

Table of Contents

List of Tables.....	v
List of Figures	v
List of Acronyms/Abbreviations	vii
Workshop Breakout Group.....	xi
Acknowledgements	xii
Preface	xv
1.0 Introduction.....	1
1.1 Purpose and Scope of this Guidance Document	1
1.2 The Correlation Between Basal Cytotoxicity and Acute Lethality	1
1.3 <i>In Vitro</i> Determination of Starting Dose for <i>In Vivo</i> Tests	3
1.4 Determination of <i>In Vitro</i> Test Performance Characteristics	4
2.0 Elements of A Standard Test For Basal Cytotoxicity.....	5
2.1 Selection of Cell Lines / Cells.....	5
2.2 Recommended Measurement Endpoints for Basal Cytotoxicity	6
2.3 Recommendations for Cytotoxicity Test Protocols.....	7
3.0 Procedure for Qualifying a Cytotoxicity Test for use with the Registry of Cytotoxicity Prediction Model	9
3.1 Procedure to Determine Whether a Candidate Cytotoxicity Test Can Use the RC Prediction Model.....	9
3.2 Recommended Reference Chemicals from the RC for Test Qualification	10
3.3 Results Obtained with the Recommended Reference Chemicals in Two Standard Tests for Basal Cytotoxicity with Human and Rodent Cells	12
4.0 Recommended Basal Cytotoxicity Tests: BALB/C 3T3 And Normal Human Keratinocyte (NHK) Neutral Red Uptake (NRU) Tests.....	15
4.1 Validation Status of the 3T3 NRU Test	15
4.2 Reliability of the 3T3 NRU Test	15
4.3 Validation Status of the NHK NRU Test	18
4.4 Reliability of the NHK NRU Test.....	19
5.0 Conclusion	19
Acknowledgement.....	19
6.0 References.....	21
Appendices
A. Registry Of Cytotoxicity	A-1
B. List of Test Protocols for Basal Cytotoxicity, European Centre for the Validation Of Alternative Methods (ECVAM) Scientific Information System (SIS)	B-1

C.	Standard Operating Procedure (SOP) for the BALB/c 3T3 Neutral Red Uptake Cytotoxicity Test - A Test for Basal Cytotoxicity.....	C-1
1.0	Standard Operating Procedure (SOP) for the BALB/c 3T3 Neutral Red Uptake Cytotoxicity Test - A Test for Basal Cytotoxicity.....	C-3
1.1	Background and Introduction.....	C-3
1.2	Rationale.....	C-3
1.3	Basic Procedure.....	C-3
1.4	Test Limitations.....	C-4
1.5	Material	C-4
1.6	Methods.....	C-6
1.7	Data Analysis	C-10
1.8	Prediction Model.....	C-11
1.9	References	C-11
D.	Standard Operating Procedure (SOP) for the Normal Human Keratinocyte Neutral Red Uptake Cytotoxicity Test - A Test for Basal Cytotoxicity	D-1
1.0	Standard Operating Procedure (SOP) for the BALB/c 3T3 Neutral Red Uptake Cytotoxicity Test - A Test for Basal Cytotoxicity	D-3
1.1	Background and Introduction.....	D-3
1.2	Rationale.....	D-3
1.3	Basic Procedure.....	D-3
1.4	Test Limitations.....	D-3
1.5	Material	D-4
1.6	Methods.....	D-6
1.7	Data Analysis	D-9
1.8	Prediction Model.....	D-10
1.9	References	D-10
E.	96-Well Plate Configuration	E-1
F.	Decimal Geometric Concentration Series	F-1
G.	Standard Test Reporting Template.....	G-1

List of Tables

1. Recommended reference chemicals for evaluating a cytotoxicity test for use with the RC prediction model	11
2. Interlaboratory reproducibility of the 3T3 NRU cytotoxicity test determined according to ISO 5725 in 12 laboratories for 29 chemicals	15

List of Figures

1. Registry of Cytotoxicity Regression Between Cytotoxicity (IC _{50x}) and Rodent Acute Oral LD ₅₀ Values for 347 Chemicals.....	3
2. Procedure for Evaluating a Cytotoxicity Test for Tiered <i>In Vitro/In Vivo</i> Testing for Acute Oral Toxicity Testing (Slightly Modified after Spielmann et. al., 1999).....	10
3. Regression Obtained by Testing the Recommended Reference Chemicals from the RC with Human Keratinocytes in the NHK NRU Cytotoxicity Test	13
4. Regression Obtained by Testing the Recommended Reference Chemicals from the RC with Mouse Fibroblasts in the BALB/c 3T3 NRU Cytotoxicity Test.....	13
5. Interlaboratory Comparability of the 3T3 NRU Cytotoxicity Test for 147 Test Chemicals in 2 Different Laboratories per Chemical.....	17

