Alternatives to the Use of Animals in Safety Testing as Required by the EU-Cosmetics Directive 2009

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Summary
Ingredients of cosmetic products are no longer allowed to be tested by animal experimentation (EU-Cosmetics Directive 76/768 EEC). For several toxicological endpoints this testing ban applies since March 11, 2009, while repeated dose toxicity tests and the test on skin sensitisation will follow on March 11, 2013. All currently available alternatives meeting the requirements of the first deadline are compiled in the following.

Keywords: Cosmetics Directive, toxicity testing, animal experiments, alternatives, OECD guidelines

1 Establishing alternative methods

Every new toxicological test method must pass through a defined procedure of validation and regulatory acceptance. This worldwide agreed procedure is modular, which allows a flexible application of only those modules that are necessary for a final implementation of a certain new method. As noted in Table 1, a formal validation process aims predominantly at defining the relevance and reliability of a new testing method. This process is usually completed by a scientific review and a statement of the ECVAM Scientific Advisory Committee (ESAC). Without a positive ESAC statement, no test may be designated as "validated". However, a test method labelled as validated is not automatically applicable for regulatory purposes. Acceptance for regulatory use can be achieved only by standardisation and implementation into a test guideline, e.g. at the OECD level. Although the process of development, validation and implementation of alternative methods is extremely time consuming, the work of several institutions worldwide has been very successful, and the German ZEBET has played an important role in this process over the last 20 years. A list of alternatives to the use of animals in safety testing, which covers the requirements of the 7th amendment of the EU Cosmetics Directive, which entered into force on March 11, 2009, is presented.

Tab. 1: Steps towards a new test method according to a harmonised OECD validation concept (1996)

	Period (approx.)
5. Regulatory implementation • Establishment of a directive (e.g. OECD)	10 years
4. Validation ESAC statement • Peer review • Relevance und reliability • Ring trial	3 years
3. Pre-Validation • Inter-laboratory comparison • Improvement of the test protocol • Improvement of the prediction model	2 years
2. Test development • Prediction model • Test protocol	5 years
1. Basic research • Applicability domain • Scientific basis	3 years

2 Available alternative methods

Dermal absorption

+ *In Vitro* Test for Percutaneous Absorption
  OECD Test Guideline 428, accepted on April 13, 2004

No animals are necessary to test the uptake of chemical substances via skin.

Acute oral toxicity

- Fixed Dose Procedure (FDP)
  OECD Test Guideline 420, accepted on December 17, 2001
- Acute Toxic Class Method (ATC)

Invited paper, reviewed for publication 16th April 2009. The author is a senior scientist at the Bundesinstitut für Risikobewertung (BfR) in Berlin, Germany. The conclusions in the paper are not necessarily in line with the official BfR position.

OECD Test Guideline 423, accepted on December 17, 2001
- Up-and-Down Procedure (UDP)
  OECD Test Guideline 425, accepted on October 03, 2008
- – Deletion of the Acute Oral Toxicity Test, Lethal Dose (LD_{50})
  OECD Test Guideline 401, deleted in 2001

Three alternatives to the classical LD_{50} test reduce the animal numbers markedly, but still require animals. On the other hand the LD_{50} test was replaced completely and could be deleted from the OECD guidelines for testing of chemicals.

Skin corrosion

+ EpiSkin™, Human Skin Model
  OECD Test Guideline 431, accepted on April 13, 2004
+ EpiDerm™, Human Skin Model
  OECD Test Guideline 431, accepted on April 13, 2004
- Rat Transcutaneous Electrical Resistance (TER)
  OECD Test Guideline 430, accepted on April 13, 2004

While the TER-test only reduces the number of animals, artificial models of human skin, EpiDerm and EpiSkin, replace animal tests on skin corrosion.

Skin irritation

+ EpiSkin™, Human Skin Model
  OECD Test Guideline, acceptance expected in 2009
+ EpiDerm™, Human Skin Model
  OECD Test Guideline, acceptance expected in 2009

EpiDerm and EpiSkin are most promising to replace animal testing on skin irritation as well. Both tests are already part of the EU collection of test guidelines and will be standardised by the OECD this year.

Eye corrosion

+ Isolated Chicken Eye (ICE)
  OECD Test Guideline in preparation
+ Bovine Corneal Opacity and Permeability (BCOP)
  OECD Test Guideline in preparation

The OECD has received 2 draft guidelines (ICE and BCOP), which will be discussed in expert groups.

Eye irritation

All existing alternative approaches are far from being validated or even standardised. Thus a test guideline is not expected soon. However, at least strong eye irritancy effects can likely be detected by ICE or BCOP. This will be a matter of discussion in the eye corrosion expert group.

Phototoxicity

+ 3T3 Neutral Red Uptake (NRU) Phototoxicity Test
  OECD Test Guideline 432, accepted on April 13, 2004

An animal-free method is available for testing of phototoxicity.

Mutagenicity

- Bacterial Reverse Mutation Test
  OECD Test Guideline 471, accepted on July 21, 1997
- *In Vitro* Mammalian Chromosomal Aberration Test
  OECD Test Guideline 473, accepted on July 21, 1997
- *In Vitro* Mammalian Cell Gene Mutation Test
  OECD Test Guideline 476, accepted on July 21, 1997
- *In Vitro* Sister Chromatid Exchange Test
  OECD Test Guideline 479, accepted on October 23, 1986
- *Saccharomyces Cerevisiae* Gene Mutation Assay
  OECD Test Guideline 480, accepted on October 23, 1986
- *Saccharomyces Cerevisiae* Mitotic Recombination Assay
  OECD Test Guideline 481, accepted on October 23, 1986
- *In Vitro* Unscheduled DNA Synthesis Test
  OECD Test Guideline 482, accepted on October 23, 1986
- *In Vitro* Micronucleus Test
  OECD Test Guideline 487, acceptance expected 2009

Several *in vitro* methods to detect different kinds of mutagenic effects, like gene mutations, chromosome mutations or genome mutations, have been accepted as guideline tests during the last decades. Unfortunately, only clear negative results are acceptable, excluding a mutagenic potential with sufficient certainty. However, the high rate of false positive results necessarily leads to follow up *in vivo* tests. Therefore, the alternatives in the field of mutagenicity testing are as yet only able to reduce the animal numbers but not replace *in vivo* tests completely.

Tab. 2: Alternatives to animal testing of different toxicolocal endpoints

Toxicological endpoint	Alternatives to replace animal tests	Alternatives to reduce animal numbers
Dermal absorption	**OECD 428**	
Acute oral toxicity		OECD 420 / 423 / 425
Skin corrosion	**OECD 431**	**OECD 430**
Skin irritation	**OECD expected soon**	
Eye corrosion	**OECD expected soon**	
Eye irritation		
Phototoxicity	**OECD 432**	
Mutagenicity		OECD 487 etc

bold = OECD-replacement methods available or expected soon
standard = OECD-reduction methods available, replacement methods or strategies in preparation
italic = no validated or standardised alternative methods available

3 Conclusion

Alternative methods to animal experiments are described in Annex IX of the EU Cosmetics Directive. This Annex should be updated as follows:
Annex IX
This Annex lists the alternative methods accepted worldwide as OECD guidelines, which are, therefore, available to meet the requirements of this Directive. As animal testing may not be replaced completely by an alternative method, it should be mentioned in Annex IX whether the alternative method fully or partially replaces animal testing (Tab. 2).

Such an Annex would keep the producers of cosmetic ingredients informed on the current status of alternative methods, especially which already meet the requirements of the Directive and which do not. Consequently, short term activities of institutions like ZEBET should focus on the following endpoints:

1. Testing of acute toxicity: a further reduction of the animal numbers or even a replacement of the animal tests appears to be possible by introducing the so-called Halle-Register.
2. Since a battery of *in vitro* methods has been available for many years, the creation of a new testing strategy combining these could make mutagenicity testing possible without animals. This is also the task of a working group of the German section of the European Environment Mutagen Society (EEMS).

It is expected that both endpoints can be ascertained without using animals in the near future!

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Annex

For additional information, parts of the articles 4a and 9 as well as the Annex IX of the EU Cosmetics Directive are cited in their present form:

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Council Directive of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products (76/768/EEC)

Article 4a

1. Without prejudice to the general obligations deriving from Article 2, Member States shall prohibit:
(a) the marketing of cosmetic products where the final formulation, in order to meet the requirements of this Directive, has been the subject of animal testing using a method other than an alternative method after such alternative method has been validated and adopted at Community level with due regard to the development of validation within the OECD;
(b) the marketing of cosmetic products containing ingredients or combinations of ingredients which, in order to meet the requirements of this Directive, have been the subject of animal testing using a method other than an alternative method after such alternative method has been validated and adopted at Community level with
due regard to the development of validation within the OECD;
(c) the performance on their territory of animal testing of finished cosmetic products in order to meet the requirements of this Directive;
(d) the performance on their territory of animal testing of ingredients or combinations of ingredients in order to meet the requirements of this Directive, no later than the date on which such tests are required to be replaced by one or more validated alternative methods listed in Annex V to Council Directive 67/548/EEC of 27 June 1967 on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances or in Annex IX to this Directive.

No later than 11 September 2004 the Commission shall, in accordance with the procedure referred to in Article 10(2) and after consultation of the Scientific Committee on Cosmetic Products and Non-Food Products intended for consumers (SCCNFP) establish the contents of Annex IX.

2. The Commission, after consultation of the SCCNFP and of the European Centre for the Validation of Alternative Methods (ECVAM) and with due regard to the development of validation within the OECD, shall establish timetables for the implementation of the provisions under paragraph 1(a), (b) and (d), including deadlines for the phasing-out of the various tests. The timetables shall be made available to the public not later than 11 September 2004 and be sent to the European Parliament and the Council. The period for implementation shall be limited to a maximum of six years after the entry into force of Directive 2003/15/EC in relation to paragraph 1(a), (b) and (d).

2.1. In relation to the tests concerning repeated-dose toxicity, reproductive toxicity and toxicokinetics, for which there are no alternatives yet under consideration, the period for implementation of paragraph 1(a) and (b) shall be limited to a maximum of 10 years after the entry into force of Directive 2003/15/EC.

2.2. The Commission shall study possible technical difficulties in complying with the ban in relation to tests, in particular those concerning repeated-dose toxicity, reproductive toxicity

and toxicokinetics, for which there are no alternatives yet under consideration. Information about the provisional and final results of these studies should form part of the yearly reports presented pursuant to Article 9.

On the basis of these annual reports, the timetables established in accordance with paragraph 2 may be adapted within a maximum time limit of six years as referred to in paragraph 2 or 10 years as referred to in paragraph 2.1 and after consultation of the entities referred to in paragraph 2.

2.3. The Commission shall study progress and compliance with the deadlines as well as possible technical difficulties in complying with the ban. Information about the provisional and final results of the Commission studies should form part of the yearly reports presented pursuant to Article 9. If these studies conclude, at the latest two years prior to the end of the maximum period referred to in paragraph 2.1, that for technical reasons one or more tests referred to in paragraph 2.1 will not be developed and validated before the expiry of the period referred to in paragraph 2.1 it shall inform the European Parliament and the Council and shall put forward a legislative proposal in accordance with Article 251 of the Treaty.

2.4. In exceptional circumstances where serious concerns arise as regards the safety of an existing cosmetic ingredient a Member Statemay request the Commission to grant a derogation from paragraph 1. The request shall contain an evaluation of the situation and indicate the measures necessary. On this basis, the Commission may, after consultation of the SCCNFP and by means of a reasoned decision, authorise the derogation in accordance with the procedure referred to in Article 10(2). This authorisation shall lay down the conditions associated with this derogation in terms of specific objectives, duration and reporting of the results.

A derogation shall only be granted if:
(a) the ingredient is in wide use and cannot be replaced by another ingredient able to perform a similar function;
(b) the specific human health problem is substantiated and the need to conduct animal tests is justified and is supported by a detailed research Protocol proposed as the basis for the evaluation.

The decision on the authorisation, the conditions associated with it and the final result achieved shall be part of the annual report to be presented by the Commission in accordance with Article 9.

Article 9

Every year the Commission shall present a report to the European Parliament and the Council on:
(a) progress made in the development, validation and legal acceptance of alternative methods. The report shall contain precise data on the number and type of experiments relating to cosmetic products carried out on animals. The Member States shall be obliged to collect that information in addition to collecting statistics as laid down by Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes. The Commission shall in particular ensure the development, validation and legal acceptance of alternative test methods which do not use live animals;
(b) progress made by the Commission in its efforts to obtain acceptance by the OECD of alternative methods validated at Community level and recognition by non-member countries of the results of the safety tests carried out in the Community using alternative methods, in particular within the framework of cooperation agreements between the Community and these countries;
(c) the manner in which the specific needs of small and medium-sized enterprises have been taken into account.

ANNEX IX
List of validated alternative methods to animal testing

This Annex lists the alternative methods validated by the European Centre on Validation of Alternative Methods (ECVAM) of the Joint Research Centre available to meet the requirements of this Directive and which are not listed in Annex V to Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. As animal testing may not be replaced completely by an alternative method, it should be mentioned in Annex IX whether the alternative method fully or partially replaces animal testing.