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This paper describes laboratory comparisons of precision and accuracy of some widely used methods for the determination of lead in blood and for lead and cadmium on filters. With the exception of the tantalum boat atomic absorption technique, the methods studied were found to be rugged for a variety of minor procedural changes. Recovery of cadmium from spiked filters was quantitative over a wide range of dilutions and for a variety of instrumental readout and calculation combinations.

A study of methods for the determination of lead and cadmium

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introduction

A large number of analytical methods have been reported for the determination of lead and cadmium in air samples and biological materials.¹⁻⁹ Until the introduction and widespread acceptance of atomic absorption spectrophotometry (AAS) in the 1960's, the primary method in use was extraction and colorimetry with dithizone. Atomic absorption methods have continued to develop rapidly in the last few years, with non-flame atomization and microsampling techniques supplementing aspiration into a flame. Among the published AAS methods for lead in blood, many techniques have been used, with sample treatment varying widely and including none,^{1,2} dilution with water,^{3,4} ashing,⁵ and chelation/extraction.^{6,7}

The NIOSH Manual of Analytical Methods contains four methods for lead (dithizone, flame AAS (2 methods), and tantalum boat AAS) and two for cadmium (both flame AAS).^{8,9} This paper reports the results obtained in a study which was initiated to compare the accuracy and precision obtained in applications of these methods and others,^{4,6} and to investigate ways in which they might be improved.

experimental

A. Apparatus. The atomic absorption

spectrophotometers used were: Perkin-Elmer Models 306* (with deuterium lamp, recorder readout and integrated digital readout), 403 (with deuterium lamp, digital and recorder readouts and tantalum boat microsampling system), and 503 (with HGA-2100 graphite furnace, deuterium lamp, and digital and recorder readout), and Varian Model AA-5 (with hydrogen lamp and digital and recorder readouts). A Beckman Model 25 spectrophotometer was also used.

B. Reagents and Standards. All reagents used were ACS Reagent Grade or better. The nitric acid used for wet-ashing was redistilled in glass prior to use. Commercial atomic absorption standards (F&J Scientific, Monroe, Conn.) were used and were found to be in agreement with standards prepared from $\text{Pb}(\text{NO}_3)_2$ and Cd metal dissolved in HNO_3 .

Standardized porcine blood samples containing 1.00 μg lead per gram (standard deviation 0.023 $\mu\text{g/g}$) were obtained from the National Bureau of Standards (NBS).¹⁰ This elevated lead content was obtained in biologically bound form by feeding lead acetate to pigs for several weeks. The anticoagulant used was EDTA. Analysis to establish the lead concentration was performed by NBS using polarography and isotope dilution mass spectroscopy. The samples were supplied in clean plastic vials and were stored at 4°C prior to use.

*The use of trade names in this article does not constitute endorsement by the U. S. Public Health Service.

TABLE I
Ruggedness Test Results — Blood Pb by Extraction/Boat AAS

Condition	Value	$\mu\text{g Pb}$ /g Blood	Difference $\mu\text{g Pb/g Blood}$
Digesting acid	A = 4 ml HNO_3 + 1 ml HClO_4	0.952	-0.094
	a = 5 ml HNO_3	1.046	
No. of acid additions to ash	B = two	1.036	0.074
	b = one	0.962	
Ash to dryness char	C = no	1.030	0.062
	c = yes	0.968	
Diluted ash vol.	D = 10 ml	1.045	0.092
	d = 5 ml	0.953	
Aliquot to boat	E = 0.2 ml	0.972	-0.054
	e = 0.1 ml	1.026	
Ignite MIBK	F = yes	1.007	0.016
	f = no	0.991	
(Dummy)	G	1.024	0.050
	g	0.974	
		SD =	0.096

Cellulose ester filters, 47 mm diameter, MF-Millipore HA (0.45 μm pore size), each containing 19.8 μg of Cd [standard deviation (SD) = 0.26 μg] and 29.7 μg of Pb (SD = 0.45 μg) were obtained from NBS.¹¹ Blank filters of the same type were spiked in this laboratory with acidic cadmium nitrate solutions to give standard filters with 10.0 μg of Cd each.

C. Analytical methods for lead in blood.

1. Dithizone. The USPHS recommended method was used.¹² Approximately 10 grams of standard blood per sample were wet-ashed with nitric acid, diluted with 1:99 HNO_3 , buffered to pH 9-10 with ammonium citrate, and extracted in the presence of KCN with dithizone in CHCl_3 . The lead was then back-extracted into 1:99 HNO_3 . The pH of this aqueous solution was adjusted to approximately 9-10 by the addition of a measured amount of ammonia, KCN was added, and the lead was extracted into CHCl_3 with a standardized dithizone- CHCl_3 solution. Absorbance was measured at 560 nm on a spectrophotometer using a zero-Pb standard as reference. This procedure appears in the NIOSH manual as P&CAM No. 102.^{8,9}

2. Extraction/tantalum boat atomic

absorption. The procedure of NIOSH P&CAM No. 101 was followed.⁸ Standard blood samples weighing approximately 2 grams were wet-ashed with a mixture of concentrated HNO_3 (4 ml) and HClO_4 (1 ml), with a second addition to complete the ashing. The ash was dissolved and diluted to volume (5 or 10 ml) with 5% HNO_3 . An aliquot was taken, the pH adjusted to 2.6 - 3.0, and the lead complexed by adding 0.4 ml of 2% ammonium pyrrolidine dithiocarbamate (APDC). The lead complex was then extracted into 3 ml of methyl isobutyl ketone (MIBK) and aliquots (0.05 to 0.2 ml) of the extract were delivered by Eppendorf pipet to the tantalum boat. The MIBK was dried and the peak absorbance of lead was measured at 283.2 nm, using simultaneous D_2 background correction and recorder readout.

In addition to the above, the effects of procedural variables were investigated in a Youden seven-factor test design.¹³ The variables studied (Table I) were selected as typical of those which, through different interpretations of the procedure or individual preferences of different analysts, would be subject to small changes. The variables and their variations studied were the

type of acid used for wet-ashing ($\text{HNO}_3 + \text{HC10}_4$ vs HNO_3 alone), the number of additions of ashing acid (1 or 2), the condition to which the ash is heated after each acid addition (taken to dryness or left moist), the volume to which the ash is diluted (5 or 10 ml), the size of the MIBK aliquot in the tantalum boat (0.1 or 0.2 ml), and the method of drying the MIBK (drying with or without ignition). One dummy variable was included as an additional estimate of reproducibility. Results shown in Table I are averages of six replicate analyses.

3. *Extraction/Aspiration.* Five-gram samples of blood were wet-ashed with three additions of 4 ml each of HNO_3 and transferred to 50-ml volumetric flasks. Ammonium hydroxide was used to adjust the pH to approximately 8.5 after adding 5 ml of ammonium citrate buffer and phenol red indicator. To this solution was added 1 ml of 10% KCN, 1 ml of 2% APDC, and 4 ml of MIBK. After shaking for 30 seconds to complete the extraction, distilled water was added until the MIBK layer was brought up into the neck of the flask. The MIBK layer was aspirated into an air-acetylene flame for analysis, using pure water-saturated MIBK to establish the baseline.⁶

4. *Graphite furnace.* Standard blood samples weighing approximately 1 gram were wet-ashed with HNO_3 , diluted to 10 ml with 5% HNO_3 , and separated into portions for analysis by the method of standard additions. The operating conditions used on the HGA-2100 furnace were: wavelength = 283.3 nm, dry 15 seconds at 105°C , ash 15 seconds at 400°C , and atomize 7 seconds at 2000°C . Simultaneous background correction with the D_2 lamp was used in all cases. An Eppendorf pipet was used to deliver 10- μl aliquots to the furnace. This procedure is that of NIOSH P&CAM No. 214.⁹

5. *Flame atomic absorption.* Eleven grams of blood per sample were wet-ashed with concentrated HNO_3 . The ash was dissolved and diluted to 10 ml with 5% HNO_3 . The ash solution was separated into three portions and known amounts of lead standard were added to two of the portions so that concentration of lead in the blood could be calculated by the method of standard additions. The solutions were aspirated into an air-acetylene flame and absorbance measured at 283.3 nm on a Varian AA-5 atomic absorption spectrophotometer. A separate measurement of absorbance was made using a hydrogen lamp in order to obtain the

TABLE II
Ruggedness Test Results — Cd and Pb on Filters

Condition	Value	$\mu\text{g Cd/filter}$	Difference, $\mu\text{g Cd/filter}$	$\mu\text{g Pb/filter}$	Difference, $\mu\text{g Pb/filter}$
Digesting acid	A = HNO_3	21.03	0.01	24.76	-0.55
	a = $\text{HNO}_3 + \text{HC10}_4$	21.02		25.31	
Amount of acid	B = 3 ml	21.15	0.25	23.63	-2.81
	b = 1 ml	20.90		26.44	
No. acid additions	C = 2	19.80	-2.44	24.72	-0.62
	c = 1	22.24		25.34	
Dissolve in	D = 1:1 HCl	20.86	-0.33	23.62	-2.83
	d = 5% HNO_3	21.19		26.45	
Dilute with	E = H_2O	21.94	1.83	27.13	4.19
	e = 5% HNO_3	20.11		22.94	
Interval, ashing to analysis	F = 4 hr	20.57	-0.91	25.16	0.25
	f = 44 hr	21.48		24.91	
Background correction	G = no	21.79	1.53	25.95	1.84
	g = yes	20.26		24.11	
		SD =	1.90	SD =	3.28

background correction, which amounted to approximately 25% of the total signal.

D. *Analytical methods for cadmium and lead on filters.* The NBS filters were analyzed by the procedure given in NIOSH P&CAM No. 173 or by P&CAM Nos. 151 and 155.⁸ The three procedures are nearly identical, differing only in the amounts of HCl and HNO₃ used in the ashing and ash dissolution steps. The filters were wet-ashed with HNO₃ in 125-ml Phillips beakers on a hot plate. A second addition of HNO₃ was made to complete the ashing, the ash was dissolved in dilute acid, diluted to 10 ml, and analyzed by aspiration into the air-acetylene flame of a Perkin-Elmer 403 or 306 instrument.

The effects of procedural variables, selected from those likely to be encountered in routine analysis, were investigated by means of a Youden seven-factor test design.¹³ The variables (Table II) were the type of ashing acid (HNO₃ + HClO₄ or HNO₃ alone), the amount of acid (1 or 3 ml), the number of acid additions (1 or 2), the type of acid used to dissolve the ash (1:1 HCl or 5% HNO₃), the solution used to dilute the dissolved ash (deionized water or 5% HNO₃), the time interval between start of ashing and analysis (4 or 44 hr.), and the optional use of a D₂ simultaneous background correction. For this study, the standard NBS filters with 19.9 μg of Cd and 29.7 μg of Pb were used.

A separate study was made of the effects of various modes of data readout and calculation methods at several dilutions of ashed filters. Each filter was spiked with 10.0 μg of Cd. Two modes of data readout were used with a Perkin-Elmer Model 306 instrument: a 2-second integrated digital average absorbance, and peak height on the recorder signal giving a "real time" absorbance tracing. The concentration of Cd in the solutions was calculated by two methods: the method of standard additions, (with and without compensation for a nonlinear calibration curve) and by comparison to a calibration curve constructed from standard solutions. Six replicates of each concentration level were analyzed, and all solutions were analyzed with and without simultaneous D₂ background correction.

results and discussion

A. *Determination of lead in blood.* A

comparison of several methods for precision and accuracy in the analysis of the standard blood for lead is given in Table III. All procedures gave acceptable accuracy. The differences between methods in precision, sensitivity, and ease of operation were significant, however. The poor precision shown by the extraction/tantalum boat method (P&CAM No. 101) renders it unsuitable for use in routine analysis. The remaining methods have acceptable precision, in agreement with previously reported values.^{12,14,15} The range of sensitivities is more than two orders of magnitude, from graphite furnace to flame AAS. Unless the quantity of blood available for analysis is limited (<1 g), all of the methods except flame AAS are sufficiently sensitive. From the standpoint of ease of operation, the methods are roughly equal, with the exception of the more complicated dithizone method.

Of the several procedures used for blood lead determination in this work, the extraction/tantalum boat method was studied most extensively. Two major contributions to variability of the results were boat aging³ and extract aging effects. When a new tantalum boat was used to analyze replicate aliquots, the signal decreased about 5% per aliquot for the first 3 or 4 aliquots. This was followed by a more gradual decrease, amounting to approximately 10% of the original absorbance value, over the next 40 to 60 analyses (Figure 1). To compensate for this effect it was necessary to run standards as frequently as every four to eight samples.

The extract aging effect was more serious. Under the conditions of the extraction, the lead complex was unstable in the MIBK layer,

TABLE III
Lead in Standard Porcine Blood¹

Method	μg Pb/g, Mean SD (N)	Sensitivity μg/ml/1%A
Dithizone (P&CAM #102)	0.97 ± 0.065 (5)	0.04
Extraction/Boat (P&CAM #101)	1.04 ± 0.23 (6)	0.02 ²
Graphite Furnace (P&CAM #214)	1.01 ± 0.060 (4)	0.004 ³
Flame AAS (P&CAM #173)	1.00 ± 0.072 (5)	0.5
Extraction/aspiration	0.99 ± 0.064 (9)	0.15

¹1.00 ± 0.023 μg Pb/g, determined by dilution mass spectroscopy.

²0.2 ml aliquot to boat.

³0.01 ml aliquot to furnace.

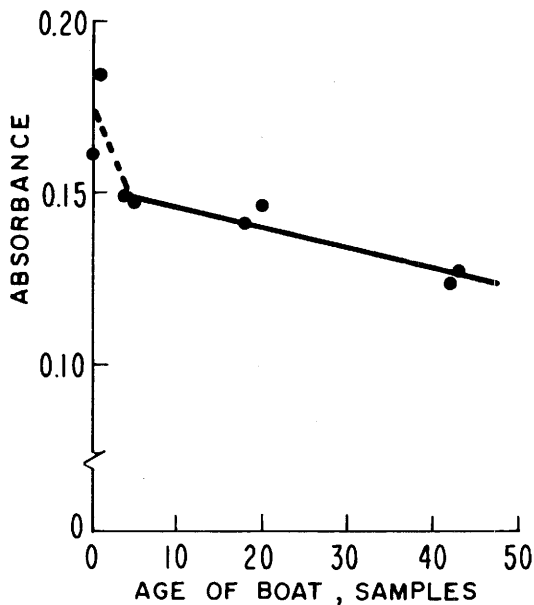


Figure 1—Absorbance of successive replicate 0.2 ml aliquots of Pb-APDC in MIBK analyzed in one tantalum boat. Each point represents a fresh extraction of a 0.2 μg Pb/ml solution.

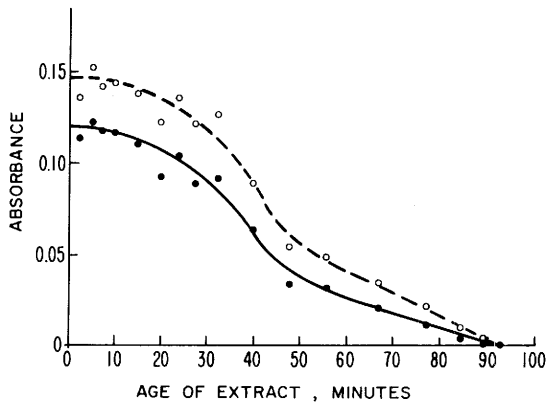


Figure 2—Extract aging effect. Successive aliquots of the same extract in a single boat over a period of time after extraction of the Pb-APDC complex. Actual absorbance values (solid curve); corrected for boat age using data of Figure 1 (dashed line).

leading to a rapid decrease in observed signal after 20 to 30 minutes, (Figure 2). Complete disappearance of signal occurred after approximately 90 minutes. In order to minimize this effect, samples and standards were extracted and analyzed in groups no larger than two or three.

In the ruggedness test performed on this method, none of the variables studied was shown

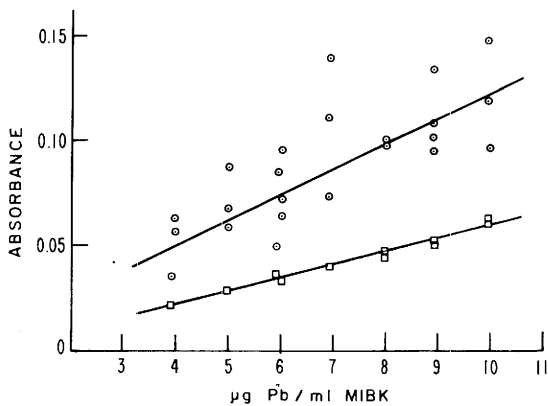


Figure 3—Comparison of tantalum boat analysis vs. aspiration. The same Pb-APDC/MIBK extracts of standard lead solutions were analyzed by two different methods. Aliquots (0.05 ml) of the extracts analyzed in the tantalum boat: upper line; extracts aspirated into the flame: lower line.

to be significant compared to the large overall standard deviation (Table I). The major source of variation arises from the use of the tantalum boat itself, aside from the boat aging effect mentioned earlier. The repeatability of successive analyses on the same extract, allowing for boat aging and extract aging effects, was observed to range from 7 to 25% RSD, with older boats showing greater variation. Further confirmation of this source of variation was obtained by analyzing the same extracts by two methods—by boat and by aspiration. The results of one of these experiments using standard lead solutions are shown in Figure 3. The only variable changed between the two calibration curves was the method of analysis of the MIBK extract, and the precision is dramatically better for the aspiration method, although sensitivity is reduced.

When the extraction/aspiration method⁶ is compared to the procedure of P&CAM No. 101, the advantages of the extraction/aspiration method are clearly evident. First, problems associated with the tantalum boat are avoided, resulting in improved precision. Second, the conditions under which the extraction is performed in the extraction/aspiration method lead to greatly increased stability for the metal complex in the MIBK layer; no change in absorbance could be detected 48 hours after extraction. It was found, however, that the extraction/aspiration method was sensitive to major changes in acid content of the diluted ash

solution. Results were erratic when perchloric acid was present, with some samples showing a 15% enhancement in absorbance due to the presence of 0.5 ml of concentrated HClO_4 in the ash solution. When nitric acid was the only acid present in the ash solution, absorbance tended to increase by about 8% when the nitric acid content was increased from 1 to 10% (w/v). In this method, as in all of the other methods involving wet-ashing, stability of the diluted ash solutions was excellent, in agreement with other reports,¹⁶ for solutions containing 1 to 5% (w/v) HNO_3 .

In the analysis for lead in blood by the graphite furnace technique, it was found necessary to use the method of standard additions in all cases, along with the use of simultaneous D_2 background correction. The slope of the standard addition curves of absorbance vs. concentration of lead were found to be roughly one-half of the slope of an aqueous standard curve, probably because of the matrix effects due to the ashed sample (Figure 4).

The aspiration of the aqueous ash solution into a flame was found to be of limited use in the determination of blood lead. The reasons for this are relatively poor sensitivity and large correction for nonspecific absorbance.

B. Determination of cadmium and lead on filters. Several variables were investigated in a ruggedness test on NBS standard filters with a 19.8 μg of Cd and 29.7 μg of Pb per filter. The results are shown in Table II. For Cd, the overall results on 24 filters gave a mean of 21.0 $\mu\text{g}/\text{filter}$, with a standard deviation of 1.9 $\mu\text{g}/\text{filter}$. No significant effects were seen in the type of acid used for wet-ashing, or in the amount of acid per addition. The number of acid additions was significant, however with higher results being obtained when only one addition was used. The reason for this may be a nonspecific absorbance by undestroyed filter organic matter. There may also be an interaction of this variable with the background correction variable; such an interaction cannot be isolated from this limited ruggedness test design. None of the other variables showed a significant influence, although the directional change caused by the use of background correction is as would be expected (Table II). For Pb analyses on the same filters, a mean of 25.0 $\mu\text{g}/\text{filter}$ and a standard

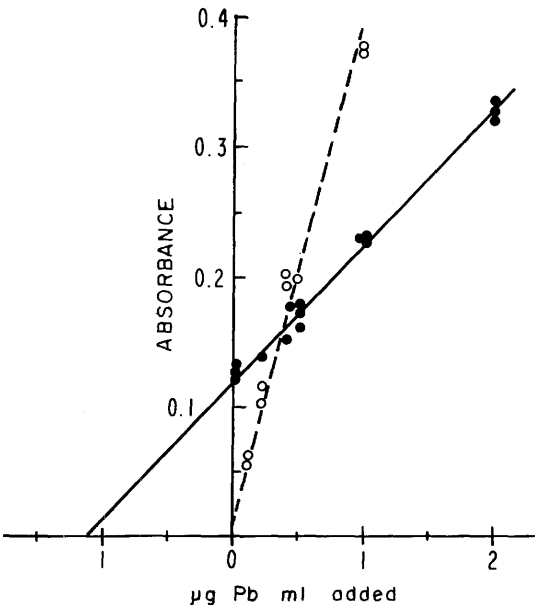


Figure 4—Calibration curves for graphite/furnace AAS method. Standard additions: solid line; Aqueous standards: dashed line. Analyzed at 283.3 nm with 10- μl aliquots.

deviation of 3.3 $\mu\text{g}/\text{filter}$ were obtained from the 24 filters in the ruggedness test. Only one factor, the diluting solvent for the ashed filters, gave a variation larger than the overall SD (Table II). The small decrease in Pb recovery noted when background corrections was used is in the direction expected, indicating that a small amount of nonspecific absorption may have occurred.

In experiments designed to measure recovery of 10 μg Cd per filter using the procedure of P&CAM No. 173, the recovery was found to be in the range $100 \pm 5\%$ over a wide range of dilutions and for a variety of readout and calculation combinations (Figure 5). Although the data shown were obtained without simultaneous D_2 background correction, they are identical with data obtained using the correction. For the digital absorbance readout, the mean recoveries at high dilutions are low, although the large variability tends to obscure the trend. Since the absorbance vs. concentration curve becomes increasingly nonlinear above approximately 1.5 μg Cd/ml, it is necessary to make use of this curve in correcting absorbances above 0.2. If this is not done, errors of 30% or more can occur. This is

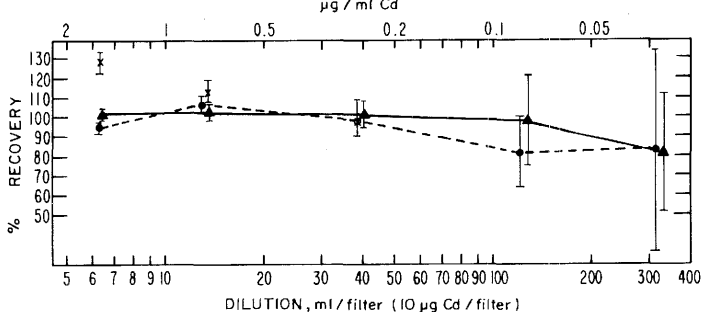


Figure 5—Recovery of Cd from filters (10 µg Cd/filter) using digital, 2-second integration calculations by: calibration curve (solid line); standard additions (broken line). Values calculated by standard additions uncorrected for curvature of standard curve, shown as "x". Brackets indicate $\pm 1\sigma$.

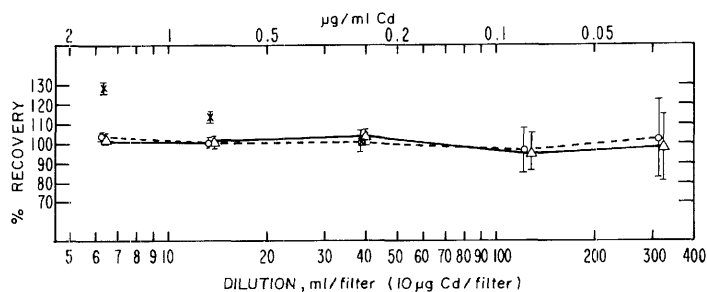


Figure 6—Recovery of Cd from filters (10 µg Cd/filter) using recorder readout. Calculations by: calibration curve (solid line); standard additions (broken line). Values calculated by standard additions, uncorrected for curvature of standard curve, shown as "x". Brackets indicate $\pm 1\sigma$.

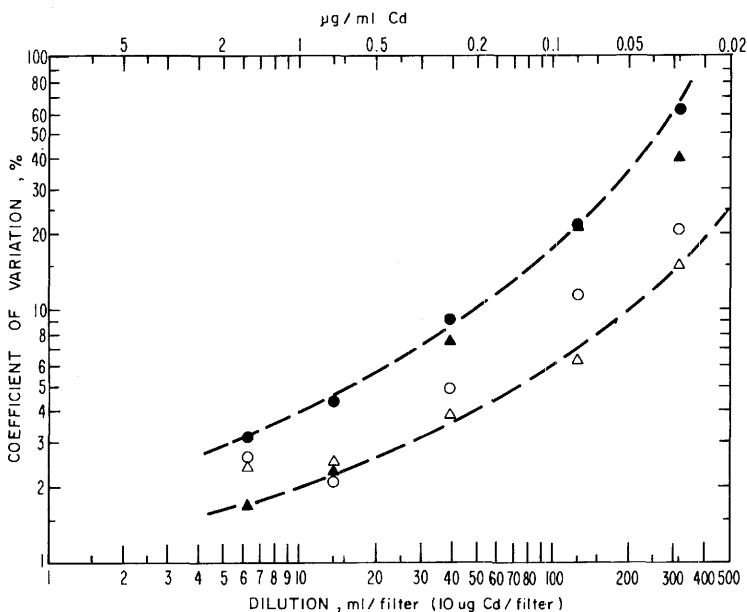


Figure 7—Coefficient of variation for Cd determinations on filters. Each filter contained 10 µg Cd. Symbols: ● - digital readout, method of standard additions; ▲ - digital readout, calibration curve; ○ - recorder readout, method of standard additions; △ - recorder readout, calibration curve.

illustrated in Figure 5, where the use of the method of standard additions at the 1.6 μg Cd/ml level leads to recoveries of 130% if the raw absorbance data are not corrected. Uncorrected absorbances could be used without error below 0.3 μg Cd/ml.

For the more dilute solutions of the ashed filters, recoveries tended to be low if digital readout was used. For these solutions, the measured absorbances were in the range 0.003 to 0.010. At these levels, the error due to rounding-off is very significant; also, an instrument baseline only slightly different from zero can be significant. However, with direct recorder readout (Figure 6), mean recoveries were nearly 100% even at 0.03 μg Cd/ml.

It is known that the coefficient of variation of the integrated absorbance is a function of integration time, with a 2-second integration yielding results which are about twice as variable as a 10-second integration.¹⁷ In the data shown in Figure 7 for various readout and calculation modes, the variability of a manually integrated, recorder trace is about one-half that of a digitally integrated, 2-second peak. The use of a digital, 10-second integration would be expected to be comparable to the manually integrated, recorder trace. Also evident from Figure 7 is the generally smaller coefficient of variation for data calculated by the use of an external calibration curve, compared to results using the method of standard additions.

conclusions and recommendations

The use of the tantalum boat method (P&CAM No. 101) for lead in blood is not recommended because of large variability. The two major sources of variation found were the instability of the extracted Pb and the irreproducibility of the tantalum boat. By extracting under different conditions, a more stable Pb complex is formed. Direct aspiration of the extract into the burner eliminates the excess variation due to the tantalum boat. Equivalent precision and accuracy are also obtained by the use of the classical dithizone method, and by a graphite furnace AAS procedure.

The determination of Cd and Pb on cellulose membrane filters by P&CAM No. 173 is fairly rugged with respect to ashing and other procedural variables. The use of at least two acid

additions for thorough ashing and provision for background correction are recommended.

For the determination of cadmium on filters, the most accurate and precise results will be obtained by the use of external calibration curves rather than the method of standard additions. Data should be obtained by long (10 second) digital integration times, or by manual integration of recorder readouts.

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Oklahoma local section is AIHA's newest

Another local section has been added to AIHA's growing roster of grass roots satellite organizations. Proposed by-laws have been approved by the AIHA Board of Directors and the inaugural meeting was held in Tulsa, Oklahoma on March 25, 1977.

President Evan E. Campbell was the featured speaker and installed the section's first roster of officers. More than 60 attended.

New president of the Oklahoma section is William J. Brown, Jr., Phillips Petroleum Company; president-elect is John W. Swanson, Cities Service Company; and secretary/treasurer is Robert Y. Nelson, Ph.D., of the University of Oklahoma.

Formation of the new local section is the culmination of many hours and long months of preparatory work by a dedicated group of Oklahoma-based AIHA national members. Invitations to join have been extended to all concerned with industrial hygiene and occupational health in Oklahoma and nearby localities.

To the new Oklahoma local section goes congratulations and best wishes for success and growth from all the other local sections and the national American Industrial Hygiene Association!