COURSE CODE: STS 463
COURSE TITLE: BIOMETRY
NUMBER OF UNIT: 3 UNIT
COURSE DURATION: THREE HOUR PER WEEK.

COURSE DETAILS:

COURSE COORDINATOR: Prof. O.E. Asiribo

LECTURER OFFICE LOCATION: COLNAS

COURSE CONTENT:

Purpose, History and Structure of Biological Assays, International Standards, Statistical Science, and Biological Assays. Terminology and Notations, Nature of Biological assays, Potency Ratio Precision of estimates, Direct and Indirect assays, Design of Bioassay, Failure in Normality assumptions, Regression Analysis, Dose Response Curves, Theory of Parallel Assays, Calibration Curves, Fieller's Theorem and its Application

COURSE REQUIREMENTS:

This is an elective course for all statistics students. Students are expected to have a minimum of 75% attendance to be able to write the final examination.

READING LIST:

1. **Biometry by Sokar**

2. **Statistical Methods in Biology by Boes R**

LECTURE NOTES

4. **Statistics for Biology by Cox , R**

1.1 Introduction

Biometry is a branch of statistical science in which statistical methods are applied to biological processes. The main objective of biometrical methods is to make general statements about the properties of groups from measurements made on individuals or samples belonging to them. This

involves estimation, interpretation and inference. In the same vein, a biological assay or bioassay is an experiment for estimating the nature, constitution or potency of a material (or of a process), by means of the reaction that follows its application to living matter.

Biological assays can be classified as either qualitative or quantitative. Qualitative assays are identified by classifications whereas quantitative assays involves identification by means of numerical assessment such as measurement of growth or change in animals or plants, increase or decrease of weight in animal tissue or other living matter.

The aim of a bioassay is to apply known treatments to subjects, measure the subject, estimate the differences between the effects of the treatments, and then use the measurement or, estimate as a foundation for comparing the potencies of the treatments applied.

An example of a bioassay is a fertilizer trial in which the yields of a crop are to be used in assessing the potency of one fertilizer compound relative to the other.

The purpose of a bioassay can thus be summarized as the estimation of the parameter representing the potency of a test preparation relative to a standard preparation.

1.2 History of Bioassay

The scientific history began in the 19^{th} century with Ehrlich's investigations into the sterilization of diphtheria antitoxin. Since then, in pharmacology, endocrinology, plant methodology and

other sciences, standardization of materials by means of the reactions of living matter has become a common practice.

The development of pharmacological standards have been described by various authors including Dale in 1939 and Gautier in 1945. Potency was first measured in animal units, the unit being the amount required for producing a specific response in an animal of a particular specie. But in their responses to, and in their tolerances of drugs, animals are very variable due to their bodily characteristics.

For example, the cat unit of digitalis and the mouse unit of insulin were discovered not to be constant.

The introduction of standard preparations of various drugs, against which others could be compared, made possible the measurement of potency on a fixed scale independent of the particular animal used.

In 1933, Gaddum's reports of research results initiated a serious statistical study of bioassay and about the same time, Bliss was independently considering the same sort of problem. The result was a large number of publications on bioassay surveys or investigations written by Irwin (1937, 1950), Bliss and Cattell (1943) and Gaddum (1953). Finney (1947) presented a systematic account of the statistical principles while Jerne and Wood (1949) published an important detailed discussion of most assay techniques.

Based on this development and the efficiency of the statistical methods employed, most pharmaceutical companies have biometricians or biostatisticians in their research laboratories or Research and Development Unit (R & D Unit).

1.3 Structure of Bioassay

A typical bioassay involves a stimulus (such as a vitamin, a hormone, a fungicide or insecticide) applied to a subject (e.g., an animal, a piece of animal tissue, a plant, a bacterial culture).

The size of the stimulus may be varied, generally in accordance with the plan of the investigator.

Also, the size or dose given to the subject can be measured as weight, volume or concentration.

The response of the subject is a measurement of the final value of some characteristic of the subject (such as body weight and kidney weight) or change in a particular characteristic (such as blood pressure) or it may be a simple record of occurrence or non occurrence of a phenomenon (such as death, recovery from disease, or muscular contraction).

The magnitude or the frequency of the response depends upon the dose. The relation between dose and response will enable the potency of a dose to be inferred from the responses it induces.

1.4 International standards

One of the early standards developed was the standardized dipthera antitoxin supplied from Ehrlich's Institute in Frankfurt, Germany and form the Hygienic laboratory in Washington, USA. In 1921, a conference conversed by a health committee of the league of Nations determined that these two standards agreed well and recommended the adoption of Erhlich's Unit as the international unit of the anti-toxin. However, by 1924, the need for other international standard was apparent and this led to the establishment of a permanent commission for biological standardization.

Lately, the expert committee on biological standardization of the WHO has become the custodian of the increasing number of standardization and in 1975, they listed over 150 international standards for antigens, antibodies, antibiotics, hormones, vitamins, enzymes and other pharmacological substances.

These standards form an indispensable part of the system of measurement necessary to modern medicine (i.e. careful control over all stages of selection, preparation, preservation and distribution for worldwide use).

International standards are chosen based on 3 main criteria.

1) The standard should be a dry preparation of an arbitrary chosen, but representative sample of the substance for which it is to serve as a standard. Since the standard must satisfy world requirement, for many years, the quantity set aside as a standard must be large.
2) The standard must be stable, a condition which is fulfilled by preparing it in the absolutely dry condition and preparing it constantly at freezing temperature, in a sealed container and protected from heat, action of light, moisture and oxygen.
3) The standard must be dispensed in such a form as to be readily accessible and capable of being brought in to use by the lab worker with minimum trouble or delay.

1.5 Role of Statistician (Statistical Science and Bioassay)

Statistical science is an indispensable tool in the study of bioassays and the statisticians contribute to the practice of bioassay in the following major ways:

- Advise on the underlying statistical principles
- Construct experimental design, that in the light of existing information, seems likely to give the most useful and reliable result.
- Analyze or instruct the investigator on how to analyze experimental data so as to make the best use of all evidence on potency.

Note that the design in (2) above, implies specification of the number and magnitude of the doses at each preparation to be tested , the number of subjects to be used at each dose level, system of allocating subjects to doses, order in which subjects are to be treated and measured and related characteristics of the experiments. This is the major function of the statistician.

Such statistical techniques as ANOVA, ANCOVA, regression analysis, estimation and hypothesis testing, etc, are quite useful in the study of bioassay.

2.0 Types of Biological Assays

There are two major types of biological assays, viz, direct and indirect assays.

2.1 Nature of Direct Assays

A direct assay is a bioassay that measures the doses of the standard and test preparations required to elicit a specified response. Direct assays are only practicable for certain stimuli and subjects, hence, their applications are limited and their use is declining. A typical example of a direct assay is the 'cat' method for the assay of digitalis. The standard or the test of preparation is infused at fixed rate into the blood stream of a cat until the heart stops beating.

The total time of infusion multiplied by the rate, measures the dose.

i.e. Dose = Rate x time

This is repeated on several cats for each preparation and the mean doses compared. By definition, the potency of the test preparation relative to the standard is the amount of the standard equivalent in extent to one unit of the test. Hence the ratio of these critical doses estimate the potency.

For the direct assay, the response must be unambiguous and easily recognized, and the dose must be administered in such a manner that the exact amount needed to produce the response can be recorded. The critical dose will generally vary from one occasion to another with the same subject. Hence, only estimate of potency is obtained and calculation from averages over a number of trials will be required.

Ideally, trials of both preparations will be made on each subject used so that estimation is independent of differences between subjects and hence, more precise. In most estimations, this is impossible, became once a subject has responded, it may not be usable again or it may be so changed as to make a second trial far from being comparable with the first one.

2.2 Notations

The true relative potency and its estimate from an assay are denoted by ρ and R respectively, while μ and M represent the logarithms of ρ and R.

i.e. $\mu = \log \rho$ and $M = \log R$

2.3 Relative Potency

Let $\bar{z}_1$ and $\bar{z}_2$ be the mean dose for test preparation and standard preparation respectively, then the relative potency, R of test preparation to the standard is given as

$$R = \frac{\bar{z}_2}{\bar{z}_1} \quad \text{.....(2.1)}$$

2.4 Precision of Estimate, R

Let $\bar{z}_i$ be the individual dose measurement within each preparation, then the variance of the mean dose can be obtained in the usual form

It is usually assumed that the variances are equal, hence a poled variance can be estimated. Pooled variance for 3 preparations is given as

$$S^2_{pooled} = \frac{(n_1 - 1)S_1^{\ 2} + (n_2 - 1)S_2^{\ 2} + (n_3 - 1)S_3^{\ 2}}{n_1 + n_2 + n_3 - 3}$$

It can be shown that the variance of R is given as

Var (R) $\approx$ $S^2/Z_1^2\,(1/N_2 + R^2/N_1)$ (for equal variances)

2.5 Application to Strophantus

Stroplantus is a cat digitalis assayed and standardized at the early stage of development of bioassay.

The following table shows the fatal doses or tolerances of 3 groups of cats for 2 preparations of strophantus and a preparation of ouabain. The doses were recorded as quantities per kg body weight.

Example 2.1 Preparations

	Strophantus 1	**Strophantus 2**	**Ouabain**
Doses	15.5	24.2	52.3
	15.8	18.5	99.1
	17.1	0.0	47.6
	14.4	22.7	65.1
	12.4	17.0	66.8
	18.9	14.7	57.6
	23.4	22.0	49.3
			45.8
			66.9
Totals	117.5	139.1	550.5
Means	16.8	19.9	61.2

Suppose that strophantus 2 and 1 are regarded as standard and test preparations respectively.

a) Estimate the relative potency of the test preparation.
b) Estimate the variance of each set in (a) above
c) Obtain the sample variance of the relative potency in (a).
d) Construct the 95% C.I. for the estimated potency.

2.6 Failure in Normality Assumption

The limits for potency described earlier depend on the frequency distribution of the tolerances or doses, particularly that they are normally distributed. However, this assumption is often violated and a possible remedy is to use a log transformation.

- The distribution of log ρ is more normal than that of ρ.
- The variances are more likely to be equal, thereby allowing for the use of pooled variance.
- Also since the estimate of relative potency is obtained as the antilog of the difference of two means, instead of the ratio of two means, ordinary simple standard error formulae can be used.

Illustration: Example 2.1 will be remarked using log transformation:

Preparation

	Strophantis 1	**Strophantis 2**	**Onabain**
Doses	0.190	0.384	0.718
	0.199	0.267	0.996
	0.233	0.301	0.678
	0.158	0.356	0.814
	0.093	0.230	0.825
	0.276	0.167	0.760
	0.369	0.342	0.693
			0.661
			0.825
Totals	1.518	2.047	6.970
Means	0.217	0.292	0.774

Exercise: Obtain relative potency of Ouabain to strophantus 2.

3.1 Advantage of Bioassays

- They are generally easy to set up
- Allow detection of responses of very low concentrations
- Provide means by which changes in sensitivity are measured from the shape of the dose-response curve
- Provide a general means by which the efficacy of a drug is measured.

3.2 Disadvantages

- Assay results may have a significant level of variability. Error in the estimate of the test preparation may result because no two organisms will respond in exactly the same way.
- Lack of chemical information; Bioassays provide information about biological activity but say little about the structure of a test preparation.
- Possibility of interference – while many bioassays are specific, it is possible that different chemicals in the preparation may influence the results.

3.3 Design of Direct Assays

The main objective of direct assay is to obtain the most precise estimate of a difference between two mean log doses. Design of direct assays is comparable to that of experimental design. The design may be simple, when only a single test preparation is to be assessed against a standard, whereas, it may be a little complex more than one test preparation is to be assayed.

In the design of an assay, we are concerned about the set of doses to be tested, the rules for allocating subjects to doses, the arrangement of all other experimental constraints such as mate control, the order in which doses given and measurements made. Choice of a design involves compromise between the conflicting interests of the precision of estimation, the quality of validity tests and the convenience, simplicity, and inexpensiveness of the experimental statistical procedures.

An assay is seldomly undertaken without some knowledge of the potency of the test preparation, T and even a very little information can help the planning. Failing this, a plot assay is greatly recommended in which few responses suffice to indicate the order of magnitude of potency.

A symmetric design is always preferred, denoted as symmetric (K, K) where K doses of each preparation equally spaced on the large scale and with equal number of responses measured at each dose. Any natural grouping of the subjects can be accommodated by block restrictions by use of randomized block design. The natural groupings such as litter differences, testes carried out in different days. When symmetry is unachievable, (due to constraints in resources, forexample), use of incomplete block design is recommended. All allocations are assumed to be at random. In a randomized block, for example, with litter as blocks, member of each litter is selected at random, for one of the doses. The order in which the units receive their doses should also be random. The user of direct assay techniques needs to be familiar with complete and incomplete randomized block designs and the factorial designs.

4.0 Dose Response Curves

Biological assay seeks to estimate equally effective doses of the standard and test preparations, that is, to measure the potency of test preparation relative to the standard. However, using direct assay techniques may result in bias result due to time lag. Also ensuring that subjects receive exactly the right dose to produce characteristic response may be difficult.

However, in an indirect assay, specified doses are given, each to several subjects. The record for each administration of a dose may state merely that a characteristic response, such as death is or ir not produced, referred to as a quantal response. Alternatively, some property of the subject (wt, wt of a given organ, blood calcium, time of survival etc) may be measured; referred to as quantitative response.

Suppose that different dose levels of a stimulus are administered to each of a series of randomly selected groups of subjects, the response elicited by each group after administration of a dose level are then observed and recorded. A graphical plot of the observed responses against the different dose levels usually produce an asymmetric, sigmoid or curve-linear relationship. Linear regression methods are much easier to deal with compared to curve-linear methods and hence, it is customary to transform curve linear relations into linear ones. The choice of a particular

transformation may be based on theoretical or empirical results. However for a biological data, log transformation has been found to be very useful for the following reasons:

- Biological processes were empirically found to use skewed frequency distributions when the responses are plotted directly with the dose level. However, it has also been found that they fit the symmetrical normal distribution better when log of the dose levels are used.
- It was also empirically established that responses to drugs tend to vary proportionately to log dose rather than to the dose.
- If the log dose is used over a considerable range of levels, increasing the dose by constant multiples cause equal linear increment in the responses.
- The proportion of the variance of the dependent variable (response) explained by the independent variable is generally increased.
- The distribution of the deviation of points around the regression line tends to be normal.
- The variances of the different groups tend to be equal or constant (homoscedacity).

If a random sample of subjects is selected from a given population, the average or experimental response to the dose is given by

$$E(\mu/Z) = U$$

A response which is to be of value for assay purposes must depend, in some manner, upon the dose;

i.e. $U = f(Z)$

$$= \alpha + \beta \log Z \qquad (4.1)$$

Example 4.1

The following data presents the measurements on the response (growth) of a bacteria using eight dose levels of vitamin B_{12}. Estimate a dose response curve for the data:

	Dose							
	0.23	0.35	0.53	0.79	1.19	1.78	2.67	4.00
Responses	0.15	0.28	0.36	0.51	0.68	0.85	1.06	1.21
	0.14	0.20	0.36	0.53	0.63	0.80	0.91	1.22
	0.19	0.23	0.34	0.54	0.64	0.71	1.09	1.29
	0.19	0.25	0.37	0.45	0.61	0.55	0.93	1.24
	0.17	0.23	0.33	0.57	0.65	0.94	1.09	1.18
	0.16	0.23	0.38	0.49	0.68	0.83	1.12	1.24
Totals	1.00	1.42	2.14	3.09	3.89	4.98	6.20	7.38
Means	0.167	0.237	0.357	0.515	0.648	0.830	1.033	1.230

For ease of computation, various transformations can be used in estimating the response-close relationship.

(a) $x = \log z$, $(b) x = 10 \log z$ $(c) \log_{1.5}(z/4)$ $(d) \log_{1.5}(z/4) + 7$

$(e) 2 \log_{1.5}(z/4) + 7$

Z	$\log Z$	X $10 \log z$	X $\log_{1.5}(z/4)$	X $2 \log_{1.5}(z/4)+7$	X $\log_{1.5}(z/4)+7$	Y
0.23	-0.6382	-6.4	-7.0	-7	0	0.167
0.35	-0.4559	-4.6	-6.0	-5	1	0.237
0.53	-0.2757	-2.7	-5.0	-3	2	0.357
0.79	-0.1024	-1.0	-4.0	-1	3	0.515
1.19	0.0755	0.8	-3.0	1	4	0.648
1.78	0.2504	2.5	-2.0	3	5	0.830
2.67	0.4265	4.3	-1.0	5	6	1.033

4.00	0.6021	6.0	0.0	7	7	1.230

All these transformations are useful in reducing the arithmetic. Since all the definitions of X are linearly related, any one of them can be used to explore the form of the response curve.

Note that the value 1.5 is the constant ratio between successive doses and 4 is the maximum dose value.

Also $\log_a X = \frac{\log_{10} X}{\log_{10} a}$ ..... (4.2)

$$\log_{1.5}(^{x}/_{4}) = \frac{\log_{10}(^{x}/_{4})}{\log_{10} 1.5}$$

For $x = 0.25$, $\log_{1.5}(^{.25}/_{4}) = \frac{\log_{10}(^{.25}/_{4})}{\log_{10} 1.5} \simeq -7.0$

Fit the dose-response relationships using regression approach.

In studying the dose-response relationship, the square root transformation rather than the log, can at times be more suitable. All possibilities should be explored.

Assignment:

A pharmaceutical company is developing a new drug. Establish a dose response relationship from the data using any suitable log information.

Dose	20	40	80	160	320
Response	2.31	4.30	9.25	18.94	34.98

(a) Fit a log transform model to the data

(b) Plot the fitted modes with the original data. Transform the dose values such that the values sum to zero.

KTC pharmaceutical company limited is developing a new antifertility drug in form of Norgestral supplemented with ethyl estradiol. The response is the mean number of subjects that are not pregnant after administration. From the results of the bioassay below:

Dose	12.5	25	50	100	200
Response	4	3	9	10	10

(a) Fit both log and square root transformation model for the dose – response relationship (b) Plot the fitted models and raw data, which model fits best?

5.0 Parallel Assays

5.1 Introduction

A parallel line bioassay is an experiment aimed at measuring the biological potency of a test preparation relative to a standard preparation of a similar substance.

Various doses of S & T preparations are administered to experimental units or subjects and in the ideal situation, the two dose-response curves are linear and parallel.

The log potency ratio of the test preparation relative to the standard preparation is defined as the horizontal displacement between the 2 dose response curves. The estimator of the potency ratio is the difference in log doses for the 2 preparations which give the same fitted response.

A symmetric parallel assay is one in which the number of dose levels of the test and standard preparations are the same. Dose levels are equispaced logarithmically and responses depend linearly on log dose.

An unsymmetrical parallel lines assay is one in which either the number of dose levels of the test and standard preparations are not the same or the dose levels are not equispaced logarithmically.

5.1 Fitting Dose-Response in Parallel Lines Assay

In parallel line assays, the dose response relation has the form $Y = \alpha + \beta(x - \bar{x})$ and the transformation is chosen with $\bar{x} = 0$ so that we have $Y = \alpha + \beta x$. This means that x values should be symmetric so that $\Sigma x = 0$. For the two lines to be parallel, they should have a common (pooled slope) but with different intercepts.

Example 5:1

The following is the result of a parallel line bioassay of vitamin D_3 in cod liver oil, by means of its antirachitic activity in chickens, using percentage bone ash as the response.

Dose of standard proportion, S				**Dose of test preparation, T**			
5.76	9.6	16		32.4	54	90	150
35	62	116		20	26	57	140
30							
24	67	105		39	60	89	133
37	95	91		16	48	103	142
28							
73	62	94		27	-8	129	118
31	54	130		-12	46	139	137
21							
-5	56	79		2	77	128	84
	48	120		31		89	101
	70	124				86	
	94						
	42						
n 9	10	8		7	6	8	7

∑Y	274	650	859	123	249	820	855
Y	30.4	65.0	107.4	17.6	41.5	102.5	122.1

Questions:

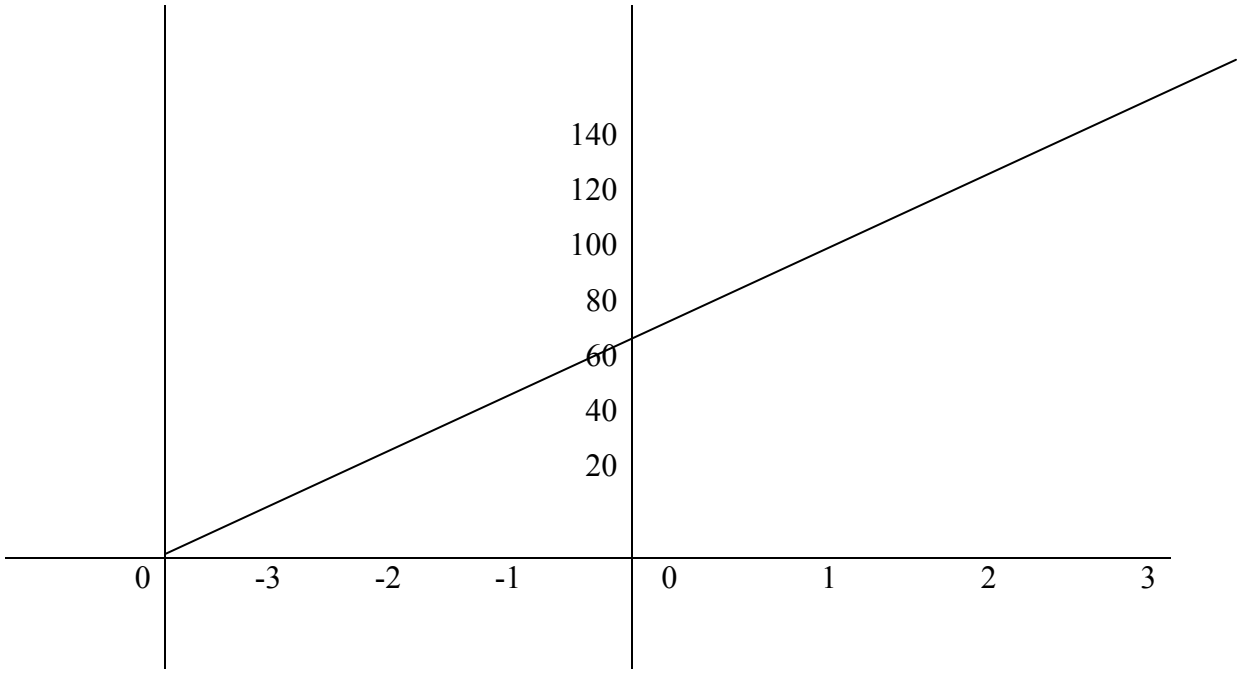
(i) Estimate the dose response relations for the test and the standard preparations

(ii) Fit the two dose response relations graphically

(iii)Use the graph to estimate M and hence the relative potency.

5.2 Estimation of Potency from graph

Two parallel line scan be drawn by eye so as to fit the points approximately. The horizontal distance between the lines roughly estimates the difference in X between doses giving equal responses: e.g. M = 0.3.



Recall that

$$X_s = \log_e Z_s - \log_e 16 + 2 = \log_e Z - \log_e 16 + \log_e e^2$$

$$= \log_e Z_s + \log_e \frac{e^2}{16} \quad \text{where } e = \sqrt{5/3}$$

$$X_t = \log_e Z_t - \log_e 150 + 3 = \log_e Z - \log_e 150 + \log_e e^3$$

where $e = \sqrt{5/3}$

Estimate of relative potency

$$M = X_s - X_t = \log_e Z_s + \log_e e^2/16 - \log_e Z_t - \log_e e^3/150$$

$$M \log_{10}^e = \log_{10} \frac{Z_s}{Z_t} + \log_{10} e^2/16 - \log_{10} e^3/150$$

$$\log R = M \log_e + \log e^3/150 - \log e^2/16$$

$$\log R = Mx \log_{10} e + \log_{10} \frac{e^3}{150} - \log_{10} \frac{e^2}{16}$$

$$= 0.1109M + \log(0.014344) - \log(0.1041666)$$

$$= 0.1109\text{M} - 1.84339 + 0998227$$

$$= 0.1109 - 0.8611 = 0.03327 - 0.8611$$

$$= \quad = \bar{1} + 1.03327 - 0.8611$$

$$= \bar{1}.1722$$

Hence, R = antilog ($\bar{1}.1722$)

$$R = 0.1487 \text{ units/mg.}$$

Potency of the cod-liver oil is estimated as 0.149 units/mg.

5.3 Estimation of potency from fitted regression

It can be shown that the horizontal displacement M can be derived as given below using the two fitted regression lines from the two preparations:

$$M = \bar{x}_s - \bar{x}_t + \frac{\bar{y}_t - \bar{y}_s}{b}$$

Recall that the regression equating obtained for each preparation were;

$$Y_s = 67.43 + 18.88x$$

$$Y_t = 71.76 + 18.88x$$

Hence from above problem, M can be obtained as

$$= -0.0741 - 0.0714 + \frac{73.1036 - 66.0296}{18.88}$$

$$= -0.155 + \frac{7.074}{18.88}$$

$$= 0.2292$$

But $\log R = 0.1109M - 0.8611(-0.2357)$

$$= 0.02542 - 0.8611 = \bar{1}.1643$$

$\Rightarrow R = 0.1460$

which is comparable with the graphical estimate of 0.149.

6.0 Fieller's Theorem

Recall that the relative log potency is given as

$$M = \bar{x}_s - \bar{x}_t + \frac{\bar{x}_t - \bar{x}_s}{b}$$

Where $\bar{x}_s - \bar{x}_t$ is a constant determined by the choice of doses and number of subjects in each preparation.

Suppose that α, β are two parameters and let $\mu = {}^{\alpha}/_{\beta}$. Also suppose that a, b are unbiased estimates of α, β, i.e. $\Sigma(a) = \alpha$ and $\Sigma(b) = \beta$. The estimated variances of a, b and their covariance are expressed as $s^2 V_{11}, s^2 V_{22}$ and $s^2 V_{12}$ respectively.

e.g. $if\ a = \bar{x}, V(a) = V(\bar{x}) = s^2({}^1/_n)$

$if\ b = regression\ coefficient, V(b) = {}^{s^2}/_{Sxx}$

If $W = a/b$ is the estimate of μ, then Fieller's theorem states that upper and lower limits of μ are

$$W_L, W_V = \left[W - \frac{V_{12}}{V_{22}} \pm \frac{t_s}{b}\left\{V_{11} - 2W_2 - g\left(V_{11} - \frac{V_{12}}{V_{22}}\right)\right\}\right]/(1-g) + V_{22}$$

where $g = \frac{t^2 s^2 v_{22}}{b^2}$ and it is the t-deviate with degrees of freedom, from t-table.

Note that when *b* is large relative to its standard error *g* will be small and if it can be neglected, *(g<.05)* then the above limits become

$$M_L, M_V = M \pm t_s(v_{11} - 2MV_{12} + m^2 v_{22})^{1/2}/6.$$

Furthermore, if the a and b can be assumed in , $\Longrightarrow cor(a,b) = V_{12} = 0$, then the limits become

$$M_L, M_V = M \pm t_s(v_{11} + w_2 v_{22})^{1/2}/b$$

(which is the same as the earlier result used for R i.e. $M_L, M_V = M \pm \frac{t_s}{b}\left(\frac{1}{N_1} + \frac{R^2}{N_2}\right)1/2$

with $R = \frac{\bar{x}_1}{\bar{x}_2}$)

7.0 Calibration Curves

Calibration is a statistical method or procedure that deals with the following procedures:

1) Evaluating the performance of individuals which can be used to predict the probability that an event of interest will occur in a specified period in the future, for example weather forcast.
2) Estimating the equation (assumed to be linear) for calibrating observations and hence; the precision of a number of measuring instruments on the basis of observations.
3) Assessing the relative calibrations and relating accuracies of a set of P instruments (P>1), each designed to measure the same characteristics on a group of individuals.

   The form of the calibration curve is given as

   $$Y - m(u,b), \quad i.e. Y - \alpha + \beta x$$

   For P instruments where

(1) $\alpha = (\alpha_1 + \alpha_2 + \cdots + \alpha_p)/P$

$\beta = (\beta_1 + \beta_2 + \cdots + \beta_p)/P$

(2) The mean $\bar{Y}$ for the P calibrated instruments is given by

$\bar{Y} = \frac{\bar{Y}_1 + \bar{Y}_2 + \cdots + \bar{Y}_p}{p}$ and has a confidence interval $\bar{Y} \pm t(v, \alpha) S_x S_y$ where $t(v, \alpha)$ is the value of the t-distribution with $v = (n_1 + n_2 + \cdots + n_p - P)$ dif. and α is the significance level.

Example: The Federal Medical Centre has just acquired the new sets of instruments for measuring blood pressure. They are developed by different companies but considered more portable, easier to operate and cheaper in terms of cost compared to the others in use. Regarding the one currently in use as a standard instrument, the systolis blood pressure readings using the three instruments were obtained as follows:

X^2	X (mmHg)	Y_1	Y_2	Y_2^2	Y_1^2	XY_1	XY_2
16129	127	131	129	16641	17161	16637	16383
20164	142	143	144	20736	20449	20306	20448
19044	138	140	140	19600	19600	19320	19320
19881	141	141	142	20164	19881	19881	20022
18769	137	139	138	19044	19321	19043	18906
14884	122	121	122	14884	14641	14762	14884
21316	146	146	144	20736	21316	21316	21024
24649	157	159	157	24649	25281	24963	24649
17689	133	135	135	18225	18225	17955	19755
22201	149	151	151	22801	22801	22499	22499
194726	**1392**	**1406**	**1402**	**197480**	**198676**	**196682**	**196090**

(a) Estimate the calibration curve for the data

(b) Construct a confidence interval for the calibration curve and hence estimate the upper and lower calibration curves.

(c) Plot the calibration chart (use scale of 1cm to 5 units on x-axis and 1cm to 10 in Y-axis.

(d) Obtain the calibration interval and have the inner and outer calibration interaction

(e) Calibrate for Y when X is 137.