

Dose-Effect and Dose-Response Relationships of Blood Lead to Erythrocytic Protoporphyrin in Young Children¹

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Dose-effect and dose-response relationships were analyzed for blood lead concentration (PbB) vs blood protoporphyrin concentration using multiple data points from 165 children, ages 3-36 months. Protoporphyrin concentrations were measured using a front-face fluorometer designed to measure zinc protoporphyrin (ZPP) and an extraction method designed to measure total protoporphyrin as the free base (FEP). Estimations were made of the threshold for PbB effects on FEP and ZPP, as well as the slopes of the PbB-FEP and PbB-ZPP interactions. There was essentially no difference in thresholds estimated using ZPP vs FEP as the effect parameter. There was no apparent effect of age on threshold. However, the slope for PbB vs ZPP was less steep than the slope for PbB vs FEP. Moreover, the average ratio FEP:ZPP was markedly elevated at 3 months (1.84:1) and decreased slowly, attaining unity at 33 months. The possible reasons for this discrepancy are discussed, as well as the implications for interpretation of lead screening program data. © 1985 Academic Press, Inc.

BACKGROUND

One of the most sensitive biochemical indices of elevated lead exposure is elevation of erythrocytic protoporphyrin (PP). The effect is due to inhibition by lead of insertion of FE^{2+} into protoporphyrin IX by heme synthetase. Iron deficiency itself also results in elevation of PP (Stockman *et al.*, 1975; Yip *et al.*, 1983). This is the final step in heme synthesis. PP elevation occurs even at levels of lead exposure below those at which a decrement of circulating hemoglobin is observed. Since this effect is viewed by many as adverse, it is important to establish the blood lead (PbB)-PP relationship (dose-effect relationship) as well as the relationship between PbB and the incidence of elevated PP (dose-response relationship). The dose-effect relationships for PbB vs PP have been reported by a number of investigators for both adults (Lamola *et al.*, 1975; Karacic, *et al.*, 1980; Hammond *et al.*, 1980) and children (Lamola *et al.*, 1975; Piomelli *et al.*, 1973; Sassa *et al.*, 1973).

Dose-effect relationships are useful in establishing the characteristics of the impact of dose on effect, but they fail to define, in and of themselves, the frequency with which a population is affected to any specified degree, e.g., ≥ 2 SD above a mean background level due to other causes. For this purpose a dose-response analysis must be derived from the dose-effect data. A dose-response

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analysis not only provides an estimate of frequency of response as a function of dose, it also provides an estimate of the threshold for interaction between the agonist and the effect, as may a segmented dose-effect model. In the specific case of PP vs PbB, there has been only one detailed analysis of dose-response with good procedural documentation (Piomelli *et al.*, 1982).

Various procedures have been used to measure PP. All of them are fluorometric procedures but are distinctly different in one respect. Some are designed to detect the presence of PP as the free base (FEP) and others are designed to measure PP as the zinc complex (ZPP). Methods of the former type involve acidification of the blood sample with subsequent extraction of the free-base form of porphyrin into an organic solvent. The latter type of method usually involves direct determination of porphyrin in the blood sample as the zinc complex, the form in which it largely occurs in circulating erythrocytes. Measurement of PP as ZPP has become very popular as a screening method for evidence of excessive lead exposure by virtue of its apparent procedural simplicity. It is not clear at this time which of the two approaches to measurement of PP more accurately reflects the impact of lead on heme synthesis or, for that matter, whether it really matters which type of method is used. There are relatively few reports concerning the correspondence between PP measured as FEP vs ZPP. Over the limited range of 0 to approximately 350 μg PP/dl whole blood, the two approaches give essentially identical results utilizing adult blood (Fischbein *et al.*, 1976). Over a more extended range of adult PP, however, a curvilinear relationship is apparent wherein as PP increases, ZPP decreases progressively in relation to FEP (Karacic *et al.*, 1980). A similar and even more pronounced progressive discrepancy has been observed in one study involving children (Kaul *et al.*, 1983). In none of the above studies have the relative merits of FEP and ZPP been established as correlates of PbB or health effects of lead exposure.

It is the purpose of this report to define dose-effect and dose-response relationships of PbB vs FEP and ZPP in a population of children less than 30 months of age. Our current prospective study of health effects of lead in children through the first 5 years of life has afforded us the opportunity to examine these relationships.

METHODS

Blood samples were drawn by venipuncture when possible (approximately 75%). The remainder were drawn by finger stick or heel stick.

Two independent measures of PP were used. One measures total porphyrin (FEP) (Chisolm and Brown, 1975) and the other measures the zinc complex of protoporphyrin by direct fluorescence reading of a blood smear (ZPP) (Blumberg *et al.*, 1977).

Proficiency for measurement of ZPP and FEP was monitored continuously by participation in the proficiency testing programs of the Centers for Disease Control, Atlanta, Georgia (CDC), and the Pennsylvania Department of Health, Lionville, Pennsylvania. Results were consistently within the acceptable limits as defined by these programs.

PbB was determined by anodic stripping voltammetry (ASV) using a Model

3010A instrument (Environmental Science Associates, Inc., Bedford, Mass.). Accuracy and precision were monitored by participation in the Centers for Disease Control and Pennsylvania proficiency testing programs. Results were within the acceptable limits most of the time as defined by these programs, 83% for CDC and 100% for Pennsylvania. In the case of instances where we fell outside the acceptable limits defined by CDC, aliquots were subsequently sent to Environmental Sciences Associates (ESA) for confirmation of our results. In most cases ESA confirmed the correctness of our results. In addition, accuracy was monitored daily using blood samples analyzed for lead by isotope dilution mass spectrometry (IDMS). These samples were obtained from the National Bureau of Standards. Accuracy and precision of our ASV method by comparison to IDMS have been reported (Que Hee *et al.*, 1985). In keeping with past practices, dose-effect analyses were made utilizing logarithmic transformation of PP data. The intersection (join point) of the regression lines for PbB-related rise in PP with the regression line for non-PbB-related PP was estimated by modifying procedures previously reported (Hudson, 1966; Hasselblad *et al.*, 1976). Specifically, the join point and the model's parameter were estimated simultaneously by minimizing the total sum of squares for error from both parts of the curve (flat and linearly increasing). An iterative procedure utilizing the Marquardt algorithm was used. Dose-response analyses of PP vs PbB utilized the Statistical Analysis System (SAS) PROBIT computer program (Finney, 1971) which includes correction for non-PbB-related contributions to PP. As no iron deficiency was noted in our study, the correction factor is accounting for background variation only. All PbBs and PPs were corrected for variations in hematocrit and are expressed as microgram per deciliter of whole blood.

In the studies involving PP response in rats the same analytical procedures were used as in the human study. Rats were exposed to lead via dams' milk to weaning and via drinking water after weaning. Mean PbBs attained ranged from 2.9 ± 1.2 to 56.7 ± 52.2 (SD) $\mu\text{g}/\text{dl}$ at 20 days of age, depending on the level of lead exposure (Hastings *et al.*, 1984).

RESULTS AND DISCUSSION

The results of the dose-effect analysis are presented in Fig. 1. Threshold for effect of PbB on PP is defined as the point of intersection of the regression line for a PbB effect on PP with the average background PP, the flat portion of the segmented curve. Although the thresholds for PbB vs FEP and PbB vs ZPP are quite similar, there is an obvious discrepancy between the two sets of lines as to the concentration of PP as a function of PbB. This is mainly due to the arbitrary manner in which fluorometers used to measure ZPP are calibrated. It also appears that the slope of the PbB-ZPP dose-effect line is not as steep as for PbB-FEP. A less steep slope for PbB-ZPP is consistent with previous observations (Fishbein *et al.*, 1976; Karacic *et al.*, 1980).

The analysis of population dose-response rate for the data is presented in Fig. 2. The definition of response is any PP value greater than 2 SD above the mean for PP in the PbB range below the point of intersection as determined by analysis of the dose-effect relationship ($\text{PbB} < 15 \mu\text{g}/\text{dl}$). The thresholds for dose-re-

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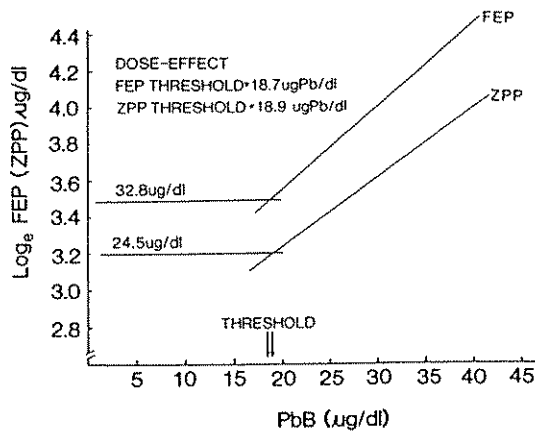


FIG. 1. Dose-effect relationship and estimation of the threshold for the effect of PbB on ZPP and FEP: $N = 1522$ and 1423 , respectively.

sponse PbB-FEP and PbB-ZPP are the same ($14.5 \mu\text{g}/\text{dl}$) and somewhat lower than in the dose-effect analysis (Fig. 1).

The dose-response curve as published by Piomelli *et al.* (1982) is included in Fig. 2 for purpose of comparison. The similarity in slope is quite striking and even the threshold is only slightly higher ($\text{PbB} = 16.5 \mu\text{g}/\text{dl}$). The Piomelli study population was appreciably older than ours (mean = 5.5 years and median = 4.7 years vs 12.5 months and 9.9 months, respectively). The Piomelli population also differed from ours in that each child was only sampled once, whereas ours were sampled repeatedly at 3-month intervals. It should also be noted that the upper 2 SD limit for background FEP in our subjects was considerably greater than for

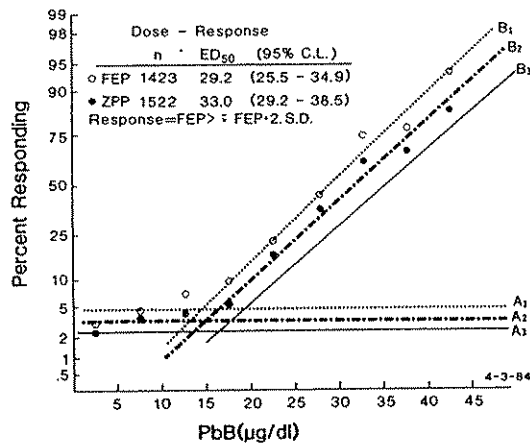


FIG. 2. Dose-response relationships depicting percentage of the cohort at any given PbB level which exhibited a ZPP or FEP value 2 SD or more above the cohort mean for each age group. A_1 = background FEP + 2 SD, this study. A_2 = background ZPP + 2 SD, this study. A_3 = background FEP + 2 SD (Piomelli *et al.*, 1982). B_1 = dose-response, FEP, this study. B_2 = dose-response, ZPP, this study. B_3 = dose-response, FEP (Piomelli *et al.*, 1982).

the older subjects in the Piomelli study. This last-named difference is probably due to the fact that in the age range of our subjects "normal" FEP levels change dramatically, especially among children whose PbBs never exceeded 15 $\mu\text{g}/\text{dl}$ (Fig. 3). This results both in a higher average background FEP and in a much greater variance in FEP averaged over the age range of our subjects.

In order to determine whether age and multiple sampling influenced the slopes and thresholds for PP vs PbB, separate dose-effect analyses were performed for each age group (Table 1). The slopes of the dose-effect relationships increased with age up to 24 months and then decreased again. By contrast, there was no systematic change in thresholds as a function of age, although these varied considerably, from 11.53 $\mu\text{g}/\text{dl}$ at 30 months to 27.86 $\mu\text{g}/\text{dl}$ at 6 months. Although data were available for cord blood samples, 10-day samples, and 3-month blood samples, they are not included in Table 1 for lack of statistically significant interaction with PbB. It is also interesting to note that the correlation of both ZPP and FEP with PbB increased progressively to age 18–24 months and then decreased as reflected in R^2 (Table 1). This may be due to the fact that in the age range of 15–24 months PbB is fairly stable, changing little over time (Fig. 3). Such a situation tends to minimize the time-related lag in the peripheral blood expression of the rise in FEP. The lead effect is exerted on erythrocyte precursors during their maturation, rather than on mature cells in the peripheral circulation. It should also be noted from Fig. 3 that the age-related rate of fall in FEP in older low-lead children is not as pronounced as at younger ages. This progressive fall in FEP during the first 2 years of life has been reported by others (Wranne, 1960; Hesse *et al.*, 1983). The remarkably elevated FEP observed at birth is not due to

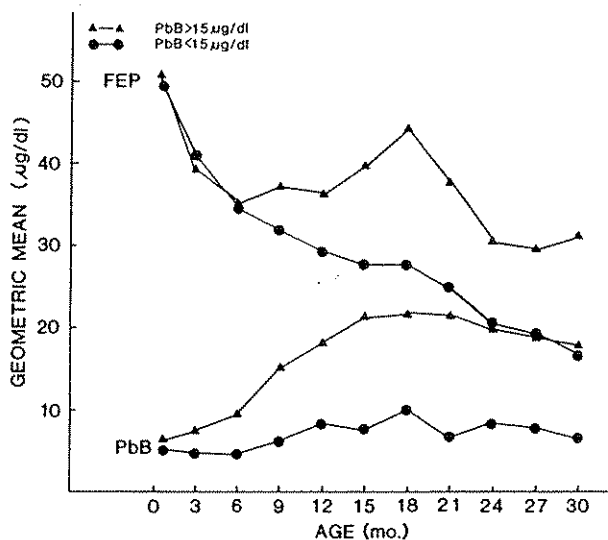


FIG. 3. Age-related changes in erythrocytic porphyrin (FEP) as related to lead exposure, comparing children whose PbBs never exceeded 15 $\mu\text{g}/\text{dl}$ to those whose PbBs did exceed 15 $\mu\text{g}/\text{dl}$ at one time or another within the 30-month period of observation. The 15 $\mu\text{g}/\text{dl}$ is arbitrary but approximates the threshold for a lead effect on FEP.

TABLE I
REGRESSION ANALYSIS: \log_e (ZPP) AND \log_e (FEP) ON PbB FOR VARIOUS AGES AND ALL
SUBJECTS COMBINED

Age (months)	Porphyrin method	<i>n</i>	α_0	β_1	R^2	Average PbB threshold ($\mu\text{g/dl}$)
6	FEP	165	3.59 ± 0.03^a	0.120 ± 0.170^a	0.044	27.86
9	ZPP	158	3.15 ± 0.02	0.015 ± 0.007	0.062	19.04
	FEP	149	3.50 ± 0.03	0.028 ± 0.008	0.153	16.72
12	ZPP	137	3.08 ± 0.04	0.023 ± 0.006	0.170	15.03
	FEP	131	3.40 ± 0.04	0.040 ± 0.008	0.276	16.24
15	ZPP	114	3.07 ± 0.04	0.029 ± 0.005	0.407	15.84
	FEP	113	3.29 ± 0.03	0.034 ± 0.005	0.438	13.49
18	ZPP	91	3.04 ± 0.05	0.040 ± 0.007	0.454	16.70
	FEP	91	3.33 ± 0.05	0.060 ± 0.012	0.486	19.60
21	ZPP	70	2.92 ± 0.08	0.042 ± 0.007	0.445	14.71
	FEP	70	3.24 ± 0.05	0.053 ± 0.011	0.498	18.50
24	ZPP	62	2.95 ± 0.06	0.041 ± 0.008	0.439	17.48
	FEP	62	3.15 ± 0.06	0.050 ± 0.009	0.497	18.44
27	ZPP	58	3.12 ± 0.06	0.030 ± 0.020	0.352	19.69
	FEP	57	3.11 ± 0.06	0.042 ± 0.012	0.357	15.16
30	ZPP	49	2.87 ± 0.05	0.030 ± 0.009	0.352	13.89
	FEP	50	3.04 ± 0.10	0.035 ± 0.009	0.318	11.53
All	ZPP	1522	3.20 ± 0.01	0.038 ± 0.003	0.273	18.94
	FEP	1423	3.49 ± 0.01	0.045 ± 0.003	0.311	18.70

Note. All FEP, ZPP, and PbB values were normalized as to hematocrit prior to regression analysis, and all R^2 are significant at $P < 0.05$. α_0 = average background level of FEP or ZPP at PbB levels below threshold expressed as \log_e FEP ($\mu\text{g/dl}$). β_1 = slope for \log_e FEP or \log_e ZPP vs PbB. ^aStandard error.

iron deficiency. A child actually is born with excess stores of iron which are not depleted until approximately the ninth month after birth.

A further analysis of dose-response was run using more stringent restrictions aimed at minimizing heterogeneity of age on the results. Thus the age range was limited to 9-18 months. These restrictions, which reduced the sample size from 1455 to 414, did not significantly alter the slope of the dose-response curve and reduced the dose-response threshold only moderately, from 14.5 to 12.5 $\mu\text{g/dl}$ (data not shown).

There is a considerable body of literature concerning various effects of lead in infant rats as related to PbB and PP, including results from this laboratory (Hastings *et al.*, 1984). We therefore analyzed the dose-effect relationship for rats 30 days of age. The results are displayed in Fig. 4 with the 24-month children included for purposes of comparison. Unlike children, infants rats had no interval of PbB over which there was no significant interaction with PbB (Table 2). Fur-

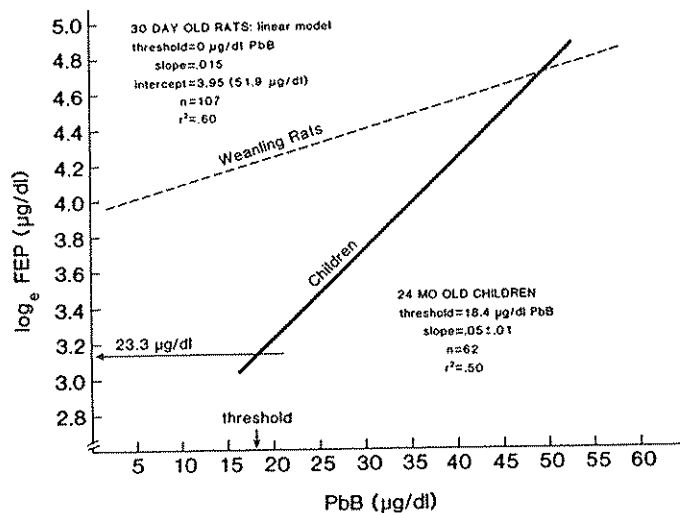


FIG. 4. Dose-effect relationship and estimation of threshold for effect of PbB on FEP in 24-month-old children and 30-day-old weanling rats.

ther, the difference in slope is striking (Fig. 4). The reasons for these differences are not clear at this time. The lesser slope in the rat FEP-PbB dose-effect curve, however, is consistent with the observation that the rat is more resistant than man to lead-induced reduction in circulating hemoglobin concentration. Thus, over the range of PbB = 2.3-104 µg/dl, no decrement in hemoglobin was observed in adult rats (Gelman *et al.*, 1978). By contrast, decrements in hemoglobin have been reported to occur at PbBs as low as 40-60 µg/dl in children (Betts *et al.*, 1973; Pueschel *et al.*, 1972) and adults (Baker *et al.*, 1979). The absence of

TABLE 2
REGRESSION ANALYSIS: log_e ZPP AND log_e FEP ON PbB, RATS

Measure	Age (days)	n	Nonthreshold model linear regression			Threshold model hockey stick				
			β ₀	β ₁	R ²	β ₀	α ₀	β ₁	Join	ΔR ²
ZPP	20	126	3.46	0.0013	0.07	2.862	3.465	0.0012	0	0
FEP	20	121	4.35	0.0023	0.07	4.074	4.417	0.0017	0	0
ZPP	30	109	3.36	0.0131	0.59	3.067	3.385	0.0125	0	0
FEP	30	107	3.95	0.0152	0.60	3.701	3.970	0.0146	0	0
ZPP	90	106	3.01	0.0240	0.71	3.019	3.019	0.024	0	0
FEP	90	91	3.41	0.0210	0.72	3.446	3.379	0.022	3.1	0

Note. β₀-y intercept for nonthreshold model. β₁-slope for log_e FEP or ZPP vs PbB for nonthreshold. α₀-average background level of FEP or ZPP at PbB levels below threshold for threshold model. α₀-y intercept for threshold model. β₁-slope for log_e FEP or ZPP vs PbB for threshold model. Join = threshold of PbB effect on log_e FEP or ZPP. ΔR² = improvement in R² over that attained with linear regression model.

a threshold for FEP-PbB in rats suggests that heme synthetase is probably the rate-limiting enzyme for conversion of protoporphyrin to heme over the full range of lead exposure levels. This may not be the case in children, as suggested by the presence of a definite threshold.

The final issue to be presented in this report concerns the discrepancy between FEP and ZPP. Much of this is probably due to difficulty in establishing correct calibration in relation to the results obtained using the extraction methods as a standard. Beyond that, however, there appears to be an age-related effect wherein the ratio of FEP to ZPP rises from approximately 1.0 shortly after birth to a peak of 1.84 at 3 months, and then gradually falls back to unity (Table 3). Interestingly, the same phenomenon was observed in infant rats. Drift in instrument performance or calibration cannot account for this age-related change. This is so because samples from children of all ages were analyzed at any one point in time, a consequence of the prospective nature of this study wherein children are continually being recruited into the study beginning shortly after birth. It is nevertheless possible that some constituent which alters fluorescence response could exist in the blood of very young children that gradually decreases with age.

The magnitude of the ratio FEP:ZPP was positively related to PbB. Approximately 10–15% of the variance in the ratio could be accounted for by PbB at 9, 12, 15, and 24 months (but not at 18 and 21 months). This is consistent with two cross-sectional studies cited earlier (Karacic *et al.*, 1980; Kaul *et al.*, 1983) showing that the discrepancy between FEP and ZPP increases as PbB increases. Whether this is due to a real difference in the concentration of the two porphyrin species is not certain. Studies are currently underway in our laboratory comparing protoporphyrin determined by extraction as the free base (FEP), as zinc protoporphyrin (ZPP) by front-face fluorometry, and as zinc protoporphyrin deter-

TABLE 3
THE CHANGING RATIO OF FEP/ZPP IN THE BLOOD OF HUMAN INFANTS AND INFANT RATS

Children			Rats ^b		
Age (months)	<i>n</i>	FEP/ZPP ^a	Age (days)	<i>n</i>	FEP/ZPP ^a
10 days	62	0.93	20	121	2.61
3	62	1.84	30	122	1.87
6	57	1.55	90	92	1.46
9	57	1.41			
12	57	1.32			
15	49	1.33			
18	45	1.40			
21	48	1.31			
24	47	1.21			
27	47	1.19			
30	31	1.19			
33	19	1.01			
36	9	0.96			

^a Expressed with correction for molecular weight differences.

^b This includes all rats, some of which had been exposed to lead and some not.

mined by an extraction method. This three-way comparison made on the same samples of blood will be reported at a later date.

Regardless of the source of the discrepancy between PP measured as FEP vs ZPP, the phenomenon has practical significance. Front-face fluorometers measuring ZPP are commonly used as screening devices to identify children with excessive lead exposure. In very young children they would underestimate the likely degree of exposure as it would have been estimated had PP been determined as FEP.

Note added in proof. The three-way comparison between FEP (by extraction), ZPP (by front-face fluorometry), and ZPP (by extraction) has been completed. Samples ($N = 750$) from children between 3 and 39 months of age were analyzed. The ratio of FEP to ZPP (by extraction) was found to be 0.95 with no age-related change in ratio. The previously reported change in ratio of FEP to ZPP (by front-face fluorometer) appears to have been an artifact of instrument calibration.

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