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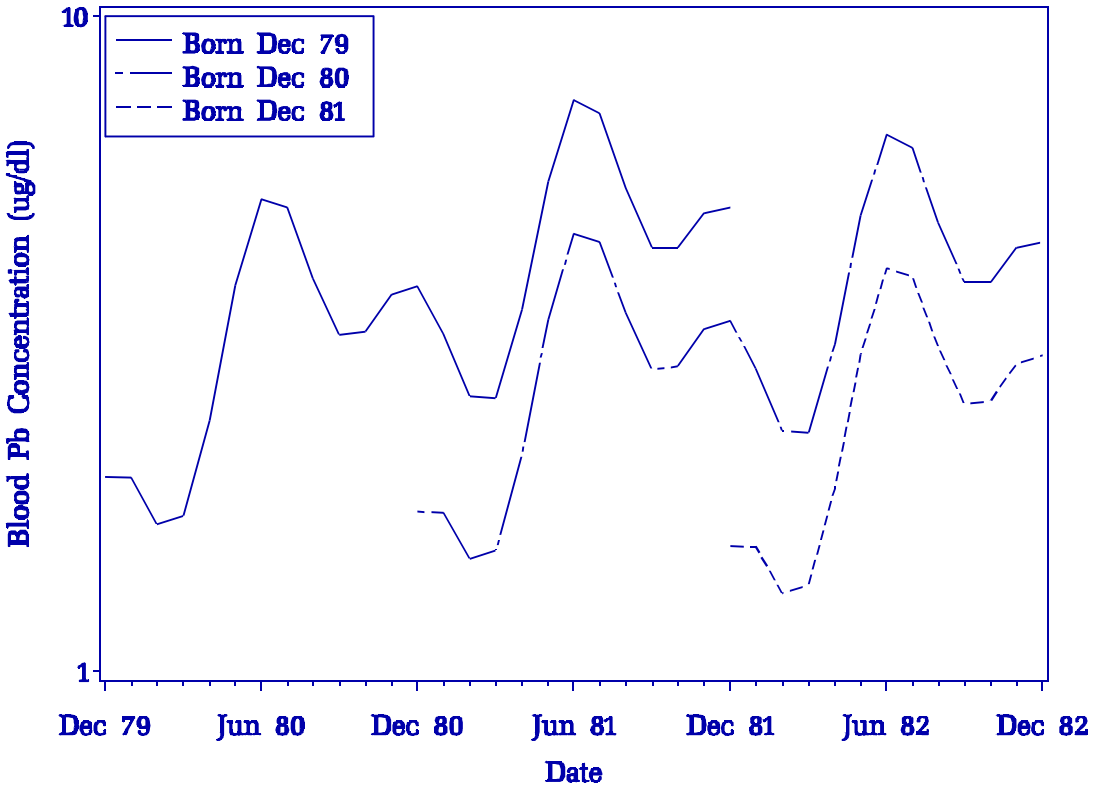
SEASONAL RHYTHMS OF BLOOD-LEAD LEVELS: BOSTON, 1979-1983

FINAL REPORT



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BOSTON, 1979-1983

Technical Programs Branch
Chemical Management Division
Office of Pollution Prevention and Toxics
Office of Prevention, Pesticides, and Toxic Substances
U.S. Environmental Protection Agency
Washington, D.C. 20460

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CONTRIBUTING ORGANIZATIONS

This study was funded and managed by the U.S. Environmental Protection Agency. The study was conducted by Battelle Memorial Institute and Midwest Research Institute under contract to the Environmental Protection Agency. Each organization's responsibilities are listed below.

Battelle Memorial Institute (Battelle)

Battelle was responsible for the development of the analysis approach, for conducting the statistical analysis of the data, and for writing the final report.

Midwest Research Institute (MRI)

Midwest Research Institute was responsible for the completion of the final report.

U.S. Environmental Protection Agency (EPA)

The Environmental Protection Agency was responsible for managing the study, for reviewing the final report, and for arranging the peer review of the final report. The EPA Work Assignment Manager was John Schwemberger. The EPA Project Officers were Jill Hacker and Phil Robinson.

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Executive Summary

Several researchers have observed increased incidence of lead poisoning during summer months. Reasons for seasonal rhythms in blood-lead levels, if such a phenomenon is real, are not immediately apparent. Altered human physiology and higher levels of lead exposure during the summer months have both been postulated as reasons for the temporal variations.

This study was undertaken to examine temporal variation in blood- and environmental-lead levels in data observed for a sample of 249 children in Boston between 1979 and 1983 at the Brigham and Women's Hospital. The two primary objectives of this study were to:

- Determine the extent to which blood-lead levels recorded in the study conducted at the Brigham and Women's Hospital exhibit seasonal variation.
- Determine if any existing seasonal trends in blood-lead levels are correlated with seasonal trends in environmental levels.

For each child in the study, blood-lead and environmental-lead measurements were collected longitudinally over a period of two years. Levels of lead in air, dust, water, and soil were included in the environmental data. Nominally, between two and five measurements were taken for each response (blood or environmental lead) in six month increments.

For the investigation of seasonal trends in the blood and environmental measures, each response was analyzed separately. For statistical reasons, responses were log transformed before analysis. In addition to seasonal variations, the child's date of birth, and age were considered for possible effects. Because significant correlations were observed between the repeated measures taken on individual children, these

correlations were estimated and incorporated into the model estimates.

In determining whether seasonal components of variation existed for each response, the first step was to model monthly averages and determine whether they exhibited systematic monthly variation. Although this approach reflected a significant source of variation, the interpretation is cumbersome. Therefore, because many of the media sampled exhibited higher levels in the summer and lower levels in the winter, a sinusoidal (Fourier) model was investigated for the seasonal component with parameters to represent the magnitude as well as the phase, or month of the peak level. This approach was sufficient for modeling lead levels in the environmental media. However, for blood, where the maximum and the minimum did not occur six months apart, a slightly more complicated Fourier model was required.

Blood-lead levels were found to have highly significant seasonal variations ($p<0.0001$), with the maximum modeled to occur in late June, and the minimum in March. The estimated maximum-to-minimum ratio was 2.5. Without adjusting for other effects, observed geometric mean blood-lead levels by month of year ranged from 2.1 µg/dl in February to 7.5 µg/dl in July. Age of child was also found to be a significant factor; the square root of age was found to be more linearly related to blood-lead levels than was age itself. Consistent with other studies, blood-lead levels in children were found to increase with age.

Air-, floor dust-, furniture dust-, and window sill dust-lead levels all exhibited highly significant seasonal variation. The estimated maximum-to-minimum ratios were 2.3 for air lead, 1.5 for floor dust lead, 1.4 for furniture dust lead, and 1.6 for window sill dust lead. Modeled lead levels for air, floor dust, and furniture dust all had peaks in July. Oddly, peak window sill dust-lead levels were modeled to occur in November. Each of these responses were also significantly

related to the date of measurement, with a decrease observed over time. This is not unexpected due to the concurrent reduction in the use of leaded gasoline.

The extent to which levels of lead in blood were correlated with levels of lead in the environment was also evaluated. As stated above, the seasonal component of variation in blood-lead levels was highly statistically significant. However, after adjusting for the linear effects of environmental measures, the (residual) blood-lead levels did not exhibit even marginally significant seasonal variation (at the 10 percent level).

These results do not necessarily imply a causal relationship between seasonal variation in environmental-lead levels and seasonal rhythms in blood-lead levels. The fact that there were arguably parallel rhythms in blood- and, say, floor-dust lead levels, doesn't imply that the blood-lead levels are *influenced* by the floor-dust lead levels. In particular, if the floor dust-lead levels were to be multiplied by two, while retaining the same blood lead levels, and models were refit, the same statistical significance levels would be reported by this analysis approach. Thus, it would be important to develop a physiological model relating levels of lead in the environment to those in blood, before proclaiming a causal relationship.

Nonetheless in this data, which was collected in the early 1980's from a specific set of children in Boston, there was abundant evidence supporting the existence and parallelism of the seasonal variations among blood-, air-, floor dust-, and furniture dust-lead levels. The three environmental-lead measures peak in July which is very near the blood-lead peak month of June. In addition, the maximum-to-minimum ratios in the environmental-lead measures, ranging from 1.4 to 2.3, are of the same order of magnitude as the blood-lead ratio of 2.5. Thus,

based on the results of this study, it is quite plausible that seasonal variations in environmental-lead levels contribute to the blood-lead rhythms.

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1.0 INTRODUCTION

Several researchers have observed elevated levels of lead contamination and/or increased incidence of lead poisoning during summer months. Reasons for seasonal rhythms in blood-lead levels, if such a phenomenon is real, are not immediately apparent. The temporal variation may result from either altered human physiology[1,2] or higher levels of lead exposure during the summer months. Determining the source of the temporal variation in blood-lead levels may enhance our understanding of the relationship between environmental-lead and its impact on body burden.

There were two primary objectives of this study:

- Determine the extent to which blood-lead levels recorded in the study conducted at the Brigham and Women's Hospital exhibit seasonal variation.
- Determine if any existing seasonal trends in blood-lead levels are correlated with seasonal trends in environmental levels.

This report examines temporal variation in blood- and environmental-lead levels in data observed on 250 children sampled in Boston between 1979 and 1983.

2.0 DATA

Umbilical cord blood samples were collected for 11,837 births at the Brigham and Women's Hospital (formerly the Boston Hospital for Women) from April 1979 to April 1981. Of these, 250 children were selected for an ongoing follow-up study involving environmental and psychological measurements. (One additional child had blood lead measured only at six months. This data was used to estimate the average lead level at six months, but does not permit assessment of seasonal variation for this child.) The selection criteria for the follow-up study included cord blood levels in the highest, lowest, and middle

deciles of the distribution of cord blood-lead levels, residence within 12 miles from the hospital, and likely to be available for two years of sampling. The resulting cohort differs from those studied in other research on lead exposure in that family incomes are relatively high, mothers were likely to be older, White, college educated, and working outside of the home. This analysis was based on the environmental and blood-lead data collected on each of these 250 children up to 24 months of age.

For each child in the study, blood-lead and environmental-lead measurements were collected at various times. The number of measurements made varied from child to child. Table 1 displays the number of observations available for analysis for blood lead and environmental lead at each age level.

TABLE 1. NUMBER OF OBSERVATIONS AVAILABLE FOR EACH RESPONSE ACROSS TIME

	Age of Child in Months					
	0	1	6	12	18	24
Blood	249		220	208	213	202
Air			217		193	125
Floor Dust		247	228		205	191
Furniture Dust		247	231		204	190
Window Sill		240	231		203	189
Dust		245	230		17	17
Water					152	148
Soil						

Typically floor dust was collected in the living room, and furniture dust was collected from the kitchen table. Kitchen sink water was collected after a 4-liter flush. A 1.0-ft^2 template was used to collect floor and furniture dust wipes, and a 0.5-ft^2 template was used to collect window sill dust wipes. Data was not available on deviations from these

protocols, so these measures were analyzed simply as µg. However, the following units can be used to interpret the results:

- Blood (µg/dl)
- Air (µg/m^3)
- Floor Dust (µg/ft^2)
- Furniture Dust (µg/ft^2)
- Window Sill Dust (µg/0.5 ft^2)

As shown in Table 1, collection of water and soil samples were mostly limited to specific sampling campaigns. For those children with replicate measurements, there was a substantial amount of variation among the water- and soil-lead concentrations made during different months for the same child. However, statistical hypothesis tests concluded that there were no systematic variations in the water- and soil-lead concentrations, and therefore, it was reasoned that water- and soil-lead concentrations remain relatively constant throughout a span of two years. Thus, replicate measurements of water- and soil-lead concentrations were averaged and used as a baseline explanatory measure for each child.

Dust-lead measurements should be interpreted as approximate loadings in µg/ft^2. However, exact dimensions of the areas sampled were not available in the data set analyzed. Therefore, in some cases they are only referred to as lead "amounts". Also, information was not available regarding the proximity of these samples to children's activity areas.

3.0 STATISTICAL MODELING APPROACH

The approach to the statistical analyses is described in this section. The five media investigated for seasonal trends in lead were blood, air, floor dust, furniture dust, and window soil dust. Since there was a significant seasonal component in

each response, it was also investigated whether a seasonal component remained present in the blood-lead levels after adjusting for the effects of lead in the environmental media.

For the investigation of seasonal trends in each of the blood and environmental measures, each response was analyzed separately. When evaluating whether there was a significant seasonal component in blood-lead levels, after controlling for differences in environmental lead levels, the data were restricted to those sampling campaigns in which measures of lead in blood, floor dust, furniture dust, window sill dust, and air were obtained (see Table 1). This restricted the data to those collected at 6, 18, and 24 months. Excluding observations with incomplete data reduced the number of observations to 461 on 193 children. The full data set contained 843 observations made on 250 children.

Each blood and environmental lead measure was log transformed before analysis. There were two main reasons for this. First, these responses varied over one to four orders of magnitude. Second, after the log transformation all of the responses were better modeled by a normal distribution, which is an underlying assumption of the statistical analyses.

Based on previous studies[3,4], factors suspected to influence children's blood-lead levels include the child's date of birth, age, and the time of year at which the sample is taken. These factors were included in the models fitted to the blood lead. For environmental lead, the dates of the measurement were included instead of the child's date of birth and age to adjust for overall trends.

3.1 FORM OF SEASONAL VARIATION

The purpose of this analysis is to investigate the presence of a systematic seasonal component of variation in

blood- and environmental-lead levels. It was not known at the outset whether such a cyclic component existed, let alone its functional form. However, it was assumed that a complete cycle for a seasonal variation, if present, would have a period of twelve months.

The information in the data on date of sampling is limited to month and year of sampling. Therefore, the simplest and most general approach is to consider month as a class variable with twelve possible levels. This approach allows each month of the year to have its own mean after adjusting for other factors in the model:

$$y_i = x_i\$ + \alpha_{m(i)} + \varepsilon_i, \qquad (1)$$

where x_i denotes the row vector of covariates for each of the other factors in the model, $ denotes the column vector of parameters for the covariates, m(i) denotes the month number for the ith observation, α_m denotes the deviation from the mean for the mth month $\left(\sum_{m=1}^{12} \alpha_m = 0\right)$ and ε_i denotes random error. A limitation to this approach is the interpretation of the 12 values of α_m; one is left with the burden of understanding 12 different monthly averages. A second limitation is in the estimation of the variance of these monthly parameters. If a simpler model with fewer parameters underlies this cyclic variation, then estimates of its parameters would be more precise.

Since for many of the media sampled, lead levels were highest during the summer and lowest during the winter, a sinusoidal (Fourier) form of the model was investigated:

$$y_i = x_i\$ + \alpha \cos((m(i)-N)*2\pi/12) + \varepsilon_i,$$

where " denotes the amplitude and N denotes the phase (in months) of the sinusoidal trend. The phase represents the time in months at which the maximum value of the sinusoidal trend, the amplitude, occurs. Freedom to vary the phase is necessary because it is not known a priori when the maximum should occur. A model with a single sinusoidal term implicitly assumes that minimum levels occur six months after maximum levels. Because this phenomenon was not observed in all media, additional Fourier terms were included in the models allowing for peaks and valleys to be less or more than six months apart. The specific forms of these models are explained in Sections 3.3 and 3.4.

Models employing the Fourier parameterization for the seasonality effect were only fitted to the data if the month-to-month variation was determined to be statistically significant based on a model utilizing the twelve levels of $"_i$. Otherwise, it was reasoned, other modeled factors satisfactorily explain the variation observed in lead levels.

3.2 CORRELATIONAL DEPENDENCE AMONG REPEATED MEASURES

It is important that the model chosen to fit to the data takes into account possible systematic correlations among repeated observations on the same child. This section describes the approach for modeling for correlational dependence.

It is sensible to assume that measurements made on the same child are correlated. However, the structure of this correlation is not known. For instance, measurements taken farther apart in time (on a given child) may be less correlated than those taken closer together in time.

The matrix presented in Figure 1 illustrates the structure of the covariance assumed among repeated blood measures taken on each child, excluding the cord blood measure.

		j=1 6 months	j=2 12 months	j=3 18 months	j=4 24 months
i=1:	6 months	F_{11}	F_{12}	F_{13}	F_{14}
i=2:	12 months	F_{21}	F_{22}	F_{23}	F_{24}
i=3:	18 months	F_{31}	F_{32}	F_{33}	F_{34}
i=4:	24 months	F_{41}	F_{42}	F_{43}	F_{44}

Figure 1. Unstructured covariance matrix.

For example, F_{23} represents the covariance between measures taken at 12 months and 18 months on the same child, after controlling for covariates. By definition $F_{ij} = F_{ji}$ for i,j = 1,2,3,4. The diagonal terms, F_{11}, F_{22}, F_{33}, F_{44} represent the variances for measures taken at each of the four increasing ages, respectively, after correcting for other factors in the model.

Without making any assumptions about the structure of the covariance matrix, Figure 1 represents the most general form possible. This is referred to as unstructured. No relationships among different covariances are assumed in the unstructured form.

Below are two more structures which were considered: autoregressive and random child effect structures.

$$F^2 \begin{vmatrix} 1 & D & D^2 & D^3 \\ D & 1 & D & D^2 \\ D^2 & D & 1 & D \\ D^3 & D^2 & D & 1 \end{vmatrix}$$

Autoregressive. The variance of blood-Pb concentration is assumed to be the same for children of all ages. The correlation between repeated measurements on the same child is assumed to decrease with time between measurements.

$$\begin{bmatrix} F^2+F_1^2 & F_1^2 & F_1^2 & F_1^2 \\ F_1^2 & F^2+F_1^2 & F_1^2 & F_1^2 \\ F_1^2 & F_1^2 & F^2+F_1^2 & F_1^2 \\ F_1^2 & F_1^2 & F_1^2 & F^2+F_1^2 \end{bmatrix}$$

Random Child Effect. Variances are assumed equal for all measures. The correlations between repeated measures on the same child are assumed to be equal regardless of time between measurements. Observations on different children are assumed to be uncorrelated.

The tradeoff among these covariance structures is that although a more general model requires fewer assumptions about correlations, it requires the estimation of more parameters. To determine an appropriate covariance structure, the Akaike Information Criterion[3] (AIC) was used. The AIC provides a means of comparing models with the same fixed effects but different covariance structures. It is defined as

$$AIC = 2p(2)-2q,$$

where p(2) is the calculated log likelihood and q is the number of parameters. This function adjusts the log-likelihood for the number of parameters in the model, including the terms for both fixed effects and covariance. The model having the largest AIC is considered to provide the best fit to the data. Due to the high AIC and the fact that variability was observed to decrease with the age of the child, the unstructured covariance was chosen as most appropriate. Table 2 displays the estimated unstructured covariance matrix for the model fit to the blood-lead data.

TABLE 2. ESTIMATED COVARIANCE MATRIX FOR (LOG) BLOOD LEAD ON AN INDIVIDUAL CHILD

Age (months)	6	12	18	24
6	3.92	0.17	0.21	0.75
12	0.17	2.85	0.49	0.47
18	0.21	0.49	1.93	0.86
24	0.75	0.47	0.86	1.93

For illustrative purposes, Table 3 displays the correlation matrix associated with the covariance matrix in Table 2.

TABLE 3. ESTIMATED CORRELATION MATRIX FOR (LOG) BLOOD LEAD ON AN INDIVIDUAL CHILD

Age (months)	6	12	18	24
6	1.00	0.05	0.08	0.27
12	0.05	1.00	0.21	0.20
18	0.08	0.21	1.00	0.45
24	0.27	0.20	0.45	1.00

For consistency, no assumptions were made about the correlation structures of repeated environmental measures at a child's home. Therefore, unstructured covariance matrices were assumed for each media.

The following sections describe the specific models fit to each of the responses.

3.3 BLOOD LEAD

Models of the following form were fit to blood-lead concentrations:

$$LB_{ia} = \$_o + \$_1 DOB_i + \$_2 a^{1/2} + S_{m(ia)} + ,_{ia}, \qquad (2)$$

where

i = child index,

a = age of child in months,

LB_{ia} = the logarithm of the measured concentration of Pb in the blood (µg/dl) for the ith child at age a,

$\$_o$ = intercept,

DOB_i = date of birth of ith child,

$\$_1$ = linear effect of date of birth,

$\$_2$ = linear effect of age,

$S_{m(ia)}$ = seasonal effect for month in which child i was age a, and

$,_{ij}$ = random error ($,_{ij}$, $,_{yz}$ correlated only if i=y; i.e., measures are from same child).

The model includes a date of birth effect to trace changes between different "birth cohorts" of children, and an age effect to reflect changes as a child grows regardless of his/her year of birth. To test whether there was confounding between the age and date of birth effects, the model was also fit without the date of birth effect. The estimates and significance levels obtained for the age effect in both cases were very similar. Therefore, it was concluded that the two factors were not confounded and both factors were included in our final model.

The seasonal effect is described by the following Fourier model,

$$S_{m(ia)} = \alpha_1 \cos((m(ia)-N_1)*2\pi/12) + \alpha_2 \cos((m(ia)-N_2)*2\pi/6),$$

where

α_1 = amplitude of annual cyclic variation,

N_1 = phase of annual cyclic variation (time when peak occurs in months),

α_2 = amplitude of biannual cyclic variation, and

N_2 = phase of biannual cyclic variation (time when first peak occurs).

The two-phase cyclic model was selected as an objective compromise between a simple sinusoidal component with unknown phase, and a model including a different term for each month of the year. Certainly there was no reason to assume, a priori, that the shape of the seasonal component would fit a sine wave perfectly. A two-phase model was chosen by repeatedly adding Fourier terms with unspecified phase and period (12/k) months, k=1,2,3... until the relative reduction of error was insignificant. For blood lead this process was halted after adding the biannual cyclic term, which cycles twice per year, to the simple annual cyclic term.

3.4 ENVIRONMENTAL LEAD

Models of the following form were fit to levels of lead in air, floor dust, furniture dust, and window sill dust:

$$(LA_{ia}, LFL_{ia}, LFU_{ia}, LWS_{ia}) = \beta_o + \beta_1 t_{ia} + S_{m(ia)} + \varepsilon_{ia}, \qquad (3)$$

where the factors not defined above for the blood-lead model are

LA_{ia} = the logarithm of the measured indoor air-lead concentration (µg/m^3) in the ith child's home at age a,

LFL_{ia} = the logarithm of the measured indoor floor dust-lead loading (µg) in the ith child's home at age a,

LFU_{ia} = the logarithm of the measured indoor furniture dust-lead loading (µg) in the ith child's home at age a,

LWS_{ia} = the logarithm of the measured window sill lead loading (µg) in the ith child's home at age a,

t_{ia} = date when ith child was *a* months old, and

$\$_1$ = linear effect of date.

The seasonal effect is described by the following Fourier model, for all four environmental media:

$$S_{m(ia)} = "_1\cos((m(ia)-N_1)*2B/12).$$

3.5 BLOOD-LEAD LEVELS ADJUSTED FOR ENVIRONMENTAL LEAD LEVELS

A model was fit to the combined blood- and environmental-lead data to determine whether there were seasonal variations in blood-lead levels above and beyond those explained by changes (perhaps seasonal) in levels of lead in surrounding environmental media. The equation for this model is as follows:

$$LB_{ia} = \$_o + \$_1\ DOB_i + \$_2 a + "_{m(ia)} + "_1 LA_{ia} + "_2 LFL_{ia} + "_3 LFU_{ia} + "_4 LWS_{ia} + "_5 LS_i + "_6 LWA_i + ,_{ia}, \quad (4)$$

where

LS_i = the logarithm of the measured concentration (ppb) of lead in soil outside the home of the ith child, and

LWA_i = the logarithm of the measured concentration (ppb) of lead in water at the home of the ith child.

Notice that the most general seasonal component, α_m, is used here, as was the approach for fitting models to each medium separately. (See equation (1).) As mentioned in Section 3.1, the convention was to first fit models with the most general seasonal formulation, but to investigate simplification only after determining that the variation was significant after controlling for other factors.

It is important to note that several of the predictor variables in this model are subject to error (e.g., each of the environmental lead measures). These errors can potentially bias estimates of these factors downward (in magnitude). The data did not permit an assessment of the magnitude of these errors, and therefore, it was not possible to adjust the estimates for the measurement error. Thus, it is reasonable to assume that our estimates of these effects are conservative.

4.0 RESULTS

This section presents the statistical analysis results. Section 4.1 provides descriptive statistics. Section 4.2 describes the outlier analysis. This is followed by the model-fitting results. Section 4.3 presents the results of fitting the seasonal model to blood-lead levels. Corresponding model-fitting results for the four environmental-lead responses are discussed in Section 4.4. Finally, in Section 4.5 we discuss results of fitting blood-lead levels to the seasonal model after controlling for environmental-lead levels.

4.1 DESCRIPTIVE STATISTICS

For each of the responses measured there was evidence of periodicity. Table 4 displays estimates of the geometric mean levels (on original scale) of lead in blood, air, floor dust, furniture dust, and window sill dust by month. The number of blood samples collected is also listed by month. Approximate log standard errors of these estimates are provided for each media, along with the observed log standard deviations. The averages are often highest in the summer months (June, July, August) and lowest in the winter months (February, March). Four of the measures, blood, furniture dust, air, and window sill dust appear to have a relative minimum in September. For reasons discussed below, cord blood measures were excluded from the calculations for blood lead.

Figure 2 displays observed geometric average blood-lead levels with 95 percent confidence bounds for each month of the study. These averages do not control for any covariates (such as age of child or date of birth). This figure reveals a slight cyclic variation, but does not show any sign of general change over time. It is not possible to distinguish between within-child effects (such as age) and between-child effects (such as date of birth) from this plot. The statistical modeling results presented next, allow this separation. It is shown that the within- and between-child effects actually counteract each other in this figure.

4.2 OUTLIER ANALYSIS

Multivariate outlier analyses were performed to identify unusual data points. A Hotelling T^2 test was applied to identify potential outliers based on the distance between an observation and the average of the remaining observations relative to the covariance matrix of the remaining observations.

The test was applied to the subset of the sampling campaigns in which each of the five measures: blood, air, floor dust,

TABLE 4. GEOMETRIC MEANS AND LOG STANDARD DEVIATIONS OF VARIOUS MEASURES BY MONTH

Month	Number of Blood Measures	Blood (µg/dl)	Air (µg/m^3)	Floor Dust (µg)	Furniture Dust (µg)	Window Sill Dust (µg)
January	72	3.10	0.07	3.06	2.46	10.54
February	54	2.13	0.06	2.58	2.12	8.03
March	100	2.87	0.05	2.39	1.85	8.54
April	74	3.87	0.06	3.54	2.58	10.49
May	68	4.64	0.10	4.43	3.27	12.25
June	63	5.13	0.11	3.65	2.68	8.94
July	77	7.52	0.10	4.40	2.58	13.87
August	51	4.05	0.11	5.58	3.92	16.78
September	96	2.49	0.09	3.95	2.63	11.64
October	73	3.06	0.10	3.52	3.53	19.39
November	53	4.51	0.07	4.98	3.36	17.32
December	62	3.61	0.05	3.32	2.90	12.44
Approximate Log Standard Error*		0.20	0.15	0.14	0.13	0.18
Log Standard Deviation**		1.65	0.97	1.16	1.08	1.54

* The actual standard error of the mean log-transformed values varied due to sample size differences across months. These numbers represent the average value of these log-standard errors.

** This represents the estimated within-month standard deviation of the log-transformed responses.

furniture dust, and window sill dust were obtained. This test identified two observations as outliers at the 10 percent level; Table 5 displays the observations. The last column of the table provides the observed significance level of the Hotelling T^2 test for the observations. A Bonferroni-type critical value is used to compensate for the numerous simultaneous tests performed and to maintain a 10% overall significance. The second to last column is the appropriate threshold, based on the Bonferroni adjustment, to compare the observed significance level with. The

6th month floor dust measure on child 804391 was 600 µg, which was the largest value of floor dust lead when all 5 responses

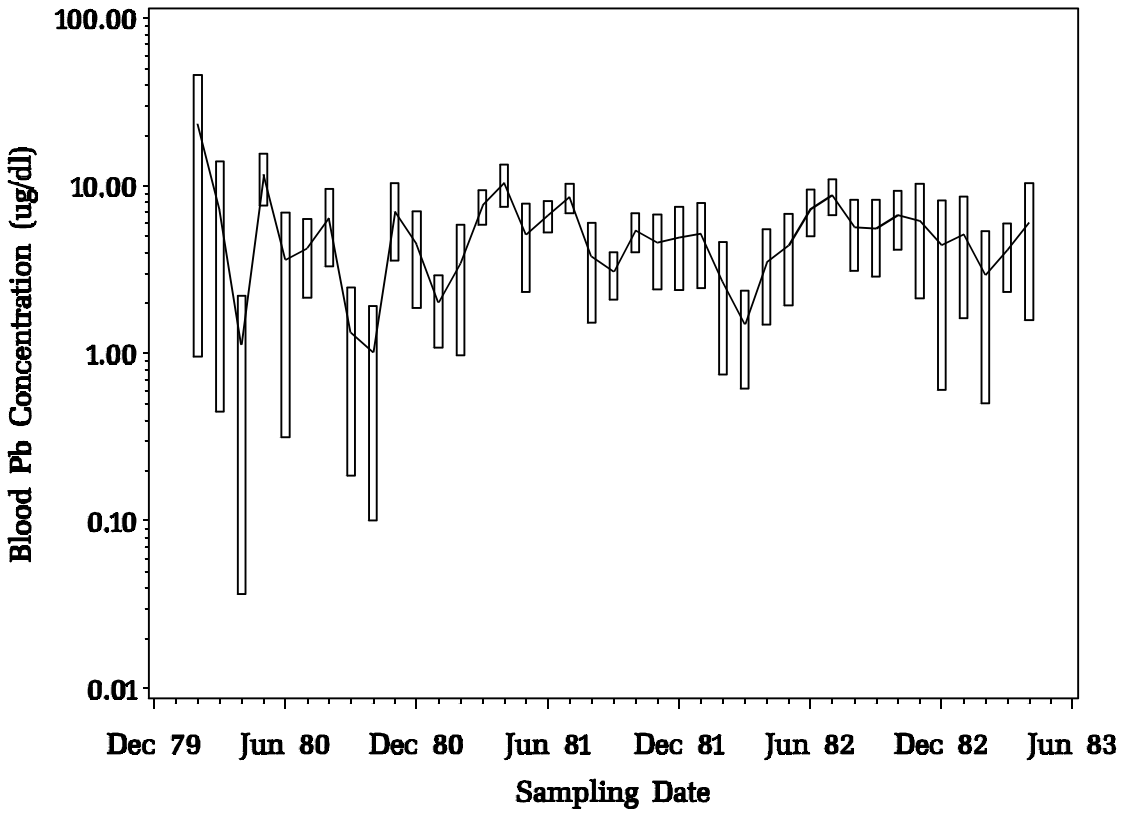

Figure 2. Geometric average blood-lead levels with 95% confidence bounds by month and year.

were measured. The 18th month blood-lead measure on child 805874 was registered as 0 µg/dl (below the detection limit), but the furniture and window sill dust-lead measures were highest among all observations with a blood measure of 0 µg/dl. Models were fit with and without these two data points to evaluate their effect on the conclusions. Since models fitted to all data yielded the same conclusions as models fitted to the data with outliers removed, results presented herein are based on analyses including all of the data.

TABLE 5. MULTIVARIATE OUTLIERS

ID	Age	Lead Levels						
		Blood (µg/dl)	Air (µg/m^3)	Floor Dust (µg)	Furniture Dust (µg)	Window Sill Dust (µg)	Bonferroni 10% Significance Cutoff	Observed Significance Level
804391	6	4	0.19	600	210	600	1.95×10^{-4}	3.29×10^{-5}
805874	18	0	0.10	1	12	160	1.95×10^{-4}	1.35×10^{-4}

Lower order principal components were also used to visually inspect the data for outliers. The lower order principal components, by construction, are linear combinations of the factors with the smallest variance. Therefore, outstanding realizations of these principal components are often examined as potential outliers. There were no unusual observations noted in a plot of the fourth and fifth of five principal components. The two outliers mentioned above were typical data points as measured by these principal components.

4.3 MODELING RESULTS FOR BLOOD LEAD

Table 6 displays estimates of mean blood-lead concentration by age, adjusting for date of birth and month of measurement. (Least-squares means are presented which represent the modeled mean for each age, holding date of birth at the average observed level, and averaging across the 12 months in which measurements were collected.) The average blood-lead level at 6 months was significantly less than those observed at the other ages, but cord blood-lead levels were actually higher than those observed at 6, 12, 18, and 24 months (though not significantly). Since cord blood lead may be more associated with the mothers' blood-lead level, and does not appear to be consistent with measurements taken at different time points, cord blood-lead levels were excluded from the subsequent analyses.

The results displayed in Table 6 also suggest that the increase in blood lead has a nonlinear relationship with age. Since at best there are only four time points on each child, only one-parameter models were considered to fit this curvature. A model containing a term for the square root of age appeared to provide an adequate fit.

TABLE 6. MEAN BLOOD-LEAD CONCENTRATION (µg/dl) BY AGE (CONTROLLING FOR RATE OF BIRTH AND MONTH OF MEASUREMENT)

Age (months)	Mean	Lower Bound	Upper Bound
0	5.2	4.5	5.9
6	2.4	1.8	3.1
12	4.0	3.2	5.1
18	4.4	3.6	5.3
24	4.3	3.5	5.1

Table 7 displays the results of fitting the model to blood-lead levels. Age of child was found to be significant, date of birth was not; the magnitude of these effects is discussed below. As described earlier, both annual and biannual cyclic components of variation were used in the model to describe seasonal variation. Both components were sinusoidal. Phase is estimated in months. For interpretation, we assumed a phase of 1.0 corresponds to January 15, phase 2.0 corresponds to February 15, etc. Added together, the peak of the systematic seasonal component occurred in late June; lowest levels occurred in early March. The magnitude of this seasonal difference was 0.93 on a log scale. This corresponds to a multiplicative increase of 2.54 in blood lead during late June over levels in early March on the same child. This number is calculated as the range of the seasonal component over a 12-month period.

TABLE 7. RESULTS OF FITTING MIXED ANOVA MODEL WITH CYCLIC SEASONAL COMPONENTS TO BLOOD MEASURES

Response	Factor		Estimate	Std. Error	Significance
Blood (843 obs.)	Intercept	$\$_0$	0.792	0.327	
	Date of Birth (in months)	$\$_1$	-0.010	0.013	0.4260
	Age (in months, square root)	$\$_2$	0.194	0.055	0.0005
	Cyclic Annual Component (Amplitude)	$"_1$	0.319	0.059	<0.0001[1]
	Cyclic Annual Component (Phase)	N_1	6.659 (Jul. 5)	0.179 (5 days)	
	Cyclic Bi-Annual Component (Amplitude)	$"_2$	0.264	0.094	0.0198[2]
	Cyclic Bi-Annual Component (Phase)	N_2	5.999 (Jun 15, Dec. 15)	0.381 (11 days)	

[1] Statistical significance of overall seasonal component.
[2] Significance of bi-annual cyclic component after controlling for annual cyclic component.

Figure 3 displays the multiplicative factor corresponding to the modeled seasonal component of variance for each month of the year connected by a solid line. The bars overlaid on this plot display the mean and confidence bounds for the residuals of observed blood-lead levels after controlling for age and date of birth. There is clearly a seasonal component present in the residuals which parallels the estimated seasonal component. To facilitate quantification of the cyclic component, the values in this plot were scaled to force the minimum multiplier to be 1.00. Reference lines were placed at 1.00 and 2.54, where the minimum and maximum occurred. The minimum appears in March, the maximum appears in June with a value close to that modeled for July. The values on this plot do not represent predicted lead levels, but rather the ratio of average lead levels for different months of the year to the level for the month with the lowest average levels, i.e. March (after controlling for age and date of birth).

Figure 4 illustrates the relative impact of each of the factors in the complete model fitted for blood lead. The figure covers a span of about three years from December 1979 through

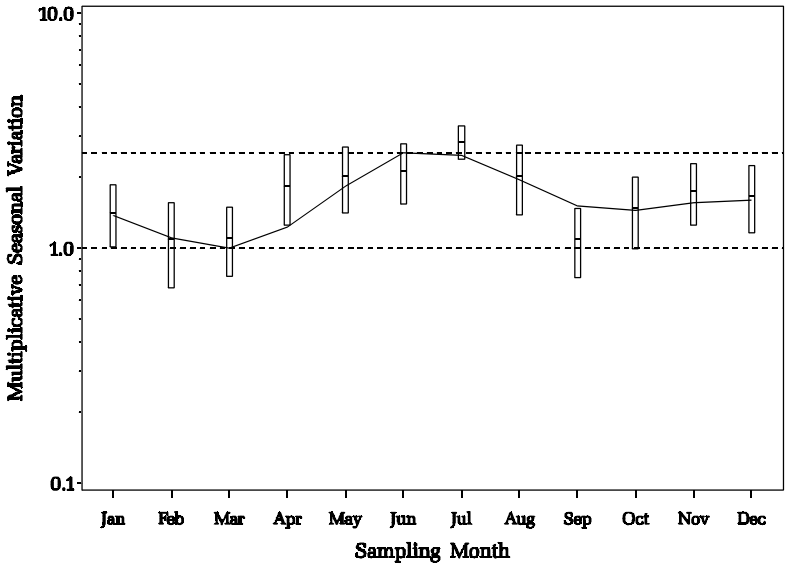

Figure 3. Modeled seasonal variation and residual blood-lead levels after controlling for age and date of birth effects.(Bars represent 95% confidential bounds for blood-lead residuals.)

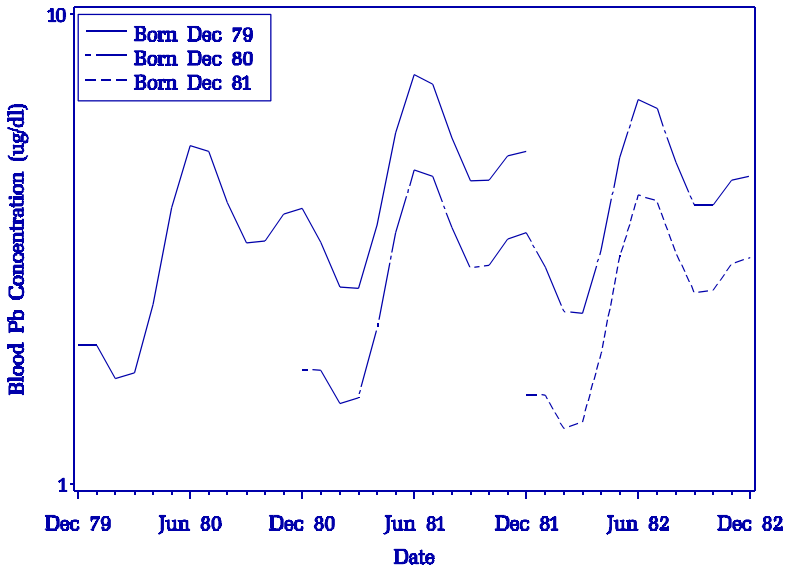

Figure 4. Modeled blood-lead levels over time,showing effects of date, child's age, and seasonal variation.

December 1982. There are three curves displayed in the plot. Each curve represents predicted blood-lead levels for a child born at a different time. The solid curve which begins earliest and represents the modeled blood-lead levels for a child born in December 1979. The most striking feature of this plot is the extensive cyclic variation about a generally increasing trend. This increase reflects the significant age effect. However, the cyclic variation outweighs the effect of age. Notice that for children born in December, although the estimated minimum seasonal variation occurs in March (Figure 3), when the age effect is added the relative minimum lead levels occur in February.

Because data was collected for only two years on each child, the solid curve terminates after two years. The second and third lines represent modeled blood-lead concentrations for children born one and two years later (in December 1980 and December 1981). There was a slight (and statistically insignificant) decrease in blood-lead levels with date of birth. This is reflected by the slightly lower starting points for children born later.

4.4 MODELING RESULTS FOR ENVIRONMENTAL LEAD

The final models fitted to the environmental media were described in Section 3.4. These models included a linear effect for date and a cyclic seasonal effect. An unstructured error variance matrix was assumed for the repeated measures at each child's home. Both the date effect and the seasonal effect were statistically significant for each of the four environmental media investigated.

Table 8 displays the estimated parameters for the fixed effects along with the standard errors and significance levels. Since the fitted model included only a single Fourier component for each media the phase listed equals the time, in months, of

the predicted maximum seasonal variation. For each medium, the significance of both parameters of the cyclic component is tested

TABLE 8. RESULTS OF FITTING MIXED ANOVA MODEL WITH CYCLIC SEASONAL COMPONENTS TO ENVIRONMENTAL MEASURES

Response	Factor	Estimate	Std. Error	Significance
Air-Lead (535 Obs.)	Intercept	1.751	0.153	
	Date	-0.025	0.005	<0.0001
	Cyclic Annual Component (Amplitude)	0.408	0.059	<0.0001
	Cyclic Annual Component (Phase)	6.815 (Jul. 9)	0.141 (4 days)	
Floor Dust-Lead (871 Obs.)	Intercept	1.503	0.123	
	Date	-0.008	0.004	0.0391
	Cyclic Annual Component (Amplitude)	0.203	0.040	<0.0001
	Cyclic Annual Component (Phase)	6.896 (Jul. 12)	0.198 (6 days)	
Furniture Dust-Lead (872 Obs.)	Intercept	1.359	0.115	
	Date	-0.012	0.003	0.0002
	Cyclic Annual Component (Amplitude)	0.184	0.038	<0.0001
	Cyclic Annual Component (Phase)	7.349 (Jul. 25)	0.217 (7 days)	
Window Sill Dust-Lead (863 Obs.)	Intercept	2.823	0.156	
	Date	-0.012	0.005	0.0107
	Cyclic Annual Component (Amplitude)	0.240	0.053	<0.0001
	Cyclic Annual Component (Phase)	10.588 (Nov. 3)	0.237 (7 days)	

simultaneously and was highly significant in all cases. Notice how close together in time the maxima are predicted for air lead, floor dust lead, and furniture dust lead. Each of these peaks are predicted to occur in July.

Figures 5 through 8 display modeled lead levels as solid lines for air, floor dust, furniture dust, and window sill dust, respectively. Overlaid on these plots are the observed geometric means by month with confidence bounds. Considering the numbers of observations represented, the models appear to fit well for so few parameters. Notice the slight decreasing trend in each response over time. The most drastic decrease was observed for air-lead levels. One obvious reason for this would be the coincident reduction in use of leaded gasoline in automobiles.

Figure 9 displays the modeled seasonal components of variation for the five responses overlaid for comparison. Each curve has been adjusted for trend effects. This figure allows direct comparison of the phase and magnitude of the estimated seasonal components between the four modeled media. The fluctuations observed in blood were larger than those observed in the environmental measures, but were similar in phase to fluctuations of lead in floor dust, furniture dust, and air. Window sill dust lead was predicted to reach its peak 4 to 5 months after blood lead.

4.5 MODELING RESULTS FOR BLOOD LEAD AFTER ADJUSTING FOR ENVIRONMENTAL FACTORS

The extent to which levels of lead in blood were correlated with levels of lead in the environment was also evaluated. Each of the measured environmental media were included in a model, along with date of birth and age effects, and a class month effect to evaluate seasonal rhythms in blood-lead levels. This model is described in detail in Section 3.5.

Whereas the seasonal component of variation in blood lead was significant before adjusting for the linear effects of the environmental measures (with class month effect, p=<.0001), it was not significant after adjusting for these effects (p=.1148). The significant predictive factors in this model were

floor dust lead, and age of the child. The effect of soil lead on blood lead was marginally significant. Recall that only

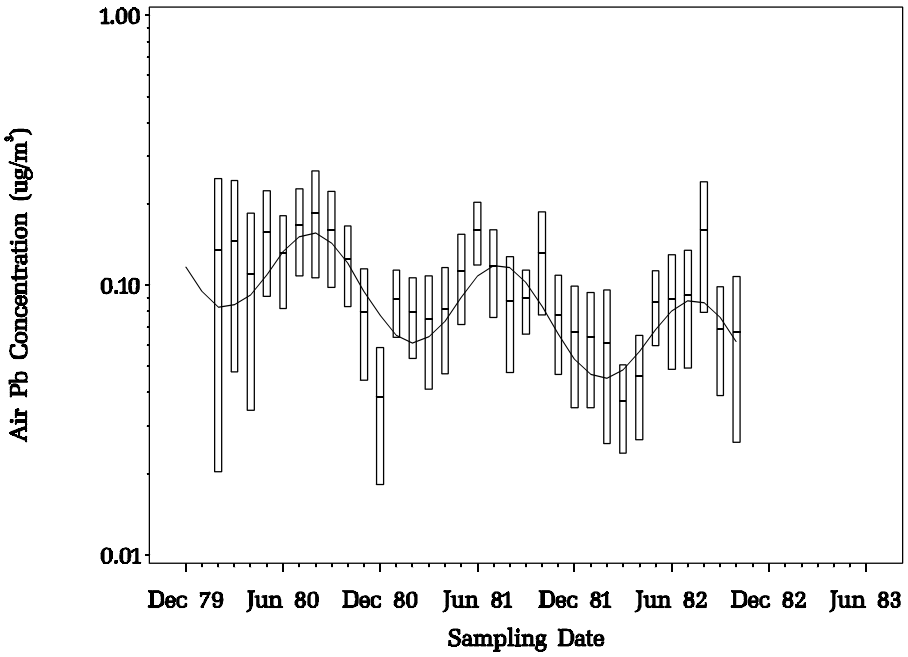

Figure 5. Modeled air-lead levels.

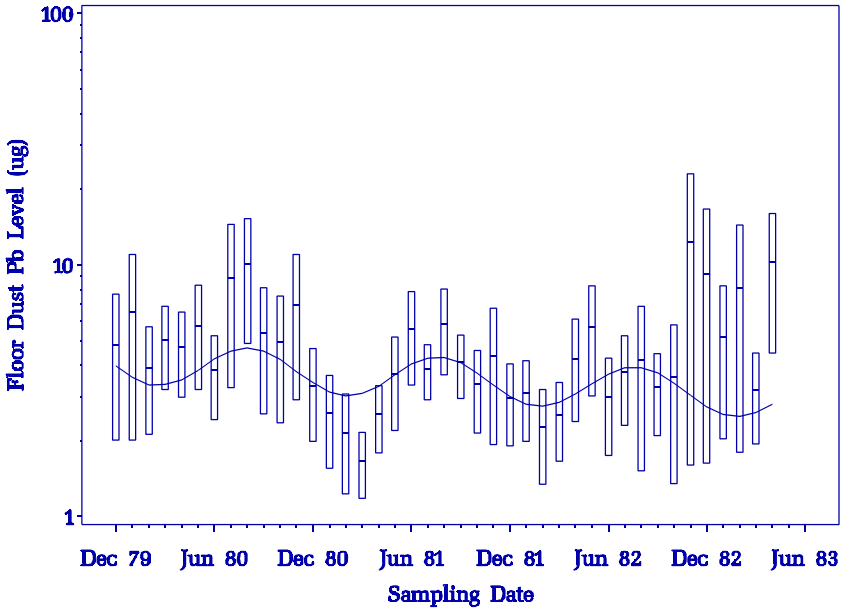

Figure 6. Modeled floor dust-lead levels.

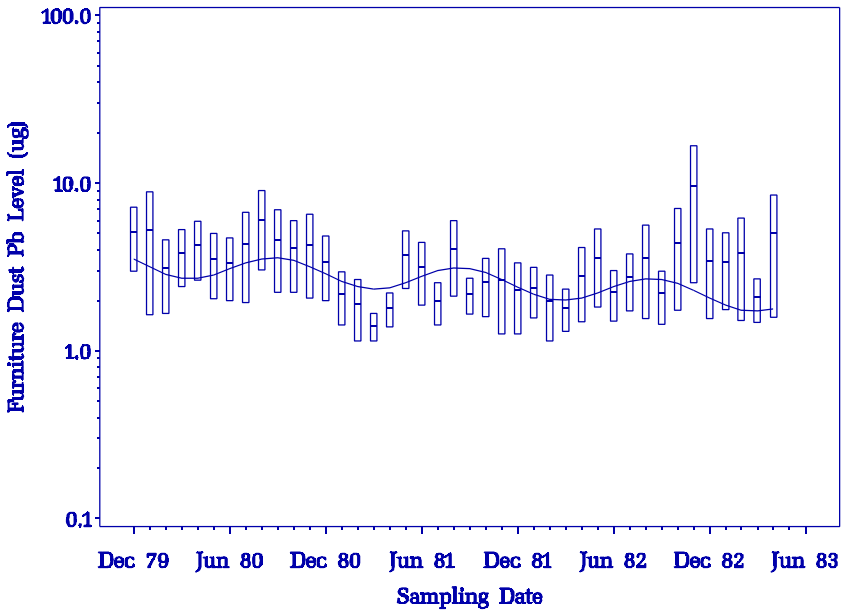

Figure 7. Modeled furniture dust-lead levels.

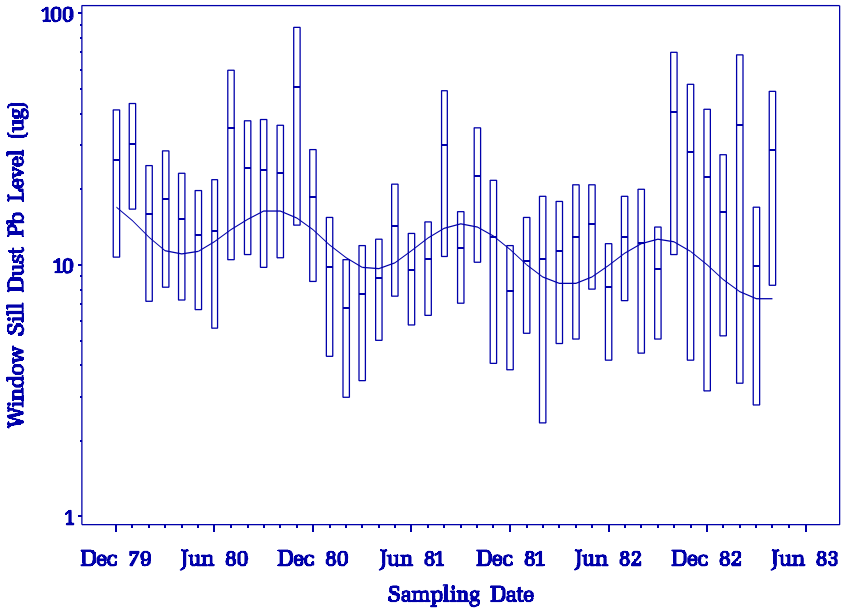

Figure 8. Modeled window sill dust-lead levels.

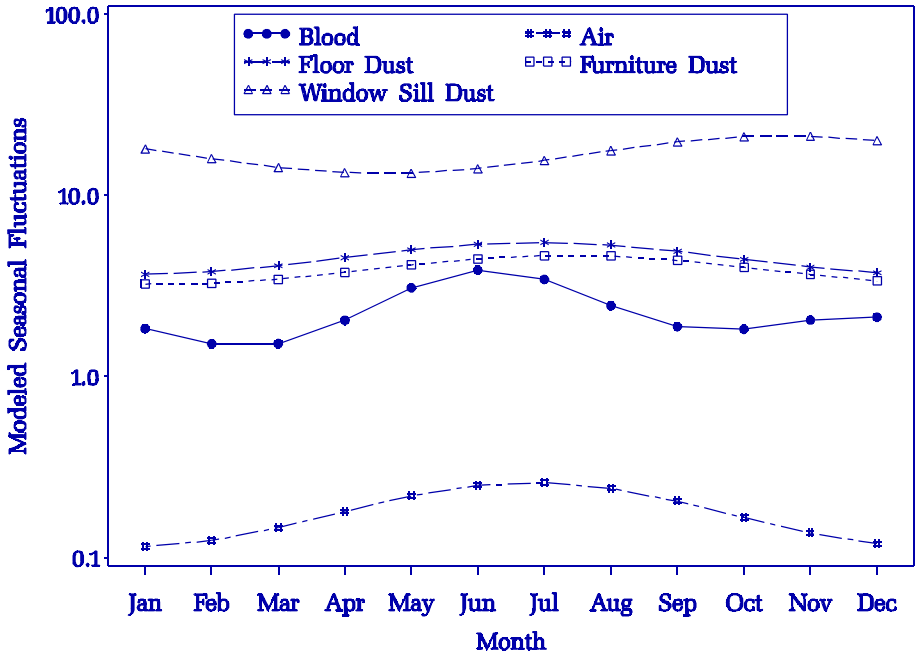

Figure 9. Estimated seasonal component of blood and environmental lead levels, overlaid.

average levels of lead in soil and water were available for each child.

Since the remaining environmental media were not observed as significant, a model was fit with age and date of birth and the significant environmental factors. Under the smaller model, there remained a significant month effect. Upon further investigation, it was found that adjusting for air-lead levels in addition to floor dust and soil lead levels reduces the monthly component of variation from very significant to marginally significant. Table 9 displays the results of the final model fit. Thus, much, but not all, of the seasonal variation in these blood-lead levels can be attributed to variations in floor dust lead, soil lead, and air lead.

One must realize that the fact that certain environmental factors were observed to be statistically significant in these models does not necessarily mean that there is a causal relationship. For example, just because floor dust-lead levels were observed as a highly significant effect in predicting blood-lead levels, one cannot conclude that seasonal increases in floor dust-lead levels cause corresponding increases in blood lead.

TABLE 9. RESULTS OF FITTING MIXED ANOVA MODEL WITH CYCLIC SEASONAL COMPONENTS TO BLOOD MEASURES ADJUSTING FOR ENVIRONMENTS LEAD MEASURES

Response	Factor		Estimate	Std. Error	Significance
Blood-lead (463 Obs.)	Intercept	$\$_0$	-0.071	0.539	
	Date of Birth	$\$_1$	0.013	0.015	0.3779
	Age	$\$_2$	0.046	0.010	<0.0001
	Floor Dust Lead	$"_2$	0.320	0.063	<0.0001
	Soil Lead	$"_5$	0.115	0.063	0.0677
	Air Lead	$"_1$	0.049	0.060	0.4099
	Month (12 level class variable)				0.1047

5.0 DISCUSSION

The magnitude of the seasonal variation observed in these data is substantial. The reader is reminded that for this study, blood- and environmental-lead levels were measured on these children every six months at best. To detect a cyclic component, one must observe levels which are systematically higher at one time during the year than at another. Since base blood-lead levels vary substantially across children, it is best to examine repeated measures on the same child. However, if the cyclic component were simply sinusoidal, the greatest within

child contrasts would be observed (in this study) for children born in the month with the highest or lowest seasonal component of variation. For example, if the seasonal variation was such that the lowest value occurred in January, and the highest value occurred in July, then children born in January or July would have the best chance of exhibiting major seasonal deviations if they were sampled at 0, 6, 12, 18, and 24 months of age. Figures 10 and 11 illustrate this phenomenon for children born in January and July. Figure 10 displays the observed blood-lead levels for five selected children born in January. Figure 11 displays blood-lead levels for five other children born in July. These figures illustrate the type of variation in blood-lead levels experienced by the children studied. Because measures were only taken six months apart, January and July were chosen because they portray the greatest seasonal contrasts.

However, if a child was born in April or October the actual differences in the blood-lead levels at the times measured, due to seasonal variation, would be near zero. Plots of these levels would be more flat. These children would provide little added value in estimating the magnitude of seasonal variation. Moreover, since measures are taken six months apart it is difficult to estimate the parameters of higher-order cyclic components. Thus, if feasible, monthly or quarterly measures would provide much better information about seasonal variation from children not born in months where the maximum or minimum occurs.

The reader is also reminded that the use of leaded gasoline was being phased out during the time this study was conducted. It is possible that leaded gasoline was the source of much of the lead in the environment - particularly in the air. Since more travelling is done in the summer than in the winter, changes in emissions from leaded gasoline may have been a significant contributor to the observed seasonal variations in environmental lead levels. Today, the use of leaded gasoline has

been virtually eliminated. Therefore, seasonal variations may be less pronounced today.

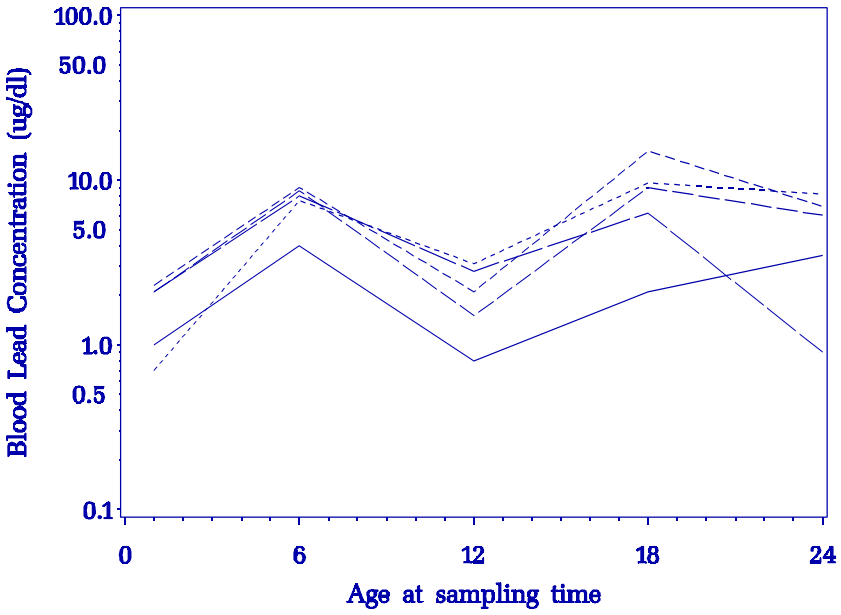

Figure 10. Blood-lead levels for five selected children born in January.

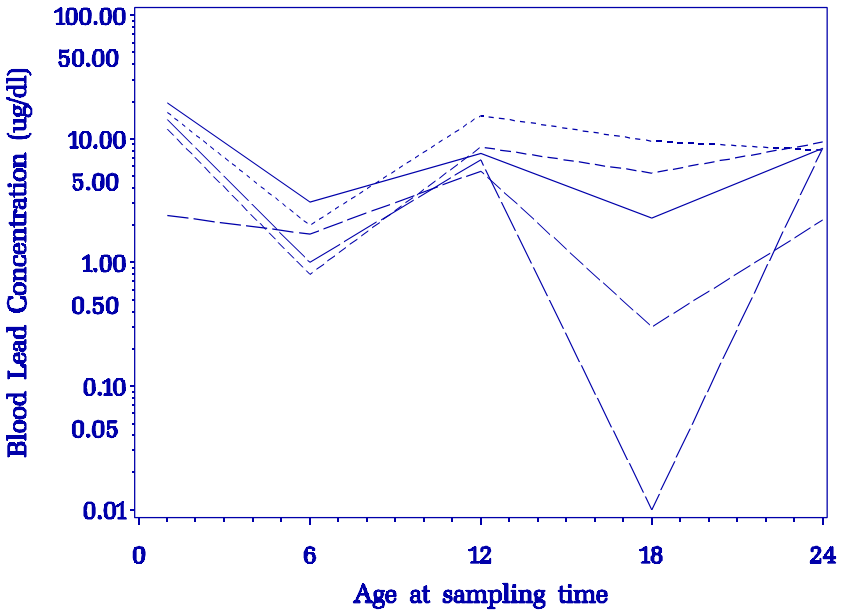

Figure 11. Blood-lead levels for five selected children born in July.

6.0 CONCLUSIONS AND RECOMMENDATIONS

This report summarized an analysis of seasonal rhythms in blood-lead levels for a longitudinal study of blood- and environmental-lead levels on 250 children sampled in Boston between 1979 and 1983. The following conclusions were arrived at:

- There was evidence of significant seasonal variation in both blood- and environmental-lead levels observed in this study.
- Lead levels in blood, air, floor dust, and furniture dust were typically highest in the summer and lowest in the late winter.
- Much of the modeled cyclic seasonal variability in blood-lead levels was explained by adjusting for the effects of environmental lead, specifically in floor dust and air.

The magnitude of the seasonal component of variation in blood lead was estimated to be 0.93 on a log scale. This corresponds to a multiplicative increase of 2.54 in blood-lead during late June over levels in early March for the same child. Thus for a child with blood-lead concentration measured in March of 2.9 µg/dl, the predicted level in June would be about 7.4 µg/dl.

If such a seasonal component of variation is confirmed to exist in blood-lead levels today, it could have a major impact on the development of health-based standards and the setting of warning levels. Specifically, it would suggest the need to take into account the month of the year in determining whether a child is at risk. The results of this study suggest that a lower blood-lead threshold should be used in February than in July, because a child with a marginal blood-lead level in the winter is anticipated to have a much higher level in the summer.

Although the results of this study show evidence of a large cyclic component of variation in blood and environmental lead levels, the reader must recognize that the children observed all lived in Boston in the early 1980s. The results suggest that seasonal variations can be very large in magnitude. However, before attempting to adjust health-based standards or blood-lead levels considered to be a health risk, more current investigations covering a broader population base over more varied geographic and socio-economic conditions should be performed. Also, to better understand the nature of seasonal blood-lead variation, more frequent measures should be taken on each child. Specifically, it would be of greater value in the assessment of seasonal rhythms in blood lead to sample fewer children at more time points than to sample more children at fewer time points.

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Bruce Buxton was the Program Manager for Battelle Memorial Institute.
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16. Abstract (Limit 200 words)

It has been conjectured that both blood-lead and environmental-lead levels are increased during summer months. Several researchers have observed elevated levels of lead contamination and/or increased incidence of lead poisoning during these months. Reasons for seasonal rhythms in blood-lead levels, if such a phenomenon is real, are not immediately apparent. The temporal variation may result from either altered human physiology or higher levels of lead exposure during the summer months. Determining the source of the temporal variation in blood-lead levels may enhance our understanding of the relationship between environmental-lead and its impact on body burden.

There were two primary objectives of this study:

- Determine the extent to which blood-lead levels recorded in the study conducted at the Brigham and Women's Hospital exhibit seasonal variation.

- Determine if any existing seasonal trends in blood-lead levels are correlated with seasonal trends in environmental levels.

This report examines temporal variation in blood- and environmental-lead levels in data observed on 249 children sampled in Boston at the Brigham and Women's Hospital between 1979 and 1983.

17. Document Analysis

a. Descriptors
Lead, lead poisoning, contamination, statistical analysis, seasonal variations, Brigham and Women's Hospital, lead levels, blood lead levels, seasonal rhythms of blood lead levels, seasonal variation in environmental lead levels.

b. Identifiers/Open-Ended Terms
Lead, lead poisoning, trend analysis, seasonal variation, Fourier analysis.

c. COSATI Field/Group

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