NISTIR 5388

# Preparation and Monitoring of Lead Acetate Containing Drinking Water Solutions for Toxicity Studies

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**Susannah B. Schiller**

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Prepared for:
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May 1994


**U.S. DEPARTMENT OF COMMERCE**
**Ronald H. Brown, Secretary**

**TECHNOLOGY ADMINISTRATION**
**Mary L. Good, Under Secretary for Technology**

**NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY**
**Arati Prabhakar, Director**

TABLE OF CONTENTS

# 1.0 INTRODUCTION

## 1.1 Objective

The objectives of the work described in this report were to design and implement a drinking water solution preparation and monitoring program as part of a collaborative interaction with the University of Maryland at Baltimore (UMAB) and the University of Maryland at Catonsville, for an animal study of lead toxicity. Drinking water solutions containing lead at concentrations of 50, 250 and 1,000 parts per million (ppm, mg/L) would be required for approximately two and one-half years during which the molecular mechanisms and tumorigenic response of low-dose lead exposure in a group of 370 male Fischer-344 rats were examined. The drinking water program had to address questions of solution preparation, transportation, storage, stability and monitoring of solution lead concentration. An additional responsibility was to provide trace metal measurement support to the project by the determination of lead concentrations in tissues from animals consuming the lead containing drinking water. Drinking water solutions were prepared from lead acetate compound. The amount of compound used was calculated to produce a lead solution with the concentration expressed as ppm lead, not ppm of lead acetate compound.

## 1.2 Background

The large number of animals involved and the lengthy duration of lead exposure in this experiment presented requirements for a large volume of lead-containing drinking water. At the initiation of the experiment, volumes of 60 to 80 liters of lead-containing solution at each of the three dose levels were required, a total of 180 to 240 liters per month. To reduce the volume of drinking water solution shipped from NIST to UMAB, an evaluation was made on the feasibility of preparing highly concentrated lead solutions (20,000 ppm) at NIST and shipping them to UMAB for dilution just prior to use. The goal was to provide a stable, concentrated lead solution that would reproducibly provide, upon dilution, twenty liter volumes of drinking water with lead concentrations of 50, 250 and 1,000 ppm, accurate to within 5 percent of the intended concentrations.

Accordingly, the first experiments undertaken for this program (1) determined the stability of aqueous inorganic lead solutions. An understanding of potential solution/container interactions such as irreversible adsorption of metal species to container walls, solubility of metal compounds in aqueous solution and the need for long term molecular stability of the solute species were critical aspects of this project. Prior experience with preparation, storage, and monitoring of dilute, aqueous metal and organometal solutions (2) provided the necessary understanding to undertake preparation of stable lead containing drinking water solutions.

Polycarbonate containers were chosen as the least likely to adsorb lead from aqueous solution. Solutions of lead acetate in deionized water, at concentrations of 20,000, 1,000, 250 and 50 ppm, buffered with acetic acid and adjusted to pH 4.5, were monitored for concentration stability. Lead concentrations in the solutions were determined by atomic absorption spectrophotometry. Both graphite furnace (GFAAS) and flame atomization (FAAS)

atomic absorption techniques were employed for solution concentration monitoring, with the vast majority of determinations made using FAAS. Monitoring periods varied from 30 days to 5 months in length, depending on the concentration of the lead solution being examined. After five months of room temperature (≈ 20 °C) storage in the dark, a concentrated lead solution, with an initial concentration determined to be 20,000 ppm with a standard deviation of 300 ppm, experienced a 5 percent loss in concentration to 19,000 ppm with a standard deviation of 100 ppm.

The stability of lead solutions at nominally 50, 250 and 1,000 ppm concentration levels was monitored for more than 11 weeks. The average lead concentration determined at the end of the stability study ranged from 99.7 to 101.6 percent of the average concentration determined at the start of the 11 week monitoring period.

Preparation of 20-liter volumes of dosing solutions by dilution of 50 mL, 250 mL and 1,000 mL volumes of 20,000 ppm concentrated lead solution was readily accomplished with an accuracy of better than ± 5 percent of the target concentration. Lead concentration determinations made on 20-liter volumes of 50, 250 and 1,000 ppm solutions approximately 30 days after the dilutions were performed revealed solution concentrations that ranged from 100.6 to 103.7 percent of their target values.

The requirements for accurate lead solution concentrations during the animal study of lead toxicity were established to be within 5 percent of the target concentration. As the above concentration monitoring experiments reveal, this criteria could be met or exceeded by the protocols developed.

## 2.0 EXPERIMENTAL

### 2.1 Labware and Bottle Cleaning

Prior to use in the weighing and subsequent dilution of lead acetate solutions, all spatulas, beakers and carboys that would come into contact with the lead acetate compound or solutions were cleaned to remove any preexisting trace metal contamination. All items were given a soap and hot water washing, rinsed in deionized water and leached with 5% aqueous nitric acid at 40 °C for a minimum of 48 hours. After 4 to 5 rinses in deionized water, the above labware was considered ready for use. The lead concentration of the deionized rinse water was determined by GFAAS. No lead could be determined in the deionized water. The deionized water lead concentration was below the level detectable by the GFAAS instrument, which was 0.6 parts per billion (0.6 $\mu$g/L) when the furnace was equipped with a conventional pyrolytically coated graphite tube.

### 2.2 Preparation of 20,000 ppm Lead Concentrate Solutions

Aqueous lead acetate solutions of 20 liter volumes, with a lead concentration of approximately 20,000 ppm were prepared as follows. A 20 liter

polycarbonate carboy (Carboy #2322, Nalge Co., Rochester, NY)[1] with polypropylene spigot and cap served as the solution reservoir. Seventeen liters of deionized water (Culligan Aqua Suma Reagent Water System, McNew Culligan Inc., Edgewater, MD) were measured into the carboy using a one liter graduated cylinder. Lead acetate compound, 732.3 g, (Fluka Chemical Corp., Ronkonkoma, NY) was weighed into a tared pyrex glass beaker and then slowly added to the carboy. During the addition of the lead acetate and for several hours afterward, the carboy contents were stirred with a heavy duty magnetic stirrer (Maxi-Stirrer Type 25500, Barnstead/Thermolyne Corp., Dubuque, IA) and a teflon or pyrex glass-covered magnetic stirring bar. The beaker and spatula used in weighing and transferring the lead acetate compound to the carboy were rinsed with four 250 mL volumes of deionized water, with all rinses being added to the carboy. After the lead acetate was completely dissolved, the pH of the solution was adjusted to a value of 4.5 by adding glacial acetic acid. The volume of acid used was recorded and subtracted from the volume of deionized water needed to bring the volume in the carboy to 20.0 liters.

After pH and total volume adjustments, the lead acetate solution was stirred for 2 to 4 hours per day for the next 3 to 4 days.

The concentration of lead in the nominal 20,000 ppm solution was determined by FAAS. The analytical instrument used was a Perkin-Elmer model 2380 atomic absorption spectrophotometer fitted with a standard burner assembly consisting of a molded plastic mixing chamber, stainless steel nebulizer and a 10 cm, single slot, titanium burner head (Perkin-Elmer Corp., Instrument Division, Norwalk, CT). An air-acetylene flame was used with a fuel flow of 2 liters per minute and an oxidizer flow of 16 liters per minute. A lead hollow cathode lamp operated at 10 milliamps provided the 283.3 nm absorbance line used for the lead determinations.

To evaluate the accuracy of lead concentration in the freshly prepared 20,000 ppm solution, a one step volumetric dilution was performed, resulting in a sample with a final lead concentration of approximately eight parts per million. Four to eight replicate samples of the concentrated solution were prepared for FAAS determination of lead concentration. Accuracy of the lead concentration to within 5% of the 20,000 ppm target concentration was considered acceptable.

The FAAS instrument was calibrated using five lead concentrations well within the 20 ppm linear working range of the instrument. Calibration concentrations were 4, 6, 8, 10, and 12 ppm, with each concentration being determined 16 times. The five calibration solutions were typically determined before and after the concentrated solution samples, with the data from both calibrations being combined in a single linear regression calculation. Calibration solutions were freshly prepared just prior to each instrument calibration by

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[1]Certain suppliers of chemicals and equipment are identified by name in order to specify the experimental conditions adequately. This does not imply endorsement or recommendation by the National Institute of Standards and Technology nor does it imply that the particular brands of chemicals and equipment named are necessarily the best for the purpose.

volumetric dilution of a commercial atomic absorption standard solution containing lead at 1,000 ppm (Fluka Chemical Corp., Ronkonkoma, NY).

Additional lead determinations of the 20,000 ppm solution were made periodically over the two to eight months that the concentration solution was being consumed. These determinations are listed in Table 1. From one 20-liter batch of concentrated lead solution, approximately 15 bottles each of 50, 250 and 1,000 mL volumes could be dispensed. The actual volumes dispensed and the dates bottles were filled are listed in Table 2.

## 2.3 Dosing Solution Preparation

The concentrated lead solution was dispensed by weight into polycarbonate containers of 50, 250 and 1,000 mL volumes at NIST and shipped by courier to the University of Maryland at Baltimore (UMAB) for use by their personnel in preparing drinking water solutions for the experimental animals. The concentrated lead solutions in the above volumes were diluted into 20-liter carboys containing deionized water to provide final lead concentrations of 50, 250 and 1,000 ppm. Although the 20,000 ppm concentrated lead solution was adjusted to pH 4.5, the acidity of the 50, 250 and 1,000 mL volumes was insufficient to bring the pH of the 20 liter drinking water solutions to pH 4.5. Additional glacial acetic acid was added to each bottle of concentrated lead solution so that upon dilution, the 20 liter volumes of solution would have a pH of 4.5. The amount of additional acid needed was determined empirically by dilution of one each of the 50, 250, and 1,000 mL volumes of concentrated solution to 20 liter volumes and adding glacial acetic acid to the 20 liter volume until pH 4.5 was reached. The dilute drinking water solutions were prepared from the concentrated lead solutions by the attached dilution procedures (Appendix).

The first carboy of 20,000 ppm lead acetate solution was prepared on the 18th of March, 1991. The solution was adjusted to pH 4.5 with glacial acetic acid. The concentrated solution was transferred into 1,000, 250 and 50 mL bottles on March 27, 1991, with additional glacial acetic acid being added to each bottle. Fifteen bottles each of the 1,000, 250 and 50 mL volumes were filled.

One bottle of each volume was used at NIST to prepare the initial 20 liter carboys of drinking water at nominal concentrations of 1,000, 250 and 50 ppm. The carboys of dilute lead solution and the remaining 14 bottles each of concentrated lead solution were delivered to UMAB on September 27, 1991. Determinations of the lead concentration in the concentrated carboy solutions were made periodically by FAAS as the solutions were consumed. These analyses are summarized in Table 1.

Additional carboys of 20,000 ppm lead acetate solution were prepared on January 6, 1992, March 19, 1992, and October 2, 1992. The March 19, 1992 preparation, and all subsequent ones, were mixed using a pyrex glass covered magnetic stirring bar. Earlier preparations had been stirred with a teflon covered stirring bar. The teflon covered stirring bar always shed wear particles into the solution and its use was therefore discontinued. The concentrated lead solutions were dispensed into 50, 250, and 1,000 mL bottles for delivery to UMAB as shown in Table 2.

## 2.4 Solution Monitoring by Flame Atomic Absorption Spectrophotometry

Following dilution of the concentrated lead solution (20,000 ppm) at UMAB to drinking water concentration levels, samples of the 50, 250 and 1,000 ppm solutions were taken from the 20 liter carboys and shipped to NIST in polycarbonate containers for FAAS determination of the lead concentration. Samples were also taken from blank control carboys and from water bottles in use in animal cages. Lead concentration in the samples was typically determined within two to six days of receipt of the samples at NIST; however, in some instances, FAAS analyses were completed approximately two weeks after receipt of the samples.

The FAAS instrument was calibrated for drinking water solution monitoring at 4, 6, 8, 10 and 12 ppm concentrations of lead . Drinking water samples were diluted to contain approximately 8 ppm of lead. Each drinking water sample dilution was prepared in duplicate. Each duplicate sample dilution was determined by FAAS 16 times. The lead concentration of the samples was calculated by entering their FAAS absorbance values into the linear regression equation generated by determination of the calibration solutions. The resulting concentration values were averaged and reported with a standard deviation.

# 3.0 DISCUSSION

## 3.1 Solutions of Lead Acetate

The volume of data accumulated during the course of this experiment was quite large. As of April, 1993, lead concentration determinations had been made for 52 carboys of 50 ppm concentration, 47 carboys of 250 ppm concentration and 42 carboys of 1,000 ppm concentration. In addition, lead determinations had been made for various solutions taken from drinking water bottles in use by the experimental animals; 19 samples of 50 ppm solutions, 15 samples of 250 ppm solutions and 8 samples of 1,000 ppm solutions.

Typically, samples were withdrawn from the carboys after approximately 48 hours of mixing following addition of the concentrated lead solution to the carboy. Samples were stored at room temperature until FAAS determinations were performed.

The 20 liter carboys used for dilution of the concentrated 20,000 ppm lead solutions to drinking water concentrations of 50, 250 and 1000 ppm were filled for the first time at NIST. Determination of the lead concentration, by FAAS, in the dilute solutions 30 days after the addition of the concentrated lead solution to the carboys gave the following values:

| Nominal Lead Concentration | FAAS Lead Concentration | | % Deviation from Target Concentration |
|---|---|---|---|
| | Pb, ppm | Std Dev | |
| 50 ppm | 49.7 | 0.2 | -0.6 |
| 250 ppm | 245 | 2.1 | -1.6 |
| 1000 ppm | 963 | 27 | -3.7 |

All subsequent lead solution data for 50, 250 and 1000 ppm concentrations are from carboy solutions that were prepared at UMAB from concentrated 20,000 ppm solutions supplied by NIST.

The four carboys of concentrated lead acetate solution (nominally 20,000 ppm) prepared during the course of this study had their lead concentrations determined by FAAS numerous times, as listed in Table 1. An analysis of variance on the lead concentration values for the carboys revealed no statistically significant differences between the concentrations in the four carboys. Additionally, there was no significant drift in the concentration over time. A plot of the concentration determinations, subdivided by vertical lines to indicate the four individual 20,000 ppm solution preparations, is shown in Figure 1.

The average lead concentration in the nominally 50 ppm carboys was 51.4 ppm. However, there were statistically significant differences between the lead concentrations delivered, which depended upon the concentrated lead solution from which the dilute lead solution was prepared. The averages in the separate preparations ranged from a low of 49.6 ppm to a high of 52.7 ppm. All except the highest of these falls within 5 percent of the 50 ppm target concentration and the highest is not significantly more than 5 percent above the target concentration. A plot of the concentration determinations, in chronological order and subdivided by dispensation date of the concentrated 20,000 ppm solutions, is presented in Figure 2.

In the nominally 250 ppm carboys, the average lead concentration was 254.9 ppm. There were also statistically significant differences between the delivered lead concentrations depending on which 20,000 ppm concentrate preparation was used for dilution. The range of delivered values was from 251.76 to 260.8 ppm, all of which fall within 5 percent of the target concentration of 250 ppm. A plot of the concentration determinations, in chronological order and subdivided by dispensation date of the concentrated 20,000 ppm solutions, is presented in Figure 3.

The average lead concentration in the nominally 1000 ppm carboys was 998 ppm. However, there were statistically significant differences between the delivered lead concentrations depending on when the 20,000 ppm concentrate was dispensed. The delivered concentration averages ranged from 972 to 1039 ppm, all within 5 percent of the targeted 1000 ppm concentration. A plot of the

concentration determinations, in time sequence and divided by dispensation date of the concentrated 20,000 ppm solutions, is presented in Figure 4. A composite plot of the 50, 250 and 1000 ppm carboy concentrations presented along a continuous concentration axis is shown in Figure 5. A summary of the above statistical discussion of carboy concentrations is presented in Table 3.

3.2 Rat Drinking Water Bottle Solutions of Lead Acetate

Monitoring of the lead concentration in the water bottles in use in the animal cages revealed a problem with lead concentration stability. Some weeks into the study, lead concentration values in the animal drinking water bottles were found to be significantly lower than that of the carboys they were being filled from. Close examination of the drinking water bottles revealed a biological contamination, possibly fungal, growing in the water bottles. A non-quantitative measurement (i.e., lead concentration not adjusted to dry weight of the biological material) of the lead concentration of the biological material revealed a concentration of several hundred ppm lead in a sample removed from a 50 ppm solution. Apparently, the biological entity was accumulating and concentrating lead from solution in its tissue. UMAB personnel, alerted to this problem, reviewed their water bottle cleaning procedure and realized a bleach treatment step had been eliminated from the washing procedure. When the bleach treatment was reinstated, the biological contamination ceased to be a problem and lead concentrations in the drinking water bottles remained stable. The average lead concentration in the nineteen 50 ppm drinking water bottles sampled, excluding the 6 bottles with concentrations affected by biological contamination, was 50.3 ppm, with a standard deviation of 2.4 ppm.

The average lead concentration in the fifteen 250 ppm drinking water bottles sampled, excluding the one bottle with concentration affected by biological contamination, was 257.1 ppm, with a standard deviation of 8.5 ppm.

The average lead concentration in the eight 1000 ppm drinking water bottles sampled, excluding two bottles with concentration affected by biological contamination, was 1,035.9 ppm, with a standard deviation of 55.6 ppm.

The lead concentration data from the rat drinking water bottle samples is presented graphically in figures 6, 7 and 8, respectively for the 50, 250 and 1,000 ppm concentrations.

The graph of the 50 ppm drinking water concentrations, by virtue of having the greatest number of samples spanning the time period, most dramatically illustrates the effect of biological contamination on the lead concentration of the drinking water; nevertheless, the effect of the contamination is also seen in the graphs of the 250 and 1000 ppm concentrations. Once the biological contamination was eliminated, lead concentrations returned to the appropriate target levels.

## 4.0 Conclusions

The solution stability studies conducted during the beginning stages of this project provided confidence that drinking water solutions containing lead acetate would not experience decreases in concentration during storage and consumption. The major challenge of this project was to provide consistent accuracy in delivering lead containing drinking water over approximately a two and one half year time period. This time span required that numerous solutions be prepared at irregular intervals, as dictated by water consumption by the experimental animals. Consequently, the solution preparation protocols needed to be easily repeatable and provide a highly accurate lead concentration in the final drinking water product. The protocols developed for the production, dispensing and dilution of concentrated lead acetate solutions (detailed in Appendix 1) worked successfully when initially tested. As the experiment progressed, the protocols proved to be very reliable for generating relatively large volumes of drinking water. By early April, 1993, a total of 141 carboys or 2820 liters of lead containing drinking water had been prepared.

When the average lead concentrations of the carboys were examined by subdividing them into groups based on the date that the 20,000 ppm lead concentrate was dispensed, all concentration averages fall within 5 percent of the target concentration values, with the exception of one data set for 50 ppm carboys prepared from concentrate dispensed on August 18, 1992. The data sets for the 50, 250 and 1000 ppm carboys describe what is statistically termed a normal data distribution around an average value, with 65 to 71 percent of the concentrations within one standard deviation of the average values and 89 to 98 percent within two standard deviations of the average values.

For the 1000 and 250 ppm carboy solutions, the average solution concentration values all fall within 5 percent of the target values. For the 50 ppm carboy solutions, all average concentration values fall within 5 percent of the target concentration with the exception of carboys prepared from 20,000 ppm concentrate dispensed on August 18, 1992. The average lead concentration in carboys prepared from the 20,000 ppm concentrate dispensed on August 18 was 52.7 ppm, 5.4 percent above the 50 ppm target concentration. This is the only set of data that does not achieve the goal of 5 percent or better accuracy in meeting target concentrations. However, this average value of 52.7 ppm, statistically, is not significantly higher than 52.5 ppm, the upper value of the plus or minus 5 percent range of acceptable 50 ppm concentrations, i.e., concentrations ranging between 47.5 and 52.5 ppm are acceptable as 50 ppm solutions.

The loss of lead from solution seen during the period of biological contamination in the drinking water bottles reinforced the need for on-going lead concentration monitoring. Had FAAS monitoring stopped after the initial analyses found no problems in meeting the desired concentrations in the first few carboy and drinking water solutions examined, the loss of lead from solution in the biologically contaminated drinking water bottles would never have been discovered.

## 5.0 References

1. Jewett, K.L., Blair, W.R., Brinckman, F.E., Wang, F.W., "Stability of Aqueous Inorganic Lead Solutions in Polycarbonate Containers", NISTIR 4725, National Institute of Standards and Technology, 1991.

2. Blair, W.R., Olson, G.J., Brinckman, F.E., Paule, R.C, "An International Butyltin Measurement Methods Intercomparison: Sample Preparation and Results of Analyses", NBSIR 86-3321, National Bureau of Standards, 1986.

## 5.1 Acknowledgements

This work was supported by the University of Maryland at Baltimore through Subcontract BS 73566-B.

## Figure Captions

Figure 1 Solution lead levels determined by FAAS for concentrated 20,000 ppm lead acetate solutions. Concentration determinations were made soon after initial solution preparation and subsequently as the solution was dispensed and consumed. Verticle lines subdivide the data into four groups based on preparation dates of the 20,000 ppm solutions. Horizontal dashed line indicates the target lead concentrations.

Figures 2, 3 & 4
The graphs show, respectively, the concentration values determined by FAAS for 50, 250 and 1000 ppm carboy solutions of lead acetate. Verticle lines subdivide the data into groups based on the date of preparation and dispensation of the 20,000 ppm concentrated lead solution. The dashed horizontal lines show the nominal target concentration of the carboy solutions and the upper and lower limits of the ± 5 percent range for solution accuracy.

Figure 5 Plot of the lead concentrations of all 50, 250 and 1000 ppm carboys plotted on a common concentration axis.

Figures 6, 7, & 8
Graphs show, respectively, the lead concentrations of 50, 250 and 1000 ppm drinking water solutions taken from water bottles in cages of the experimental animals. The loss of solution lead concentration due to water bottle contamination by biological growth, reaching a maximum effect at about 200 days into the study, is clearly seen.

## APPENDIX

## DILUTION INSTRUCTIONS for LEAD ACETATE DRINKING WATER SOLUTIONS

### General Information and Instructions:

Use clean graduate cylinders for all volumetric measurements. Cylinders of 1000 or 2000 mL volumes are suggested.

After a soap and water wash and rinse, further clean graduate cylinders by allowing them to stand overnight full of 5% aqueous nitric acid. Rinse 4 to 5 times with distilled water. Keep the tops of the cylinders covered when they are not in use.

Carboys of 20 liter capacity will be used to contain lead acetate drinking water solutions. Individual carboys will be filled with lead acetate solutions at 50, 250 and 1000 ppm concentrations at NIST prior to their use at UMAB. The initial contact with lead acetate solutions will minimize any loss of solution concentration due to container wall adsorption. Do not wash the carboys before making dilutions of the concentrated lead solutions by the procedures described in the accompanying instructions.

The concentrated lead solutions have been pH adjusted at NIST to result in a pH of 4.5 in the diluted lead solutions.

All empty polycarbonate bottles should be returned to NIST for reuse.

DILUTION INSTRUCTIONS
for
LEAD ACETATE DRINKING WATER SOLUTIONS

50 ppm Concentration

1) Fill 20 liter carboy with 15 liters of distilled water.

2) Pour the contents of a 50 mL bottle containing concentrated lead solution into the carboy.

3) Add 25 mL of distilled water to the 50 mL bottle, put on the cap and shake the bottle. Pour this rinse water into the 20 liter carboy. Repeat this rinsing operation four times using 25 mL of distilled water for each rinse and add the rinse water to the carboy each time.

4) The carboy now contains:

| | |
|---|---|
| | 15 liters of distilled water |
| | 50 mL of conc. lead solution |
| | 100 mL of rinse water |
| Volume in Carboy: | 15.15 liters |

5) Add 4 liters plus 850 mL of distilled water to bring the final carboy volume to 20 liters.

6) After through mixing, the solution is ready for use.

DILUTION INSTRUCTIONS
for
LEAD ACETATE DRINKING WATER SOLUTIONS

250 ppm Concentration

1) Fill 20 liter carboy with 15 liters of distilled water.

2) Pour the contents of a 250 mL bottle containing concentrated lead solution into the carboy.

3) Add 125 mL of distilled water to the 250 mL bottle, put on the cap and shake the bottle. Pour this rinse water into the carboy. Repeat this rinsing operation a total of four times using 125 mL of distilled water for each rinse and add the rinse water to the carboy each time.

4) The carboy now contains:

| | |
|---|---|
| | 15 liters of distilled water |
| | 250 mL of conc. lead solution |
| | 500 mL of rinse water |
| Volume in Carboy: | 15.75 liters |

5) Add 4 liters plus 250 mL of distilled water to bring the final carboy volume to 20 liters.

6) After through mixing, the solution is ready for use.

DILUTION INSTRUCTIONS
for
LEAD ACETATE DRINKING WATER SOLUTIONS

1000 ppm Concentration

1) Fill 20 liter carboy with 15 liters of distilled water.

2) Pour the contents of a one liter bottle containing concentrated lead solution into the carboy.

3) Add 250 mL of distilled water to the one liter bottle, put on the cap and shake the bottle. Pour this rinse water into the 20 liter carboy. Repeat this rinsing operation four times using 250 mL of distilled water for each rinse and add the rinse water to the carboy each time.

4) The carboy now contains: 15 liters of distilled water
1 liter conc. lead solution
1 liter of rinse water
---
Volume in Carboy: 17 liters

5) Add 3 liters of distilled water to bring final carboy volume to 20 liters.

6) After through mixing, the solution is ready for use.

TABLE 1

Determination of Lead Concentration in 20,000 ppm Solutions

| Date of Carboy Preparation | Date of FAAS Analysis | Pb Conc. (ppm) | Std Dev | Number of Independent Dilutions | Number of Replicate Analyses |
|---|---|---|---|---|---|
| March 18, 1991 | March 20, 1991 | 19,146 | 1151 | 2 | 10 |
| | March 22, 1991 | 19,159 | 1185 | 2 | 20 |
| | April 15, 1991 | 20,260 | 847 | 5 | 38 |
| | August 2, 1991 | 18,995 | 130 | 3 | 96 |
| January 6, 1992 | January 13, 1992 | 20,199 | 76 | 3 | 96 |
| | January 23, 1992 | 19,109 | 44 | 3 | 96 |
| | January 27, 1992 | 19,065 | 366 | 3 | 96 |
| March 19, 1992 | March 26, 1992 | 19,776 | 401 | 8 | 128 |
| | June 17, 1992 | 18,469 | 202 | 4 | 64 |
| | June 25, 1992 | 19,813 | 492 | 4 | 64 |
| | June 26, 1992 | 18,949 | 127 | 4 | 64 |
| | August 20, 1992 | 18,437 | 509 | 4 | 64 |
| | September 2, 1992 | 18,870 | 193 | 4 | 64 |
| October 27, 1992 | November 10, 1992 | 20,200 | 587 | 8 | 128 |
| | April 14, 1993 | 20,434 | 118 | 4 | 64 |
| March 18, 1991 | Average Concentration | 19,390 | 585 | | |
| January 6, 1992 | Average Concentration | 19,458 | 642 | | |
| March 19, 1992 | Average Concentration | 19,052 | 611 | | |
| October 27, 1992 | Average Concentration | 20,317 | 166 | | |
| Average of all 15 determinations: | | 19,392 | 663 | (RSD = 3.4%) | |

TABLE 2

Dispensing Dates and Volumes of 20,000 ppm Concentrated Lead Solutions

| Preparation Date | Dispensing Date | 50 mL Bottles | 250 mL Bottles | 1000 mL Bottles |
|---|---|---|---|---|
| 3/18/91 | 3/27/91 | 15 | 15 | 15 |
| 1/6/92 | 1/21/92 | 12 | 8 | 8 |
| | 2/10/92 | 0 | 9 | 7 |
| 3/19/92 | 4/13/92 | 12 | 10 | 8 |
| | 8/18/92 | 12 | 12 | 0 |
| 10/27/92 | 12/18/92 | 8 | 0 | 8 |
| | 2/02/93 | 0 | 4 | 8 |
| | 3/31/93 | 2 | 6 | 0 |
| | 6/24/93 | 10 | 0 | 0 |

TABLE 3

SUMMARY of CARBOY DILUTIONS

| Target Concentration | Number Prepared | Average Lead Concentration | |
|---|---|---|---|
| | | Pb, ppm | Std Dev |
| 50 ppm | 52 | 51.4 | 2.2 |
| 250 ppm | 47 | 254.9 | 8.1 |
| 1000 ppm | 42 | 998.2 | 31.1 |

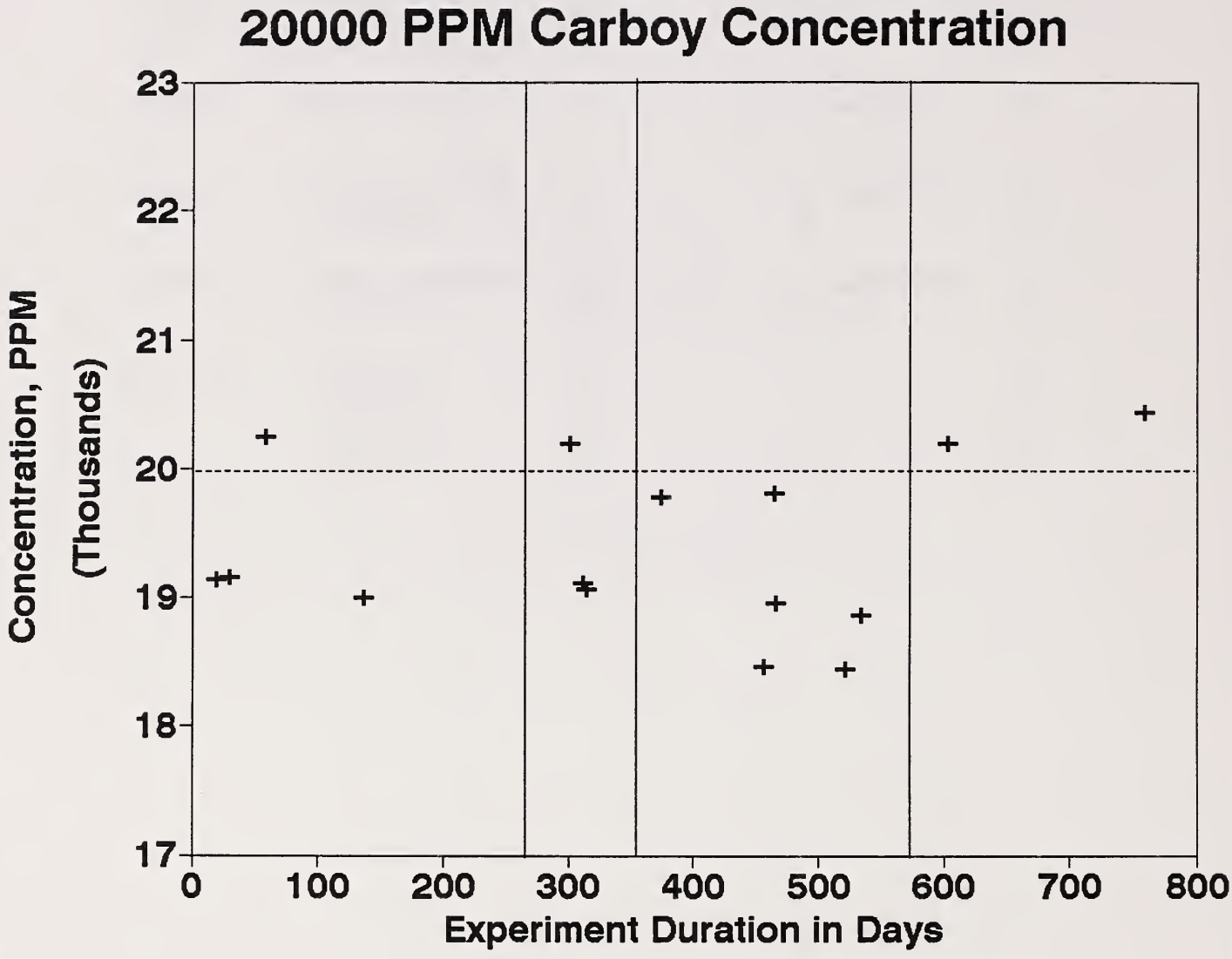
20000 PPM Carboy Concentration
Concentration, PPM
(Thousands)
23
22
21
20
19
18
17
0
100
200
300
400
500
600
700
800
Experiment Duration in Days

FIGURE 1

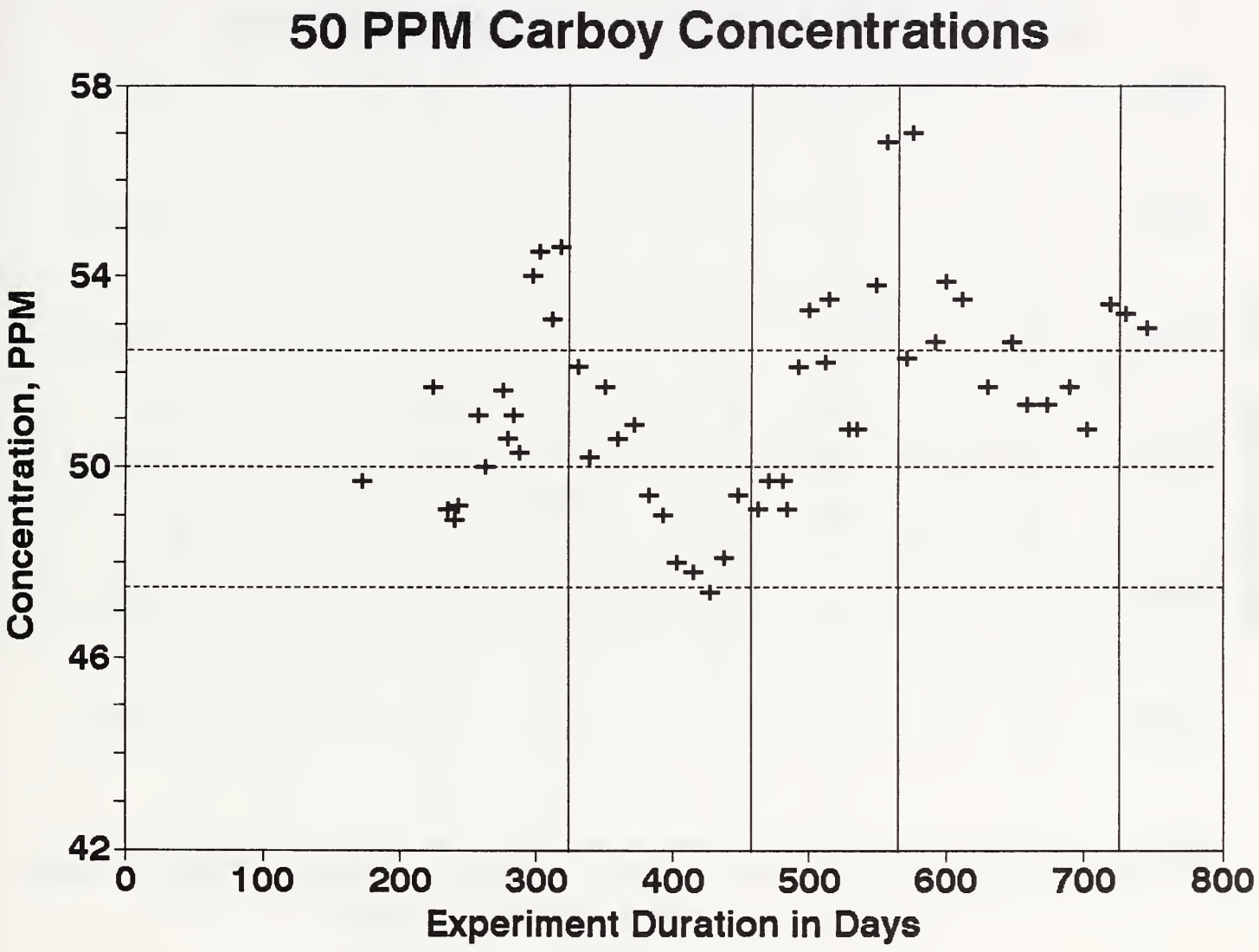
50 PPM Carboy Concentrations
Concentration, PPM
58
54
50
46
42
0
100
200
300
400
500
600
700
800
Experiment Duration in Days

FIGURE 2

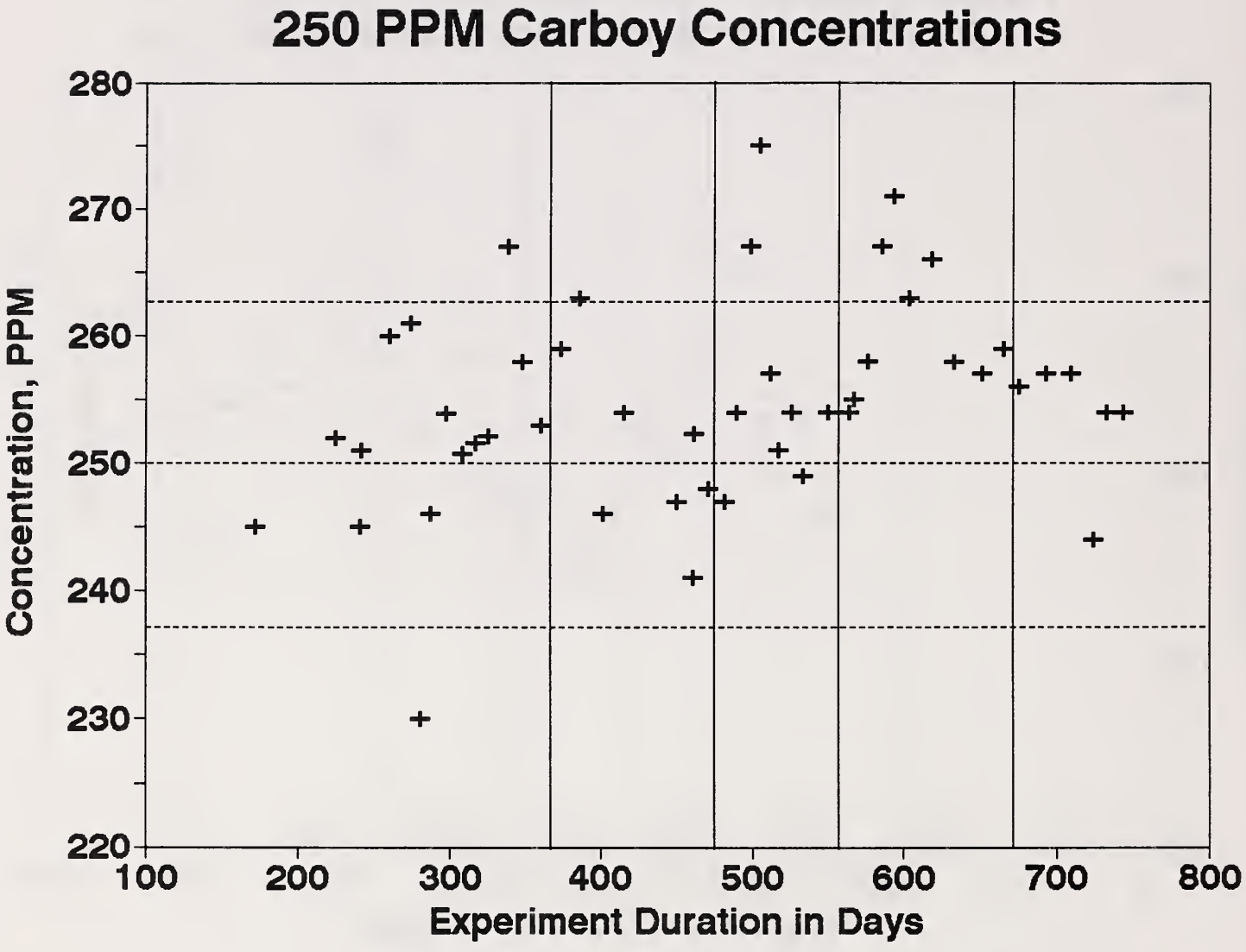
250 PPM Carboy Concentrations
Concentration, PPM
280
270
260
250
240
230
220
100
200
300
400
500
600
700
800
Experiment Duration in Days

FIGURE 3

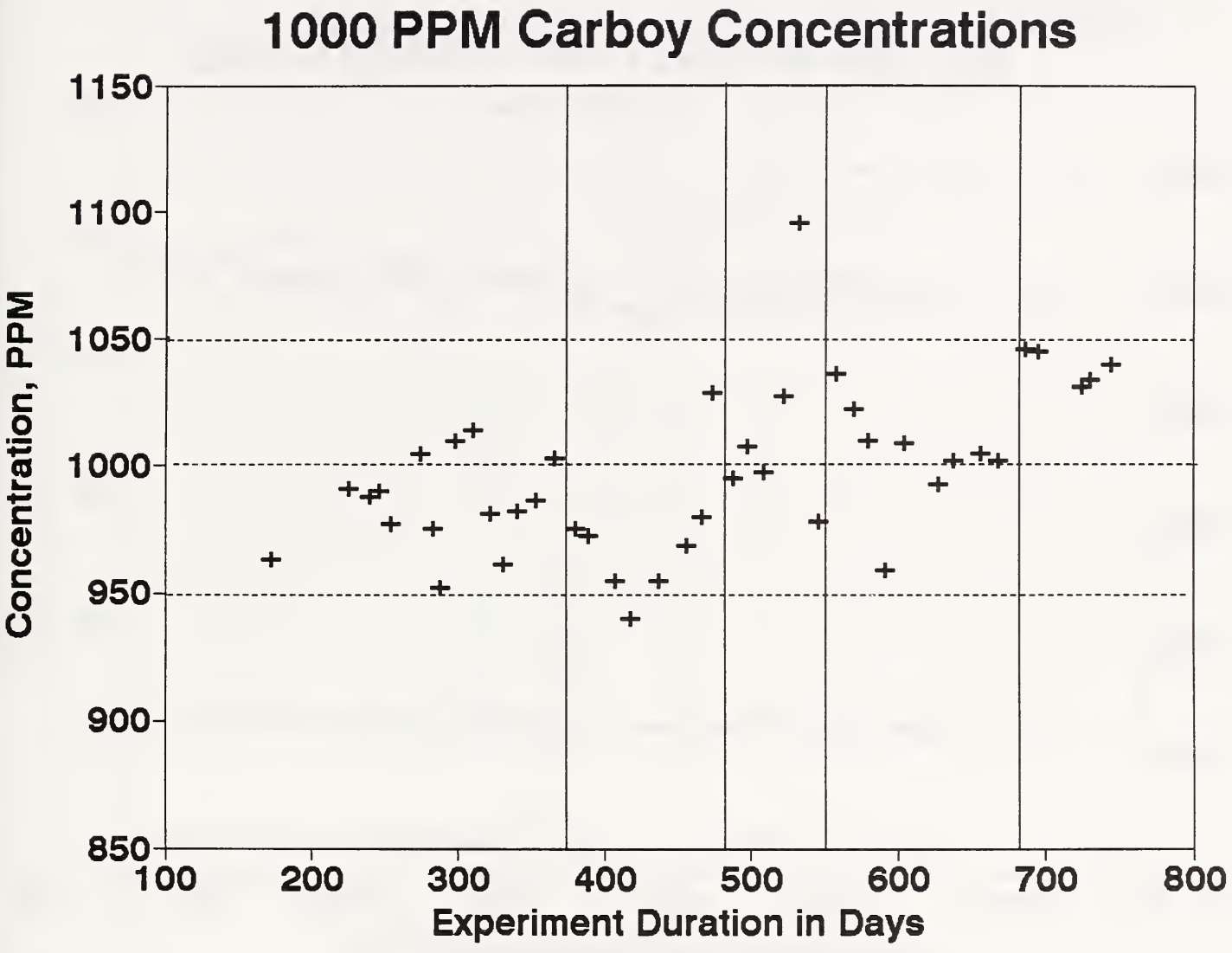
1000 PPM Carboy Concentrations
Concentration, PPM
1150
1100
1050
1000
950
900
850
100
200
300
400
500
600
700
800
Experiment Duration in Days

FIGURE 4

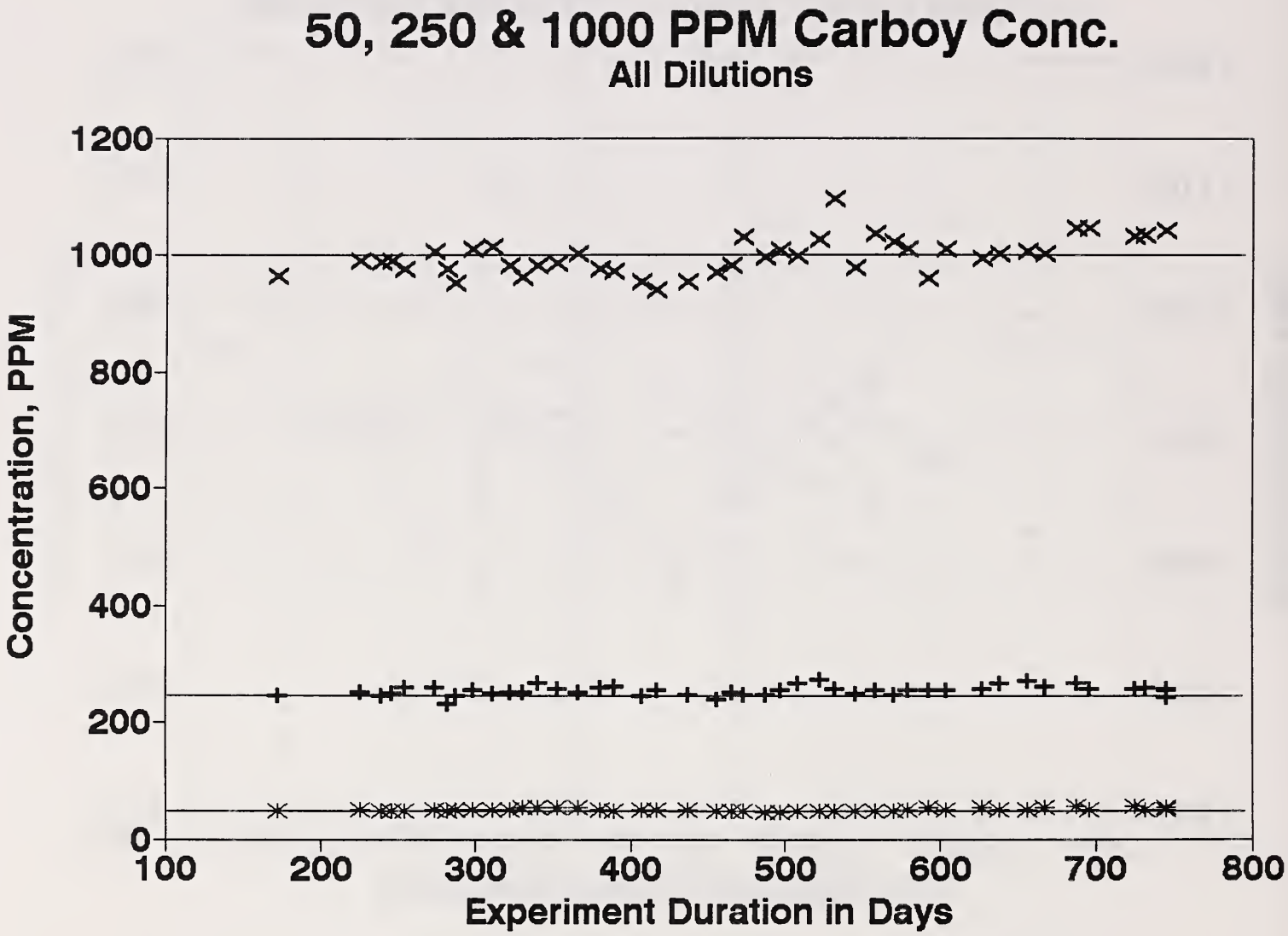
50, 250 & 1000 PPM Carboy Conc.
All Dilutions
Concentration, PPM
1200
1000
800
600
400
200
0
100
200
300
400
500
600
700
800
Experiment Duration in Days

FIGURE 5

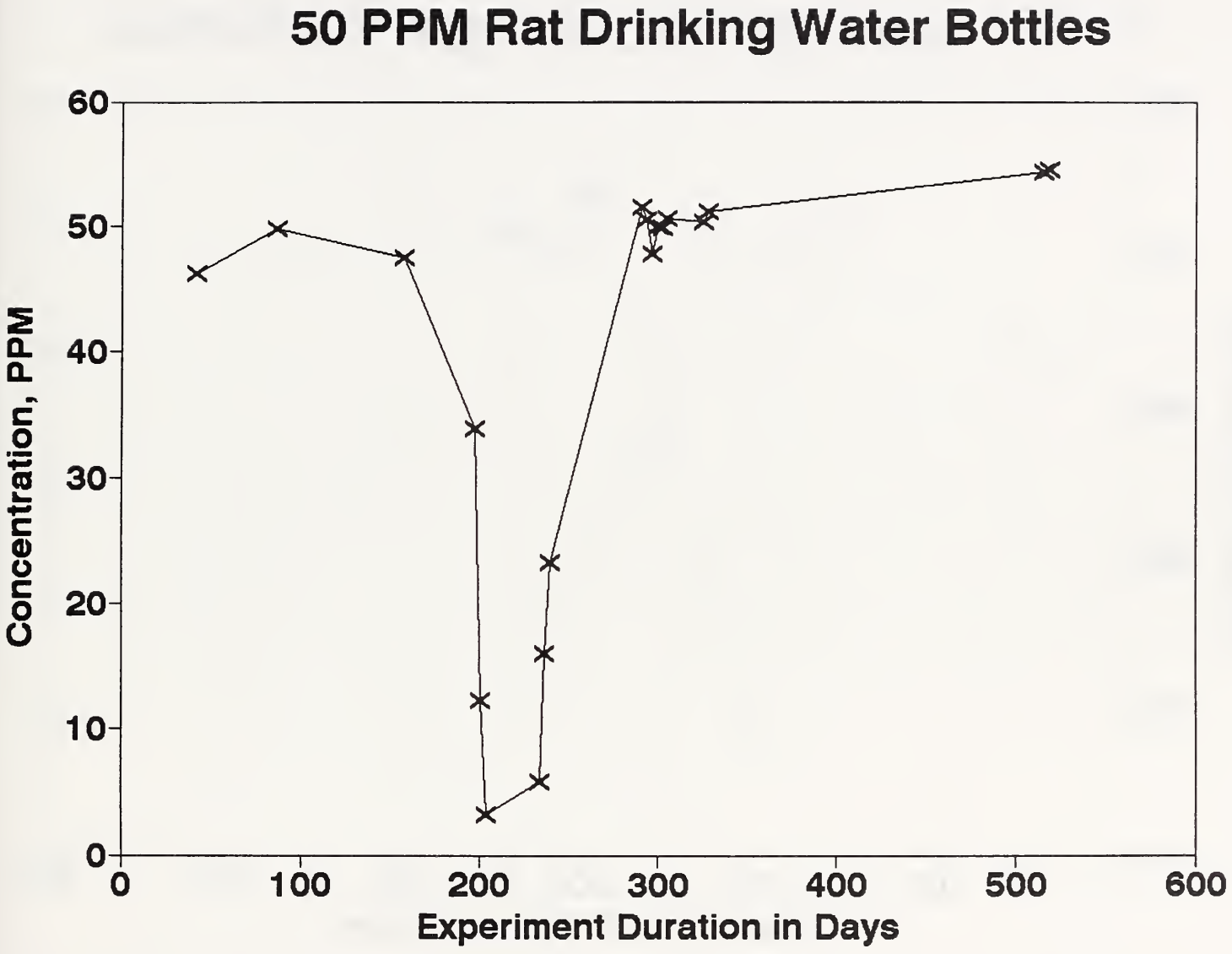
50 PPM Rat Drinking Water Bottles
Concentration, PPM
60
50
40
30
20
10
0
0
100
200
300
400
500
600
Experiment Duration in Days

FIGURE 6

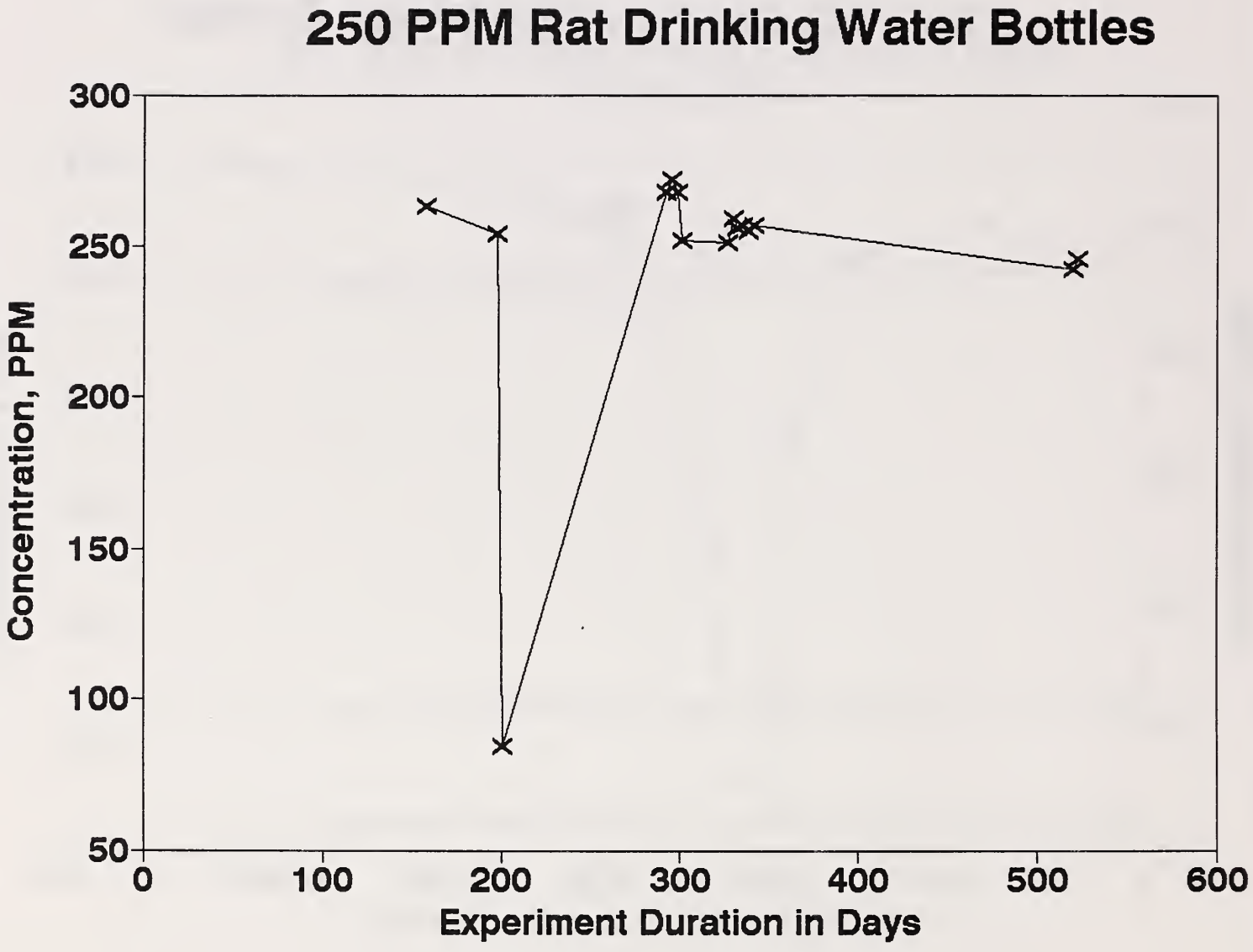
250 PPM Rat Drinking Water Bottles
Concentration, PPM
300
250
200
150
100
50
0
100
200
300
400
500
600
Experiment Duration in Days

FIGURE 7

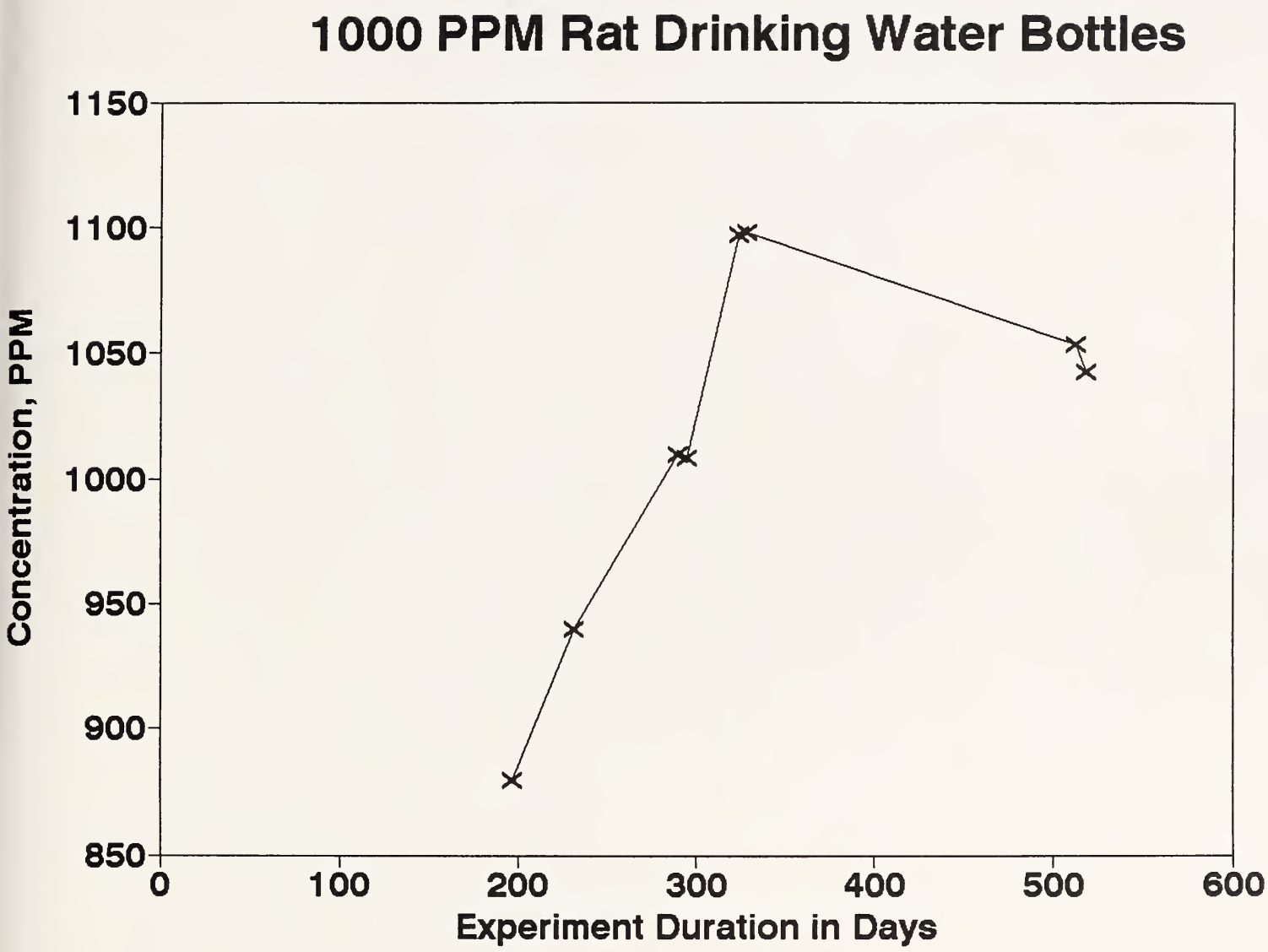
1000 PPM Rat Drinking Water Bottles
Concentration, PPM
1150
1100
1050
1000
950
900
850
0
100
200
300
400
500
600
Experiment Duration in Days

FIGURE 8