A REVIEW OF THE RECENT LITERATURE CONCERNING THE RELATIONSHIP OF FREE ERYTHROCYTE PROTOPORPHYRIN, ZINC PROTOPORPHYRIN AND BLOOD LEAD LEVEL

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Lead intoxication (PbI) in humans dates to antiquity. This is because lead was readily available, had a low-melting point, and was easily cast. Lead being relatively soft as well, could be fabricated by simple means. Early history shows that humans worked with and used lead, and that they were poisoned by it[1].

In early mining, lead deposits were often found near the surface of the ground. The lead occurred as oxides and carbonates. These compounds were readily absorbed and did cause lead intoxication in great numbers of lead miners.[2]

Today, acute severe cases of lead poisoning are less apt to be seen although they still occur. Instead, chronic low-level lead intoxication is more apt to be encountered. However, the population risk is much greater at the present time than in the past due in part to the wide scale use of leaded gasolines, leaded plastics, and the wide distribution of automobile batteries.

The effects of lead intoxication are varied: gastro-intestinal sequelae, peripheral and central nervous system effects; encepalopathy, including psychosis, neuropathy, anemia; and interference with the synthesis of heme, causing an accumulation of heme precusors in the blood. These precusors are suspected of being toxic in their own right[3]. It is the interference with the heme biosynthetic pathway that produces a number of substances useful for measuring the degree of lead intoxication, as well as the quantity of lead absorbed into the body.

The problem of measurement of lead in the human body is a critical one. Much of the historical data on lead intoxication is related to measurements of blood

levels of lead. This constitutes a large pool of information on the subject. Recent experimentation by Rabinowitz et al[4] and Riegert and Whitlock[5] show that there are significant short-term variations of approximately plus or minus 10 percent in a given individual's blood-lead level, even under the best of laboratory conditions. These variations are thought to be brought about by illness, dehydration, acidosis, or parathyroid hormone, as well as dietary variations in calcium and phosphorous ratios[6,7,8,9]. Further, blood lead levels tend to represent recent absorption. To add to these biological difficulties, the direct analysis of blood for blood-lead content is hampered by the high level of skill required in analytical techniques, and the great care demanded to avoid the risk of sample contamination. Generally, the proficiency record of laboratories in blood-lead determinations has been poor[10,11,12, 32,33]. It, therefore, appears that the measurement of blood-lead level for an individual presents difficult problems. However, most clinical measures of lead toxicity have been related to blood-lead measurements, making blood-lead levels a commonly used measure of lead toxicity and absorption. Carefully done blood-lead procedures are therefore valuable on a statistical basis and can be used to evaluate and calibrate other methods of lead intoxication measurement provided chelation therapy is taken into account.

Research to date has shown that the most biologically significant, sensitive, and stable measurements of lead intoxication are the by-products of blocked heme biosynthesis. The most conveniently measured of these by-products are: delta-aminolevulinic acid (ALA), delta-aminolevulinic acid dehydratase (ALAD), free erythrocyte protoporphyrin (FEP) and zinc chelated erythrocyte protoporphyrin (ZPP). The heme biosynthetic pathway is shown in Figure 1. This figure is taken from Perbroth et al[3]. The exact manner that lead inhibits the synthesis is unknown, but it is apparent from the effects on precusors that the interference takes place in at least 2 sites. One site lowers the production of the enzyme ALAD

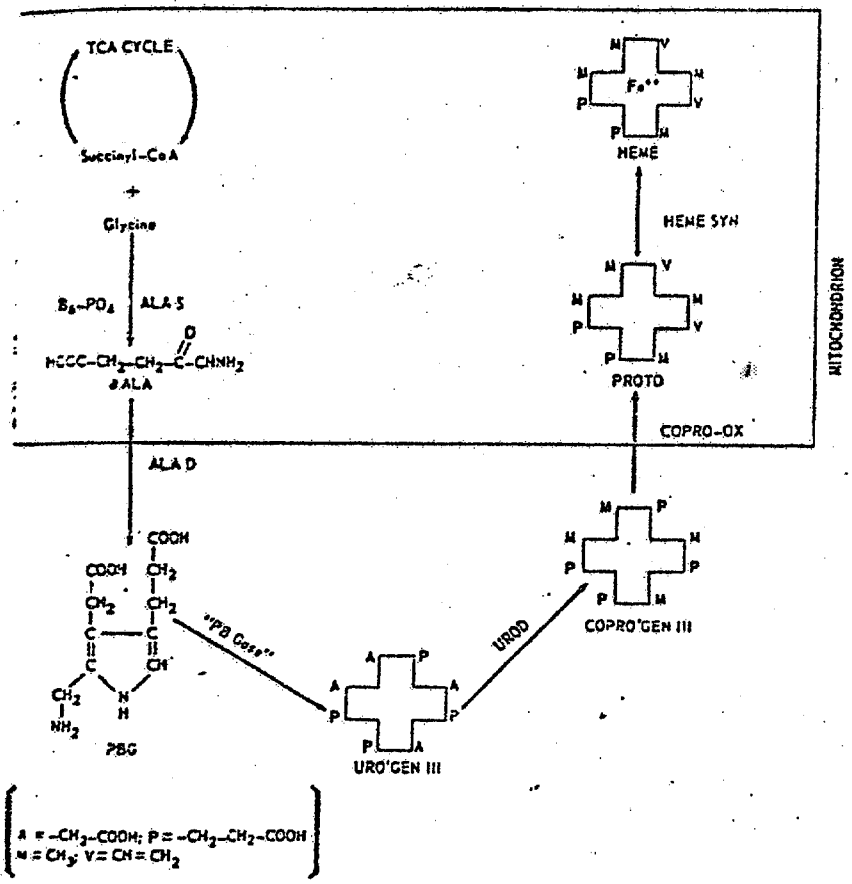

FIG. 1. Diagrammatic representation of the major steps in porphyrin biosynthesis. TCA cycle = tricarboxylic acid cycle; B_6 — PO_4 = pyridoxal phosphate; ALA S = δ-aminolevulinic acid synthetase (ALA synthetase); δ-ALA = δ-aminolevulinic acid; ALA D = δ-aminolevulinic acid dehydrase (ALA dehydrase); PBG = porphobilinogen; "PBGase" is a term used to represent the enzymatic activity involved in the conversion of PBG to uroporphyrinogen III. Two enzymes are actually involved in this conversion, PBG deaminase and an isomerizing enzyme [20]; URO'GEN III = uroporphyrinogen III (hexahydrouroporphyrin III); UROD = URO'GEN decarboxylase; COPRO'GEN III = coproporphyrinogen III; COPRO-OX = COPRO'GEN oxidase. The reaction shown probably involves two enzymatic steps [20]; PROTO = protoporphyrin; HEME SYN = heme synthetase (ferrochelatase). Enzymes represented within the rectangle are located within mitochondria.

leading to an increase of ALA. At the other site, lead interferes with the insertion of iron in the protoporphyrin ring, leading to an accumulation of protoporphyrins in the blood. Both ALAD and the substrate ALA have been used as parameters in determinations of exposure to lead. Blood levels of the enzyme and substrate, or urine levels of the substrate (ALA-U) are sometimes utilized in determining the amount of exposure to lead. The protoporphyrins are generally either extracted from the red blood cells (free erythrocyte protoporphyrin) or are measured directly in the blood in the form of zinc protoporphyrin. The protoporphyrins, therefore, measure the same phenomena; their ratio of magnitude with respect to lead is determined by the method of measurement.

Since the correlation of blood-lead level with individual lead intoxication is difficult and often does not reflect the true biological status of the individual,[12] a logical approach to lead intoxication would be to examine the more sensitive and stable by-products of heme biosynthesis caused by lead interference. The most sensitive of these is the enzyme ALAD [13,14]. Research by Hernberg et al, has shown that there is a direct relationship between ALAD and blood lead. The health significance of ALAD, however, remains to be proven[15]. In addition, the test for ALAD, while accurate, is difficult and time-consuming.

The next most sensitive detectable biological disturbance induced by lead in vivo is the increased concentration of FEP[12]. Research has shown that a significant increase of free circulating porphyrins is not well tolerated[16,3] in the body. FEP is detected by fluoresence. The concentration of FEP is affected by hematocrit levels and iron deficiency anemia, and therefore, hematocrit and Hb readings should be taken in conjunction with positive FEP results[17,18].

Extensive research has shown that FEP has a logarhythmic or exponential relationship with blood lead[14, 19,20,21,22]. The logarhythmic or exponential relationship has been shown to be true in both male and female adults, as well as in children, although the slope of the response curve is different for each of these groups[16]. The largest data base is on children, with male adults, and female adults having considerably less data in that order.

Recent investigations show that free erythrocyte protoporphyrin in patients with lead intoxication is, in fact, not free but is chelated with zinc[23]. Research by Lamola, Joselow, and Yamane[24]; and Fischbein, Eisinger, and Blumberg[25] has shown that the measurement of fluorescence at 495 nm wave length of blood directly, without any extraction steps, can serve as a simple and specific screening test for lead intoxication.

The outstanding advantage of using zinc protoporphyrin for lead intoxication measurements is that it can be detected with a portable field instrument called a hematoflurometer[25]. This instrument only requires that a drop of the subject's whole blood be placed on a slide and be directly assayed for lead effects. The read out of the instrument is not subject to dilution errors or extraction errors, as in FEP measurement; nor is it as subject to contamination errors, laboratory technique errors, or day-to-day biological variability, as in the base of blood lead levels. The only significant sources of variability are the calibration samples, which can be rigidly controlled on a broad scale, plus individual hematocrit and hemoglobin levels. Low hematocrit and hemoglobin would manifest themselves as a small percentage of false positives which can be easily segregated. The hematoflurometer was specifically designed by Bell Laboratories to give an almost instantaneous reading of the biological effects of lead intoxication. It has been field-tested by Fischbein et al, and found to be convenient and accurate.

Thus, the methodology exists to simply and inexpensively monitor the biological effects of lead intoxication for those workers exposed to lead hazards. The question now arises, "Do we have enough dose-response information for screening purposes to determine the acceptable upper limits of ZPP in the blood due to lead intoxication?"

A search of the literature shows a considerable inconsistency in the relationship between FEP and blood-lead levels between different investigators (See Tables I, II, and III). The variations of these ratios between the investigators, is apparently due to the following:

a) mean age
b) sex or ratio of sexes in studies of children
c) race or ratio of races
d) extraction method
e) type of regression equation fitted to data

Observation of Table III indicates that the combination of extraction method and regression equation is capable of producing an order of magnitude variation between similar age and sex groups.

If the uniform method of ZPP analysis using the hematoflurometer is adopted, the regression equation could be the last large source of variability.

The regression equations used in the major studies of lead intoxication using protoporphyrin thus far take three forms:

1. quadratic
2. logarhythmic
3. logistic

In the quadratic and logarhythmic protoporphyrin vs. blood-lead level equations, the protoporphyrin tends toward infinity with increasing blood lead. Living systems generally do not exhibit this type of dose-response relationship; instead the response tends to level off with increasing dose. The logistic equation exhibits this leveling off characteristic at relatively high lead doses while exhibiting a rapid rise at medium doses similar to the quadratic and logarhythic equations. In some recent studies, the data exhibits the logistic form[20,22,34].

A number of the investigators listed in Tables I and II have been able to recommend upper limits of FEP tnat are consistent with blood-lead limits to prevent lead intoxication; however, because of the variations, it is not possible to directly translate these recommendations into limits for ZPP. Therefore, to make the FEP information useful, it must be correlated directly with ZPP readings with respect to blood-lead levels. Fischbein et al[25,26] are in process of establishing this relationship. (See Figure 2). In addition, Lamola et al[30] have made a quanitative determination of erythrocyte zinc protoporphyrin. This work should lead to a standardized base line for all investigators in this field.

In plotting the regression equations of Lamola et al[24] for ZPP (See Fig. 3), an obvious inflection point occurs on these curves at about 13.5 ug/100ml ZPP for both children and adult males. This would appear to be the threshold biological response (as determined by ZPP) to lead intoxication. In children, this would be approximately 22 ug/100 ml Pb.B and for adult males, it would be approximately 35 ug/100ml Pb.B. The 35 ug/100ml of blood lead coincides with Zielhuis[16] lower estimate of the threshold of biological response for adult males (as determined by protoporphyrin in erythrocytes, method of Schwartz & Wikoff).

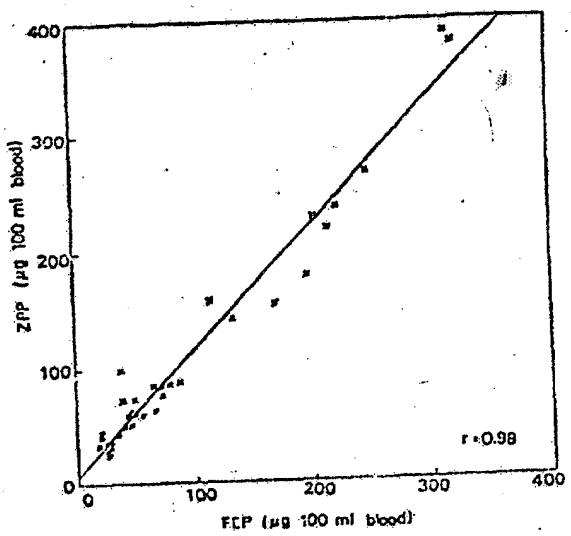

FIG. 2. Correlation between zinc protoporphyrin, determined by the hematofluorometer, and free erythrocyte protoporphyrin, determined by acidic extraction and spectrofluorometry (8).

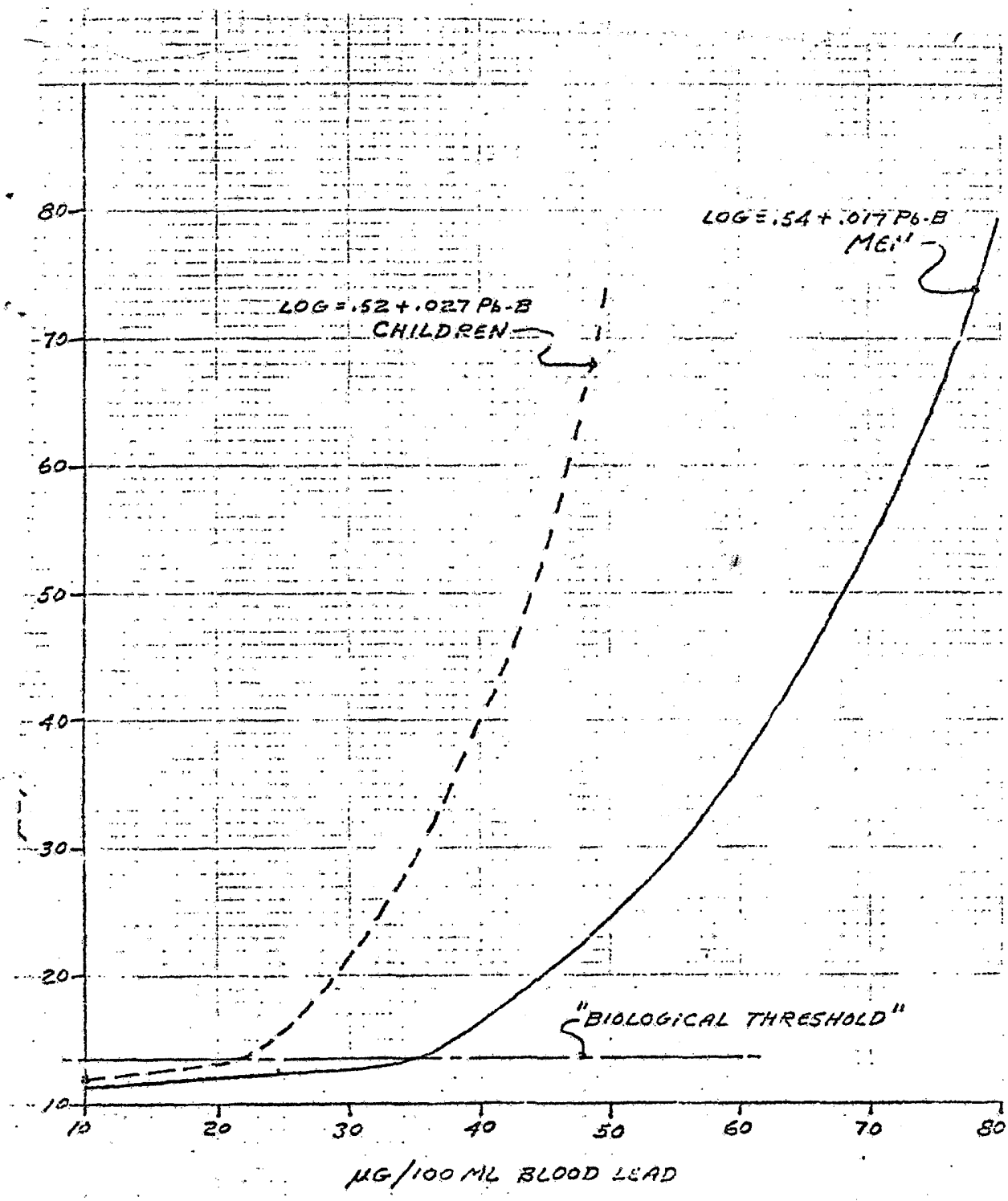

FIGURE 3

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A PHS, CDC Report[35] dated March 1977 in a study of five lead smelters found the threshold of clinically detectably pathology to be a blood-lead level of 60 ug/100 ml. The ZPP equivalent level should be established by the results of current research.

There is adequate evidence that female adults show increased sensitivity to lead intoxication as compared with male adults[19,27,28]. This is based upon responses of the hemopoietic system to increased blood-lead levels. The relationship of ZPP and blood lead in adult famales is yet to be well-established however.

In summary, all current evidence suggests that an advantageous way to assess lead intoxication is by analysis of the by-products of lead interference with the heme biosynthetic pathway; and that the by-product of choice for analysis is zinc protoporphyrin. In addition, the common methodology used in ZPP analysis should allow pooling of the data of different investigators for statistical analysis.

AVAILABLE DATA, FEP vs BLOOD LEAD

Table I

1. Roels, Arch. Env. Hlth., Dec. 76 Method: modified Granick	Children Log FEP = 1.321 + 0.025 (Pb-B) Graph, Fig. 2, pg. 313, N=51, r=.728
2. Roels, Int. Arch. Arbeitsmed 34:97-108 1974 Method: Granick	Female adult Log FEP = 1.243 + 0.0259 (Pb-B) N=26, r=.743
	Male adult Log FEP = 1.4777 + 0.0106 (Pb-B) N=40, 4=.544
	Children Log FEP = 1.454 + 0.0211 (Pb-B) N=37, 4=.592 Graph, Fig. 2, pg. 102
3. Tomokuni Arch. Env. Hlth., Dec. 75 Method: mod. Piomelli	Adult males Graph, Fig. 1, pg. 589, N=65, r=.72
4. Piomelli, Pediatrics 51:2, Feb. 1973 Method: Piomelli	Children Log FEP = 1.623 + .0177 (Pb-B) Graph, Fig. 1, pg. 255, N=1038, 4=.72
5. Landrigan, Pediatrics 89:6, Dec. 76 Method: Granick	Children FEP = 0.043 x $(Pb\text{-}B)^2$ + 0.45 (Pb-B)-2.14 Graph, Fig. 3, pg. 907, N=1056, r=.79
6. Reigart, Pediatrics 57:1, Jan.76 Method: Chisolm	Children Tables IV, V, pg. 57
7. Chisolm, Pediatrics, 84:4, Apr. 76 Method: Chisolm	Children Relationship Linear between log FEP and Pb-B Graph, Fig. 1, pg. 492, N=115, r=.60
8. Joselow, NIOSH Symp. 76 Method: Piomelli	Children, Male adults Adapted from Piomelli Pediatrics 51:254-257, 1973 Fig. 5, pg. 33, Graph Ln FEP=3.0219 + .0245 Pb-B
9. CDC, EP 1-76-33-2 Method: Piomelli	Adult Males Graph, Fig. 2, pg. 7 N=78, 4=.76 Ln FEP = 3.686 + .0276 Pb-B

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AVAILABLE DATA, FEP vs. BLOOD LEAD (Cont.)

10.	CDC, EPI-76-83-2 Method: Piomelli	Adult males Table 2, pg. 5 Table 3,4, pg. 6 Table 5, pg. 7 N=38, 4=.79
11.	Levine, et al, Am. J Pub. Hlth, 66:6, June 76 Method: Granick	Adult Males Tables 1,2,pg. 549
12.	L. Alessio, et al Int. Arch. Occ. Environ.Hlth 37:73-88, 1976 Method: Schwartz & Wikoff	Adult Males $FEP = \frac{230}{1 + \exp(5.34 - .079\ Pb\text{-}B)}$ N=201, 4=.904

AVAILABLE DATA, ZPP vs BLOOD LEAD

Table II

1.	Joselow, NIOSH Symp. 76 Method: Lamola	Graphs Fig. 6, pg. 34 Adapted from Lamola, Joselow, Yamane, Clin. Chem. 21:92-97, 1973
2.	Lamola, Clin. Chem. 21:93-97, 1975 Method: Lamola	Equa. Children Log ZPP = 0.52 + 0.027 Pb-B N=250, r=.77 Equa. Men Log ZPP = 0.54 + 0.017 Pb-B N=35, r=.87
3.	Fischbein, Mt. Sinai J. Med. 43:3, May, June 76 Methods: Lamola, Sassa, (Granick)	Graph, Fig. 4, pg. 298 Correlation ZPP and FEP N=33, 4=.98
4.	Lilis & Fischbein Report to NIOSH, ES-00928, May 15, 1976 Method: Lamola	Chelated Lead Smelter Workers Graph, Fig. 8, pg. 59 N=158
5.	Fischbein, Proceedings Int. Conf. on Heavy Metals in Envirn., Toronto, Oct., 1975 Method: Lamola, Sassa (Granick)	Correlation ZPP & FEP
6.	Kellogg Report NIOSH March 1977 Method: Lamola	Equa. Women Log ZPP = .946 + (.019 x Pb-B) N=189, r=.59 Equa. Men Log ZPP = .820 + (.018 x Pb-B) N=710, r=.79

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TABLE III

COMPARISON OF BLOOD LEAD vs. FEP FOR DIFFERENT INVESTIGATORS

	FEP									
	Children				Women	Men				
Pb-B	74 Roels	76 Roels	Piomelli	Landrigan	Roels	Roels	Tomokuni (est.)	EPI 76-33-2	EPI 76-83-2	Alessio
10	42.5	37.2	63	6.7	31.8	38.4	31	26.2	52.6	2.92
20	75.2	66.2	93	24.1	57.7	49	72	33.5	69.4	6.37
25	-	-	-	-	-	-	-	-	-	9.35
30	122	111	142	50.1	105	62.5	120	42.8	91.2	13.7
35	-	-	-	-	-	-	-	-	-	19.8
40	198	209	214	84.7	190	79.9	190	54.6	102	28.4
50	323	372	322	128	354	100	270	69.9	159	55.8
60	525	662	484	180	626	130	400	89.2	209	99.2
70	854	1108	729	241	1104	166	-	114	276	153.
80	1388	2090	1094	309	2066	212	-	142	364	204.
90	2256	3720	1644	387	3742	271	-	186	479	-
100	3665	6620	2464	473	6810	345	-	238	631	260.

NOTE: 1. All numbers in micrograms/100 milliliters

2. All calculations based on regression equations in Tables I & II

TABLE IIIA

COMPARISON OF BLOOD LEAD vs. ZPP FOR DIFFERENT INVESTIGATORS

	ZPP			
	MEN		WOMEN	CHILDREN
Pb-B	Lamola	Kellogg	Kellogg	Lamola
10	11.8	10.1	13.4	12.0
20	12.2	15.7	20.4	13.0
25	12.5	19.1	25.5	15.7
30	12.7	24.4	31.7	21.4
35	13.6	30.3	39.4	29.2
40	16.6	37.7	49.1	39.8
50	24.6	58.4	76.0	74.0
60	36.4	90.4	118	138
70	53.7	140	174	257
80	79.5	217	282	478
90	118	336	438	892
100	174	520	677	1660

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