Statistical evaluation of mutagenicity test data

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UKEMS sub-committee on guidelines for mutagenicity testing. Report. Part III

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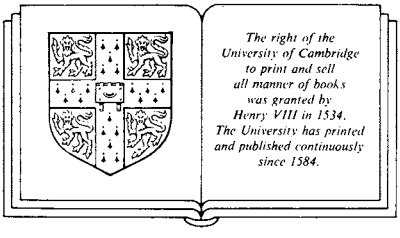

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PREFACE

D.J. KIRKLAND

1 OBJECTIVES

In March 1982 the United Kingdom Environmental Mutagen Society appointed a Sub-Committee to determine the minimal professional criteria that should be applied to mutagenicity testing in order to meet the requirements of UK authorities. The tests recommended in the 'Guidelines for Testing of Chemicals for Mutagenicity' which was published by the Department of Health and Social Security (DHSS, 1981) formed the initial basis of the first volume which dealt with the most commonly used mutagenicity tests (UKEMS, 1983). A second volume (UKEMS, 1984), which also had to take account of other published guidelines, addressed a series of supplementary tests.

Very few of the chapters in these first two volumes adequately tackled the statistical aspects, either in terms of experimental design, or in terms of data analysis. As many guidelines were employing phrases like 'Data should be analysed using appropriate statistical methods' the UKEMS Sub-Committee decided that Part III of their reports should address the statistical evaluation of mutagenicity test data. This report therefore attempts to do that and, where appropriate, to highlight the statistical implications of experimental design. The topics covered include bacterial and mammalian cell colony and fluctuation assays, *in vitro* and *in vivo* chromosomal aberration tests, sister chromatid exchange tests, *Drosophila* and dominant lethal assays.

2 TERMS OF REFERENCE

The terms of reference of the Sub-Committee were to assess the various statistical approaches available for their suitability in evaluating data from the most widely used mutagenicity tests, such that practising genetic toxicologists would be able to better understand what was required of them by regulatory authorities in this respect, and be better advised as to

which forms of analysis were preferred, and why. Specifically for each of the test types, the following items were to be considered:

2.1 How to determine the suitability of the data obtained from an assay for fitting a distribution; when the data are unsuitable; when and how data should be transformed.

2.2 The types of statistical analyses that can be used with the assay data under consideration; which, if any, factors govern the choice of analysis; an order of preference if several types of analysis may be used.

2.3 Some worked examples using real data to help the reader understand 2.1 and 2.2.

3 STRUCTURE

The Sub-Committee consisted of a Steering Group with the task of assessing and reporting on the papers submitted by a series of individual Working Groups (the exception was the introductory paper which was written by one person).

3.1 Steering Group

The main sections of UKEMS were represented by seven individuals and this group was supplemented by three statisticians used to dealing with genetic toxicology data on a regular basis.

3.2 Working Groups

Eight Working Groups were established and chaired by UKEMS members with relevant expertise. The Working Groups comprised between five and nine members, and each group included at least two statisticians.

4 SAFETY CONSIDERATIONS

The safety of staff involved in the conduct of mutagenicity tests described in this and earlier reports has been a fundamental consideration of the Sub-Committee who wish to emphasise that such staff should be fully trained in techniques for handling hazardous chemicals and should be fully aware of the nature of the hazards by reference to the appropriate handbooks (NCI, 1975; IARC, 1979; MRC, 1981).

5 TIMETABLE

The terms of reference of the Sub-Committee require the provision of information that reflects the current state of knowledge of the field.

Reports I and II (UKEMS, 1983, 1984), dealing with genetic toxicology methods in a rapidly developing field, were therefore completed to strict timetables. It was recognised with this report that, although the format or type of genetic toxicology data presented for statistical analysis may change fairly rapidly with time, the statistical approaches would be likely to change less rapidly. It was also recognised that a familiarisation period was required during which genetic toxicologists and statisticians on Working Groups and the Steering Group learned more of each other's disciplines and languages such that communication could be effective. A rigid timetable was not therefore enforced and the entire project spanned from Summer 1985 to Spring 1988.

6 ACKNOWLEDGEMENTS

On behalf of the Sub-Committee and the Society I would like to express my profound thanks to all of the participants in this project and in particular to the statisticians, many of whom were not UKEMS members yet exhibited immense enthusiasm and commitment. I would also like to thank the UK Health and Safety Executive for their financial and moral support of this project.

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David J. Kirkland
Sub-Committee Chairman

LIST OF ABBREVIATIONS

$d.f.$ = degrees of freedom
MS = mean square
F or VR = variance ratio
P = probability
NS = not significant
SCP = sum of cross product
D or d = independent variable, e.g. dose
SS = sum of squares
x = dependent variable, e.g. colony count
EMS = error mean square
R = rank
L_j = sum of ranks to cut point 'j'
$S.D.$ = standard deviation
$S.E.$ = standard error
W = weight
MF = mutant frequency
V or Var = variance
OUA = ouabain
6TG = 6-thioguanine
TFT = trifluorothymidine
CHO = Chinese hamster ovary
SCE = sister chromatid exchanges
$ANOVA$ = analysis of variance
z or Z = standard or normal deviate
PE = polychromatic erythrocyte
MPE = micronucleated polychromatic erythrocyte
MTD = maximum tolerated dose
MI = mitotic index
H_0 = null hypothesis
H_1 = alternative hypothesis
α = type I error or probability of false positive conclusion
β = type II error or probability of a false negative conclusion
$1-\beta$ = the power of a statistical test