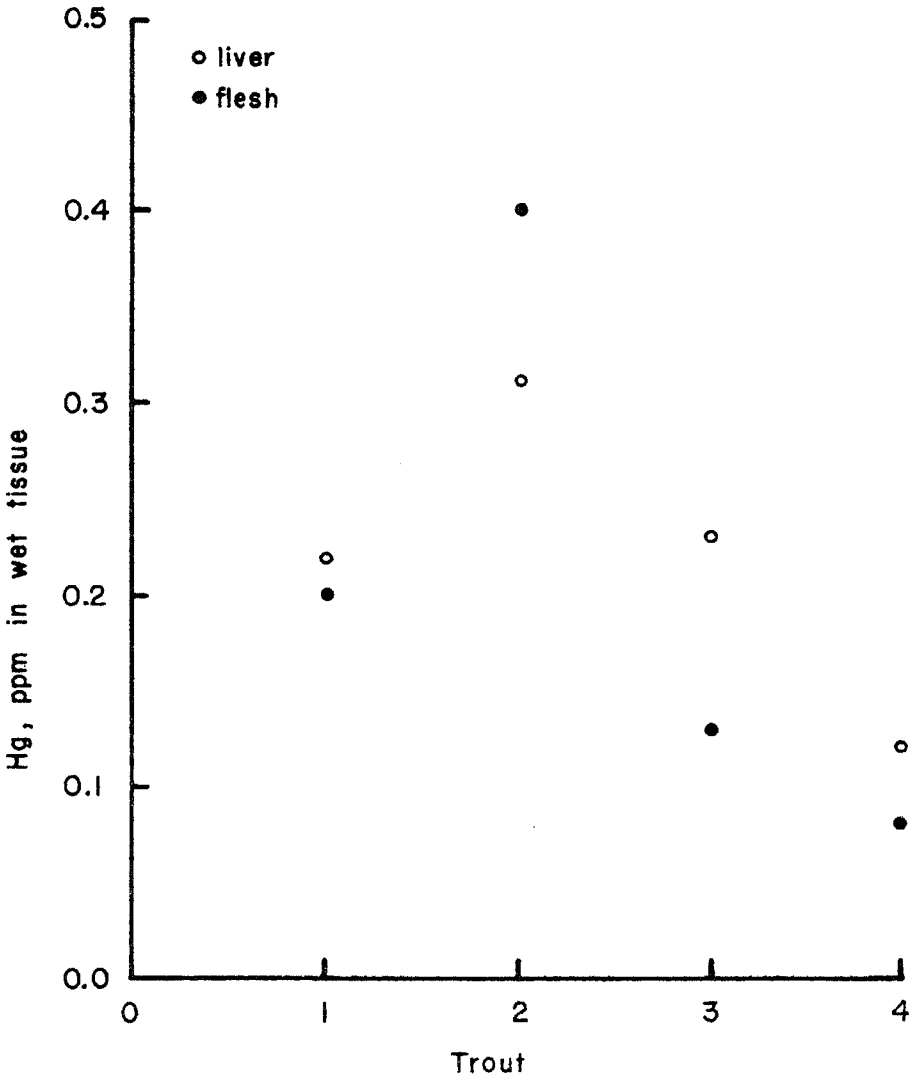


Fig. 5. A correlation of Mercury in flesh and in liver

be concluded that mercury levels in the 25 to 30 year time span since 1945 have diminished substantially.

Stocking procedures now in effect in southern Missouri can contribute to decreased mercury accumulation in trout. Trout taken from stocked areas spend a greater portion of their lives in hatcheries feeding on artificial foods than they do feeding off of natural foods. This should result in the stocked trout having lower mercury levels than native trout. With the exception of Roaring River, trout were taken from the trout parks in February assuring that they had been in the streams since the close of the previous trout season in November. These trout had been feeding on natural foods for four months. This study indicates that there was no significant accumulation of mercury in these fish, concluding these streams to have a relatively low background level of mercury.

Analytical procedures were simultaneously carried out on the liver and flesh of the same fish. Suzuki *et al.* (1974) report that levels of total mercury concentrations are elevated in the liver and the kidney. Figure 5 correlates mercury levels in the flesh with that of the liver. In this study the mercury levels in the liver were slightly higher than those in the flesh.

In biological samples containing mercury, the destruction of organic material without loss of mercury is a major problem due to the volatility of mercury and covalent mercury compounds. Koirtyohann (1974) checked for possible loss of mercury compounds under the same digestion procedure used in this study. To check for loss of mercury,

the fumes excaping from the reflux condenser were passed through a nitric acid trap and analyzed. In no case was the mercury found in the trap as much as 1% of the total for that sample. Recovery studies using phenyl mercuric acetate and methyl mercury have been checked using this digestion procedure. Recoveries were in excess of 95%.

In addition to the mercury absorption line at 2537 Å, there is another line at 1849 Å. This second line is 30 times more sensitive than the 2537 Å line, however it can be used only when absorption by atmospheric constituents such as oxygen have been removed. Therefore this line is not applicable to standard flameless mercury techniques involving the aeration of mercury vapor into the absorption cell.

Initially, some problems associated with the precision of the data were encountered. For example, the range of mercury concentrations may be from 0.2 to 0.7 ppm for six analyses of the same fish. The source of the problem was narrowed down to either the sample not being homogeneous or interference from the insoluble hydrated oxides of manganese. Either problem would result in inconsistent data and would yield results of unsatisfactory precision.

At this time, the fillets were being homogenized in a one part fish to two parts water mixture in a blender. The fillets were chosen for analysis since that is the part of the fish consumed. After examining this aspect of the procedure, the possibility existed that the samples were not truly homogeneous. Either the blending time was not sufficient to achieve homogeneity or the samples were

separating before being weighed. It was decided that a more homogeneous sample could be attained by cutting the fillets into small pieces and manually mixing them. After changing to this procedure, the results were more consistent.

The involvement with hydrated manganese oxides is the result of their formation when potassium permanganate was added to the sample. These oxides could occlude mercury(II) during their formation, therefore hindering the reduction of the mercury ions.

Although attempts were made to remove the insoluble materials by filtration and centrifugation, both methods seemed unsatisfactory. The modification which proved to be most satisfactory involved increasing the hydroxylamine hydrochloride concentrations from 5% (initially used) to 10%. This completely reduced all the manganese to manganese(II) which is soluble in acidic media. This makes the standards and the samples similar. After addition of the more concentrated hydroxylamine hydrochloride solution, the absorbance was observed. There was no change in absorbance, therefore the hydroxylamine solution was not reducing any mercuric ions.

The data in Table 1 was collected after the necessary modifications were made in the procedure which imporved the precision of the data. Analysis of the fish were done in triplicate. Examination of some of the analyses, taken at random from the data, shows standard deviations of ±0.025 at the 0.1 ppm level and ±0.038 at the 0.2 ppm level. Uthe *et al.* (1970), using a slightly different analytical procedure, reports standard deviations of ±0.039 at the 0.1 ppm level and ±0.051 at the 0.5 ppm level.

CONCLUSION

The mercury level in trout of southern Missouri taken from the stocked trout parks as well as the streams where natural populations are maintained are considerably lower than the 0.5 ppm maximum level placed on fish by federal regulation. The results of this study indicate levels in the range of 0.1 to 0.3 ppm mercury.

The mercury levels were relatively constant and showed no variation or high level accumulation with increased size. This indicates there is a source of background mercury in these streams but that there is very little fluctuation in the abundance of mercury. Levels of mercury in trout spending at least four months in the trout park feeding on natural food were consistent with the levels of mercury in the trout that were fed trout food and recently released.

The results of the analysis on the liver and flesh of the same trout showed that the levels in the liver were slightly higher than the levels in the flesh. This type of correlation would enable analysis of the liver to be used as an indicator to the mercury level of the edible flesh.

The mercury levels obtained from this study are considerably lower than the levels of mercury in fish taken 25 to 30 years ago from the same area. This is the result of a substantial decrease in the mercury levels in the streams or of the stocking procedures

now practiced in the streams. The public awareness of mercury as an environmental problem has resulted in its removal from such commercial products as paints, fertilizers, and insecticides. This has aided in the lowering of mercury levels in the streams of southern Missouri.

ACKNOWLEDGEMENTS

The assistance of Dr. Raymond L. Venable for his instruction in the use of the Perkin-Elmer Atomic Absorption Spectrophotometer 303 and of Dr. Gary L. Bertrand for his many contributions to this project are gratefully acknowledged.

The author wishes to thank Mr. George E. Smith, Director of the Water Resources Research Center, Columbia, Missouri and the United States Department of the Interior whose financial assistance supported the work upon which this publication is based.

Thanks to the management of the three trout parks in Missouri for allowing the collection of samples prior to the opening of the 1975 trout fishing season. Their cooperation is gratefully acknowledged.

LIST OF FIGURES

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