PubH 7405:
REGRESSION ANALYSIS

MLR: BIOMEDICAL APPLICATIONS

Multiple Regression allows us to get into **two new areas** that were not possible with Simple Linear Regression:

(i) Interaction or Effect Modification, and
(ii) Non-linear Relationship.

This lecture today is devoted to biomedical applications; we cover two topics:
(1) For interactions, we re-visit and expands the topic of **bioassays**, and
(2) As an example of non-linear models, I'll show you how to study "**seasonal diseases**"- a case similar to that of quadratic regression - with **two predictor terms representing the same "predictor source"** where we search for an optimal condition for the outcome.

DEFINITION

"Biological assays" or "bioassays" are a set of methods for estimating the potency or strength of an "agent" by utilizing the "response" caused by its application to biological material or experimental living "subjects".

COMPONENTS OF A BIOASSAY

- The subject is usually an animal, a human tissue, or a bacteria culture,
- The agent is usually a drug,
- The response is usually a change in a particular characteristic or even the death of a subject; the response could be binary or on **continuous** scale.

DIRECT ASSAYS

- In **direct assays**, the doses of the standard and test preparations are "directly measured" for an "event of interest".
- When an (pre-determined) event of interest occurs, e.g.. the death of the subject, and the variable of interest is the **dose** required to produce that response/event for each subject.
- In other words: (Binary) **Response is fixed**, **Dose is a Random Variable**.

INDIRECT ASSAYS

- In **indirect assays**, the doses of the standard and test preparations are applied and we observe the response that each dose produces; for example, we measure the tension in a tissue or the hormone level or the blood sugar content. For each subject, the dose is fixed in advance, the variable of interest is not the dose but the **response** it produces in each subject.
- **Doses are fixed, Response is a Random Variable**; statistically, indirect assays are more interesting and also more difficult.

For Indirect Assays, depending on the "measurement scale" for the response – our Random Variable, we have:

(1) Quantal assays, where **the response is binary**: whether or not an event (like the death of the subject) occur,

(2) Quantitative assays, where measurements for the response are on a **continuous** scale. This is our main focus; the dependent variable Y.

The common indirect assay is usually one in which the ratio of equipotent doses is estimated from “curves” or “lines” relating quantitative responses and doses for the two preparations. **The shape of these curves or lines further divides Quantitative Indirect Assays into:**

(1) **Parallel-line assays** are those in which the response is linearly related to the **log dose**,

(2) **Slope ratio assays** are those in which the response is linearly related to the **dose** itself.

PARALLEL-LINE ASSAYS

- Parallel-line assays are those in which the response is linearly related to the log dose.
- From the definition of "relative potency" ρ, the two equipotent doses are related by $\mathbf{D_S = \rho D_T}$.
- The model: $E[Y_S|\mathbf{X_S}=\log(D_S)] = \alpha + \boldsymbol{\beta} X_S$, for Standard and, for same dose of Test we have $E[Y_T|\ \mathbf{X_S}=\log(D_S = \rho D_T) = (\alpha + \beta \log\rho) + \boldsymbol{\beta} X_T$
- We have 2 parallel lines with a **common slope** β and **different intercepts**.

$$\text{Intercept}_S = \alpha$$

$$\text{Intercept}_T = \alpha + \beta M$$

$$M = \frac{\text{Intercept}_T - \text{Intercept}_S}{\text{Common Slope}};$$

$$M = \log \rho \text{ is estimated by m}$$

Doing correctly, we should fit the **two straight lines with a common slope**. Here, each line was fitted separately – not right but can use to see if data fit the model.

When we learn Simple Linear Regression, **we solved the problem by calculating the weighted average of the two estimated slopes**. Another approach, which turns out more simple, is Multiple Linear Regression.

MULTIPLE REGRESSION

- An alternative approach is pooling data from both preparations and using "Multiple Regression";
- Dependent Variable: **Y = Response**;
  Two Independent Variables or predictors are:
  X = log(Dose) &
  P = Preparation (a "dummy variable" coded as P = 1 for "Test" and P = 0 for "Standard")

From the Model:

$$E(Y) = \beta_0 + \beta_1 X + \beta_2 P$$

Standard Preparation:

$$E(Y_S) = \beta_0 + \beta_1 X$$

Test Prepararation:

$$E(Y_T) = \beta_0 + \beta_1 X + \beta_2$$

$$= (\beta_0 + \beta_2) + \beta_1 X$$

Difference of Intercepts $= \beta_2$

Common Slope $= \beta_1$

Multiple Regression Model:

$$E(Y) = \beta_0 + \beta_1 X + \beta_2 P$$

β_1 is the common slope and

β_2 is the "difference of intercepts";

$$M = \log \rho = \frac{\beta_2}{\beta_1}$$

X = log(Dose) & P = Preparation

DELTA METHOD

If Y is a function of two random variables X_1 and X_2 then we have approximately, with partial derivatives evaluated at the mean values:

$$Var(Y) \cong \left[\frac{\delta y}{\delta x_1}\right]^2 Var(X_1) + 2\left[\frac{\delta y}{\delta x_1}\right]\left[\frac{\delta y}{\delta x_2}\right] Cov(X_1, X_2) + \left[\frac{\delta y}{\delta x_2}\right]^2 Var(X_2)$$

In the current application: **The point estimate of log(Relative Potency) is b_2/b_1**

Multiple Regression Model:

$$E(Y) = \beta_0 + \beta_1 X + \beta_2 P$$

β_1 is the common slope and

β_2 is the "difference of intercepts";

$$M = \log \rho = \frac{\beta_2}{\beta_1}; m = \frac{b_2}{b_1}$$

$$Var(m) \cong \frac{b_2^2}{b_1^4} Var(b_1) + 2(-\frac{b_2}{b_1^2})(\frac{1}{b_1}) Cov(b_1, b_2) + \frac{1}{b_1^2} Var(b_2)$$

SLOPE RATIO ASSAYS

- Slope-ratio assays are those in which the response is linearly related to the dose itself.
- From the definition of "relative potency" ρ, the two equipotent doses are related by $D_S = \rho D_T$.
- **The model**: $E[Y_S|\mathbf{X_S}=D_S)] = \boldsymbol{\alpha} + \beta X_S$, for its equipotent dose $E[Y_T|\ \mathbf{X_S}=D_S = \boldsymbol{\alpha} + \beta\rho X_T$; the lines have the same intercept - the mean response at zero dose.
- **Result**: We have two straight lines with **a common intercept** and **different slopes**.

$$\text{Common Intercept} = \alpha$$

$$\text{Slope}_S = \beta$$

$$\text{Slope}_T = \beta\rho$$

$$\rho = \frac{\text{Slope}_T}{\text{Slope}_S};$$

$$\rho \text{ is estimated by } r = \frac{b_T}{b_S}$$

MULTIPLE REGRESSION

- An alternative approach is pooling data from both preparations and using "Multiple Regression";
- Dependent Variable: **Y = Response**;
  Two Independent Variables are:
  X = Dose &
  P = Preparation (a "dummy variable" coded as P = 1 for "Test" and P = 0 for "Standard")

Multiple Regression Model #1:

$$E(Y) = \beta_0 + \beta_1 X + \beta_2 PX$$

β_0 is the common intercept and slopes are:

$$\beta_T = \beta_1 + \beta_2$$

$$\beta_S = \beta_1$$

$$\rho = \frac{\beta_1 + \beta_2}{\beta_1}$$

X = Dose & P = Preparation

MULTIPLE REGRESSION #2

Let Y be the response, X_S and X_T the doses. Consider the following model in which for any observation on S, set $X_T=0$, for any observation on T, set $X_S=0$; the model may include control observations for which we set $X_S= X_T= 0$:

$$E(Y) = \beta_0 + \beta_S X_S + \beta_T X_T;$$

$\beta_0 =$ Common Intercept

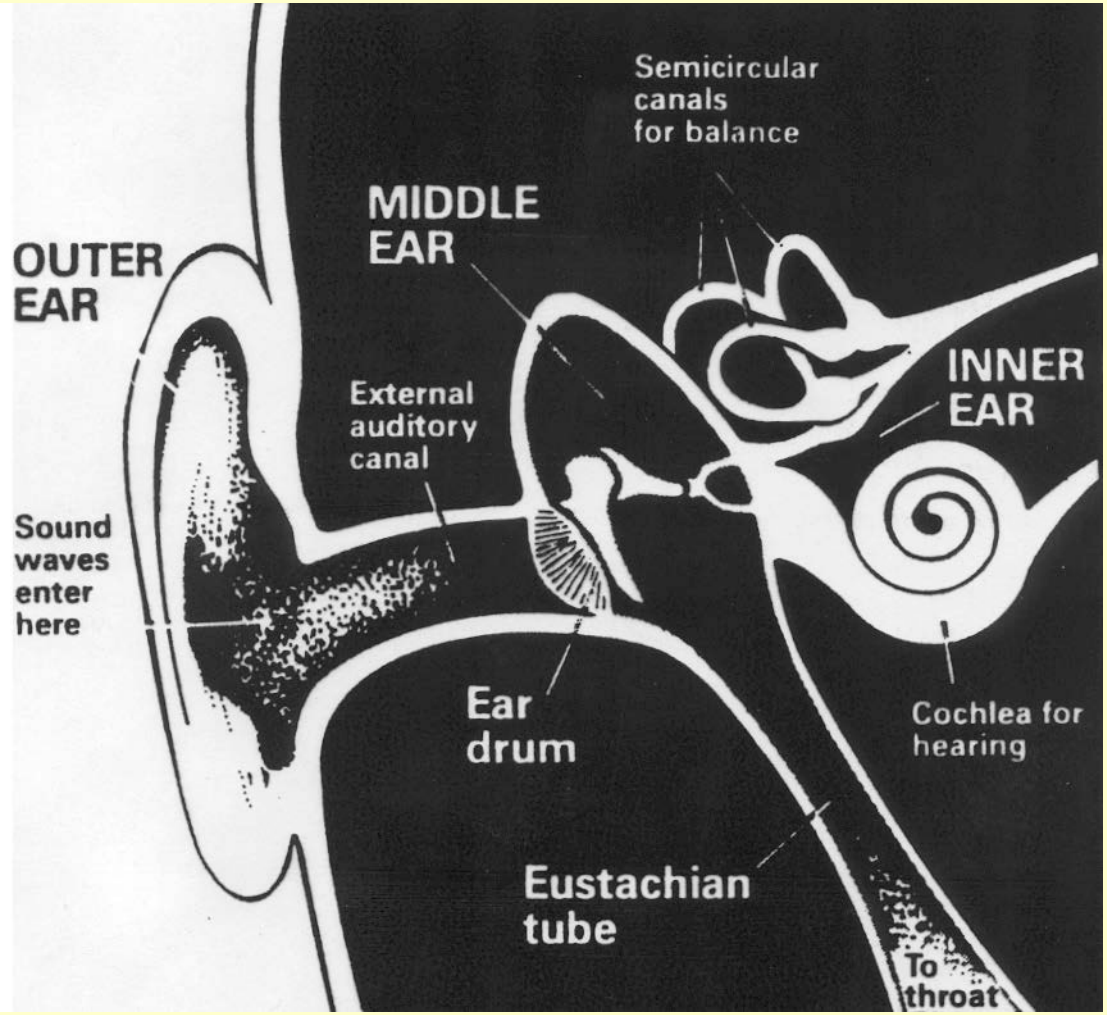
$$\rho = \beta_T / \beta_S$$

In both Multiple Regression models for Slope-Ratio assays, point estimate of Relative potency is obtained from computer output; and **we can use Delta Method to calculate Variance/Standard Error.** That is possible with the option "COVB" in PROC REG.

POLYNOMIAL REGRESSION

- The second-order or quadratic model could be used when the true relationship may be unknown but the second degree polynomial provides a better fit than a linear one.
- If a quadratic model fits, perhaps an useful application would be **to maximize/minimize the "Mean Index"**, $\beta_1 x + \beta_2 x^2$, in order to determine the value of X at which the Mean of Y attains its maximum or minimum value (depending on the sign of β_2).
- The following application is based on this idea of optimization involving **two related predictor variables**.

OTITIS MEDIA: INFLAMMATION OF THE MIDDLE EAR



OTITIS MEDIA

- Is Inflammation of the middle-ear space, often referred to as “Children Ear Infection”.
- Is the **2nd most prevalent disease on earth**, affects 90% of children by age 2 (in the US).
- **Costs 3.8 billions in direct costs** (physician visits, tube placements, antibiotics, etc…) and 1.2 billions in indirect costs (lost works by mothers, etc...); in 1995 dollars.
- Causes hearing loss, **learning disabilities**, and other middle-ear sequelae.

AS A CHILDREN DISEASE

- It is the most common diagnosis at physician visits ahead of well-child, URI, injury, and sore throat.
- It is responsible for 24.5 million physician visits in 1990, increased 150% from 1975; probably due to increased awareness and more aggressive diagnoses.

Perhaps it is the most typical “public health problem”; and its most interesting characteristic is that it is a “**seasonal disease**” – just like the most prevalent disease on earth, the **common cold**.

DOB AS A DISEASE INDICATOR

- Other examples
- Nilsson et. al. Season of births as predictor of atopic manifestations. *Archives of Disease in Childhood*, 1997. (food allergies, asthma, etc…)
- Torrey et. al. Seasonal birth patterns of neurological disorders. *Neuro-epidemiology*, 2000.

LITERATURE REVIEWS

- Most or all investigators, with emphasis on "**season**", often **divide the year into four seasons** and compare them using either a **Chi-square test** or a **One-way Analysis of Variance F-test** depending on the endpoint being discrete or continuous!
- What are the **problems** with this vastly **popular** approach to investigating seasonality?

POINTS TO CONSIDER

- There is no universal agreement on the definition of seasons, plus regional differences (for example, the Midwest's winter is more than 3 months; we have 2 seasons a year, **Winter** & **Road Repair**).
- There are **no reasons to believe** that the risk associated with time of the year is **similar within seasons and different between seasons**. If there is any risk associated with time of the year, that **level of risk must change gradually** , not abruptly, as time progresses.

A NEWER APPROACH

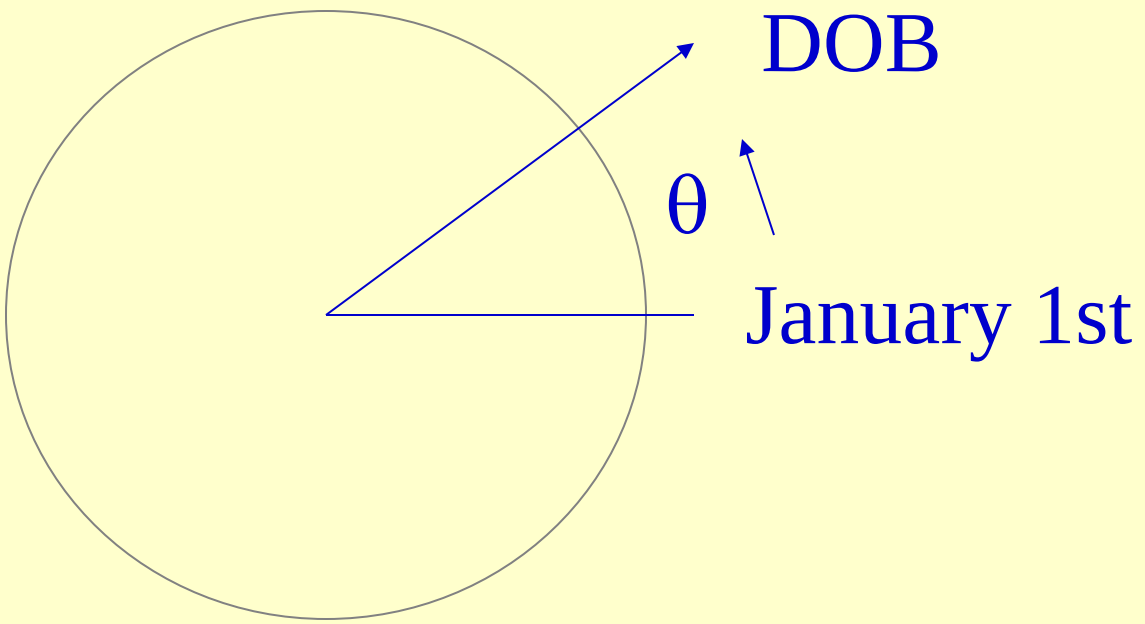
- DOB will be treated as a continuous variable with a circular distribution.
- Many endpoints may be considered but let focus on a continuous endpoint (representing a "possible cause") as a show case of MLR
- I like to see this as a study of an important public health problem (a disease with high prevalence) focusing on a widespread exposure (**indoor pollution**).

DATA FOR ILLUSTRATION

- Study population: 592 infants within Health Partners system. Pregnant women were enrolled, and followed. **Cord blood** was collected at time of birth and **blood samples** were analyzed by ELISA for pneumococcal IgG antibody for 7 serotypes.
- **Specific Aims**: **we focus on DOB and see how it's related to the disease by seeing how it's related to antibodies.**

DOB REPRESENTATION

The DOB for each infant is represented by an angle, called θ, between 0^0 and 360^0:



CONVERSION FORMULA

- The angle θ representing each DOB is calculated using the following conversion:

$\theta = (\text{DOB - January } 1^{st})/(365 \text{ or } 366)*360^{0}$

- Each θ is an angle ranging from 0^{0} to 360^{0}
- For example, if an infant was born on October 2^{nd}, 1991, then:

$\theta = (10/02\text{-}01/01)/(365)*360^{0}$; or $\mathbf{270.25^{0}}$

PARAMETERIZATION: RESULT

- The DOB, represented by θ, becomes a continuous variable with a **circular** distribution without a true zero origin point (that is, any other date can be used as time origin in the place of January 1st ; **we would have the same results regardless of choices**).
- The DOB, represented by θ, is characterized by two (2) component: sine (**sinθ**) & cosine (**cosθ**).

LOW ANTIBODY: A CAUSE?

- Otitis Media is often referred to as "**Ear Infection**"; bacteria have a definite role here.
- The disease occurs early in life before infants have their own completed **immune system**; their ability to fight infection consist only of what they are inherited from their mother.
- Streptococcus pneumoniae (Spn) causes about 50 percent of recurrent Acute Otitis Media episodes.
- Therefore, the role of cord blood Pneumococcal **IgG antibody** is important.

HYPOTHESIS

- Low **maternal concentration of IgG** antibody results in low **neonatal antibody and early onset of OM**, **which leads to recurrent and chronic diseases.**
- I'll only use **type 19F (Ant19F), on log scale -** because skewed distribution), one of the seven serotype, for illustration.

In other words:

The dependent variable is:
Y = Antibody, type 19F – on log scale

Target predictors? **Sine(θ) and Cos(θ)**, together they represent the DOB.

MULTIPLE REGRESSION

- We are interested in whether there is a relationship between infants' DOBs and their type 19F IgG antibody concentrations, so we fit a linear multiple regression model.
- **Y= Ant19F** is the response variable and $\mathbf{X_1}$**=sin(θ)** and $\mathbf{X_2}$**=cos(θ)** are two covariates:

Model:

$$\mathbf{Mean(Ant19F) = \beta_0+\beta_1*sin(\theta)+\beta_2*cos(\theta)}$$

This model is **similar to a polynomial regression**; just like (X and X^2), the two predictors Sin(θ) and Cos(θ) are representing the **same "predictor source"–** so that coefficients β_1 and β_2 do not follow the usual interpretation. Therefore, **we will investigate their roles together**.

MULTIPLE REGRESSION RESULTS

Factor	Coefficient Estimate	St Error	p-value
Intercept	b_0=.57	.08	<.001
sin(θ)	b_1=.38	.11	<.001
cos(θ)	b_2=.01	.11	.095

The p-value for sin(θ) is very small, indicating that **DOB, part of which is represented by sin(θ),** is a significant factor in predicting infants' antibody 19F concentration.

ANTIBODY: RESULTS

- Since DOB is represented by two variables (sin(θ) and cos(θ)), it is difficult to interpret the two regression coefficients individually.
- By taking the derivative of:

$$\text{Mean(Ant19F)} = b_0 + b_1*\sin(\theta) + b_2*\cos(\theta)$$

relative to θ and set it to zero, we get two angles in (0,360): 88.5^0 (max.; Ant19F=2.59) and 268.5^0 (min.; Ant19F=1.21), which correspond to **April 1st & September 30th .**

An Illustration of The Horoscope

- I divide the year into 4 seasons : (i) **February 16 - May 15** (centered at April 1, date with maximum antibody), (ii) **May 16 - August 15**, (iii) **August 16 - November 15** (centered at September 30, date with minimum antibody), and (iv) **November 16 - February 15**. The following Table shows a rather symmetric distribution of antibody level, type 19F

Season	GM of Antibody	95% Confidence Interval
2/16-5/15	2.264	(1.714,2.992)
5/16-8/15	1.942	(1.432,2.633)
8/16-11/15	1.375	(0.930,2.032)
11/16-2/15	1.735	(1.290,2.333)

RESULTS/IMPLICATION

- The results imply that infants born during the Spring season (as April 1st suggests) tend to have a higher antibody concentration than infants born during the Fall season (as September 30th suggests).
- Therefore infants born in Spring should have a lower risk of disease than infants born in Fall.

ANTIBODY: INTERPRETATION

- The finding that infants born in the Fall have low antibody levels compared to those born in the Spring probably due to different levels of **maternal exposure preceding the infant's birth.**
- Pregnant women have the greatest exposure potential to pneumococcal bacteria during winter, peak antibody levels would follow that exposure, resulting in greater amounts of antibody transferred to infants born in the Spring.
- Result: amount of time a pregnant women staying indoor would be predictive of cord blood antibody.

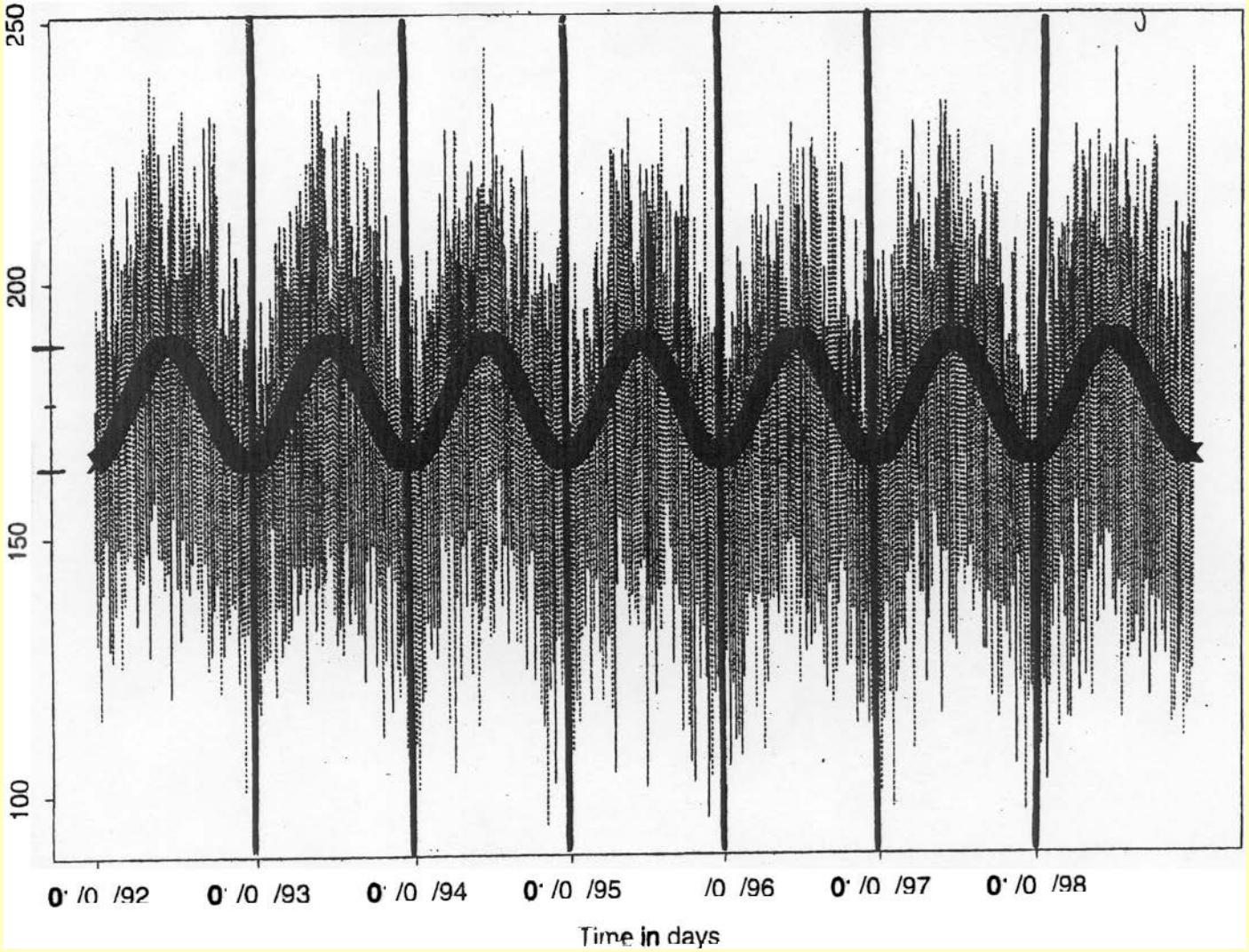
SOURCES OF EXPOSURE

- Some difference in the findings (Low-antibody kids in **late September**, representing **the lack of exposure** by the **mothers).** It's not sole determinant.
- The other source is the **exposure by the newborns** (houses are closely sealed in the Winter, starting in **November to March**, likely leading to more severe indoor pollution when newborns suffer).
- Disease occurrence due to both sources, lack of exposure by mother (Fall) and exposure by the child (Winter): total combined effect is peaked in **late October to late December** when disease occurs.

MORE GOOD NEWS: SEASONAL BIRTHS PATTERN

188

165



There are other smaller cycles:

(1) **Hours of the Day**: Blood Pressure or Time to take medication?

(2) **Days of the Week**: “Weekend Effects” in scheduling surgeries, say C-sections?

Due As Homework

#15.1 Refer the first data set below, a 4-point assay of Corticotropin and find a point estimate of the Relative Potency.

Dose				
Standard		Test		
0.015	0.045	0.015	0.045	Total
45.07	60.2	49.75	66.35	221.37
44.12	62.93	35.83	45.58	191.46
39.64	48.44	44.94	54.26	187.28
31.48	48.95	34.76	56.39	171.58
160.31	220.52	165.28	225.58	771.69

#15.2 Use the data set in the following Table,

a) Use data from the Standard Preparation & Scatter diagram to verify the linear regression of Y versus X = log(dose) fits better than Y versus X = Dose;

b) Formally test (at α = .05) that the lines are parallel;

c) Find a point estimate of the log(Relative Potency);

d) Calculate the Standard Error of the estimate in (c).

	Vitamin D3 (Standard)			Cod-liver Oil (Test)			
Dose	5.76	9.6	16	32.4	54	90	150
Response	33.5	36.2	41.6	32	32.6	35.7	44
	37.3	35.6	37.9	33.9	37.7	42.8	43.3
	33	36.7	40.5	30.2	36	38.9	38.4
		37	42			40.3	44.2