

## Mission and accomplishments of ZEBET, the national centre for alternatives in Germany at the BfR (Federal Institute for Risk Assessment)

Horst Spielmann, Barbara Grune, Manfred Liebsch,  
Andrea Seiler and Richard Vogel

ZEBET (National Centre for Documentation and Evaluation of Alternative Methods to Animal Experiments) at the  
Federal Institute for Risk Assessment (BfR = Bundesinstitut für Risikobewertung)

Corresponding author: Professor Horst Spielmann

c/o BfR Berlin, Germany, ZEBET at the BfR, Diederisdorfer Weg 1, D-12277 Berlin, Germany

Phone: +(49)-1888-412-2270, Fax: +(49)-1888-412-2958, horst.spielmann@bfr.bund.de & horstspielman@aol.com

---

### Abstract

A short description is given of the history of ZEBET, the National Centre for the Documentation and Evaluation of Alternatives to Animal Experiments of the Federal Government of Germany at the Federal Institute of Risk Assessment (BfR). ZEBET was established in 1989 as the first government centre focusing on reducing testing in animals. In 1993 the EU followed this example by establishing ECVAM (European Centre for the Validation of Alternative Methods) at the Joint Research Centre (JRC) in Ispra (Italy), in 1997 the federal government agencies of the USA formed ICCVAM (Interagency Coordinating Center for the Validation of Alternative Methods) and in 2005 the Japanese government has established JaCVAM (Japanese Centre for the Validation of Alternative Methods) at the National Institute of Health in Tokyo. The decrease in experimental animal numbers during the past decade in Europe is illustrated by the situation in Germany and the contribution of international harmonisation of test guidelines on reducing animal numbers in regulatory testing is described. A review of the development of the principles of experimental validation is given and the 3T3 NRU in vitro phototoxicity test is used as an example for a successful validation study, which led to the acceptance of the first in vitro toxicity test for regulatory purposes by the OECD. Finally, the currently accepted alternative methods for standardisation and safety testing of drugs, biologicals and medical devices are summarised.

**Keywords:** ZEBET, history, guideline, validation

---

### Introduction

In 1959, William Russell and Rex Burch published their book, *The Principles of Humane Experimental Technique* (Russell and Burch, 1959), in which they put forward the Three Rs (3Rs) concept (*reduction, refinement and replacement*) in relation to the humane treatment of experimental animals. Their concept was little recognised outside the UK for about 20 years, until the animal welfare movement, the general public, some committed politicians and, finally, the international scientific community, raised concerns about the suffering of experimental animals. The Three Rs concept has become the generally accepted scientific basis of institutions serving the development of alternatives to animal experiments. In 1989 ZEBET ((National Centre for Documentation and Evaluation of Alternative Methods to Animal Experiments = Zentralstelle zur Erfassung und Bewertung von Ersatz- und Ergänzungsmethoden

zum Tierversuch) was established at the Federal Health Institute BfR as the first government agency promoting the development and validation of non-animal testing procedures. In 1993 the EU followed this example by establishing ECVAM (European Centre for the Validation of Alternative Methods) at the Joint Research Centre (JRC) in Ispra (Italy), in 1997 the federal government agencies of the USA formed ICCVAM (Interagency Coordinating Center for the Validation of Alternative Methods) and in 2005 the Japanese government has established JaCVAM (Japanese Centre for the Validation of Alternative Methods) at the National Institute of Health in Tokyo.

The activities of these institutions are focusing on replacing regulatory animal tests that have to be conducted to identify the toxic properties of chemicals to which humans or the environment are exposed, when the chemicals are used in a specific product or for a specific purpose. The 3-Rs concept

has proven successful in reducing the suffering of laboratory animals used in regulatory safety testing. At the international level the harmonisation of test guidelines has proven successful to reduce safety testing in animals for regulatory purposes, in particular since there are several examples proving that regulatory animal tests can indeed be replaced, when the mechanistic basis of the specific area of toxicology is well understood and an appropriate in vitro model is available. Using the validation of the 3T3 NRU in vitro phototoxicity test as an example, it will be illustrated how the first non-animal toxicity tests was experimentally validated and accepted for regulatory purposes by EU Member-States and in 2004 also at a world-wide level by the OECD. Additional examples are given for animal tests, which have been replaced by advanced non-animal methods.

### Establishing ZEBET, the national centre for the documentation and evaluation of alternatives to animal experiments in Germany

To promote the implementation of the EU Directive 86/609/EEC (EU Commission, 1986) on the use of experimental animals the European Commission and several Member States have established centres for the validation of alternative methods, e.g. in 1989 ZEBET, the German centre for the documentation and evaluation of alternative methods, was established at the Federal Health Institute BGA in Berlin and in 1993 ECVAM, the European Centre for Validation of Alternative Methods.

To give an example of the duties assigned to validation centres, the national German centre ZEBET is serving the following mission:

- to establish a database & information service on alternatives at the national and international level
- to develop alternatives according to the 3Rs principle of Russel and Burch
- to fund research on alternatives
- to co-ordinate validation studies
- to co-operate with national & international funding agencies and validation centres
- to provide a forum for information on alternatives to animal testing.

At the Federal Institute for Risk Assessment BfR ZEBET's main activity is the reduction of animal tests conducted for regulatory purposes. The EU validation centre ECVAM is also focusing its activity on reducing regulatory safety testing in animals and the US validation agency ICCVAM is serving a similar mission. ECVAM and ZEBET have funds to support the development and validation of alternative methods, while ICCVAM has so far limited its activity to evaluate the results of validation studies conducted elsewhere.

### Decrease in experimental animal numbers during the past decade in Europe as illustrated by the situation in Germany

EU Directive 86/609/EEC has in 1989, for the first time allowed government authorities in EU Member States to collect test animal numbers according to a standardised procedure (European Commission 1986). Since 1989 the annual numbers of experimental animals in Germany have decreased from 2.7 Mio. In 1989 to 2 Mio. in the year 2004. A closer analysis shows that the decrease was predominantly due to a reduction in animal numbers used for the development of drugs, which went down by more than 50% in 15 years from 1.4 Mio in 1989 to 0.5 Mio in 2004. The decrease is even more impressive, when taking into account earlier data provided by the German association of drug manufacturers, which show that in 1977 4.4 Mio. experimental animals had been used for the development and safety testing of new drugs in Germany (Fig. 1) (BPI 1981).

This dramatic decrease in laboratory animal numbers is not due to the high priority, which EU Directive 86/609 gives to the development of in vitro alternatives but to the general change of methodology in the life sciences from animal models to molecular biology and genetics including cell and tissue culture models. In the field of drug development the new technology allows high throughput screening (HTS) of thousands of new drug candidates. This approach is, of course, more predictive, faster and cheaper than animal models, which had successfully been used for drug development in the past. However, at the same time it has proven extremely difficult to reduce animal numbers in regulatory safety testing by non-animal methods, since the established endpoints in toxicity tests in laboratory animals are usually organ specific and covering endpoints that are quite similar to the situation in humans.

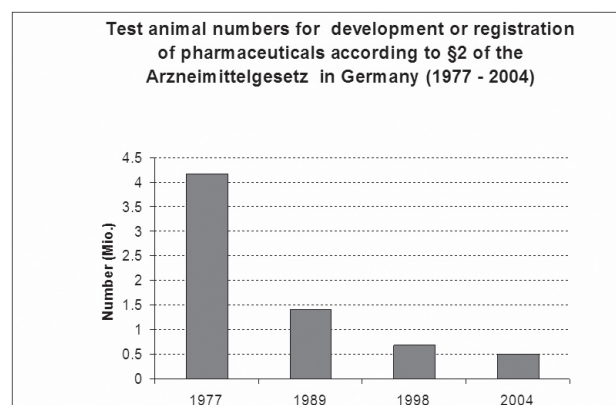


Fig. 1. Test animal numbers used by the German drug industry 1977-2004

The data for 1977 were collected by the German Drug Manufacturers Association (BPI, 1981) and the data for 1989 and 2004 were officially compiled by the German Federal Minister of Agriculture according to EU Directive 86/609/EEC (EU Commission, 1986).

### Reducing animal numbers in regulatory testing by international harmonisation of test guidelines

For the past 50 years toxicity testing has been developed empirically in many laboratories around the world. For a given specific area of toxicology, e.g. eye and skin irritation or embryotoxicity, the standard animal procedures differed considerably between countries, e.g. as far as species, regimen of treatment, numbers of animals per treatment group etc. In addition, the way in which the information from a specific animal test for classification and labelling, e.g. the Draize eye test, was used in different ways by regulatory agencies in various countries and even by different agencies of one country.

### Harmonisation of OECD guidelines for the testing of chemicals

The international harmonisation of toxicity tests by the OECD in 1982 (OECD 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. The 30 Member States of the OECD 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) (D'Arcy and Harron, 1995), 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.

**Table 1** summarises the most important areas, which require safety testing in animals, and in which the test guidelines have been harmonised at the international level. **Table 2** shows that

Table 1. International harmonisation of guidelines for toxicity testing

The table summarises the different fields of safety testing, for which international harmonisation was implemented both for economical reasons, to reduce the cost of testing, and for ethical reasons, to reduce suffering of laboratory animals.

- |  |
|--|
| <ul style="list-style-type: none"><li>• <b>Industrial chemicals, pesticides, cosmetics, food additives etc.</b><br/>OECD-Guidelines for the Testing of Chemicals</li><li>• <b>Drugs and medical devices</b><br/>International Conferences on Harmonisation (ICH)</li><li>• <b>Safety and efficacy of hormones and biologicals</b><br/>Pharmacopoeias (European and US Pharmacopoeia)</li><li>• <b>Vaccines and other immunologicals</b><br/>WHO recommendations, European and US Pharmacopoeia</li></ul> |
|--|

in addition to drugs, industrial chemicals and pesticides, international test guidelines have also been harmonised for hormones and biologicals by the pharmacopoeias and for vaccines by the WHO. So far the harmonisation of international test guidelines for toxicity and safety testing has been the most successful approach to reduce animal testing for regulatory purposes.

### Regulatory acceptance of the successfully validated 3T3 NRU *in vitro* phototoxicity test at a world-wide level by the OECD in 2004

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 program to develop and validate *in vitro* photoirritation tests. In the prevalidation study conducted with 20 test chemicals quite unexpectedly, the 3T3 NRU PT *in vitro* phototoxicity test, which is a photo-cytotoxicity test using the mouse fibroblast cell line 3T3 and neutral red uptake (NRU) as the endpoint for cytotoxicity, was the only *in vitro* test in which all of the 20 test chemicals were correctly identified as phototoxic or non-phototoxic (Spielmann et al., 1994). 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. A representative set of test chemicals covering all major classes of phototoxins was selected according to results from standardised photopatch testing in humans (Spielmann et al., 1998a). 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. Therefore, the ECVAM Scientific Advisory Committee (ESAC) concluded in 1998, that the 3T3 NRU PT is a scientifically validated test which is ready to be considered for regulatory acceptance (ESAC 1998).

However, the Scientific Committee on Cosmetology and Non-Food-Products (SCCNFP), criticised that an insufficient number of UV-filter chemicals (widely used as sunblockers) were tested in the formal validation study. In a subsequent blind trial on UV filter chemicals the phototoxic potential of all test chemicals was predicted correctly in the 3T3 NRU PT *in vitro* phototoxicity test (Spielmann et al., 1998b). 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. In 2000 the European Commission has officially accepted and published

Table 2. *In vitro* tests replacing test animals in the quality control of biological drugs and in toxicology  
*In vitro* tests, which have been sufficiently validated to replace regulatory animal tests are given and also the degree of replacement and the specific regulatory authority.

<i>in vitro</i> method	animal test	degree of replacement	accepted by regulatory authorities (country)
<b><u>QUALITY CONTROL - BIOLOGICAL DRUGS</u></b>			
<b>Pregnancy test</b> <i>immune assay</i>	<i>frog test</i>	complete replacement	world-wide
<b>Pyrogenicity test</b> <i>limulus-(LAL)-test</i>	<i>rabbit test</i>	replacement for protein free solutions	US-, EU- and Japan-Pharmacopoe
<i>human cytokine TNF-<math>\alpha</math> (ELISA)</i>		replacement for all applications	EU-Pharmacopoe submitted
<b>Vitamins and hormones</b> <i>(vitamines, oxytocin, calciitonin, parathorm. sexual hormones)</i> <i>HPLC, immune assays</i>	bioassay in <i>chicken, rats &amp; mice</i>	complete replacement	EU-Pharmacopoe
<b>Insulin-determination</b> <i>HPLC</i>	convulsion test <i>mouse</i>	complete replacement	US- and EU-Pharmacopoe
<b>Insulin-determination</b> <i>HPLC</i>	blood glucose determination <i>mouse &amp; rabbit</i>	complete replacement	US- and EU-Pharmacopoe
<b><u>TOXICITY TESTING</u></b>			
<b>Eye irritation</b> <i>HET-CAM test</i> <i>BCOP test</i> <i>isolated chicken eye</i> <i>isolated rabbit eye</i>	Draize test <i>rabbit's eye</i>	replacement for severely irritating materials	EU according to OECD Test Guideline 405 <i>Germany, Belgium</i> <i>Netherlands, U.K.</i>
<b>Phototoxicity</b> 3T3 NRU phototoxicity test	Phototoxicity tests <i>rabbit, rat &amp; guinea pig</i>	complete replacement	OECD Test Guideline 432
<b>Skin corrosion</b> <i>Human skin constructs</i>	corrosivity testing on <i>rabbit skin</i>	complete replacement for corrosive materials	OECD Test Guideline 431
<b>Skin penetration</b> <i>human skin</i>	Skin penetration test <i>on the skin of rats</i>	Complete replacement	OECD Test Guideline 428
<b>Delayed neurotoxicity</b> <b>Of organo-phosphates</b> <i>NTE-esterase determination in neuroblastoma cells</i>	OECD: neurotoxicity test of organo-phosphates in <i>Chicken</i>	partial replacement for esterase inhibitors	World-wide According to OECD Guidelines 418 and 419

the 3T3 NRU PT phototoxicity test in Annex V of Directive 67/548 EEC on the Classification, Packaging and Labelling of Dangerous Substances (EU Commission 2000b). 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. Meanwhile, in the year 2004 the OECD has accepted the 3T3 NRU PT phototoxicity test at the world-wide level as the first *in vitro* toxicity test into the OECD Guidelines for the testing of chemicals (OECD 2004b).

### More-recent Successful ECVAM Validation Studies on *In Vitro* Toxicity Tests

#### 1. Validation of two *in vitro* skin corrosivity tests

Two *in vitro* tests for skin corrosivity, employing the human skin model EPISKIN and excised rat

skin, were successfully validated in an ECVAM validation study conducted from 1996 to 1998. The results obtained with the EPISKIN model and the rat skin transcutaneous electrical resistance (TER) test were reproducible both within and among the laboratories that performed the test. The two *in vitro* tests were able to distinguish between corrosive and non-corrosive chemicals for all the types of chemicals studied. The ESAC therefore concluded that the EPISKIN test and the TER test are scientifically validated and ready to be used as replacements for the animal test for distinguishing between corrosive and non-corrosive test chemicals. As a result, the two *in vitro* corrosivity tests were accepted in 2000 by the EU as official methods for chemicals testing, and added to Annex V of Directive 67/548/EEC (European Commission, 2000a). Meanwhile, the OECD put the acceptance of these two *in vitro* toxicity tests

on its agenda, and finally, in 2004, two new test guidelines, No. 430, *In vitro* corrosivity test: rat skin transcutaneous electrical resistance (TER) test, and No. 431, *In vitro* corrosivity test using human skin models, were accepted by the national experts of the OECD test guidelines programme (OECD 2004a). It is now up to the European Commission to accept the two new OECD test guidelines for skin corrosivity testing into Annex V of *Directive 67/548/EEC*.

## 2. The ECVAM validation study on *in vitro* tests for acute skin irritation

To replace the Draize skin irritation test ECVAM, the European Centre for the Validation of Alternative Methods, has sponsored a formal validation study of three *in vitro* tests, two employing reconstituted human epidermis models (EPISKIN, EpiDerm) and the skin integrity function test (SIFT) employing *ex vivo* mouse skin. It was the goal of the study to assess if the *in vitro* tests would predict *in vivo* classification according to the EU classification "R 38" and "non-irritant". 58 chemicals (25 irritants and 33 non-irritants) were tested. These were selected to give broad coverage of physico-chemical properties, and an adequate distribution of irritancy scores derived from *in vivo* rabbit skin irritation tests.

In phase I, 20 of these chemicals (9 irritants and 11 non-irritants) were tested under blind conditions by a single (lead) laboratory for each of the methods, to confirm the suitability of the protocol improvements introduced after prevalidation. Using cytotoxicity (MTT) as endpoint, the predictive ability of both EpiDerm and EPISKIN was considered sufficient to justify them proceeding to phase II, while the predictive ability of the SIFT was inadequate. Since both skin models provided false predictions around the *in vivo* classification border (rabbit Draize score 2), the release of the cytokine Interleukin-1 $\alpha$  (IL-1 $\alpha$ ) was also determined. In phase II each human skin model was tested in three laboratories with 58 chemicals. The main endpoint measured for both EpiDerm and EPISKIN was cell viability (MTT reduction). In samples from chemicals providing MTT results above the threshold of 50% viability IL-1 $\alpha$  release was also measured to determine if the additional endpoint would improve the predictive ability of the tests.

For EPISKIN, sensitivity was 79% and specificity was 82% (MTT only); with the combination of MTT and IL-1 $\alpha$ , sensitivity increased to 91% with a specificity of 79%. For EpiDerm, sensitivity was 61% and specificity was 88% (MTT only) while the predictive potential of was not improved by IL-1 $\alpha$ . Following independent peer review, in April 2007 ECVAM's Scientific Advisory Committee endorsed the scientific validity of the EPISKIN test as a replacement for the rabbit skin irritation method, and of the EpiDerm method for identifying skin irritants

as part of a tiered testing strategy (ECVAM 2007).

After the report of the ECVAM validation study of *in vitro* skin irritation tests has recently been published (Spielmann et al., 2007), the new assay will most probably be the first *in vitro* toxicity tests to replace the Draize rabbit skin irritation test in Europe and internationally as new OECD Test Guideline in the very near future.

## Conclusions

ZEBET, the National Centre for the documentation and evaluation of alternatives to animal experiments in Germany, was in 1989 the first government centre dedicated on implementing the 3Rs concept into regulatory testing. It was the first task of ZEBET to establish a database and information service of alternative methods to animal experiments for scientists in industry, universities, research institutions and regulatory agencies both at the national level and internationally. At the international level had the development of *in vitro* toxicity tests and the successful management of experimental validation studies of *in vitro* toxicity tests had the highest impact of ZEBET's activities, since several of the validated tests have been accepted for regulatory purposes at the international level by the EU Commission and by the OECD. Thus ZEBET has proven that the concept of establishing national centres dedicated to development and validation of alternatives to animal experiments is a promising way forward to implement the 3Rs concept successfully at the international level. Moreover, by establishing national centres for alternatives governments can financially and scientifically contribute to reducing animal experiments and it provides visibility of these activities both at the national and international level.

## Acknowledgements

Our work at ZEBET has been depending on the generous funding by ECVAM, the EU Centre for Validation, and the Federal Government of Germany via the BMBF, the Department for Research and Technology of the Federal Government of Germany, and via the BMELV, Department of Agriculture and Consumer Protection.

## References

- Bundesverband der Pharmazeutischen Industrie e.V. (BPI) (1981) Tiere in der Arzneimittelforschung. Nutzen und Grenzen von Tierversuchen und anderen experimentellen Modellen. Frankfurt, Germany: BPI Abt. Presse und Öffentlichkeitsarbeit, 32 pp.
- D'Arcy, P.F. & Harron, D.W.G. (Eds.) (1995) Proceedings of the Third International Conference on Harmonisation (ICH) Yokohama 1995. Belfast, UK: The Queens University of Belfast.
- ECVAM (2007). ESAC Statement on the validity of *in vitro*-tests for skin irritation. Available at <http://ecvam.jrc.it/index.htm>, accessed on 14 October 2007.

- ESAC (ECVAM Scientific Advisory Committee) (1998) Statement on the scientific validity of the 3T3 NRU PT test (an *in vitro* test for phototoxic potential). *ATLA* **26**, 7–8.
- ESAC (ECVAM Scientific Advisory Committee) (2000) Statement on the application of the EpiDerm( human skin model for skin corrosivity testing. *ATLA* **28**, 365-367.
- European Commission (1983) Directive 83/467/EEC adapting to technical progress for the fifth time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. Brussels, Belgium: EU DG Environment.
- European Commission (1986) EU Directive 86/609/EEC on protection of animals used for experimental and other scientific purposes. Brussels, Belgium: EU DG Environment.
- European Commission (2000a) EU Directive 2000/33/EU for the 21<sup>st</sup> Amendment of Annex V of the EU Directive 86/906/EEC for classification and labelling of hazardous chemicals: Test guideline B-40 "skin corrosivity - *in vitro* method". O. J. European Communities June 8 2000, **L136**, 85-97.
- European Commission (2000b) EU Directive 2000/33/EU for the 21<sup>st</sup> Amendment of Annex V of the EU Directive 86/906/EEC for classification and labelling of hazardous chemicals: Test guideline B-41 "phototoxicity - *in vitro* 3T3 NRU phototoxicity test". O. J. European Communities June 8 2000, **L136**, 98-107.
- OECD (Organisation for Economic Co-operation and Development) (1982) OECD Guidelines for Testing of Chemicals. Paris, France: OECD Publication Office.
- OECD (2004a) OECD guidelines for the testing of chemicals: Test Guideline 431 "*In Vitro* corrosivity test using human skin models". Paris, France, OECD Publication Office.
- OECD (2004b) OECD guidelines for the testing of chemicals: Test Guideline 432 "*In vitro* 3T3 NRU phototoxicity test". Paris, Frankreich, OECD Publication Office.
- Russell W.M.S. and Burch R.L. (1959) The principles of humane experimental technique. London, UK: Methuen.
- Spielmann, H., Balls, M., Brand, M., Döring, B., Holzhütter, H.G., Kalweit, S., Klecak, G., L'Epattenier, H., Liebsch, M., Lovell, W.W., Maurer, T., Moldenhauer, F., Moore, L., Pape, W.J.W., Pfannenbecker, U., Potthast, J., De Silva, O., Steiling, W. and 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.
- Spielmann, H., Lovell, W.W., Hölzle, E., Johnson, B.E., Maurer, T., Miranda, M., Pape, W.J.W. Sapora, O. and Sladowski, D. (1994) *In vitro* phototoxicity testing. The report and recommendations of ECVAM Workshop 2. *ATLA* **22**, 314-348.
- Spielmann, H., Balls, M., Dupuis, J., Pape, W.J.W., Pechovitch, G., de Silva, O., Holzhütter, H.G., Clothier, R., Desolle, P., Gerberick, F., Liebsch, M., Lovell, W.W., Maurer, T., Pfannenbecker, U., Potthast, J.M., Csato, M., Sladowski, D., Steiling, W. & Brantom, P. (1998a) 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.
- Spielmann, H., Balls, M., Dupuis, J., Pape, W.J.W., de Silva, O., Holzhütter, H.G., Gerberick, F., Liebsch, M., Lovell, W.W. and Pfannenbecker U. (1998b) A study on UV filter chemicals from Annex VII of European Union Directive 76/768/EEC, in the *in vitro* 3T3 NRU phototoxicity test. *ATLA* **26**, 679–708.
- Spielmann, H., Hoffmann, S., Liebsch, M. Botham, P., Fentem, J., Eskes, C., Roguet, R., Cotovio, J., Cole, T., Worth, A., Heylings, J., Jones, P., Robles, C., Kandárová, H., Gamer, A., Remmele, M., Curren, R., Raabe, H., Cockshott, A., Gerner, I. and Zuang, V. (2007). The ECVAM international validation study on *in vitro* tests for acute skin irritation: report on the validity of the EPISKIN and EpiDerm assays and on the skin integrity function test. *ATLA* **35**, 559-601.