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Water quality — Guidance on statistical interpretation of ecotoxicity data

Qualité de l'eau — Lignes directrices relatives à l'interprétation statistique de données écotoxicologiques



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Contents

Page

Foreword.....	xii
Introduction	xiii
1 Scope	1
2 Normative references	1
3 Terms and definitions	1
4 General statistical principles.....	8
4.1 Different statistical approaches	8
4.1.1 General.....	8
4.1.2 Hypothesis-testing methods	8
4.1.3 Concentration-response modelling methods	10
4.1.4 Biology-based methods	11
4.2 Experimental design issues	11
4.2.1 General.....	11
4.2.2 NOEC or EC _x : Implications for design.....	12
4.2.3 Randomization	12
4.2.4 Replication.....	13
4.2.5 Multiple controls included in the experimental design.....	13
4.3 Process of data analysis.....	14
4.3.1 General.....	14
4.3.2 Data inspection and outliers.....	14
4.3.3 Data inspection and assumptions	15
4.3.3.1 Scatter	15
4.3.3.2 Heterogeneous variances and distribution	15
4.3.3.3 Heterogeneous variances and true variation in response.....	16
4.3.3.4 Consequences for the analysis	16
4.3.4 Transformation of data.....	16
4.3.5 Parametric and non-parametric methods	17
4.3.5.1 General	17
4.3.5.2 Parametric methods.....	17
4.3.5.3 Generalized linear models (GLMs)	18
4.3.5.4 Non-parametric methods.....	18
4.3.5.5 How to choose?.....	18
4.3.6 Pre-treatment of data.....	19
4.3.7 Model fitting.....	19
4.3.8 Model checking	20
4.3.8.1 Analysis of residuals	20
4.3.8.2 Validation of fitted dose-response model	21
4.3.9 Reporting the results.....	21
5 Hypothesis testing.....	21
5.1 Introduction	21
5.1.1 General.....	21
5.1.2 NOEC: What it is, and what it is not.....	25
5.1.3 Hypothesis used to determine NOEC	25
5.1.3.1 Understanding the question to be answered	25
5.1.3.2 One-sided hypothesis.....	26
5.1.3.3 Two-sided trend test	26
5.1.3.4 Trend or pair-wise test.....	26
5.1.4 Comparisons of single-step (pair-wise comparisons) or step-down trend tests to determine the NOEC	28

This is a preview of "ISO/TS 20281:2006". [Click here to purchase the full version from the ANSI store.](#)

5.1.4.1	General discussion	28
5.1.4.2	Single-step procedures.....	28
5.1.4.3	Step-down procedures.....	29
5.1.4.4	Deciding between the two approaches	30
5.1.5	Dose metric in trend tests	31
5.1.6	Role of power in toxicity experiments	31
5.1.7	Experimental design	32
5.1.8	Treatment of covariates and other adjustments to analysis	33
5.2	Quantal data (e.g. mortality, survival).....	34
5.2.1	Hypothesis testing with quantal data to determine NOEC values	34
5.2.2	Parametric versus non-parametric tests	35
5.2.2.1	Basis	35
5.2.2.2	Single-step procedures.....	36
5.2.2.3	Step-down procedures.....	36
5.2.2.3.1	Choice of step-down procedure.....	36
5.2.2.3.2	Test for monotone dose response	36
5.2.2.3.3	Analysing the monotonic response for quantal data — Step-down procedure	37
5.2.2.3.4	Possible modifications of the step-down procedure.....	37
5.2.2.4	Alternative procedures	37
5.2.2.4.1	Parametric and non-parametric procedures.....	37
5.2.2.4.2	Pair-wise ANOVA-based methods	38
5.2.2.4.3	Jonckheere-Terpstra trend test.....	38
5.2.2.4.4	Poisson tests	38
5.2.2.5	Assumptions of methods for determining NOEC values	38
5.2.3	Additional information.....	39
5.2.4	Statistical items to be included in the study report.....	40
5.3	Hypothesis testing with continuous data (e.g. mass, length, growth rate) to determine NOEC	40
5.3.1	General.....	40
5.3.2	Parametric versus non-parametric tests	41
5.3.3	Single-step (pair-wise) procedures	42
5.3.3.1	General	42
5.3.3.2	Dunnnett's test.....	42
5.3.3.3	Tamhane-Dunnnett test.....	42
5.3.3.4	Dunn's test	42
5.3.3.5	Mann-Whitney test.....	43
5.3.4	Step-down trend procedures	43
5.3.5	Determining the NOEC using a step-down procedure based on a trend test	43
5.3.5.1	General	43
5.3.5.2	Preliminaries	43
5.3.5.3	Step-down procedure.....	43
5.3.5.3.1	Preferred approach	43
5.3.5.3.2	Williams' test.....	44
5.3.5.3.3	Jonckheere-Terpstra test.....	44
5.3.6	Assumptions for methods for determining NOEC values	44
5.3.6.1	Small samples — Massive ties.....	44
5.3.6.2	Normality	45
5.3.6.3	Variance homogeneity	45
5.3.7	Operational considerations for statistical analyses.....	46
5.3.7.1	Treatment of experimental units.....	46
5.3.7.2	Identification and meaning of outliers	46
5.3.7.3	Multiple controls.....	46
5.3.7.4	General	47
5.4	Statistical items to be included in the study report.....	47
6	Dose-response modelling	48
6.1	Introduction	48
6.2	Modelling quantal dose-response data (for a single exposure duration).....	49
6.2.1	General.....	49
6.2.2	Choice of model	50

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6.2.2.1	General	50
6.2.2.2	Probit model	51
6.2.2.3	Logit model	53
6.2.2.4	Weibull model	54
6.2.2.5	Multi-stage models	55
6.2.2.6	Definitions of EC ₅₀ and EC _x	55
6.2.3	Model fitting and estimation of parameters	56
6.2.3.1	Software and assumptions	56
6.2.3.2	Response in controls	56
6.2.3.3	Analysis of data with various observed fractions at each dose group	57
6.2.3.4	Analysis of data with one observed fraction at each dose group	58
6.2.3.5	Extrapolation and EC _x	58
6.2.3.6	Confidence intervals	58
6.2.4	Assumptions	59
6.2.4.1	General	59
6.2.4.2	Statistical assumptions	59
6.2.4.3	Evaluation of assumptions	59
6.2.4.3.1	Evaluation of basic assumptions	59
6.2.4.3.2	Evaluation of the additional assumption	59
6.2.4.4	Consequences of violating the assumptions	60
6.2.4.4.1	Consequences of violating basic assumptions	60
6.2.4.4.2	Consequences of violating the additional assumption	60
6.3	Dose-response modelling of continuous data (for a single exposure duration)	60
6.3.1	Purpose	60
6.3.2	Terms and notation	60
6.3.3	Choice of model	61
6.3.3.1	First distinctions	61
6.3.3.2	Linear models	62
6.3.3.3	Threshold models	62
6.3.3.4	Additive versus multiplicative models	63
6.3.3.5	Models based on "quantal" models	63
6.3.3.6	Nested non-linear models	64
6.3.3.7	Hill model	67
6.3.3.8	Non-monotone models	67
6.3.4	Model fitting and estimation of parameters	68
6.3.4.1	Software and assumptions	68
6.3.4.2	Response in controls	68
6.3.4.3	Fitting the model assuming normal variation	68
6.3.4.4	Fitting the model assuming normal variation after log-transformation	68
6.3.4.5	Fitting the model assuming normal variation after other transformations	69
6.3.4.6	No individual data available	69
6.3.4.7	Fitting the model using GLM	69
6.3.4.8	Covariates	70
6.3.4.9	Heterogeneity and weighted analysis	71
6.3.4.10	Confidence intervals	73
6.3.4.11	Extrapolation	73
6.3.4.12	Analysis of data with replicated dose group	73
6.3.5	Assumptions	74
6.3.5.1	General	74
6.3.5.2	Statistical assumptions	74
6.3.5.3	Additional assumption	74
6.3.6	Evaluation of assumptions	75
6.3.7	Consequences of violating the assumptions	75
6.3.7.1	Basic assumptions	75
6.3.7.2	Additional assumption	76
6.4	To accept or not accept the fitted model?	77
6.4.1	Can the fitted model be accepted and used for its intended purpose?	77
6.4.2	Is the model in agreement with the data?	77
6.4.3	Do the data provide sufficient information for fixing the model?	77

This is a preview of "ISO/TS 20281:2006". [Click here to purchase the full version from the ANSI store.](#)

6.5	Design issues	81
6.5.1	General	81
6.5.2	Location of dose groups	81
6.5.3	Number of replicates	81
6.5.4	Balanced versus unbalanced designs	82
6.6	Exposure duration and time.....	82
6.6.1	General	82
6.6.2	Quantal data.....	82
6.6.3	Continuous data.....	83
6.6.3.1	General	83
6.6.3.2	Independent observations in time	83
6.6.3.3	Dependent observations in time	85
6.7	Search algorithms and non-linear regression	85
6.8	Reporting statistics.....	86
6.8.1	Quantal data.....	86
6.8.2	Continuous data.....	87
7	Biology-based methods	87
7.1	Introduction	87
7.1.1	Effects as functions of concentration and exposure time.....	87
7.1.2	Parameter estimation.....	89
7.1.3	Outlook.....	89
7.2	Modules of effect-models.....	90
7.2.1	General	90
7.2.2	Toxico-kinetic model	91
7.2.3	Physiological targets of toxicants.....	91
7.2.4	Change in target parameter	92
7.2.5	Change in endpoint.....	93
7.3	Survival	93
7.3.1	Relationship between hazard rate and survival probability	93
7.3.2	Assumptions of survival probability at any concentration of test compound	94
7.3.3	Summary	94
7.4	Body growth	97
7.4.1	Routes for affecting body growth.....	97
7.4.2	Assumptions.....	97
7.4.3	Von Bertalanffy growth curve	98
7.5	Reproduction	99
7.5.1	Routes that affect reproduction.....	99
7.5.2	Assumptions.....	100
7.5.3	Implication	100
7.6	Population growth.....	101
7.6.1	General	101
7.6.2	Assumptions.....	101
7.7	Parameters of effect models	103
7.7.1	General	103
7.7.2	Effect parameters	103
7.7.2.1	Toxicity and dynamic parameters	103
7.7.2.2	Killing rate, b_k	104
7.7.3	Discussion	105
7.7.4	Eco-physiological parameters	107
7.8	Recommendations	109
7.8.1	Goodness of fit.....	109
7.8.2	Choice of modes of action	110
7.8.3	Experimental design	110
7.8.4	Building a database for raw data.....	110
7.9	Software support.....	110
7.9.1	General	110
7.9.2	DEBtox	111
7.9.3	DEBtool	111

This is a preview of "ISO/TS 20281:2006". Click here to purchase the full version from the ANSI store.

8	List of existing guidelines with references to the subclauses of this Technical Specification.....	112
Annex A	(informative) Analysis of an “acute immobilization of <i>Daphnia magna</i>” data set (OECD GL 202 — ISO 6341) using the three presented approaches.....	115
A.1	Data set (see Table A.1)	115
A.2	Examples of data analysis using hypothesis testing (NOEC determination)	115
A.3	Example of data analysis by dose-response modelling	120
A.4	Example of data analysis using DEBtox (biological methods).....	125
Annex B	(informative) Analysis of an “algae growth inhibition” data set using the three presented approaches.....	127
B.1	General.....	127
B.2	Examples of data analysis using hypothesis testing (NOEC determination)	128
B.3	Example of data analysis by dose-response modelling	135
B.4	Examples of data analysis using DEBtox (biological methods).....	139
Annex C	(informative) Analysis of an “<i>Daphnia magna</i> reproduction” data set (OECD GL 211 – ISO 10706) using the three presented approaches	142
C.1	Examples of data analysis using hypothesis testing (NOEC determination)	143
C.2	Example of data analysis by dose-response modelling	148
C.3	Examples of data analysis using DEBtox (biological methods).....	155
Annex D	(informative) Analysis of a “fish growth” data set (OECD GL 204/215 – ISO 10229) using the three presented approaches	160
D.1	Data set.....	160
D.2	Examples of data analysis using hypothesis testing (NOEC determination)	162
D.3	Example of data analysis by dose-response modelling	172
D.4	Examples of data analysis using DEBtox (biological methods).....	177
Annex E	(informative) Description and power of selected tests and methods.....	180
E.1	Description of selected methods for use with quantal data	180
E.2	Power of the Cochran-Armitage test	189
E.3	Description of selected tests for use with continuous data	198
E.4	Power of step-down Jonckheere-Terpstra test	218
Annex F	(informative) Annex to Clause 7 “Biology-based methods”.....	231
F.1	General.....	231
F.2	Effects on survival.....	231
	Bibliography	237
	Figure 1 — Conceptual illustration of accuracy and precision.....	2
	Figure 2 — Illustration of a concentration-response relationship and of the estimates of the EC _x and NOEC/LOEC	5
	Figure 3 — Analysis of quantal data: Methods for determining the NOEC	23
	Figure 4 — Analysis of continuous data: Methods for determining the NOEC.....	24
	Figure 5 — Analysis of continuous data: Methods for determining the NOEC (<i>continued</i>)	24
	Figure 6 — Flow-chart for dose-response modelling.....	50

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Figure 7 — Probit model fitted to observed mortality frequencies (triangles) as a function of log-dose	52
Figure 8 — Logit model fitted to mortality dose-response data (triangles)	53
Figure 9 — Weibull model fitted to mortality dose-response data (triangles)	54
Figure 10 — Logit model fitted to mortality dose-response data (triangles), with background mortality	57
Figure 11 — Two members from a nested family of models fitted to the same data set.....	66
Figure 12 — Cholinesterase inhibition as a function of dose at three exposure durations.....	71
Figure 13 — Relative liver masses against dose, plotted on log-scale	72
Figure 14 — Dose-response model fitted to the data of Figure 13, showing that the heterogeneous variance was caused by males (triangles) and females (circles) responding differently to the chemical ..	73
Figure 15 — Model fitted to dose-response data with and without an outlier in the top dose	76
Figure 16 — Two different models (both with four parameters) fitted to the same data set resulting in similar dose-response relationships.....	79
Figure 17 — Two data sets illustrating that passing a goodness-of-fit test is not sufficient for accepting the model.....	80
Figure 18 — Observed biomasses (marks) as a function of time, for nine different concentrations of Atrazine	84
Figure 19 — Growth rates as derived from biomasses observed in time (see Figure 18) at nine different concentrations (including zero), with the Hill model fitted to them.....	84
Figure 20 — Estimated growth rates as a function of (log-)concentration Atrazine	85
Figure 21 — Fluxes of material and energy through an animal, as specified in the DEB model.....	92
Figure 22 — Time and concentration profiles of the hazard model, together with the data of Figure 27	95
Figure 23 — Time and concentration profiles for effects on growth of <i>Pimephalus promelas</i> via an increase of specific maintenance costs by sodium pentachlorophenate (data by Ria Hoofman, TNO-Delft).....	98
Figure 24 — Time and concentration profiles for effects on growth of <i>Lumbricus rubellus</i> via a decrease of assimilation by copper chloride (data from Klok and de Roos 1996)	99
Figure 25 — Effects of cadmium on the reproduction of <i>Daphnia magna</i> through an increase of the costs per offspring — Data from the OECD ring-test	101
Figure 26 — Example of application of the DEBtox method.....	102
Figure 27 — The effect of a mixture of C,N,S-compounds on the growth of <i>Skeletonema costatum</i> via an increase of the costs for growth — Data from the OECD ring test	103
Figure 28 — A typical table of data that serves as input for the survival model, as can be used in the software package DEBtox (Kooijman and Bedaux 1996).....	108
Figure 29 — This profile likelihood function of the NEC (right panel) for the data in Figure 28 results from the software package DEBtox (Kooijman and Bedaux 1996)	108
Figure A.1 — Probit model fitted to mortality response at day 2 — $CED = EC_{10}$	122
Figure A.2 — The Weibull (left panel) and the two-stage LMS model fitted to the mortality data at day 2	123
Figure A.3 — Probit model fitted to mortality data on day 1 (left panel), and fitted to both day 1 and day 2 simultaneously	124
Figure A.4 — DEBtox example: Parameters and asymptotic standard deviations (ASD).....	125
Figure A.5 — Graphical test of model predictions against data.....	125
Figure B.1 — Exponential growth model fitted to biomass, assuming a constant initial biomass (a), and growth rate (b) dependent on concentration (0, 0,01, 0,02, 0,03, 0,06, 0,1, 0,2, 0,3, 0,6 mg/l)	136
Figure B.2 — Exponential growth model fitted to biomass, assuming a constant initial biomass (a), and growth rate (b) dependent on each individual flask (six for the control group, and 2 for each nonzero concentration)	136

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Figure B.3 — Estimated growth rates (from individual flasks, see Figure B.2) as a function of the concentration of Atrazine	137
Figure B.4 — Regression residuals from analysis on log-scale (upper panels) and from analysis without transformation (lower panels)	138
Figure B.5 — Growth rate plots	139
Figure B.6 — DEBtox example: Data for effects of Atrazine in micrograms per litre on the growth of <i>Selenastrum capricornutum</i> in cells per millilitre	139
Figure B.7 — DEBtox example: Parameter estimates and asymptotic standard deviations (ASD).....	139
Figure B.8 — DEBtox example: Time profile (Population growth, growth model, CAS 1912-24-9).....	140
Figure B.9 — DEBtox example: Concentration profile (Population growth, growth model, CAS 1912-24-9) .	140
Figure B.10 — DEBtox example: Profile likelihood for NEC estimate (Population growth, growth model, CAS 1912-24-9).....	141
Figure C.1 — Number of life young as a function of concentration (on log-scale to improve visibility), counted over the first two weeks (triangles) and over the third week (circles).....	149
Figure C.2 — Total live young (TLY) in third week plotted against TLY in first two weeks, showing the correlations between these counts.....	149
Figure C.3 — Exponential model fitted to the number of life young counted over week three	151
Figure C.4 — Plots of regression residuals	152
Figure C.5 — Means of number of young, plotted cumulatively against time	153
Figure C.6 — Estimated ET_{50} values from Figure C.5, plotted against the concentration, with a fitted dose-response model.....	154
Figure C.7 — Number of young, plotted cumulatively against time	154
Figure C.8 — ET_{50} s estimated per replicate (see Figure C.7) as a function of the concentration with a fitted dose-response model.....	155
Figure C.9 — ET_{50} s estimated per replicate (see Figure C.7) as a function of the concentration, with two outliers removed	155
Figure C.10 — DEBtox example: Data for the cumulative number of offspring per female as affected by an unknown compound	156
Figure C.11 — DEBtox example: Parameter estimates and asymptotic standard deviations (ASD).....	156
Figure C.12 — DEBtox example: Time profile (reproduction, maintenance model, ISO repro set).....	157
Figure C.13 — DEBtox example: Concentration profile (reproduction, maintenance model, ISO repro set) .	157
Figure C.14 — DEBtox example: Profile likelihood for NEC estimate (reproduction, maintenance model, ISO repro set).....	158
Figure C.15 — DEBtox example: Body length at 21 days.....	158
Figure D.1 — Exponential model, $y = a \exp(bx)$, fitted to masses at 28 days	174
Figure D.2 — Plots of regression residuals for the analysis of Figure D.1	175
Figure D.3 — Plots of regression residuals	175
Figure D.4 — Dose-response analysis of the fish masses, but without log-transformation	176
Figure D.5 — Exponential model fitted to the body lengths	177
Figure D.6 — DEBtox example: Parameter estimates and asymptotic standard deviations (ASD).....	177
Figure D.7 — DEBtox example: Concentration profile	178
Figure D.8 — DEBtox example: Profile likelihood for NEC estimate.....	179
Figure E.1 — Cochran-Armitage test: Plot showing that 5 subjects per concentration would give very low power.....	190

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Figure E.2 — Cochran-Armitage test: Design with 20 subjects per concentration..... 191

Figure E.3 — Cochran-Armitage power versus maximum rate change: Power at Dose 5 in 5-dose study, with trend shape linear, lag = 0, sample size = 20..... 192

Figure E.4 — Cochran-Armitage power versus maximum rate change: Power at Dose 4 in 5-dose study, with trend shape linear, lag = 0, sample size = 20..... 192

Figure E.5 — Cochran-Armitage power versus maximum rate change: Power at Dose 3 in 5-dose study, with trend shape linear, lag = 0, sample size = 20..... 193

Figure E.6 — Cochran-Armitage power versus maximum rate change: Power at Dose 5 in 5-dose study with trend shape linear, lag = 0, sample size = 40..... 193

Figure E.7 — Cochran-Armitage power versus maximum rate change: Power at Dose 4 in 5-dose study with trend shape linear, lag = 0, sample size = 40..... 194

Figure E.8 — Cochran-Armitage power versus maximum rate change: Power at Dose 3 in 5-dose study with trend shape linear, lag = 0, sample size = 40..... 194

Figure E.9 — Cochran-Armitage power versus maximum rate change: Power at Dose 5 in 5-dose study with trend shape linear, lag = 0, sample size = 60..... 195

Figure E.10 — Cochran-Armitage power versus maximum rate change: Power at Dose 4 in 5-dose study with trend shape linear, lag = 0, sample size = 60..... 195

Figure E.11 — Cochran-Armitage power versus maximum rate change: Power at Dose 3 in 5-dose study with trend shape linear, lag = 0, sample size = 60..... 196

Figure E.12 — Cochran-Armitage power versus maximum rate change: Power at Dose 5 in 5-dose study with trend shape linear, lag = 0, sample size = 80..... 196

Figure E.13 — Cochran-Armitage power versus maximum rate change: Power at Dose 4 in 5-dose study with trend shape linear, lag = 0, sample size = 80..... 197

Figure E.14 — Cochran-Armitage power versus maximum rate change: Power at Dose 3 in 5-dose study with trend shape linear, lag = 0, sample size = 80..... 197

Figure E.15 — Power of step-down Jonckheere test: 6 doses at Step 1, N = 10..... 219

Figure E.16 — Power of step-down Jonckheere test: 6 doses at Step 2 versus 5 doses at Step 1, N = 10... 220

Figure E.17 — Power of step-down Jonckheere test: 6 doses at Step 3 versus 5 doses at Step 2 versus 4 doses at Step 1, N = 10..... 220

Figure E.18 — Power of step-down Jonckheere test: 6 doses at Step 2 versus 5 doses at Step 1, N = 5.... 221

Figure E.19 — Power of step-down Jonckheere test: 6 doses at Step 1, N = 5..... 221

Figure E.20 — Power of step-down Jonckheere test: (Doses,Step) = (6,4) (5,3)(4,2) (3,1), N = 5..... 222

Figure E.21 — Power of step-down Jonckheere test: 6 doses at Step 3 versus 5 doses at Step 2 versus 4 doses at Step 1, N = 5..... 222

Figure E.22 — Power of step-down Jonckheere test: 6 doses at Step 1, N = 10..... 223

Figure E.23 — Power of step-down Jonckheere test: 6 doses at Step 2 versus 5 doses at Step 1, N =10.... 223

Figure E.24 — Power of step-down Jonckheere test: 6 doses at Step 3 versus 5 doses at Step 2 versus 4 doses at Step 1, N = 10..... 224

Figure E.25 — Power of step-down Jonckheere test: (Doses, Step) = (6,4) (5,3) (4,2) (3,1), N = 10..... 224

Figure E.26 — Power of step-down Jonckheere test: 6 doses at Step 1, N = 20..... 225

Figure E.27 — Power of step-down Jonckheere test: 6 doses at Step 2 versus 5 doses at Step 1, N = 20... 225

Figure E.28 — Power of step-down Jonckheere test: 6 doses at Step 3 versus 5 doses at Step 2 versus 4 doses at Step 1, N = 20..... 226

Figure E.29 — Power of step-down Jonckheere test: (Doses, Step) = (6,4) (5,3) (4,2) (3,1), N = 20..... 226

Figure E.30 — Power of step-down Jonckheere test: 6 doses at Step 1, N = 40..... 227

This is a preview of "ISO/TS 20281:2006". [Click here to purchase the full version from the ANSI store.](#)

Figure E.31 — Power of step-down Jonckheere test: 6 doses at Step 2 versus 5 doses at Step 1, N = 40 ..	227
Figure E.32 — Power of step-down Jonckheere test: 6 doses at Step 3 versus 5 doses at Step 2 versus 4 doses at Step 1, N = 40	228
Figure E.33 — Power of step-down Jonckheere test: (Doses, Step) = (6,4) (5,3) (4,2) (3,1), N = 40	228
Figure E.34 — Power of step-down Jonckheere test: 6 doses at Step 1, N = 80	229
Figure E.35 — Power of step-down Jonckheere test: 6 doses at Step 2 versus 5 doses at Step, N = 80	229
Figure E.36 — Power of step-down Jonckheere test: 6 doses at Step 3 versus 5 doses at Step 2 versus 4 doses at Step 1, N = 80	230
Figure E.37 — Power of step-down Jonckheere test: (Doses, Step) = (6,4) (5,3) (4,2) (3,1), N = 80	230

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

In other circumstances, particularly when there is an urgent market requirement for such documents, a technical committee may decide to publish other types of normative document:

- an ISO Publicly Available Specification (ISO/PAS) represents an agreement between technical experts in an ISO working group and is accepted for publication if it is approved by more than 50 % of the members of the parent committee casting a vote;
- an ISO Technical Specification (ISO/TS) represents an agreement between the members of a technical committee and is accepted for publication if it is approved by 2/3 of the members of the committee casting a vote.

An ISO/PAS or ISO/TS is reviewed after three years in order to decide whether it will be confirmed for a further three years, revised to become an International Standard, or withdrawn. If the ISO/PAS or ISO/TS is confirmed, it is reviewed again after a further three years, at which time it must either be transformed into an International Standard or be withdrawn.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO/TS 20281 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

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Introduction

Ecotoxicity tests are biological experiments performed to examine if either a potentially toxic compound, or an environmental sample (e.g. effluent, sediment or soil sample) causes a biologically important response in test organisms. If so, the goal is to determine the concentration that produces a given level of effects or produces an effect that cannot be distinguished from background variation.

In a test, organisms are exposed to different concentrations or doses of a test substance or a test substrate (e.g. waste water, sludge, or a contaminated soil or sediment), sometimes diluted in a test medium. Typically, at least one group of test organisms (the control group) is not exposed to the test substance or substrate, but is otherwise treated in the same way as the exposed organisms.

The endpoint(s) observed or measured in the different batches may be the number of surviving organisms, size or growth of organisms, number of eggs or offspring produced or any relevant biochemical or physiological variable that can be reliably quantified. Observations are made after one or several predefined exposure times. The endpoint's relationship with the concentration of the tested chemical or substrate is examined. The way statistics are applied may have a considerable impact on the results and conclusions from ecotoxicity tests, and consequently on the associated policy decisions. Various documents (Williams 1971, Piegorsch and Bailer 1997, Tukey *et al.* 1985, Pack 1993, Chapman *et al.* 1995, Hoekstra 1993, Kooijman and Bedaux 1996, Laskowkj 1995, Chapman 1996, OECD 1998, ASTM 2000) exist on the use of available statistical methods, the limitations of these methods and how to cope with specific problematic data. Discussions of statistical principles and commonly used techniques are found in general references such as Armitage and Berry (1987) [basic information on hypothesis testing and regression, transformations], Finney (1978) [analysis of quantal data, especially probit models], Hochberg and Tamhane (1987) [thorough treatment of multiple comparison methods], Newman (1994) [information related to biology-based models, EC_x], and Sparks (2000) [a collection of articles covering field and laboratory experiments, multivariate techniques, risk assessment, and environmental monitoring].

When problematic data are encountered or critical decisions depend upon inferences from ecotoxicity tests, consultation with a qualified statistician is useful. (Statisticians should be consulted before beginning the experiment to ensure proper design, sample size, limitations, and to be sure that the study is actually able to answer the research question that the experimenter poses. Once bad data have been collected, there is little a statistician can do to rectify the problem.)

Clause 8 contains a table listing all the existing ISO and OECD ecotoxicity standards/guidelines that could be analysed using this guidance document. For each standard/guideline, reference is made to the adapted clauses of this Technical Specification.

Clause 4 details the different statistical approaches that can be used for the analysis of ecotoxicity data, depending on the aim. In particular, it gives the assumptions made when using hypothesis-testing methods, concentration-response modelling methods or biology-based methods and their limitations. It also gives some indication on experimental design issues. Some general principles and advice are also given for the process of data analysis.

Clause 5 deals with hypothesis testing, Clause 6 with dose-response modelling and Clause 7 with biology-based methods.

There was an ISO resolution (ISO TC 147/SC 5/WG 10 Antalya 3), as well as an OECD workshop recommendation (OECD 1998), that the NOEC should be phased out from International Standards.

However, the NOEC is still required in many regulatory standards from many countries, and in some cases, where a detailed determination of an EC_x is not relevant and the alteration of the study design is too costly to fulfil the requirements for regression models. Therefore guidance is provided on the statistical methods for the determination of the NOEC.

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In the annexes, examples of analyses with the three main methods (hypothesis testing for NOEC estimation, dose-response modelling and biology-based modelling) of four different data sets are given. They concern:

- acute toxicity on *Daphnia magna*;
- inhibition of algae growth;
- reproduction of *Daphnia magna*; and
- fish growth.