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.

2 Available alternative methods

Dermal absorption

\[ \text{In Vitro Test for Percutaneous Absorption} \]
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

\[ \text{Fixed Dose Procedure (FDP)} \]
OECD Test Guideline 420, accepted on December 17, 2001
\[ \text{Acute Toxic Class Method (ATC)} \]
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 toxicological endpoints

<table>
<thead>
<tr>
<th>Toxicological endpoint</th>
<th>Alternatives to replace animal tests</th>
<th>Alternatives to reduce animal numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dermal absorption</td>
<td>OECD 428</td>
<td>OECD 420 / 423 / 425</td>
</tr>
<tr>
<td>Acute oral toxicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin corrosion</td>
<td>OECD 431</td>
<td>OECD 430</td>
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<tr>
<td>Skin irritation</td>
<td>OECD expected soon</td>
<td></td>
</tr>
<tr>
<td>Eye corrosion</td>
<td>OECD expected soon</td>
<td></td>
</tr>
<tr>
<td>Eye irritation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phototoxicity</td>
<td>OECD 432</td>
<td>OECD 487 etc</td>
</tr>
<tr>
<td>Mutagenicity</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**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:


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 validat-

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...
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.