STATEMENT ON THE SCIENTIFIC VALIDITY OF THE 3T3 NRU PT TEST
(AN IN VITRO TEST FOR PHOTOTOXIC POTENTIAL)

At its 9th meeting, held on 1-2 October 1997 at the European Centre for the Validation of Alternative Methods (ECVAM), Ispra, Italy, the ECVAM Scientific Advisory Committee (ESAC) unanimously endorsed the following statement:

The results obtained with the 3T3 NRU PT test in the blind trial phase of the EU/COLIPA international validation study on in vitro tests for phototoxic potential were highly reproducible in all the nine laboratories that performed the test, and the correlations between the in vitro data and the in vivo data were very good. The Committee therefore agrees with the conclusion from this formal validation study that the 3T3 NRU PT is a scientifically validated test which is ready to be considered for regulatory acceptance.

The ESAC has been regularly kept informed of the progress of the study, and this endorsement was based on an assessment of various documents, including, in particular, the report on the performance of the 3T3 NRU PT test in a multilaboratory blind trial on 30 coded chemicals, which is to be published in Toxicology in Vitro.

This validation study was conducted in accordance with the general principles laid down in the report of the CAAT/ERGATT workshop held in 1990, guidelines contained in the report of an ECVAM/ERGATT workshop held in 1995, criteria laid down by ECVAM and the ECB, criteria recommended at an OECD workshop held in 1996, and the US ICCVAM report on validation and regulatory acceptance.

In order for this method to be considered for use for legislative and other purposes, a draft guideline incorporating the standard protocol for the 3T3 NRU PT test will be prepared by 31 December 1997, according to OECD guidance on the preparation of test guidelines.

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3 November 1997
1. The ESAC was established by the European Commission, and is composed of representatives of
the EU Members States, industry, academia and animal welfare, together with representatives of
the relevant Commission services. The following members of the ESAC were present at the
meeting on 1-2 October 1997:

Dr B Blaauboer (ERGATT) Dr P Botham (ECETOC)
Professor J Castell (Spain) Dr D Clark (UK)
Dr B Garthoff (EFPIA) Professor A Guillouzo (France)
Dr C Hendriksen (The Netherlands) Professor G Papadopoulos (Greece)
Professor V Rogiers (Belgium) Dr O de Silva (COLIPA)
Professor H Spielmann (Germany) Dr O Svendsen (Denmark)
Professor H. Tritthart (Austria) Dr M Viluksela (Finland)
Professor E Walum (Sweden) Dr F Zucco (Eurogroup for Animal Welfare)

Professor M Balls (ECVAM) Mrs M Bernard (DGIII)
Mr G Corelle (DGXI) Dr J Fentem (ECVAM)
Dr B Lucaroni (DGXII) Ms S Louhimies (DGXI)
Professor JM Martin (EI) Mr J Vogelgesang (DGXI)

2. CAAT: Center for Alternatives to Animal Testing, Baltimore, USA; ECB: European Chemicals
Bureau, Ispra, Italy; COLIPA: European Cosmetic, Toiletry and Perfumery Association;
ERGATT: European Research Group for Alternatives in Toxicity Testing, Utrecht, The
Netherlands; ICCVAM: ad hoc Interagency Coordinating Committee on the Validation of
Alternative Methods, Research Triangle Park, USA; OECD: Organization for Economic
Cooperation and Development, Paris, France.

3. Spielmann H, Balls M, Dupuis, J, Pape WJW, Pechovitch G, de Silva O, Holzhütter HG, Clothier,
R, Desolle P, Gerberick F, Liebsch M, Lovell WW, Maurer T, Pfannenbecker U, Potthast JM,
phototoxicity validation study: results of Phase II (blind trial); part 1: the 3T3 NRU test for
phototoxic potential. Toxicology in Vitro, in press.

4. Balls M, Blaauboer B, Brusick D, Frazier J, Lamb D, Pemberton M, Reinhardt C, Roberfroid M,
recommendations of the CAAT/ERGATT workshop on the validation of toxicity test procedures.
ATLA 18: 303-337.

5. Balls M, Blaauboer BJ, Fentem JH, Bruner L, Combes RD, Ekwall B, Fielder RJ, Guillouzo A,
Practical aspects of the validation of toxicity test procedures. The report and recommendations of
ECVAM workshop 5. ATLA 23: 129-147.


the ad hoc Interagency Coordinating Committee on the Validation of Alternative Methods.
105pp. Research Triangle Park: NIEHS.
General information about the study:

A. The study was managed by a Management Team consisting of representatives of the European Commission and COLIPA, under the chairmanship of Professor Horst Spielmann (ZEBET, BgVV, Berlin, Germany). The following laboratories participated in the blind trial on the 3T3 NRU PI test: ZEBET (the lead laboratory), Beiersdorf (Hamburg, Germany), University of Nottingham (Nottingham, UK), Henkel (Düsseldorf, Germany), Hoffman-La Roche (Basel, Switzerland), L’Oréal (Aulnay-sous-Bois, France), Procter & Gamble (Cincinnati, USA), Unilever (Sharnbrook, UK), and Warsaw Medical School (Warsaw, Poland).

B. This study began in 1991, as a joint initiative of the European Commission and COLIPA. Phase I of the study (1992-93) was designed as a prevalidation phase, for test selection and test protocol optimisation. Phase II (1994-95) involved a formal validation trial, conducted under blind conditions on 30 test materials which were independently selected, coded and distributed to nine laboratories. The results obtained were submitted to an independent statistician for analysis. Data analysis and preparation of the final report took place during 1996-97.

C. A number of tests at different stages of development were included in the study, but the 3T3 NRU PT test was found to be the one most ready for validation. It is a cytotoxicity test, in which Balb/c mouse embryo-derived cells of the 3T3 cell line are exposed to test chemicals with and without exposure to UVA under carefully defined conditions. Cytotoxicity is measured as inhibition of the capacity of the cell cultures to take up a vital dye, neutral red. The prediction model requires a sufficient increase in toxicity in the presence of UVA for a chemical to be labelled as having phototoxic potential.

D. Two versions of the prediction model were applied by the independent statistician. The phototoxicity factor (PTF) version compared two equi-effective concentrations (the IC₅₀ value, defined as the concentration of test chemical which reduces neutral red uptake by 50%) with and without UV light. However, since no IC₅₀ value was obtained for some chemicals in the absence of UVA, another version was devised, based on the Mean Phototoxic Effect (MPE), whereby all parts of the dose-response curves could be compared.

The two versions of the prediction model were applied to classify the phototoxic potentials of the 30 test chemicals on the basis of the in vitro data obtained in the nine laboratories. Comparing these in vitro classifications with the in vivo classifications independently assigned to the chemicals before the blind trial began, the following overall contingency statistics were obtained for the 3T3 NRU PT test:

<table>
<thead>
<tr>
<th></th>
<th>PIF version</th>
<th>MPE version</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specificity</td>
<td>90%</td>
<td>93%</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>82%</td>
<td>84%</td>
</tr>
<tr>
<td>Positive predictivity</td>
<td>96%</td>
<td>96%</td>
</tr>
<tr>
<td>Negative predictivity</td>
<td>64%</td>
<td>73%</td>
</tr>
<tr>
<td>Accuracy</td>
<td>88%</td>
<td>92%</td>
</tr>
</tbody>
</table>
E. Other methods in the study included the human keratinocyte NRU PT test, the red blood cell PT test, the SOLATEX PT test, the histidine oxidation test, a protein binding test, the Skin² ZK1350 PT test, and a complement PT test. The other methods showed varying degrees of promise, e.g. as potential mechanistic tests for certain kinds of phototoxicity, and this will be the subject of further reports.
ECVAM European Centre for the Validation of Alternative Methods

STATEMENT ON THE APPLICATION
OF THE 3T3 NRU PT TEST TO UV FILTER CHEMICALS

At its 10th meeting, held on 31 March 1998 at the European Centre for the Validation of Alternative Methods (ECVAM), Ispra, Italy, the ECVAM Scientific Advisory Committee (ESAC) unanimously endorsed the following statement:

The outcome of the study with UV filter chemicals further confirms the validity of the 3T3 NRU PT test, which has now been demonstrated to be applicable for testing these types of chemicals for their phototoxic potential.

The ESAC has been regularly kept informed of the progress of the special study, and this endorsement was based on the assessment of various documents and a verbal report to the ESAC by Professor Horst Spielmann, Chairman of the Management Team for the study.

This special study was conducted in accordance with the general principles for validation laid down in the report of the CAAT\textsuperscript{2}/ERGATT\textsuperscript{2} workshop held in 1990, guidelines contained in the report of an ECVAM/ERGATT workshop held in 1995, criteria laid down by ECVAM and the ECB, criteria recommended at an OECD\textsuperscript{2} workshop held in 1996, and the US ICCVAM\textsuperscript{2} report on validation and regulatory acceptance.

A detailed report on the study will be published in \textit{ATLA} during 1998, and the report on Phase II of the EU/COLIPA validation study on the 3T3 NRU PT test will shortly be published in \textit{Toxicology in Vitro}. The ESAC formally endorsed the method as a scientifically validated test at its meeting on 1-2 October 1997.

The experience and results obtained during the study on UV filters have been taken into account in the drafting of a proposed test guideline on the \textit{in vitro} 3T3 NRU PT test.

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20 May 1998
1. The ESAC was established by the European Commission, and is composed of representatives of the EU Member States, industry, academia and animal welfare, together with representatives of the relevant Commission services. The following members of the ESAC were present at the meeting on 31 March 1998:

Dr B Blaauboer (ERGATT)          Dr P Botham (ECETO)  
Professor J Castell (Spain)      Dr D Clark (UK)        
Dr B Garthoff (EFPIA)            Professor A Guillouzo (France)  
Dr C Hendriksen (The Netherlands) Dr R Lorenzini (Italy)  
Professor G Papadopoulos (Greece) Dr V Rogiers (Belgium)    
Dr B Rusche (Eurogroup for Animal Welfare) Dr O de Silva (COLIPA)  
Professor H Spielmann (Germany)  Dr O Svendsen (Denmark)    
Professor H. Tritthart (Austria) Dr M Viluksela (Finland)   
Professor E Walum (Sweden)       Mr G Corcelle (DGXI)    
Professor M Balls (ECVAM)        Dr G Fracchia (DGXII)   
Dr J Fentem (ECVAM)              Ms S Louhimies (DGXI)   
Ms S Fentem (ECVAM)              Dr M Robert (DGIII)     
Mr A Van Elst (DGXXIV)           


