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ECVAM European Centre for the Validation of Alternative Methods

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)¹ unanimously endorsed the following statement:

The results obtained with the 3T3 NRU PT test in the blind trial phase of the EU/COLIPA² 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*.³

This validation study was conducted in accordance with the general principles laid down in the report of the CAAT²/ERGATT² workshop held in 1990,⁴ guidelines contained in the report of an ECVAM/ERGATT workshop held in 1995,⁵ criteria laid down by ECVAM and the ECB,^{2,6} criteria recommended at an OECD² workshop held in 1996,⁷ and the US ICCVAM² report on validation and regulatory acceptance.⁸

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 Corcelle (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, Csato M, Sladowski D, Steiling W & Brantom P (1997) The international EU/COLIPA *in vitro* 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, Rosenkranz H, Schmid B, Spielmann H, Stamatou AL & Walum E (1990) Report and 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, Lewis RW, Lovell DP, Reinhardt CA, Repetto G, Sladowski D, Spielmann H & Zucco, F (1995) Practical aspects of the validation of toxicity test procedures. The report and recommendations of ECVAM workshop 5. *ATLA* 23: 129-147.
6. Balls M & Karcher W (1995) The validation of alternative test methods. *ATLA* 23: 884-886.
7. Anon (1996) *Final Report of the OECD Workshop on Harmonization of Validation and Acceptance Criteria for Alternative Toxicological Test Methods*. 60pp. Paris: OECD.
8. Anon (1997) *Validation and Regulatory Acceptance of Toxicological Test Methods. A Report of 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:

	<i>PIF version</i>	<i>MPE version</i>
Specificity:	90%	93%
Sensitivity:	82%	84%
Positive predictivity:	96%	96%
Negative predictivity:	64%	73%
Accuracy:	88%	92%

- 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.