Altern. Animal Test. EXperiment. 7 (4),81-89 (2001)

- Plenary Lecture -

Current Validation Studies on Alternatives to Animal Experiments in Europe

Horst Spielmann and Manfred Liebsch

National Center for Documentation and Evaluation of Alternative Methods to Animal Experiments (ZEBET) at the Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV), Berlin, Germany

Abstract

Since no scientific approach or regulatory guidelines existed for the experimental validation of *in vitro* toxicity tests, in 1990 a US/European validation workshop agreed in Amden (Switzerland) on a simple definition of the validation process. Several international validation studies failed, although they were conducted according to these recommendations. Taking into account the lessons learned from this experience, a second validation workshop was held by ECVAM in Amden in 1994 to develop a more precisely defined validation concept. Prevalidation and the development of biostatistically defined prediction models were added as essential elements to the validation process. In 1995/1996 the ECVAM validation procedure was officially accepted by EU member countries and at the international level by the US regulatory agencies and the OECD.

The improved validation concept was immediately introduced into ongoing validation studies. In 1996 the ECVAM/COLIPA validation study of *in vitro* phototoxicity test, which was conducted according to the ECVAM/OECD validation concept, was finished successfully and in 1998 a supporting study on UV-filter chemicals. In 1998 the 3T3 NRU PT *in vitro* phototoxicity test was the first experimentally validated *in vitro* toxicity test that was recommended for regulatory purposes by ESAC, the ECVAM Scientific Advisory Committee, and by the DG ENV of the EU Commission. Meanwhile two *in vitro* skin corrosivity tests have successfully been validated by ECVAM. Finally, in June of the year 2000 the three experimentally validated tests were accepted by EU member states for regulatory purposes as the first *in vitro* toxicity tests.

Examples will be given of successful validation studies during the past decade with particular reference to *in vitro* toxicity tests that were evaluated for regulatory purposes either by the US validation centre ICCVAM or ECVAM in the fields of sensitisation, phototoxicity and embryotoxicity.

Correspondence: Dr. Horst Spielmann
Director and Professor, Head of Department ZEBET at the BgVV, Diedersdorfer Weg 1 D-12277 Berlin, Germany
Tel: +49-1888-412-2270 Fax: +49-1888-412-2958; e-mail: zebet@bgvv.de

1. International harmonisation of animal test in regulatory toxicology

1989, ZEBET was established at the Federal Health Office (BGA) in Berlin as the National German Centre for Documentation and Evaluation of Alternatives to Animal Testing. ZEBET's mission is to reduce animal testing for regulatory purposes. The only concept available in 1989 to reduce testing in animals was the Three Rs principles of Russell and Burch [1].

It is the goal of regulatory toxicology in the field of chemicals to ensure the occupational safety of workers in the process of producing chemicals, to ensure the safety of food and beverages, to protect patients against possible hazards represented by drugs and medical devices, and to protect humans and the environment against possible hazards posed by residues of chemicals, e.g. pesticides. The standard approach in regulatory toxicology to assess the toxicity of chemicals is the determination of toxic properties in standardised animal tests, as described in the *OECD Guidelines for Testing of Chemicals* [2]. This information is then used by regulators to classify each chemical according to internationally harmonised guidelines in the first step, e.g. as harmful, toxic, irritant, then to label them in the second step according to EU risk (R) phrases, e.g. "R-41: risk of serious damage to the eye". The consequences of classification and labelling are the restricted use of the tested chemical in finished products (depending on exposure), and safety and labelling recommendations.

The international harmonisation of toxicity tests by the OECD in 1982 was the first, and so far, the most effective step in reducing duplication of testing in animals for regulatory purposes, since a toxicity test conducted according to the OECD guidelines will be accepted by regulatory agencies in all OECD Member States. These Member States are the world's major industrial nations. A similar approach has thereafter been used for the safety and efficacy testing of drugs by the International Conference on Harmonisation (ICH), which represents the three major economic regions namely, Europe, Japan and the USA. Since 1990, the ICH has accepted harmonised guidelines for efficacy and safety testing of drugs and medicines, including animal tests. Again, the harmonisation of test guidelines has led to significant reduction of testing in animals, since regulatory agencies around the world now accept the results of a test conducted according to ICH guidelines.

2. Evolution of the principles of scientific validation I: 1st Amden workshop on validation

Regulators will only accept alternatives to animal tests in toxicology, if the new tests will allow them to classify and label chemicals in the same way as the results of current animal tests allow them to do. The OECD has therefore indicated that *in vitro* toxicity tests can be accepted for regulatory purposes only after a successful experimental validation study. This procedure is essential to prove that the new *in vitro* toxicity tests will provide the same level of protection as the animal tests are currently providing.

To approach this problem scientifically, European and American scientists interested in the validation of toxicity tests met in Amden, Switzerland, to agree on a definition of experimental validation and to define the essential steps in this process. In the workshop report of the 1st Amden validation workshop, validation was defined as the process by which reproducibility and relevance of a toxicity testing procedure are established for a particular purpose [3], regardless of whether the method is an *in vitro* or *in vivo* test. In addition, at this workshop, the essential steps of the experimental validation process were defined in the following manner:

1. test development in a single laboratory;
2. experimental validation under blind conditions in several laboratories in a ring trial;
3. independent assessment of the results of the validation trial; and
4. regulatory acceptance.

Steps 2 and 3 were identified as the core part of a formal validation study conducted for regulatory purposes. The report of the 1st Amden workshop on validation encouraged scientists to start formal validation studies. Since the Draize eye test has been the most widely criticised toxicity tests, several international validation studies on alternatives to the Draize eye test were initiated:

1. BGA/BMBF study: national validation study in Germany 1988-1995 [4].
2. IRAG study: retrospective international study, organised by US regulatory agencies 1991- 1994 [5].
3. EC/HO study: international validation study organised by the UK, sponsored by EU 1992-1995 [6].
4. Japanese study: national validation study 1991-1995 [7].
5. COLIPA study: international validation study 1994-997 [8].

The management team of the EC/HO validation study, in which nine alternatives to the Draize eye test were tested under blind conditions with 60 carefully selected test chemcials in 36 laboratories, concluded at the end of the study in 1995 that none of the in vitro alternatives was able to completely replace the Draize eye test, and that the validation process had to be improved [6].

3. Evolution of the principles of scientific validation II: 2nd Amden workshop on validation

Thus, despite the joint efforts of many scientists around the world, the first attempt at validation failed, and the leading scientists involved met for a 2nd validation workshop in Amden in 1994, to learn from the unsuccessful attempts and to improve the validation procedure. Taking this experience into account, the participants in the 2nd Amden validation workshop recommended the inclusion of new elements into the validation process [9], which had not sufficiently been identified in the 1st Amden validation workshop. The three essential elements recommended were the definition of a biostatistically based *prediction model*, the inclusion of a *prevalidation stage* between test development and formal validation under blind conditions, and a well-defined *management structure*.

A *prediction model* should allow the prediction *in vivo* endpoints in animals or humans from the endpoints determined in the *in vitro* test. The prediction model must be defined mathematically in the standard operation procedure of the test that will undergo experimental validation under blind conditions with coded chemicals [9]. In order to assess the limitations of a new test before it will be evaluated in a validation study, the test should be standardised in a *prevalidation study* with a few test chemicals in a few laboratories [10]. This will ensure that the *in vitro* test method, including the prediction model, is robust and that the formal validation study with coded chemicals is likely to be successful. Finally, the goal of a validation study has to be defined clearly, and the *management structure* has to ensure that within the study the scientists who are responsible for essential tasks can conduct their duties independently from the sponsors and the managers of the study, e.g. biostatistical analysis, and the selection, coding and shipment of the test chemicals.

The improved concept of experimental validation for regulatory purposes defined in the 2nd validation workshop in Amden was accepted by the EU validation centre, ECVAM, in 1995, and in 1996 it was accepted by US regulatory agencies [11] and also by the OECD [12]. After this agreement at the international level, scientists have tried to follow

the ECVAM/US/OECD principles for validation in new validation trials. The improved validation concept was immediately introduced into ongoing validation studies, such as the ECVAM/COLIPA validation study on *in vitro* phototoxicity tests.

4. Successful validation and regulatory acceptance of *in vitro* toxicity tests

4.1 Validation of the 3T3 NRU in vitro phototoxicity test

Phototoxicity is an acute reaction, which can be induced by a single treatment with a chemical and UV or visible radiation. Since no standard guideline for the testing of photoirritation potential, either *in vivo* or *in vitro*, had been accepted for regulatory purposes at the international level by the OECD, in 1991, the European Commission (EC) and the European Cosmetics, Toiletry and Perfumery Association (COLIPA) established a joint programme on developing and validating *in vitro* photoirritation tests. In the first phase of the study, which was funded by DG XI of the EC and co-ordinated by ZEBET, *in vitro* phototoxicity tests established in laboratories of the cosmetics industry were evaluated, and a new assay, the 3T3 NRU PT test, which is a photocytotoxicity test using the mouse fibroblast cell line 3T3 and neutral red uptake (NRU) as the endpoint for cytotoxicity.

In the prevalidation study, which was conducted with 20 test chemicals (11 phototoxic and 9 non-phototoxic ones) quite unexpectedly, the 3T3 NRU PT *in vitro* phototoxicity test was the only *in vitro* test in which all of the test chemicals were correctly identified as phototoxic or non-phototoxic [13]. Independently of this prevalidation exercise, a laboratory in Japan subsequently obtained the same correct results in the 3T3 NRU PT, when testing the same set of 20 test chemcials.

In the second phase of the study, which was funded by ECVAM and co-ordinated by ZEBET, the 3T3 NRU PT test was validated with 30 carefully selected test chemicals in 11 laboratories in a blind trial on the 3T3 NRU PT test. A special ECVAM workshop was held to independently select a representative set of test chemcials covering all major classes of phototoxins was selected according to results from standardised photopatch testing in humans [14]. The results obtained in this *in vitro* test under blind conditions were reproducible, and the correlation between *in vitro* and *in vivo* data was almost perfect [15]. Therefore, the ECVAM Scientific Advisory Committee (ESAC) concluded, that the 3T3 NRU PT is a scientifically validated test which is ready to be considered for regulatory acceptance [16].

However, the EU expert committee on the safety of cosmetics, the Scientific Committee on Cosmetology and Non-Food-Products (SCCNFP), criticised the fact that there was an insufficient number of UV-filter chemicals (widely used as sunblockers) tested in the formal validation study. In the blind trial on UV filter chemicals, which was again funded by ECVAM and co-ordinated by ZEBET, the phototoxic potential of all of the 20 test chemicals (10 UV-filter chemicals, which were non-phototoxic, and 10 phototoxic test chemicals) was predicted correctly in the 3T3 NRU PT *in vitro* phototoxicity test [17].

Therefore, in 1998, the EU, having accepted the 3T3 NRU PT test as the first experimentally validated *in vitro* toxicity test for regulatory purposes, officially applied to the OECD for world-wide acceptance of this *in vitro* toxicity test. Early in 2000 the European Commission has officially accepted and published the 3T3 NRU PT phototoxicity test in *Annex V* of *Directive 67/548 EEC on the Classification, Packaging and Labelling of Dangerous Substances* [18]. Thus, this *in vitro* test is the first formally validated *in vitro* toxicity test that has been accepted into Annex V, and it is the only phototoxicity that is accepted for regulatory purposes in Europe. However, the OECD has

so far not taken the acceptance of this in vitro toxicity test on their agenda during the past two years.

4.2 Validation of two in vitro skin corrosivity tests

Two *in vitro* test for skin corrosivity testing applying a human skin model EPISKIN™ and excised rat skin, were successfully validated in an ECVAM validation study from 1996-1998 [19]. The ECVAM Scientific Advisory Committee ESAC concluded in 1998 [20]: The results obtained with the EPISKIN™ test involving the use of a reconstructed human skin model and the rat skin transcutaneous electrical resistance (TER) test in the international ECVAM validation study on *in vitro* tests for skin corrosivity were reproducible, both within and among laboratories that performed the test. The tests were able to distinguish between corrosive and non-corrosive chemicals for all of the chemical types studied. ESAC therefore agrees with the conclusions from the fornmal validation study that the EPISKIN™ test and the TER test are scientifically validated to be used as replacement for the animal test for distinguishing between corrosive and non-corrosive test chemicals, and that the tests are ready to be considered for regulatory acceptance. As a result, the two *in vitro* corrosivity test have been accepted by the European Commission for regulatory purposes in the year 2000 [21].

4.3 Validation of the Epiderm™ human skin model for corrosivity testing

Since the EPISKIN™ human skin model was not commercially available any more after it had been experimentally validated, a second human skin model the EpiDerm™ was validated in an ECVAM study from 1998-2000. This short study, which was co-ordinated by ZEBET and conducted in three laboratories with chemicals from the previous validation study, proved that the EPISKIN™ human skin model met the acceptance criteria of the TER and EPISKIN™ *in vitro* corrosivity tests. Therefore, ESAC concluded at its last meting in March of 2000 that the EpiDerm™ human skin model can be used for distinguishing between corrosive and non-corrosive chemicals within the context of the EU test guideline for skin corrosion [22].

4.4 ECVAM validation study of three in vitro embryotoxicity tests

In an ECVAM validation study, three *in vitro* embryotoxicity test were validated under blind conditions from the years 1997-2000. In the EU, there is a strong demand for validated *in vitro* tests in developmental toxicity testing using mammalian embryos as well as primary cultures of embryonic cells and permanent cell lines. As the most important result of the present validation study, for the first time, three *in vitro* embryotoxicity tests have been established that are backed by validated test protocols, which will be available through ECVAM as INVITTOX protocols 1. the whole embryo culture (WEC) test using cultures of whole rat embryos, 2. the micro mass (MM) test employing primary cultures of dissociated limb bud cells of rat embryos and 3. the embryonic stem cell test (EST), which is using two established mouse embryonic cell lines and which does not require to sacrifice pregnant animals.

In the ECVAM validation study 20 test chemicals were tested that are backed by high quality *in vivo* data in humans and animals. Each *in vitro* test was experimentally validated evaluated under blind conditions in four laboratories. All of the *in vitro* embryotoxicity tests met three essential criteria of validated alternative toxicity tests. Firstly, standard operation procedures (SOPs) were established, which are now available to public. Secondly, sound biostatistical prediction models (PMs) have been established and validated [23] The PMs for all of the three tests provide an accuracy of close to 80% and, more importantly, 100% predictivity for strong embryotoxic chemicals. Thus,

they can routinely be used to identify strongly embryotoxic chemicals, e.g. when screening new substances. Thirdly, the three *in vitro* tests were experimentally validated in a blind ring trial according to the validation scheme recommended by the EU, the OECD and the US NIEHS [2, 3, 11, 12]

Thus, this study clearly demonstrated, that the ECVAM strategy for prevalidation and validation of *in vitro* toxicity tests is sound. This conclusion based on the final report of the study was were accepted by ECVAM in June of the year 2000.

4.5 Validation of the Local Lymph Node Assay (LLNA) for sensitising properties

The Local Lymph Node Assay (LLNA) for the evaluation of sensitising properties, which was developed an validated in laboratories of the chemical industry in the UK (England), was accepted for regulatory purposes in 1999 by the federal regulatory authorities of the USA under the chairmanship of the US Validation Centre ICCVAM at the NIEHS [24]. In the year 2000, ESAC, the ECVAM Scientific Advisory Committee, concluded from reviewing this report that the LLNA is a scientifically validated test which can be used to assess the skin sensitisation potential of chemicals. Therefore, ESAC recommended that the LLNA should be the preferred method for sensitisation testing since it used fewer animals and causes less distress than the conventional guinea-pig methods [25]. However, in some instances and for scientific reasons ESAC accepted the use of the conventional methods.

4.6 Ban of the ascites method for the production of monoclonal antibodies

Taking into account studies conducted in several EU member states, ESAC has also recommended banning the production of monoclonal antibodies using the *in vivo* ascites mouse technique. Several companies have developed bioreactors, which allow to culture mouse hybridoma cells *in vitro*, which are producing monoclonal antibodies. A few EU member states are strictly enforcing the ban of the ascites mouse method, which causes pain, suffering and death of the mice, e.g. Germany, the Netherlands, Sweden and the UK, while other EU member states have not yet implemented the ban. Taking into account the progress in Europe, meanwhile the US NIH has stated that any research institute that continues to approve the routine use of ascites in producing monoclonal antibodies will no longer be eligible to receive a US Government research grant. is also recommending to use bioreactors rather than ascites mice for the production of monoclonal antibodies [25]..

4.7 Regulatory acceptance of four alternatives to the Draize eye irritation test

Several validation studies of *in vitro* alternatives to the Draize eye test have been conducted in Europe during the past decade. As a result, four *in vitro* alternatives have been accepted for regulatory purposes to identify severely eye irritating materials according to EU Directive 86/906/EEC for the classification and labelling of hazardous chemicals: the HET-CAM assay on the embryonated chicken egg, the BCOP test on the isolated bovine cornea from slaughterhouse, and two *in vitro* test on isolated chicken and rabbit eyes from animals that have been sacrificed for other purposes. Chemicals, which provide a negative reaction in any of the four *in vitro* tests still have to be tested in the Draize eye test in 1-3 rabbits in order to confirm the absence of exe irritation potential. In several EU member states, e.g. France an Germany, the HET-CAM test is accepted by the national authorities for the safety testing of cosmetics.

5. Conclusions and recommendations

The successful validation and regulatory acceptance of several *in vitro* toxicity test in the European Union proves that the validation

procedure recommended by ECVAM and the OECD [2, 3, 11, 12] is most appropriate for the validation of *in vitro* toxicity tests. However, taking into account both time frame and costs, e.g. of the ECVAM/COLIPA validation study of *in vitro* embryotoxicity tests, the formal validation procedure must be improved in order to reduce both costs and duration of the studies. To illustrate the problem, this particular study required funding of more than 1 million ECU (≅ US $) and the ECVAM validation study of three *in vitro* embryotoxicity was funded with a budget of 1.6 million ECU. The two examples illustrate that validation studies are very expensive and time-consuming, since it appears to take, on an average, 10 years from test development to regulatory acceptance.

Until today under the chairmanship of Professor Michael Balls and ECVAM the European Union has taken the lead in the experimental validation of *in vitro* test methods for regulatory purposes. In Japan several validation studies have been conducted on alternatives to the Draize eye test and on acute local irritation testing on the skin. In contrast, the US validation centre ICCVAM has focused its activity on reviewing the results of validation studies that were funded by other institutions, since money of the National Toxicology Program has so far not been set aside for the validation of *in vitro* methods but the situation may improve in the very near future.

Taking into account the lessons learned during the past decade, progress in the acceptance of *in vitro* toxicity tests at an international level will be achieved only, if Europe, Japan and the USA would share the burden of funding validation studies in a co-ordinated manner. The OECD might provide an appropriate forum for this important international activity although, due to other priorities, the OECD has not yet accepted the in vitro toxicity test that are used for regulatory purposes in Europe after successful validation.

Acknowledgements

The progress in the validation and acceptance of *in vitro* methods for toxicity testing could only be achieved with the continuous support of the staff of ZEBET, and the high priority that our work has been given both by ECVAM and COLIPA. I am particularly indebted to my colleagues in the management team of the ECVAM/COLIPA validation project on *in vitro* phototoxicity tests, namely Michael Balls (ECVAM, Ispra, Italy), Wolfgang Pape (Beiersdorf, Hamburg, Germany), Odile de Silva (L'Oréal, Paris, France) and Jack Dupuis (COLIPA, Brussels, Belgium), who passed away quite unexpectedly one month ago and to whom we want to dedicate the present manuscript.

References

1. Russell WMS, Burch RL (1959) The principles of humane experimental technique. London, Methuen.
2. OECD (1983) OECD Guideline for Testing of Chemicals. OECD Publications Office , Paris, France.
3. Balls M, Blaauboer B, Brusik D, Frazier J, Lamp D, Pemberton M, Reinhardt C, Robertfroid M, Rosenkranz H, Schmid B, Spielmann H, Stammati AL, Walum E. (1990) Report and recommendations of the CAAT/ERGATT workshop on the validation of toxicity test procedures. *ATLA* 18, 313-337.
4. Spielmann H, Liebsch M, Kalweit S, Moldenhauer F, Wirnsberger T, Holzhütter H-G, Schneider B, Glaser S, Gerner I, Pape WJW, Kreiling R, Krauser K, Miltenburger HG, Steiling W, Luepke NP, Müller N, Kreuzer H, Mürmann P, Spengler J, Bertram-Neis E, Siegemund B, Wiebel FJ. (1996) Results of a validation study in Germany on two *in vitro* alternatives to the Draize eye irritation test, the HET-CAM test and the 3T3 NRU cytotoxicity test. *ATLA* 24, 741-858.
5. Spielmann H, Liebsch M, Moldenhauer F, Holzhütter H-G, Bagley DM, Lipman JM, Pape WJW, Miltenburger HG, de Silva O, Hofer H, Steiling W (1997) IRAG working group 2 report: CAM-based assays. *Food & Chemical Toxicology* 35, 39-66.

6. Balls M, Botham PA, Bruner LH, Spielmann H (1995) The EC/HO international validation study on alternatives to the Draize eye irirtation test. *Toxicology in Vitro* 9, 871-929.
7. Ohno Y, Kaneko T, Kobayashi T, Inoue T, Kuroiwa Y, Yoshida T, Momma J, Hayashi M, Akiyama J, Atsumi T, Chiba K, Endo T, Fujii A, Kakishima H, Kojima H, Masamoto K, Masuda M, Matsukawa S, Ohkoshi K, Okada J, Sakamoto K, Takano K, Takanaka A (1994) First-phase validation of the *in vitro* eye irritation test for cosmetic ingredients. *In Vitro Toxicology* 7, 89-94.
8. Brantom PG, Bruner LH, Chamberlain M, de Silva O, Dupuis J, Earl LK, Lovell DP, Page WJW, Uttley M, Bagley DM, Baker FW, Bracher M, Courtellemont P, Declercq L, Freeman S, Steiling W, Walker AP, Carr GJ, Dami N, Thomas G, Harbell J, Jones PA, Pfannenbecker U, Southee JA, Tcheng M, Argembeaux H, Castelli D, Clothier R, Esdaile DJ, Itigaki H, Jung K, Kasai Y, Kojima H, Kristen U, Larnicol M, Lewis RW, Marenus K, Moreno O, Peterson A, Rasmussen ES, Robles C, Stern M. (1997) A summary report of the COLIPA international validation study on alternatives to the Draize rabbit eye irritation test. *Toxicology in Vitro* 11, 141-179.
9. Balls M, Blaauboer BJ, Fentem J, Bruner L, Combes RD, Ekwal B, Fiedler RJ, Guillouzo A, Lewis, RW, Lovell DP, Reinhartd 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.
10. Curren RD, Southee JA, Spielmann H, Liebsch M, Fentem J, Balls M (1995) The role of prevalidation in the development, validation and acceptance of alternative methods. *ATLA* 23, 211-217.
11. National Institute of Environmental Health (NIEHS) (1997) Validation and Regulatory Acceptance of Toxicological test Methods: a report of the Ad hoc Interagency Co-ordinating Committee on the Validation of Alternative Methods. NIEHS NIH Publication No. 97-3981. Research Triangle Park, USA.
12. OECD (1996) Final Report of the OECD Workshop on Harmonization of Validation and Acceptance Criteria for Alternative Toxicological Tests Methods. OECD Publication Office, Paris, France.
13. Spielmann H, Balls M. Brand M, Döring B, Holzhütter HG, Kalweit S, Klecak G, L'Epattenier H, Liebsch M, Lovell WW, Maurer T, Moldenhauer F, Moore L, Pape WJW, Pfannenbecker U, Potthast J, De Silva O, Steiling W, Willshaw A (1994). EC/COLIPA project on *in vitro* phototoxicity testing: first results obtained with the Balb/c 3T3 cell phototoxicity assay. *Toxicology in Vitro* 8, 793-796.
14. Spielmann H, Lovell WW, Hölzle E, Johnson BE, Maurer T, Miranda M, Pape WJW, Sapora O, Sladowski D (1994) *In vitro* phototoxicity testing. The report and recommendations of ECVAM Workshop 2. *ATLA* 22, 314-348.
15. 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 (1998) The international EU/COLIPA *in vitro* phototoxicity validation study: results of Phase II (blind trial); part 1: the 3T3 NRU phototoxicity test. *Toxicology in Vitro* 12, 305-327.
16. Balls M, Corcelle G (1998) Statement on the scientific validity of the 3T3 NRU PT test (an *in vitro* test for phototoxic potential). *ATLA* 26, 7-8.
17. Spielmann H, Balls M, Dupuis J, Pape WJW, de Silva O, Holzhütter HG, Gerberick F, Liebsch M, Lovell WW, Pfannenbecker U (1998) A study on UV filter chemicals from Annex VII of Euroepan Union Directive 76/768/EEC, in the *in vitro* 3T3 NRU phototoxicity test. *ATLA* 26, 679-708.
18. European Commission (2000a) Test guideline B-41 "phototoxicity - *in vitro* 3T3 NRU phototoxicity test" of Annex V of the EU Directive 86/906 /EEC for classification and labelling of hazardous chemicals O.J. of the European Communities, June 8 2000, L136, 98-107.
19. Fentem JH, Archer, GEB, Balls M, Botham PA, Curren RD, Earl LK, Esdaile DJ, Holzhütter HG, Liebsch M (1998) The ECVAM interbational validation study on *in vitro* test for skin corrosivity. 2. Results and evaluation by the Managment Team. *Toxicology in Vitro* 12, 483-524.
20. ESAC - ECVAM Scientific Advisory Committee (1998) Statement on the scientific validity of the rat skin transcutaneous resistance (TER) test and the EPISKIN™ test (*in vitro* tests for skin corrosivity). *ATLA* 26, 275-280.
21. European Commission (2000b) Test guideline B-40 "skin corrosivity - *in vitro* method" of Annex V of the EU Directive86/906/EEC for classification and labelling of hazardous chemicals O.J. of the European Communities, June 8 2000, L136, 85-97.
22. ESAC - ECVAM Scientific Advisory Committee (2000a) Statement on the application of the Epi-Derm™ human skin model for skin corrosivity testing. *ATLA* 28, 365-367.
23. Genschow E, Scholz G, Brown N, Piersma A, Brady M, Clemann N, Huusjkonen H, Paillard F, Bremer S, Becker K, Spielmann H (2000) Devel-

opment of prediction models for three *in vitro* embryotoxicity tests in an ECVAM validation study. *In Vitro and Molecular Toxicology* 13, 51-65.

24. National Institute of Environmental Health (NIEHS) (1999) The murine local lymph node assay. The results of an independent peer review evaluation co-ordinated by the interagency Co-ordinating Committee on the Validation of Alternative Methods (ICCVAM) and the National Toxicology Program Center for the Evaluation of Alternative Toxicological methods (NICEATM). NIEHS NIH Publ. No. 99-4494. Research Triangle Park, USA.
25. ESAC - ECVAM Scientific Advisory Committee (2000b) Statement on the validity of the local lymph node assay for skin sensitisation testing. *ATLA* 28, 365-367.
26. US NIH (2000) NIH Guide to grants and contracts. Published on 3 February 2000 on the internet: http://grants.gov/grants/guide/notice.-files/NOT-OD-00-019.html