1. Alcohol consumption is a problem in college fraternities. One possible strategy to affect alcohol consumption is to create incentives for reducing consumption at parties. The incentive may be a prize awarded to the fraternity with the lowest average blood alcohol level towards the end of the party (between Midnight and 1AM). There is interest in evaluating the effectiveness of such an intervention.

A study is designed that randomly selects three fraternities from a specified college campus. Parties are ‘randomly’ selected by listing planned parties in a fraternity in a time period, and then randomly selecting a party. The time periods correspond Sept 1 and Oct 15; Oct 15 to Jan 1; Jan 1 to March 1, and from March 1 to May 25th. At a specified time at a party (between midnight and 1AM), blood alcohol is measured on a simple random sample of 25% of the party attendees. There are three treatments, and a control. One treatment (T1) corresponds to announcing prior to a party that blood alcohol levels will be monitored. The second treatment (T2) corresponds to announcing blood alcohol level monitoring prior to the party, and announcing the lottery as an incentive for limiting alcohol consumption. The third treatment announces the lottery and blood alcohol level monitoring, but also gives the party attendees specific guidelines (tailored to weight and height) for the relationship between blood alcohol and drinking at the parties start. The control measures blood alcohol at a party without announcement (C=control).

This design randomly selects three fraternities from a specified college campus, and then uses the following design.

<table>
<thead>
<tr>
<th>Fraternity</th>
<th>Party 1</th>
<th>Party 2</th>
<th>Party 3</th>
<th>Party 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C</td>
<td>T2</td>
<td>T1</td>
<td>T3</td>
</tr>
<tr>
<td>2</td>
<td>C</td>
<td>T3</td>
<td>T2</td>
<td>T1</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
</tbody>
</table>

a. Write an analysis plan that includes a clear definition of the study population, all parameters. Define parameterizations for the problems in terms of fixed effects, and identify the main effects of interest.
b. Use the model in a) to develop a mixed model (or models) that would be appropriate for analyzing the data. Define fixed and random effects in the model, including variance components. Using your model, describe the variance matrix for the response vector for a randomly selected fraternity.
Below Background Exposure To Lead Is Associated with a Marked Acceleration in Puberty Onset in Female Mice (I. Iavicoli\textsuperscript{a}, G. Carelli\textsuperscript{b}, E.J. Stanek III\textsuperscript{c}, N. Castellino\textsuperscript{d}, & E.J. Calabrese\textsuperscript{*})

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**ABSTRACT**

Female Swiss mice typically display signs of puberty at about 33-37 days of age. In the present investigation (96 female mice tested in eight Pb exposure levels, n=12 per exposure level), the time to puberty onset was markedly influenced by exposure to dietary lead. While modest increases in blood lead concentration from a normal background of 2-3 \( \mu \text{g/dl} \) to 13.2 \( \mu \text{g/dl} \) delayed the onset of puberty by 15-20\% to about 40-43 days, reducing blood lead from 2-3 \( \mu \text{g/dl} \) to 0.7 \( \mu \text{g/dl} \) was associated with a striking acceleration of puberty to 21 days, an enhancement by over 30\%. This dose response relationship represents novel findings of possible ecological as well as public health significance and indicates that lead is able to induce biologically significant changes at blood lead levels previously thought to be without effect.

**KEYWORDS:** hormesis, lead, puberty, U-shaped, dose-response, low dose, opposite effects

**INTRODUCTION**

Lead (Pb) is widely known for its capacity to cause reproductive toxicity in male and female rodents. With respect to females, a number of studies indicate that Pb exposure during pregnancy and the neonatal period may affect a delay in sexual maturity, irregular estrus, and reduced numbers of corpora lutea. These investigations have employed exposures to Pb that far exceed (i.e., \(~15\) to \(100\)-fold) the current background (i.e., 2-3 \( \mu \text{g/dl} \)) blood lead concentration (PbB) in children in the US (Dearth et al., 2002; Kimmel et al., 1980; McGivern et al., 1991; Ronis et al., 1996). Of considerable concern has been whether there are biological responses of public health significance at and below the CDC standard of 10 \( \mu \text{g/dl} \) (Kaufman, 2001a; Kaufman, 2001b; Neddleman and Bellinger, 2001). Of additional interest has been what is the nature of the dose response in the low dose zone. The present investigation assessed not only the effects of Pb at PbB as low as 0.7 \( \mu \text{g/dl} \) but also provides insight on the nature of the dose response for Pb.
induced pre/postnatal effects in female mice since opposite biological effects were observed below and above the typical blood background concentration of 2 µg/dl.

METHODS AND MATERIALS

One day after mating (evaluated by vaginal plug - 1st day of gestation) 24 female Swiss mice (from the Experimental Animal Production Plant of the Catholic University of Sacred Heart, Roma, Italy) were randomly divided in eight groups of three mice. Each group was given the solid purified rodent diet DP 1000 (Altromin Rieper A. S.p.A., Vandoies, Italy) at 8 different Pb levels: 0.02, 0.06, 0.11, 0.2, 2, 4, 20, 40 ppm. We describe the 40 ppm Pb diet group and 20 ppm Pb diet group as “high and relatively high exposure”; the 4 ppm Pb diet group and a 2 ppm Pb diet group, as “low exposures”; the 0.2 ppm Pb diet group (considered the normal background “control” group); and the 0.11 ppm Pb diet group, and the 0.06 ppm Pb diet group, rated as “very low exposure”. Finally we describe the 0.02 ppm Pb diet group, as a “Pb-free” environment. Diets and drinking water were administered ad libitum. All diets, > 0.02 ppm Pb diet, were obtained by addition of Pb acetate trihydrate to this diet. After the birth, four female mice were randomly selected from each litter and housed in Macrolon® cages manufactured by Tecniplast S.p.A. (Buguggiate, Italy). During lactation the mothers received the same feed given during pregnancy and the same diets were given to the litter after weaning.

During the experiment the fathers were removed from the rearing room after successful mating. The pregnant females remained in the rearing room through pregnancy, parturition and weaning. After weaning, the male offspring, non-selected female offspring and mothers were removed from the rearing room. After weaning the fathers were then returned to the rearing room and placed into the cages of female offspring on day 18 with one male/four females.

**Body Weight.** Data were collected on pup weight at days 1, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, and 90.

**Reproductive Function and Fertility.** Female 18-day-old offspring from each group were housed with sexually mature adult 3-month-old male. In all animals, the age at vaginal opening, estrus (determined by vaginal smear according to Nelson et al. (1982) and by estradiol measurement), vaginal plug, first parturition and the number of pups were recorded.

**Blood Collection.** On the day of estrus, as determined by vaginal smear, each mouse was anesthetized with ketamine (10 mg/kg) and xylazine (100 mg/kg). About 110 µl of blood from each animal was removed by retro-orbital bleeding and collected in heparinized tubes for estradiol and PbB determinations.

**Estradiol Determinations.** Determinations of estradiol in 50 µl aliquots of serum were made using the DSL-10-4300 ACTIVE™ Estradiol EIA kit supplied by Diagnostic System Laboratories, Inc. (Webster, Texas, USA).

**Air Sampling.** Twenty airborne particulate samples were collected at regular intervals during the study for Pb determinations. At a distance of approximately 1 m from the cages, environmental air was drawn for 24 hours through cellulose
nitrate filters (Sartorius AG, Göttingen, Germany; diameter 25 mm, pore size 0.45 µm) inserted in IOM holders (ISO, 1995; Mark and Vincent, 1986).

**Pb Analyses.** Pb analyses in blood, water, diets, and airborne particulate were performed by flameless atomic absorption spectroscopy at 283.3 nm with the Model 4100ZL (PerkinElmer, Bodensee, Germany) atomic absorption spectrophotometer equipped with longitudinal Zeeman-effect background correction and transversely heated graphite atomizer (Iavicoli et al., 2001).

**Statistical Methods.** Four female mice from a litter for each of 24 mothers contributed to the 96 mice in the study, with three mothers randomly assigned to each of the diets.

Data on the weight of the offspring are given in an accompanying spreadsheet. (see fertilitydata.xls) Write a report (10 pages or less) that analyzes the relationship between growth of the mice (weight) and lead.