# SYMPOSIUM ON "THE ROLE OF STATISTICS IN BIOLOGICAL ASSAYS"*

DR. B. MUKERJI, Director, Central Drug Research Institute, Lucknow, presided over the Symposium. In his opening remarks he said that careful design of experiment is vital to investigational work in all branches of science, and more particularly in biology. Uncontrolled factors are usually present in the material under investigation and may magnify the errors in the results and lead to misinterpretations. The contribution of statistics to experimental design in biological assays is to help furnish logical schedules for taking measurements that will hold potential sources of bias in check and increase the accuracy of the comparisons to be made.

Three types of variation are likely to appear in biological assays. The first type might be called ' deliberate variation ' and arises from the different factors or treatments introduced by the experimenter himself for purposes of comparison. The second type of fluctuation arises from unintentional and sometimes unknown changes in experimental conditions. The third type, usually called ' experimental error ' or ' normal variation', is the chance fluctuation that no amount of skilful planning can eliminate. Curbing sources of unplanned variation is a major function of the statistical design of experiments.

He then emphasized the need for the adoption of such basic statistical techniques as randomisation, supplementary measurements and replication in designs for biological assays, and concluded with the remark that the modern methods of statistical analysis and interpretation could be of major help to workers in the field of biological sciences where comparisons of responses in animals are sometimes extremely difficult.

Dr. P. V. Krishna Iyer (Defence Science Laboratory, New Delhi) pointed out that statistical methods enabled the experimenters to conduct their investigations economically and efficiently. Finney, Bliss, Gaddum and others have shown that the statistical designs used in agriculture are equally applicable in bioassays with some modifications. The analysis mainly consists in testing the regression coefficients of the response on the dose metameter for the various preparations.

---

* Symposium held on 22nd January 1957, at the Central Drug Research Institute, Lucknow, on the occasion of the 10th Annual Meeting of the Indian Society of Agricultural Statistics.

When the responses are quantal, the analysis is best done by using the maximum likelihood procedure.

The analyses of bioassays data are mostly based on the assumption that the observations are distributed normally. It may be worthwhile to examine how far non-parametric methods which are independent of the form of the distributions are applicable for analysing data on bioassays. He emphasised that efficient non-parametric methods can be devised for analysing bioassay data. As an instance he cited the non-parametric tests designed by Iyer and Singh (*J. Ind. Soc. Agri. Stat.*, Vol. VII) for testing the regression coefficients of the response to test and standard preparations. Further investigations on determining the non-parametric confidence interval for a given sample, using the statistics considered in the above paper, were in progress.

Shri M. N. Das (Indian Council of Agricultural Research, New Delhi) said that in recent years the need for the use of statistical methods in bioassays is being gradually recognised. This was mainly due to the work of Dr. Finney who supplied the underlying theory to connect all the techniques which were outwardly disconnected, filled in the existing gaps and wrote two valuable books for the guidance of both the statisticians and bioassayers.

The object of bioassays is mainly to measure the potency of some unknown preparation of a substance by means of the reactions which it produces in living matter. It is to get an estimate of this potency together with a measure of its precision, after taking into account the variation among test subjects, that the science of statistics becomes indispensable.

The application of statistical techniques can be ensured by properly designing the assay. The main purpose of designing the trial in bioassay is first to ensure the validity of the estimate of potency and secondly to decrease the error of estimation. The precision of an assay can be increased by using homogeneous groups of animals.

The assay techniques can be divided into several broad classes. Certain preparations allow direct measurements of their doses required to produce certain specified responses. Such assays are known as direct assays. In cases where this is not possible, indirect methods are adopted. These indirect assays may be further divided into parallel line and slope ratio assays. If the response happens to be quantal, the technique of probit analysis is most profitably adopted in such cases.

Shri S. G. Mohanty (Indian Council of Agricultural Research, New Delhi) remarked that one of the primary problems in biological

assays is to estimate the ED 50 (Effective medium dose) in quantal response data. Probit analysis is the standard technique employed for the purpose where a direct sampling procedure of testing groups of subjects at preassigned levels of intensity of stimulus is followed. It may, however, be necessary to evolve a procedure of allocation of the available supply of test subjects at the various preassigned levels, so as to maximise the precision of the estimate of ED 50. Bartletts' inverse sampling technique is a procedure of this type. Dixon and Mood have developed another technique known as 'Up and Down' method which may be preferred in certain situations to the probits. The technique is to choose a series of levels fixed throughout the experiment and the first subject is tested on any of the levels selected at random. Then at any stage of the process, any subject will be tested at the level immediately below or above the level on which the previous test was made according as the previous subject responded or not. The estimation is also simple provided the sample is large and a rough estimate of the standard deviation of the normally distributed transformed variate is available in advance.

The chief advantage of the 'Up and Down' method is that the allocation of subjects at various levels is automatic and the tests tend to concentrate near ED 50. This increases the accuracy with which ED 50 can be estimated. The method has been proposed by Brownlee, Hodges and Rosemblatt for small samples provided the standard deviation of the normalised variate is known beforehand.

T. V. Narayana has discussed two other procedures, *viz.*, the 1-rule and the 3-rule. For allocating subjects at various levels at any stage, the 1-rule uses all the previous results of the test on the level employed at that stage, while the 3-rule uses all the previous results of the test on the two neighbouring levels as well.

Under certain general conditions it has been proved that after a large number of tests have been made under this procedure, all the tests will be confined to the 3 closest levels to the ED 50 with a probability approaching as near to the unity as we please. Besides, a modified version of the 3-rule may be applied in situations where more precise information is available regarding the distribution of the variate.

Shri P. A. George (Central Drug Research Institute, Lucknow) pointed out that in the usual situation in bioassay with quantal responses, a certain number of test subjects, say '$r$', respond out of '$n$' subjected to the test dose. Here both '$n$' and '$r$' are known exactly but there are situations where this may not be so. For example, in testing the effect of temperature or of some bactericidal preparation on

living bacteria, it may be necessary to take a small volume of the bacterial suspension subjected to the treatment, and determine the number of surviving bacteria, say by the plating technique. Here the total number of bacteria is not known, only the survivors are counted exactly.

The number of survivors '$s$' in a sample will be a Poisson variate, and with a series of values of '$s$' at different test doses, the ED 50 of the bactericide (or the temperature) can be calculated by a process similar to the usual probit analysis. Since the probability of function is different the weights and the working equivalent deviate will be different. Wadley (*Ann. Appl. Bio.*, **36**, 1949) has considered this problem and a computation technique similar to the probit analysis has been developed. Anscombe (*Ann. Appl. Bio.*, **36**, 1949) has also considered the problem where the distribution is taken to be a negative binomial instead of the Poisson.

Shri George then considered an extension of Wadley's problem, when the number '$S$' is not exactly counted but estimated by the 'fertile place' technique. If $P(x)$ is the percentage kill at dose $x$, $(1 - P = Q)$ and '$m$' is the expected number of bacteria in the sample, the probability of a sample being fertile is $[1 - \text{Exp}(-mQ)]$. The ED 50 and other parameters (*e.g.*, the slope) can be calculated by Finney's general maximum likelihood solution. If $P(x)$ is taken to be a normal distribution function, a computational scheme similar to probit analysis can be derived. However, because of the parameter '$m$' additional tabulation will be necessary for obtaining the weighting function $w$ and the working deviate. For maximum efficiency at any test dose it was found that $mq = 1 \cdot 5936$.

Shri George in conclusion demonstrated that the same distribution can arise in quite different situations also, *e.g.*, where a particular organism takes time to develop inside a host and we are interested in the mean or medium time required for the development of a single organism inside the host, assuming the development time (or its logarithm) to be normally distributed.

Shri N. Sen (Central Drug Research Institute, Lucknow) said that instead of comparing the magnitude of the effects of different treatments, biological assays are used to determine the relative potencies of test and standard preparations. These assays are of two kinds, *viz.*, quantal and quantitative. Quantal assays may also be of two types, *viz.*, (i) number killed is observed out of a known number, as in insecticidal trials, (ii) number surviving is observed out of unknown

numbers, as in bacterial assays. The role of the statistician in a bio-assay experimental programme is to give the statistical basis of the assay, to give the design of the assay experiment, to analyse the data and to suggest further lines of investigation.

Shri Sen then considered some of the requirements in the design of toxicity assays like the number of doses to be taken, number of insects to be assigned to each dose, number of replications with each test preparation, factors to be varied and overall cost of experimentation.

The choice of doses depends upon what percentage point we are interested in estimating. It is advisable to get a rough estimate by a pilot assay and then the doses are chosen so that the quantal responses are symmetric about the percentage point in question. For example, in estimating LD 50, the doses are chosen such that the variance of log (LD 50) is minimum. For estimation of LD 90 or LD 05 the doses should be so chosen as to give responses near about the stated extreme percentages.

The tolerances shown by insects vary within wide limits and are generally very susceptible to environmental factors. The distributions in original units are skew but may be normalised by the use of suitable transformations.

Relative toxicity is determined from the dosage mortality curves for the test and standard preparations by using the method of maximum likelihood, and the error of estimate is also obtained. When excessive variation is observed, it is advisable to assign the insects at random in small groups. Variances from separate replications may be combined to give a pooled estimate. On repeated experimentation the relative toxicities may be combined by giving weights equal to the reciprocal of the variances.

Test insects generally vary in susceptibility both in mean and variance. The susceptibility depends very much on the species, sex, age and the environmental conditions. Its variability was observed to depend very much on the method of administration. Shri Sen concluded from a series of experiments conducted by him that if in repeated experiments position is more stable, it is advisable to estimate the error of assay from the observed log (LD 50)'s in the different experiments, and if position is variable and the slope is stable, 'internal estimates' may provide better estimate error. Shri Sen quoted some results from another series of experiments conducted by him and concluded that if the experimental control is such as to give quite consistent estimates of the parameters in repeated experiments, the standard curve estimated

from observed log (LD 50)'s is a better estimate of the population standard deviation of log (LD 50) than the estimate from 'internal estimates'.

Shri R. S. Asthana (Department of Agriculture, U.P., Lucknow) said that a physician is not so much interested in the theraputic ratio $\frac{\text{LD } 50}{\text{ED } 50}$ of a new medicinal agent as in the index of standard safety margin (S.S.M.) given by $\left(\frac{\text{LD } 1}{\text{ND } 99} - 1\right) 100$, (ND stands for Narcotic Dose), as recommended by Foster. The index appeared to be good enough but he cautioned the research workers in its use since the probit transformation is good enough for the estimation of LD 50 or ED 50, but the estimates of LD 1 or ND 99 may not be sufficiently accurate.

He then said that the assays of mixtures of drugs have also posed a new set of problems both for statisticians and biologists. The joint action of two drugs has been classified into similar and dissimilar actions with further divisions in the presence and absence of interaction. No quantitative and mathematical representation of the various joint action phenomenon has been given so far. Prof. Gaddum has, however, given a qualitative representation. Finney has given a mathematical form for the synergistic action which has been proved to be not satisfactory by Plackett and Hewlett. The latter have constructed a model with seven parameters but no experimental evidence has been reported. These developments are new and open new lines of investigations.

He concluded with the remark that both in the design and analysis of bioassay data, a thorough knowledge of statistical principles is essential in order to arrive at inferences which may have some scientific value.