April 16, 1956

Mr. Manfred Bowditch
Director of Health and Safety
Lead Industries Association
420 Lexington Avenue
New York 17, New York

Dear Manfred:

I attach hereto a memo from Cholak to me on the subject of the method of analysis referred to in your letter of April second. To this I have added two comments, the second of which is in some respects the more important. If the first seems dogmatic, I ask you to consider one's clinical dilemma when the result of the analysis of the blood of a very sick small child or that of a workman whose job is at stake is somewhere between 0.075 and 0.085 mg. per 100 grams. This is an extremely critical situation in view of the fact that the least error we can have in the best routine analytical practice is of the order of $\pm$0.01 mg. If the blood value is actually 0.07 the child or workman is certainly free of symptoms of lead intoxication (that is; if this result is obtained in close time relationship to the last period of exposure), but if it is 0.08 or more, the symptoms (if not obviously due to something else) may be those of lead intoxication. How in God's name does one decide such a matter when the possible error of the method is $\pm$0.02, $\pm$0.03 or $\pm$0.04 mg. in this range of findings? The dubious range under these circumstances extends over a wide band of values. If this is true in the case of the blood, how much more is it true of the urine with its wide intrinsic physiological variability. It is possible to make a minute incision with an axe, but why in hell should any one wish to do so?

Sincerely,

Robert A. Kehoe, M. D.

RAK:jw

Attachment

To: Dr. R. A. Kehoe

From: Mr. J. Cholak

Subject: Bessman and Layne: Lead Analysis

Date: April 11, 1956

This method, except for the ashing step, is an adaptation of our screening method although reference is made only to Amdur's modification of this method. The ashing step is used to destroy the chelate. I believe a separatory funnel would be more convenient than the tube that is also used in this modification to ash the sample. However, as used by the authors, the method is suitable only for relatively high concentrations of lead in the blood and urine.

It is stated that the calibration curve for a 1 cm. cell is linear in the range of 1 to 10 γ per tube. With the size of the sample used, this means that the lower limit of certain detectability of lead in blood is 0.05 mg. per 100 grams (2 grams blood used) and in urine is 0.10 mg. per liter (10 ml. used). Although the color is concentrated in a volume of 3 ml. (increasing the sensitivity), I would expect the reproducibility to be approximately ±0.5 microgram per tube. This, when calculated on the basis of the size of sample employed, would be ±0.025 mg. per 100 grams of fresh blood, and ±0.05 mg. per liter of urine.

I fail to see where this method, because of the small quantities of material employed, offers any advantage over the screening method, which can be applied to larger samples than the authors use. I hardly think that it will revolutionize the procedures for the analysis of lead in biological materials.

The recoveries reported in the paper have been based upon samples to which 4 γ of lead have been added. This quantity is equivalent to 40 mg. per liter of urine or to 0.20 mg. per 100 grams of blood. Other recovery data refer only to samples of urine or blood from poisoned individuals. I doubt that this method would be satisfactory for the regular screening of samples obtained from persons whose exposure to lead is within or only a little above normal levels.

Further Comment by R.A.K.

1. I have no hesitation in condemning any screening method or any adaptation of a screening method for diagnostic purposes. There is no proper substitute for a highly precise, strictly quantitative and specific method. The screening methods are acceptable, only for certain types of industrial control. The reasons for this are so fundamental to good medical practice as to permit of no real controversy.

2. The analysis of samples of blood of small volume (or weight) is satisfactory from the physiological viewpoint, if the method of analysis is sufficiently sensitive and accurate, since the concentration of lead in the blood varies little except in response to the absorption of lead and to certain types of therapy. That is to say that physiological variability is slight. The analysis of small volumes of urine, on the other hand, is fraught with very great practical disadvantages (unless the sample analysed is a representative portion of a much larger volume of urine which is homogenous with reference to the distribution of lead therein - a difficult condition to achieve because of the frequency and extent

of flocculation and sedimentation in urine on simple cooling) since there is a wide variation in the rate of excretion of lead in the urine from hour to hour and from day to day in consequence of certain factors not all of which are known and subject to interpretation. The two present fairly common practices of correcting the concentration in terms of a standard "normal" specific gravity, or of calculating the output of lead in the urine on a time base, are both unsatisfactory, being seriously in error especially in those situations in which the interpretation of the results has the greatest clinical significance, i.e. those associated with wide deviations from mean normal values of the specific gravity and the lead content of the urine.

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It is stated that the calibration curve for a 1 cm. cell is linear in the range of 1 to 10 γ per tube. With the size of the sample used, this means that the lower limit of certain detectability of lead in blood is 0.05 Mg/100 Grams (2 grams blood used) and in urine is 0.10 Mg/Liter (10 ml. used). Although the color is concentrated in a volume of 3 ml. (increasing the sensitivity), I would expect the reproducibility to be approximately ±0.5 microgram per tube. This, when calculated on the basis of size of sample employed, would be ±0.025 Mg/100 Grams fresh blood, and ±0.05 Mg/Liter of urine.

I fail to see where this method, because of the small quantities of material employed, offers any advantage over the screening method, which can be applied to larger samples than the authors use. I ~~do not believe that this method is going to revolutionize the field of biologic lead analysis.~~

Recoveries in the paper have been established by the addition of 4 γ of lead, equivalent to 40 Mg/liter of urine or 0.20 Mg/100 grams of blood. Other recovery data refer only to samples of urine or blood from poisoned individuals. I doubt whether this method would be satisfactory for regular routine screening purposes.