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Workshop Report

Regulatory assessment of *in vitro* skin corrosion and irritation data within the European framework: Workshop recommendations

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Abbreviations: A.I.S.E, International Association for Soaps, Detergents and Maintenance products; BfR, German Federal Institute For Risk Assessment; Cat, category; ECHA, European Chemicals Agency; ECVAM, European Centre for the Validation of Alternative Methods; ESAC, ECVAM Scientific Advisory Committee; EU, European Union; EU CLP, EU Classification, Labelling and Packaging Regulation (EC, 2008a); EU DPD, EU Dangerous Preparation Directive 1999/45/EC (EC, 1999); EU DSD, EU Dangerous Substance Directive classification 67/548/EEC; FOPH, Swiss Federal Office of Public Health; GHS, Globally Harmonised System for Classification and Labelling (UN, 2009); GLP, Good Laboratory Practices; ITS, integrated testing strategies; IUCLID, International Uniform ChemicaL Information Database; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide, Thiazolyl blue, EINECS number 206-069-5, CAS number 298-93-1; OD, Optical Density; OECD, Organisation for Economic Co-operation and Development; OHP, Orange House Partnership; PG, packaging group; (Q)SAR, (Quantitative) Structure–Activity Relationship; REACH, EU Regulation 1907/2006 on the Registration, Evaluation, Authorisation and restriction of CHemicals (EC, 2006); RhE, Reconstructed human Epidermis; SD, Standard Deviation; SeCAM, Services & Consultation on Alternative Methods; SIT, Skin Irritation Test; SOP, Standard Operating Procedures; TER, Transcutaneous Electrical Resistance test; TG, Testing Guidelines; UN, United Nations; US, United States of America; ZEBET, Centre for Alternative Methods to Animal Experiments, BfR.

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ABSTRACT

Validated *in vitro* methods for skin corrosion and irritation were adopted by the OECD and by the European Union during the last decade. In the EU, Switzerland and countries adopting the EU legislation, these assays may allow the full replacement of animal testing for identifying and classifying compounds as skin corrosives, skin irritants, and non irritants. In order to develop harmonised recommendations on the use of *in vitro* data for regulatory assessment purposes within the European framework, a workshop was organized by the Swiss Federal Office of Public Health together with ECVAM and the BfR. It comprised stakeholders from various European countries involved in the process from *in vitro* testing to the regulatory assessment of *in vitro* data. Discussions addressed the following questions: (1) the information requirements considered useful for regulatory assessment; (2) the applicability of *in vitro* skin corrosion data to assign the corrosive subcategories as implemented by the EU Classification, Labelling and Packaging Regulation; (3) the applicability of testing strategies for determining skin corrosion and irritation hazards; and (4) the applicability of the adopted *in vitro* assays to test mixtures, preparations and dilutions. Overall, a number of agreements and recommendations were achieved in order to clarify and facilitate the assessment and use of *in vitro* data from regulatory accepted methods, and ultimately help regulators and scientists facing with the new *in vitro* approaches to evaluate skin irritation and corrosion hazards and risks without animal data.

1. Background

European legislations strongly call for the use of alternative test methods. The seventh amendment to the Cosmetics Directive (EU Directive 2003/15/EC; EC, 2003) taken up by the new Cosmetics Regulation 1223/2009 (EC, 2009a), prohibited testing of finished cosmetic products on animals since 2004 and the testing of cosmetic ingredients in animals since 2009. This animal testing ban was reinforced by a marketing ban of cosmetic finished products tested on animals from 2004, and of cosmetic products containing ingredients tested on animals from 2009 except for more complex toxicological effects, such as repeated-dose toxicity (including skin sensitization and carcinogenicity), toxicokinetics and reproductive toxicity, for which the marketing ban shall apply from 2013. Furthermore, the EU Regulation for the Registration, Evaluation, Authorisation and restriction of CHemicals (REACH; EC, 2006) requires *in vitro* testing for eye and skin irritation for substances marketed in volumes between 1 and 10 tonnes per year. It also lays down general rules for adaptation to the standard regimen, which comprise the use of validated *in vitro* assays as full or partial replacements of the animal test (EC, 2006; ECHA, 2008a, 2010a). Finally, the new Directive 2010/63/EU on the protection of animals used for scientific purposes (EC, 2010a) establishes that testing on animals should not be carried out "*if another method or testing strategy for obtaining the result sought, not entailing the use of a live animal is recognised under the legislation of the Union*".

Based on such regulatory requests and on the advancements in the validation of alternative test methods, today in the European Union, Switzerland and countries that adopt the EU legislation, regulatory accepted *in vitro* test methods are available that allow for the full replacement of the animal test for skin corrosion and irritation. Two detailed reviews on the application of validated alternative methods available for the regulatory assessment of chemical safety related to human skin and eye corrosion/irritation were carried out following a request from the Swiss Federal Office for Public Health (Eskes, 2010a,b). Each document included a review on the current status of alternative methods, how they relate to the *in vivo* assay, future prospects and a list of elements for consideration when evaluating data generated by the adopted *in vitro* test methods. In particular, a number of open questions were identified regarding the regulatory assessment of data generated from the adopted *in vitro* test methods for skin and eye irritation and corrosion. In the case of skin irritation and corrosion, these included (Eskes, 2010a):

1. the usefulness of having harmonized recommendations for the regulatory assessment of *in vitro* data on skin irritation/corrosion within the European framework;
2. the needs and possibilities of using the adopted *in vitro* assays to classify substances according to the skin corrosion sub-categories 1A, 1B and 1C as introduced by the EU Classification, Labelling and Packaging (CLP) Regulation (EC, 2008a);
3. the need to identify and/or assess suitable integrated testing strategies for skin corrosion and irritation, and the usefulness of maintaining the *in vivo* test as a last step in the strategy;
4. the applicability of the validated and adopted *in vitro* assays for skin irritation and corrosion to test mixtures, preparations and dilutions.

In order to address the above-mentioned issues, the Swiss Federal Office of Public Health (FOPH) and Services & Consultation on Alternative Methods (SeCAM) organised together with the European Centre for the Validation of Alternative Methods (ECVAM), the German Federal Institute For Risk Assessment (BfR), and the Orange House Partnership (OHP) the Workshop entitled "Regulatory assessment of *in vitro* data on skin corrosion and irritation within the European framework". The main objective was to develop harmonised recommendations for the regulatory assessment of *in vitro* data on skin corrosion and irritation within the European framework. The workshop took place on the 14 and 15 September 2010 in Bern, Switzerland, and comprised 38 participants from various European countries representing major stakeholders involved in the process from testing to the assessment of *in vitro* data, including regulatory agencies, European Commission, OECD, various industry sectors, contract test laboratories, European associations and scientists with expertise on skin irritation and corrosion *in vitro* methods.

The present manuscript addresses the current status of adopted *in vitro* test methods for skin corrosion and irritation, their