

**Evaluation and Validation of Reconstructed Human Skin  
Models as Alternatives to Animal Tests in  
Regulatory Toxicology**

**DISSERTATION**

to obtain the academic degree  
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by

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**Dedicated to:**

*My parents and Dušan.*

*Thank you for your love and support.*

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## LIST OF PUBLICATIONS RELATED TO THE THESIS

1. Trauer S., **Kand'árová H.**, Schmidt E., Spielmann H., Traue D., Balizs G., Rozycki C., Schaefer U., Lehr C.-M. and Liebsch M. (2006). *In vitro* test methods to examine the phototoxic potential and the skin permeation of Butyl Methoxy-dibenzoylmethane (BM-DBM). *In preparation*.
2. **Kand'árová H.**, Liebsch M., Schmidt E., Genschow, E., Traue D., Spielmann H., Meyer K., Steinhoff C., Tornier C., De Wever B. and Rosdy M. (2006). Assessment of the skin irritation potential of chemicals using the SkinEthic reconstructed human epidermis model and the "common skin irritation protocol" evaluated in the ECVAM skin irritation validation study. ATLA, *Accepted for publication*.
3. **Kand'árová H.**, Liebsch M., Spielmann H., Genschow E., Schmidt E., Traue D., Guest R., Whittingham A., Warren N., Gamer A., Remmele M., Kaufmann T., Wittmer E., De Wever B. and Rosdy M. (2006). Assessment of the SkinEthic reconstituted human epidermis for skin corrosion testing according to OECD Test Guideline 431. *Toxicology in Vitro* 20, 547-559.
4. **Kand'árová H.**, Liebsch M., Gerner I., Schmidt E., Genschow E., Traue D. and Spielmann H. (2005). EpiDerm skin irritation test protocol - an assessment of the performance of the optimised test. ATLA 33 (4), p. 351-367.
5. **Kand'árová H.**, Liebsch M., Genschow E., Gerner I., Traue D., Slawik B. and Spielmann H. (2004). Optimisation of the EpiDerm test protocol for the upcoming ECVAM validation study on *in vitro* skin irritation tests. ALTEX 21, 3/04, p. 107-114.

## OTHER PUBLICATIONS

6. Schreiber S., Mahmoud A., Vuia A., Rübhelke M.K., Schmidt E., Schaller M., **Kand'árová H.**, Haberland A., Schäfer U.F., Bock U., Korting H.C., Liebsch M. and Schäfer-Korting M. (2005). Reconstructed epidermis versus human and animal skin in skin absorption studies. *Toxicology in vitro* 19 (6), p. 813-822.
7. **Kand'árová H.**, Jantová S. and Hojerová J. (2004). New approaches to safety evaluation of cosmetic products and ingredients. (Review in Slovak language) *Czechoslovak Dermatology* 79 (2), p. 75-83.
8. **Kand'árová H.**, Hojerová J. and Jantová S. (2002). Safety of cosmetic products - *in vitro* methods. (Mini-review in Slovak language) *Derma* 4, p. 15-17.

## POSTERS RELATED TO THE THESIS

1. **Kand'árová H.**, Liebsch M. and Spielmann H. (2006). Development and evaluation of a robust, common protocols for reconstructed human skin models: *in vitro* skin irritation and corrosion testing. 13<sup>th</sup> Congress on Alternatives to Animal Testing. June 2-4, Linz, Austria. Abstract In: ALTEX. In Press.
2. **Kand'árová H.**, Kejlová K., Jírová D., Bendová H., Tharmann J., Traue D., Spielmann H. and Liebsch M. (2006). ECVAM Feasibility Study: Can the prevalidated *in vitro* skin model phototoxicity assay be upgraded to quantify phototoxic potency of topical phototoxins? 47. Frühjahrstagung der DGPT. April 4-9, Mainz, Germany. Abstract In: Naunyn - Schmiedeberg's Archives of Pharmacology. In Press.

3. Trauer S., **Kand'árová H.**, Schmidt E., Traue D., Schaefer U., Lehr C.-M. and Liebsch M. (2006). *In vitro* skin absorption of Buthyl Methoxydibenzoylmethane determined in human epidermis and in a reconstructed human epidermis model. 47. Frühjahrstagung der DGPT. April 4-9, Mainz, Germany.  
Abstract In: Naunyn - Schmiedeberg's Archives of Pharmacology. *In Press*.
4. Jírová D., Kejlová K., Bendová H., **Kand'árová H.**, Weidenhoffer Z. and Liebsch M. (2005). Bergamot oil intended for topical use: attempts at a risk assessment of phototoxicity. The 15<sup>th</sup> International Symposium on Contact Dermatitis (ISCD) in conjunction with 5<sup>th</sup> International Symposium on Irritant Contact Dermatitis (ISICD). November 6-9, Paphos, Cyprus.  
Abstract In: Exogenous Dermatology. Vol. 3, No. 4., 2004, p. 193.
5. Jírová D., Kejlová K., Bendová H., **Kand'árová H.**, Weidenhoffer Z. and Liebsch M. (2005). Bergamot oil intended for topical use. International Cosmetological Conference. October 19-21, Brno, Czech Republic.  
Abstract In: Proceedings of the International Conference on Cosmetology, Brno - Czech Republic, p.18.
6. **Kand'árová H.**, Liebsch M., Spielmann H., Genschow E., Schmidt E., Traue D., Guest R., Whittingham A., Warren N., Gamer A. O., Remmele M., Wittmer E., Kaufmann T., Rosdy M. and De Wever B. (2005). Skin corrosion *in vitro*: assessment of the SkinEthic™ reconstituted human epidermal model for *in vitro* skin corrosion testing according to OECD TG 431. 5<sup>th</sup> World Congress on Alternatives & Animal Use in the Life sciences. Session 5.12: Workshop - Strategies for prioritising and streamlining the Validation Process. August 21–25, Berlin, Germany.  
Abstract In: ALTEX 22, Special Issue 2005, p. 157.
7. **Kand'árová H.**, Kejlová K., Jírová D., Tharmann J. and Liebsch M. (2005). ECVAM Feasibility Study: Can the pre-validated *in vitro* skin model phototoxicity assay be upgraded to quantify phototoxic potency of topical phototoxins? 5<sup>th</sup> World Congress on Alternatives & Animal Use in the Life sciences. Session 5.3: Progress and Needs for Developing and Validating Alternatives for Dermal Toxicity Testing. August 21–25, Berlin, Germany.  
Abstract In: ALTEX 22, Special Issue 2005, p. 156.
8. **Kand'árová H.**, Liebsch M., Spielmann H., Schmidt E., Traue D., Meyer K., Steinhoff C., Tornier C., Rosdy M. and De Wever B. (2005). Assessment of the SkinEthic reconstituted human epidermal model for the prediction of the dermal irritation potential of chemicals. 5<sup>th</sup> World Congress on Alternatives & Animal Use in the Life sciences. Session 5.3: Progress and Needs for Developing and Validating Alternatives for Dermal Toxicity Testing. August 21–25, Berlin, Germany.  
Abstract In: ALTEX 22, Special Issue 2005, p. 157.
9. Jírová D., Kejlová K., Bendová H., **Kand'árová H.**, Weidenhoffer Z. and Liebsch M. (2005). Bergamot oil intended for topical use - attempts at a risk assessment of phototoxicity. 5<sup>th</sup> World Congress on Alternatives & Animal Use in the Life sciences. Session 5.3: Progress and Needs for Developing and Validating Alternatives for Dermal Toxicity Testing. August 21–25, Berlin, Germany.  
Abstract In: ALTEX 22, Special Issue 2005, p. 257.
10. Steinhoff C., **Kand'árová H.**, Steger-Hartmann T., Schweinfurth H., Liebsch M. and Meyer K. (2005). Occupational safety assessment of skin corrosion/irritation using human reconstituted epidermis models. 5<sup>th</sup> World Congress on Alternatives & Animal Use in the Life sciences. Session 5.3: Progress and Needs for Developing and Validating Alternatives for Dermal Toxicity Testing. August 21–25, Berlin, Germany.  
Abstract In: ALTEX 22, Special Issue 2005, p. 167.

11. **Kand'árová H.**, Liebsch M., Spielmann H., Guest R., Whittingham A., Warren N., Gamer A.O., Kaufmann T., Remmele M. and De Wever B. (2004). *In vitro* skin corrosion testing: validation of the SkinEthic reconstructed human epidermis for the assessment of skin corrosion potential of chemicals. GD Symposium. November 25, Berlin, Germany.
12. **Kand'árová H.**, Liebsch M., Spielmann H., Genschow E., Schmidt E., Guest R., Whittingham A., Warren N., Gamer A., Remmele M., Kaufmann T., Wittmer E. and De Wever B. (2004). Assessment of the human epidermis model SkinEthic RHE™ for *in vitro* skin corrosion testing according to OECD TG 431. 12<sup>th</sup> Congress on Alternatives to Animal Testing. October 16-17, Linz, Austria.  
Abstract In: ALTEX 21, 3/04, p. 160-161.
13. Liebsch M., **Kand'árová H.**, Spielmann H., Guest R., Whittingham A., Warren N., Gamer A.O., Kaufmann T., Remmele M. and De Wever B. (2004). Validation of the human epidermis model SkinEthic for the skin corrosion testing according to new OECD Test Guideline 431. 43<sup>rd</sup> Annual SOT meeting. March 21-25, Baltimore, USA.
14. Liebsch M., **Kand'árová H.**, Slawik B., Jírová D., Kejlová K. and Spielmann H. (2003). ECVAM Machbarkeitsstudie: Kann der prävalidierte *in vitro* Hautmodell Phototoxizitätstest zum Test für die Wirkungsstärke (Potenz) topischer Phototoxine ausgebaut werden? 11<sup>th</sup> Congress on Alternatives to Animal Testing. September 19-21, Linz, Austria.  
Abstract In: ALTEX 20, 3/03, p. 179

#### ORAL PRESENTATIONS RELATED TO THE THESIS

1. Engelke M. and **Kand'árová H.** (2006). Eye irritation test *in vitro*. Practical Training Course for the identification of ocular irritancy and corrosivity. FU-Berlin, Institute for Pharmacy. March 24-29, Berlin, Germany.
2. Spielmann H., **Kand'árová H.**, and Traue D. (2006). HET-CAM Test. Kurs und Seminar Tierversuche: Versuchstiere & Alternativmethoden. Charité - Berlin. March 7<sup>th</sup>, Berlin, Germany.
3. Spielmann H. and **Kand'árová H.** (2005). HET-CAM Test. Kurs und Seminar Tierversuche: Versuchstiere & Alternativmethoden. Charité - Berlin. September 27<sup>th</sup>, Berlin, Germany.
4. **Kand'árová H.** (2005): Skin corrosion test *in vitro* using reconstructed human skin models. Practical Training Course on Skin Models. FU-Berlin, Institute for Pharmacy. September 26–28, Berlin, Germany.
5. **Kand'árová H.**, Liebsch M., Gerner I., Schmidt E, Genschow E, Traue D and Spielmann H. (2005). Skin irritation *in vitro*: EpiDerm test protocol developed and optimised for an ECVAM validation study on skin irritation testing of chemicals. 5<sup>th</sup> World Congress on Alternatives & Animal Use in the Life sciences. Session 5.3: Progress and Needs for Developing and Validating Alternatives for Dermal Toxicity Testing. August 21–25, Berlin, Germany.  
Abstract In: ALTEX 22, Special Issue 2005, p. 156.
6. **Kand'árová H.**, Liebsch M., Spielmann H., Genschow E., Schmidt E., Guest R., Whittingham A., Warren N., Gamer A., Remmele M., Kaufmann T., Wittmer E., De Wever B. (2005). Validation study with the SkinEthic reconstituted human epidermis for skin corrosion testing according to OECD Test Guideline 431. 46. Frühjahrstagung der DGPT. March 15-17, Mainz, Germany.  
Abstract In: Naunyn - Schmiedeberg's Archives of Pharmacology, Supplement 1 to Volume 371, February 2005, No. 519, p.124.

7. **Kandárová H.**, Liebsch M. and Spielmann H. (2005). *In vitro* skin irritation testing: development and evaluation of a robust, common protocol for 3D reconstructed skin models. Skin *In vitro* 2005 - Cell Systems workshop. January 27<sup>th</sup>, Cologne, Germany.  
Abstract In: Proceedings of the Cell Systems Workshop - Skin *in vitro* 2005. Maritim Hotel, Cologne, p. 8.
8. **Kandárová H.**, Liebsch M., Gerner I., Schmidt E., Genschow E., Traue D. and Spielmann H. (2004). Optimised EpiDerm™ test protocol for the assessment of skin irritation. INVITOX 2004: XIII<sup>th</sup> Workshop of the European Society *In vitro* in Cooperation with SSCT. September 8-11, Zegrze, Poland.  
Abstract In: Abstract book of the INVITOX 2004: XIII<sup>th</sup> Workshop of the European Society *In vitro* in Cooperation with SSCT. Zegrze, Poland, p. 38.
9. **Kandárová H.**, Liebsch M., Spielmann H., Tornier C. and De Wever B. (2004). Assessment of the Skin Irritation Potential of Chemicals using the SkinEthic Reconstituted Human Epidermis. 3rd International SkinEthic Workshop. October 7-8, Nice, France.  
Presentation In: Workshop book from 3<sup>rd</sup> International Workshop on *In vitro* Reconstituted Human Tissue Models in Applied Pharmacology and Toxicology Testing. p. 171-183.
10. **Kandárová H.**, Liebsch M., Spielmann H., Genschow E., Schmidt E., Guest R., Whittingham A., Warren N., Gamer A., Remmele M., Kaufmann T., Wittmer E. and De Wever B. (2004). Outcome of the validation study with the SkinEthic reconstituted human epidermis for skin corrosion testing according to OECD Test Guideline 431. October 7-8, Nice, France.  
Presentation In: Workshop book from 3<sup>rd</sup> International Workshop on *In vitro* Reconstituted Human Tissue Models in Applied Pharmacology and Toxicology Testing. p. 153-165.
11. **Kandárová H.**, Liebsch M. and Spielmann H. (2003). Assessment of the Skin Corrosion Potential of Chemicals using SkinEthic Reconstituted Human Epidermis. October 16-17, Nice France.  
Presentation In: Workshop book from 3<sup>rd</sup> International Workshop on *In vitro* Reconstituted Human Tissue Models in Applied Pharmacology and Toxicology Testing. p. 233-249.
12. **Kandárová H.**, Liebsch M. and Spielmann H. (2003). Untersuchungen zur Entwicklung eines gemeinsamen Testprotokolls für die Hautmodelle EPISKIN und EpiDerm im Rahmen der geplanten ECVAM Validierung von *in vitro* Hautirritationstests. 11<sup>th</sup> Congress on Alternatives to Animal Testing. September 19-21, Linz, Austria.  
Abstract In: ALTEX 20, 3/03, p. 179.