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Estimation of the Dose-Response Model of Principle Biological Assays

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ABSTRACT

In this research bioassay technique was used which is rarely applied in scientific research. The problem was how to determine the properties of the drug, and efficiency of the active ingredients. Some industry needs to determine the potency of new batches relative to a standard sample, also needs to know what preparation is better and the relation between dose- responses. The main purpose of this study was to test differences between the means of test and standard preparations, and whether there was an effect of doses on the responses of insects, and also to determine if potency of test preparation is better than standard preparation. It is found that there was an effect of doses on responses of insects (ants), and the potency of test preparation was better than the test of standard preparation.

INTRODUCTION

A biological assay or bioassay is employed to determine the effect of a substance on a certain type of living matter. A biological test system, for example animals, tissue, etc. is expected to a particular stimulus like a drug, whose concentration (dose) is usually varied. The magnitude of the response of the biological system depends on the dose. In contrast with physical or chemical methods, detailed information of the drug activity as a function of the dose is obtained (Finney, 1978). A special characteristic of bioassays is that one of the largest sources of variation in the outcome is the difference between the test units, and since the response is dependent on living matter this introduces large variability between measurements obtained by identical operation (Rocke, 2004).

The response can be a characteristic like body, weight, or the occurrence of a certain phenomenon e.g. death. For example, in toxicological assays several doses of a substance are given to rats and the response variable is the survival of the rats (Finney, 1978). Biological assays are usually comparative; the capacity of a substance to cause a specific effect is estimated relative to a standard. The standard and the test preparations are identical in their biological activity

principle and differ only in extend to which they are diluted by solvents. This type of bioassay is used to determine the potency of new batches relative to a standard sample (Tarding *et al.,* 1969)

Serious scientific history of biological assay began at the close of 19th century with Ehrlich's investigations into the standardization of diphtheria antitoxin. Since then, the standardization of materials by means of the reactions of living matter has become a common practice, not only in pharmacology, but in other branches of science also, such as plant pathology. However the assays were put on sound bases only since 1930's when some statisticians contributed with their refined methods to this area (Saha, 2002). The aim of this study was to identify the nature of bioassay and to compare test substance with the standard preparation.

MATERIALS AND METHODS:

An experiment of two preparations was used to give the samples to insects and recorded the responses of each insect to find out the differences between preparations and fit the dose-response regression model of two preparations to estimate the potency of test preparation; the statistical package was used to find out the results and to fit the regression model for standard and test preparation. 5 samples of each insect was taken to test what is better test or response preparations represent the fit equation model. All we need are estimates of the β's to assess the effect of explanatory variables of interest and the measure of effect, which is called potency.

PRINCIPLES OF BIOLOGICAL ASSAYS:

The typical bioassay involves a stimulus (for example, a vitamin, a drug, a fungicide), applied to a subject (for example, an animal, a piece of animal tissue, a plant, a bacterial culture). The intensity of the stimulus (USP General Chapter (2008) is varied by using the various "doses" by the experimenter. Application of stimulus is followed by a change in some measurable characteristic of the subject, the magnitude of the change being dependent upon the dose. A measurement of this characteristic, for example, a weight of the whole subject, or of some particular organ, an analytical value such as blood sugar content or bone ash percentage, or even a simple record of occurrence or non-occurrence of a certain muscular contraction, recovery from symptoms of a dietary deficiency, or death-is the response of the subject (Saha, 2002).

Types of biological assays: There are two types of quantitative biological assays: direct and indirect assays (Finney, 1978) .The principle of a direct assay is to measure the doses of the standard and the test preparations that produced a specified response. The potency of the test preparation relative to the standard is defined as the amount of standard equivalent in effect to one unit of the test (Finney, 1978). In other words, the potency of the test defined as how much standard it is needed to produce the same effect as with one unit of the test substance .A direct assay is of limited applicability since it requires measuring the exact dose needed, not merely one that is large enough to produce a certain effect. The response must be easily recognized and the dose must be administered in a manner that the exact amount needed to produce the response is recorded (Finney, 1978).

In an indirect assay, several doses are given to different biological units. The

response is recorded for each dose. The relative potency of the test sample is determined relative to that of the standard by statistical of the dose-response relation.

The dose- response relation: In order to compare the biological response caused by a test sample relative to standard preparation, the test sample should contain the same active substances as the standard preparation. Furthermore, the test sample should not contain any other chemical, like for example impurities, which can affect the response variable. This implies that the test substance behave as a dilution or concentration of the standard preparation (Aldana Rosso, 2010).

A random sample of several identical biological units receives a dose z of the standard preparation, which produces a response of size u_s. The dose-response function is: $F_S(z)$ which is a real single valued function in the dose range used in the assay (Govindarajulu, (2001). The same procedure is repeated for the test preparation. The response function for the test preparation is: $F_T(z)$. For a certain response U, the doses are Z_S and Z_T.

Since the test preparation behaves as a dilution of the standard preparation, the mathematical form for the response is the same for both preparations. This condition is known as similarity, and it is a prerequisite assays. For all doses Z is fulfilled that:

$$Z_T * \rho = Z_S \ldots\ldots\ldots\ldots (1)$$

This expression in log-scale is $\log(Z_T) * \log(\rho) = \log(Z_S).$ the logarithm of the relative potency is the horizontal distance between the two responses.

Doses –responses regression: The regression of response on stimulus may be represented graphically as curve when the stimulus is in the form of a "dose" (e.g. of a drug, or possibly of applied force, or some other source), this may be called a "dose response curve"

In its simplest form, a dose –response curve is a simple linear or polynomial regression. More complex "dose – response" curves may involve; For example, a transcendental function .Other may involve transformation of the dose in the regression (Motulsky and Christopoulos, (2003)). This function of the dose is called the dose metameter. In some cases, the response is quantal (yes/no) and the dose-response technique is a probit analysis or logit analysis.

The determination of a threshold dose is also a dose –response problem .here the response is A below a dose x_0 and B above that point .That is the model is $E(Y|x) = A$ if $X < \chi_0$ and $E(Y|x) = B$ if $x \geq \chi_0$ where $E(Y|x)$ is the expected value of the response y given X=x. The model requires that we estimate the value of x_0 ,A and B.in this research we will consider function of simple linear model

$$E(Y|x) = A + Bx \ldots\ldots (2)$$

The step in conducting a dose –response (Montgomery, (2005) study (in idealized form) consists of the following:

(a) Assume a form or model for the curve (for the example, linear, quadratic, or threshold).

(b) Select the dose metameter (dose or log (dose)).

(c) Design the study so "good" information is obtained; this includes obtaining estimates of the model coefficient with small standard errors and having the ability to test for model

failures (such as testing for a quadratic, when a linear one has been assumed or testing for non-normal errors).

(d) Collect the data.

(e) Perform the analysis.

(f) Prepare a report describing the steps in the study, including the limitations of the study.

Dose -response curve: Dose –response curve is a simple X-Y graph relating the magnitude of a stressor (e.g. amount of a drug, temperature) to the response of the receptor (e.g. organism under study) .The response may be physiological or biochemical response, or even death (mortality), and thus can be count (or proportion, e.g. mortality rate),ordered descriptive categories (e.g., severity of a lesion),or continues measurement (e.g., blood pressure) (Altshuler,1981) ,A number of effects (or endpoints)can be studied ,often at different organization levels (e.g., population whole animals , tissue, cell) .

The measurement dose (usually milligrams, micrograms, or grams per kilograms of body- Wight for oral exposures or milligrams per cubic meter of ambient air for inhalation exposures) is generally plotted on the X axis and the response is plotted on the Y axis. Other dose unit includes moles per body-weight, moles per animal, and for dermal exposure, moles per square centimeter. In some cases, it the logarithm of the dose that is plotted on the X axis, and in such cases the curve is typically sigmoidal, with the steepest portion in the middle. Biological based models using dose are preferred over the use of log (dose) because the latter can visually imply a threshold dose when in fact there is none.

The first point along the graph where a response above zero (or above the control response) is reached is usually referred to as a threshold –dose. For most beneficial or recreational drugs, the desired effects are found at doses slightly greater than the threshold dose. At higher doses, undesired side effects appear and grow stronger as the dose increases. The more potent a particular substance is, the steeper this curve will be , In quantitative situations ,the Y-axis often is designated by percentage ,which refer to the percentage of exposed individual registering a standard response (which may be death , as in LD_{50}). Such a curve is referred to as a quantal dose-response curve, distinguishing it from a graded dose – response curve, where response is continuous (Pingel, et al. (1985).

The LC_{50}/LD_{50} represent the concentration LC_{50} or dose LD_{50} at which 50% of the population responds.

Parallel line assays: In general, dose response function is not linear but they can be transformed to become linear. One of the most frequently used transformations is the log-transformation (Finney, 1978). In the most widely used type of assay, the transformed response has a homogeneity linear regression on log-dose, that is the variance of the log-response is independent of the log-dose. For such an assay the condition of similarity is equivalent to the condition that the test and the sample transformed response curves are parallel (Aldana Rosso, 2010).

The dose-response curves of the test and the standard preparation are:

$$Y_T = a_T + b * \log(doses) \ldots\ldots\ldots\ldots (3)$$

$$Y_S = a_s + b * \log(doses) \ldots\ldots\ldots\ldots\ldots (4)$$

The relative potency is defined as the amount of standard sample needed to produce the same response as with the test preparation, therefore the relative log-potency is the horizontal distance between the two responses (Christophe Hurlin, (2013):

$$a_S + b*\log(dose_S) = a_T + b*\log(dose_T) \ldots\ldots\ldots(5)$$

Therefore:

$$\log(\rho) = \log(dose_T) - \log(dose_S) = \frac{a_s - a_t}{b} \ldots\ldots\ldots(6)$$

Where:

b =pooled slope and calculate from:

$$b = \frac{(S_{XY})_S + (S_{XY})_T}{(S_{XX})_S + (S_{XX})_T} \ldots\ldots\ldots\ldots\ldots(7)$$

RESULTS AND DISCUSSION

The data of two preparations (test preparation –standard preparation) each of them have five doses (0.024, 0.027,0.03, 0.033, and 0.036) for test preparation, and (0.009, 0.012, 0.015, 0.018, 0.021,) for standard preparation was taken to analyzed, each dose has 9 responses for insects (ants) to determine the relative potency of test preparation.

Test of differences between two means:

$$H_0 : \mu_T = \mu_S$$

$$H_1 : \mu_T \neq \mu_S$$

Table (1) shows the descriptive statistics for the tolerances of chlordane which given to the insects to known the responses till to kill it, the mean for test preparation is (0.7507). The mean for standard preparation is (0.2554) means that the test preparation is kill quickly than the standard preparation, the standard deviation for test preparation is (0.1448) and (0.1681) for the standard preparation. The test mean is greater than standard mean, also the values in standard preparation is more variate one.

Table 1: Preparations descriptive Statistics

Preparations		N	Mean	Std. Deviation
Response	Test	45	0.7507	0.1448
	standard	45	0.2554	0.1681

From Table (2) found that the value of t-test was (14.976) with degree of freedom was (88), and significance probability was (0.000) this value is less than (0.05) means that there is the difference between the two preparations (is not same). By the other words the response in test preparation varies much more than the response in standard preparation. And the mean difference between two preparations was (0.4953) with confidence interval (0.4296, 0.5611).

Table 2: The result of t-test

t-test	d.f	Sig (2-tailed)	Mean difference	Std. error of mean	95% confidence interval of the difference	
					lower	upper
14.976	88	0.000	0.4953	0.0331	0.4296	0.5611

This means that there is a statistically significance difference between mean response of test preparation and mean response of standard preparation. Since the table of group statistic revealed that the mean for the test preparation was greater than the mean for the standard

preparation (clearly in the positive value of mean).

Test for effect of doses and preparation on responses of insects:

H_0:There was no significant effect from doses on responses.

H_1: There was significant effect from doses on responses.

For preparation:

Hypotheses:

For doses:

H_0:There was no significant effect from preparation on responses.

H_1: There was significant effect from preparation on responses.

Table 3: The effect of doses and preparations on responses of (ants)

Source	Sum of square	d.f	Mean square	F	Sig
Corrected model	59.010	2	29.505	136.907	0.000
Intercept	15.291	1	15.291	70.953	0.000
Log doses	9.313	1	9.313	43.212	0.000
Preparation	1.598	1	1.598	7.717	0.030
Error	1.509	7	0.216		
Total	265.473	10			
Corrected model	60.519	9			

Source: researcher using SPSS 2016.

Table (3) indicated that there is a significant main effect for the log doses (0.000), and there was also significant main effect for the preparation (0.003), the two main effects were qualified, however, by a significant interaction between (preparation*log doses) (0.000).

CONCLUSION

There are significant differences between test preparation and standard preparation. (Sig (0.000)), with value (t =14.976). The mean differences between two preparations are (0.4953). Also there is effective significant of doses on responses of insect (ants) the (Sig = 0.000) with value (F = 43.212), this implies that when there is a positive relationship between doses and responses. To check there is effective significant from preparation on responses of insects (ants) we found that the (Sig = 0.030) with value (F = 7.717). means that the efficiency of test preparation is better than standard preparation. The potency of test preparation is (0.206) in other words, (1) cc of the standard preparation is equivalent to (0.206) cc of test preparation. The test preparation is more effective than the standard preparation.

We recommended the investigation to use the bioassays method analysis because it is new method an unknown. Also utilize the techniques of potency to determine how the test preparations do well. And we advised the Medical factories owners to use bioassay to determine dose-response relation.

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