
A Report Prepared in the Context of the 7th Amendment to the Cosmetics Directive for Establishing the Timetable for Phasing Out Animal Testing

Edited by Chantra Eskes and Valerie Zuang

This report was made possible by the cooperation and support of: the European Centre for the Validation of Alternative Methods (ECVAM) the European Cosmetic Toiletry and Perfumery Association (COLIPA) the European Federation for Cosmetic Ingredients (EFFCI) and Eurogroup for Animal Welfare

July 2005
# Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foreword</td>
<td>1</td>
</tr>
<tr>
<td>Preface</td>
<td>3</td>
</tr>
<tr>
<td>Overview</td>
<td>5</td>
</tr>
<tr>
<td><strong>Executive Summary</strong></td>
<td>7</td>
</tr>
<tr>
<td>ES.1 The 7th amendment to the cosmetics directive</td>
<td>7</td>
</tr>
<tr>
<td>ES.2 Safety data requirements for the purposes of the cosmetics directive</td>
<td>7</td>
</tr>
<tr>
<td>ES.3 Expert reviews and time estimation for phasing out animal tests</td>
<td>7</td>
</tr>
<tr>
<td>ES.3.1 Acute toxicity</td>
<td>8</td>
</tr>
<tr>
<td>ES.3.2 Skin irritation and corrosion</td>
<td>9</td>
</tr>
<tr>
<td>ES.3.3 Eye irritation</td>
<td>9</td>
</tr>
<tr>
<td>ES.3.4 Skin sensitisation</td>
<td>9</td>
</tr>
<tr>
<td>ES.3.5 Skin absorption and penetration</td>
<td>10</td>
</tr>
<tr>
<td>ES.3.6 Subacute and subchronic toxicity</td>
<td>10</td>
</tr>
<tr>
<td>ES.3.7 Genotoxicity and mutagenicity</td>
<td>10</td>
</tr>
<tr>
<td>ES.3.8 UV-induced toxic effects</td>
<td>10</td>
</tr>
<tr>
<td>ES.3.9 Toxicokinetics and metabolism</td>
<td>11</td>
</tr>
<tr>
<td>ES.3.10 Carcinogenicity</td>
<td>11</td>
</tr>
<tr>
<td>ES.3.11 Reproductive and developmental toxicity</td>
<td>12</td>
</tr>
<tr>
<td>ES.4 Conclusions</td>
<td>12</td>
</tr>
<tr>
<td>ES.5 Summary table</td>
<td>13</td>
</tr>
</tbody>
</table>

## Chapter 1

1.1 The 7th amendment to the cosmetics directive | 19 |

1.2 References | 20 |

## Chapter 2

2.1 Safety data requirements for the purposes of the cosmetics directive | 21 |
2.1.1 Requirements of the Cosmetics Directive | 21 |
2.1.2 Toxicological data requirements for cosmetics ingredients, based on the SCCNFP guidelines | 21 |
2.1.3 Toxicological data requirements for chemicals | 22 |
2.1.4 Cosmetic safety assessment: specific data demands | 23 |
2.2 From animal testing to alternative methods | 23 |
2.2.1 Definitions used with regard to alternative methods | 23 |
2.2.2 Definitions used with regard to the development, validation and acceptance process of alternative methods | 24 |
2.3 Considerations with regard to the estimated timings | 25 |
2.4 References | 26 |

## Chapter 3

3.1. Acute Toxicity | 27 |
3.1.1 Inventory of methods currently available | 27 |
3.1.2 Inventory of alternative methods currently available | 29 |
3.1.3 Identified steps or tests with no alternative methods available | 31 |
3.1.4 Summary of alternative methods currently available and foreseeable time to achieve peer reviewed validation | 31 |
3.1.5 Conclusions | 31 |
3.1.6 References | 33 |
Contents continued

3.5 Skin Absorption and Penetration 105
  3.5.1 Current data requirements and current tests 105
    3.5.1.1 Data required for cosmetic ingredients 105
    3.5.1.2 Data required for cosmetic finished products 105
    3.5.1.3 Available human and animal tests 105
    3.5.1.4 Available alternative methods 105
  3.5.2 Identified areas or endpoints for which no validated/"valid"
      alternative methods are available 105
  3.5.3 References 105

3.6 Subacute and Subchronic Toxicity 109
  3.6.1 Inventory of methods currently available 109
  3.6.2 Inventory of alternative methods currently available 109
    3.6.2.1 Introduction 109
    3.6.2.2 Target organ and target system toxicity models at the basic research
          level 110
  3.6.3 Identified steps or tests with no alternative methods available 112
  3.6.4 Summary of alternative methods currently available and
       foreseeable time to achieve ESAC endorsement 112
  3.6.5 Conclusions 114
  3.6.6 References 114

3.7 Genotoxicity and Mutagenicity 117
  General considerations 117
  3.7.1 State of the art in the field of genotoxicity and mutagenicity tests
       in the light of the 7th Amendment to the Cosmetics Directive 117
  3.7.2 Proposed strategy 118
  3.7.3 Inventory of methods currently available 119
  3.7.4 Inventory of alternative methods currently available 119
  3.7.5 Recommendations for achieving reduction in animal use 124
  3.7.6 Summary of alternative methods currently available and foreseeable
       time to achieve ESAC endorsement 125
  3.7.7 Final comments 125
  3.7.8 References 128

3.8 UV-Induced Effects 131
  Summary 131
  3.8.1 Acute phototoxicity 132
  3.8.2 Photogenotoxicity 136
  3.8.3 Photoallergy (photosensitisation) 140
  3.8.4 Summary of alternative methods currently available and foreseeable
       time to achieve ESAC endorsement 141
  3.8.5 References 141

3.9 Toxicokinetics and Metabolism 147
  Objectives to be achieved 147
  3.9.1 Inventory of methods currently available 147
  3.9.2 Inventory of alternative methods currently available 149
    3.9.2.1 Introduction 149
    3.9.2.2 Tier 1 and Tier 2 stand-alone methods 151
    3.9.2.3 Tier 1 and Tier 2 integrated in vitro and in silico approaches 159
    3.9.2.4 Integrated human volunteer-based approaches 163
  3.9.3 Identified steps or tests with no alternative methods available 163
    3.9.3.1 Tier 1 163
    3.9.3.2 Tier 2 164
Contents continued

3.9.4 Summary of alternative methods currently available and foreseeable time to achieve ESAC endorsement 164
3.9.5 Recommendations 164
3.9.6 Conclusions 171
3.9.7 References 171

3.10 Carcinogenicity
General considerations 177
3.10.1 Inventory of alternative methods currently available 177
   3.10.1.1 Cell transformation assays 177
   3.10.1.2 Gap junction intercellular communication (GJIC) 178
3.10.2 Reduction and refinement 178
   3.10.2.1 Transgenic mouse models 178
   3.10.2.2 Recommendations for use in relation to animal replacement 179
3.10.3 Summary of alternative methods currently available and foreseeable time to achieve ESAC endorsement 179
3.10.4 Final comments 181
3.10.5 References 181

3.11 Reproductive and Developmental Toxicity
3.11.1 Introduction 183
3.11.2 Inventory of alternative methods currently available 183
   3.11.2.1 State of the art 183
   3.11.2.2 Successfully validated tests 184
   3.11.2.3 Advanced tests 185
   3.11.2.4 Identified areas or endpoints with no validated alternative methods available 190
3.11.3 Summary of alternative methods currently available and foreseeable time to achieve ESAC endorsement 194
3.11.4 Conclusions 194
3.11.5 References 194

Appendices 211

Appendix 1: Participating stakeholders and commission services 211
Appendix 2: Participating scientific experts nominated by commission services and stakeholders 213
Appendix 3: Glossary of terms 215
Appendix 4: Methods adopted by the EU for testing chemicals 217
   A.4.1 The EU testing methods adoption process 217
   A.4.2 The testing methods of Annex V to Directive 67/548/EEC 220
   A.4.3 Proposed testing methods for inclusion in the 30th adaptation to technical progress 221
Appendix 5: OECD guidelines for the testing of chemicals 223
   A.5.1 The OECD test guideline submission and adoption process 223
   A.5.2 Overview of currently available and draft TGs of Section 4: health effects 228
   A.5.3 Reference 228