

Statistics in Toxicology Using R

Ludwig A. Hothorn

Institut für Biostatistik, Leibniz Universität
Hannover, Germany



CRC Press

Taylor & Francis Group

Boca Raton London New York

CRC Press is an imprint of the
Taylor & Francis Group, an **Informa** business
A CHAPMAN & HALL BOOK

Contents

List of Figures	ix
List of Tables	xiii
Preface	xvii
1 Principles	1
1.1 Evaluation of short-term repeated toxicity studies	1
1.2 Selected statistical problems	1
1.2.1 Data visualization by barcharts or boxplots?	1
1.2.2 How to present tests' outcomes: Stars, letters, p -values, or confidence intervals?	5
1.2.3 Proof of hazard or proof of safety?	7
1.2.4 Sample size matters	10
1.2.5 Multiplicity occurs	11
1.2.6 Several types of endpoints occur	11
1.2.7 Directional decisions	11
1.2.8 Specific designs	12
1.2.9 Mixing distribution and outliers	12
1.2.10 The phenomenon of conflicting decisions	13
1.2.11 Decision tree approaches	13
1.2.12 The special importance of control groups	14
1.2.13 Statistical significance and biological relevance	16
1.3 Proof of hazard using two-sample comparisons	17
1.3.1 Normal distributed continuous endpoints	17
1.3.2 Log-normal distributed continuous endpoints	19
1.3.3 Non-normal distributed continuous endpoints	20
1.3.4 Proportions	21
1.3.5 Counts	23
1.3.6 Further endpoint types	24
2 Simultaneous comparisons versus a negative control	25
2.1 Proof of hazard using simultaneous comparisons versus a negative control	25
2.1.1 Normally distributed continuous endpoints: The Dunnett procedure	25
2.1.2 Normally distributed continuous endpoints: The Williams procedure	30
2.1.3 Normally distributed continuous endpoints: Ratio-to-control procedures	34
2.1.4 Nonparametric approaches for comparisons versus a negative control	38

2.1.5	Simultaneous comparisons versus a negative control for proportions	39
2.1.6	Trend tests for proportions	48
2.1.7	Multinomial endpoints: Evaluation of differential blood count . . .	53
2.1.8	Analysis of graded histopathological findings	55
2.1.9	Comparisons versus a negative control for transformed endpoints .	62
2.1.10	Testing mixed responder/non-responder data	64
2.1.11	Testing non-inferiority: The evaluation of recovery period data . .	65
2.2	Trend tests	67
2.2.1	Aims and limitations	67
2.2.2	Closed testing procedure and order restriction	71
2.2.3	Trend tests for different endpoint types and different designs . . .	71
2.3	Reference values	73
2.4	Analysis of complex designs	75
2.4.1	Analysis of interactions: Evaluation of sex by treatment interaction	76
2.4.2	Analysis of designs between one- and two-way layouts	77
2.4.3	Analysis of block designs	79
2.4.4	Analysis of covariance: Evaluation of organ weights	81
2.4.5	Repeated measures: Evaluation of body weights	88
2.5	Proof of safety	92
2.5.1	One-sided hypotheses: Test on non-inferiority	93
2.5.2	Two-sided hypotheses: Test on equivalence	95
3	Evaluation of long-term carcinogenicity assays	99
3.1	Principles	99
3.2	Analysis of mortality	100
3.2.1	Common NTP-style	100
3.2.2	A Williams-type trend test for the comparison of survival functions	102
3.3	Analysis of crude tumor rates	103
3.3.1	Analysis of crude tumor rates using a Williams-type test	103
3.3.2	Analysis of crude tumor rates using historical control data	104
3.4	Mortality-adjusted tumor rates with cause-of-death information	105
3.4.1	Analysis of incidental tumors	106
3.4.2	Analysis of fatal tumors	108
3.5	Mortality-adjusted tumor rates without cause-of-death information	110
3.6	More complex analyzes	111
3.6.1	Multiple tumors	111
3.6.2	Multivariate response	116
3.6.3	The combined analysis over sex	117
3.6.4	Time-to-event data with litter structure	118
4	Evaluation of mutagenicity assays	121
4.1	What is specific in the analysis of mutagenicity assays?	121
4.2	Evaluation of the Ames assay as an example for dose-response shapes with possible downturn effects	122
4.3	Evaluation of the micronucleus assay as an example for nonparametric tests in small sample size design	125
4.4	Evaluation of the SHE assay using trend tests on proportions	128
4.4.1	The Cochran-Armitage trend test for proportions	129

4.4.2	Trend tests followed by pairwise tests	130
4.4.3	Evaluation using Dunnett-type procedure for proportions	131
4.5	Evaluation of the <i>in vivo</i> micronucleus assay as an example of the analysis of proportions taking overdispersion into account	132
4.6	Evaluation of the <i>in vivo</i> micronucleus assay as an example of the analysis of counts taking overdispersion into account	133
4.7	Evaluation of HET-MN assay for an example of transformed count data	136
4.8	Evaluation of cell transformation assay for an example of near-to-zero counts in the control	136
4.8.1	Profile likelihood	137
4.8.2	FT-transformation	138
4.8.3	Zero-inflated Poisson model	139
4.9	Evaluation of the LLNA as an example for <i>k</i> -fold rule	140
4.10	Evaluation of the HET-MN assay using historical control data	141
4.11	Evaluation of a micronucleus assay taking the positive control into account	143
4.12	Evaluation of the Comet assay as an example for mixing distribution	144
4.13	Evaluation of the <i>in vitro</i> micronucleus assay as an example for comparing cell distributions	149
5	Evaluation of reproductive toxicity assays	153
5.1	The statistical problems	153
5.2	Evaluation of the continuous endpoint pup weight	154
5.2.1	Possible simplification?	156
5.3	Evaluation of proportions	157
5.3.1	Possible simplification?	161
5.3.2	Analysis of multiple binary findings	162
5.4	Analysis of different-scaled multiple endpoints	166
5.5	Analysis of female-specific endpoints	168
5.6	Behavioral tests	169
5.6.1	Behavioral tests on selected pups	169
5.6.2	Behavioral tests with time-to-event data	172
5.6.3	Morris water maze test using juvenile rats	173
6	Ecotoxicology: Test on significant toxicity	177
6.1	Proof of safety	177
6.2	Two-sample ratio-to-control tests	178
6.2.1	Two-sample ratio-to-control tests for non-inferiority for normal distributed endpoints, allowing heteroscedasticity	179
6.2.2	Two-sample ratio-to-control tests for proportions	181
6.3	Ratio-to-control tests for several concentrations	182
7	Modeling of dose–response relationships	185
7.1	Models to estimate the ED_{xx}	185
7.2	Benchmark dose estimation	189
7.3	Is model selection toward LOAEL an alternative?	192
8	Further methods	197
8.1	Toxicokinetics	197
8.2	Toxicogenomics	199
8.3	Evaluation of interlaboratory studies	201

9 Conclusions	205
Appendix: R Details	207
A.1 Selected packages containing specific statistical approaches	207
A.2 Packages containing toxicological data	208
A.3 Packages containing specific graphics and data manipulation	208
References	209
Index	233