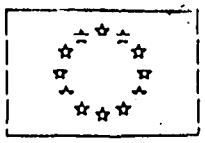

COMMISSION OF THE EUROPEAN COMMUNITIES

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REPORT FROM THE COMMISSION

DEVELOPMENT, VALIDATION AND LEGAL ACCEPTANCE OF ALTERNATIVE METHODS TO ANIMAL EXPERIMENTS IN THE FIELD OF COSMETIC PRODUCTS

1995

DEVELOPMENT, VALIDATION AND LEGAL ACCEPTANCE OF ALTERNATIVE METHODS TO ANIMAL EXPERIMENTS IN THE FIELD OF COSMETIC PRODUCTS

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SUMMARY

I INTRODUCTION

The Commission's obligations

Article 4(i) of Directive 76/768/EEC, as amended by Directive 93/35/EEC, specifies that *"Member States shall prohibit the marketing of cosmetic products containing ... ingredients or combinations of ingredients tested on animals after 1 January 1998..."*.

The ambiguity of the expression "combinations of ingredients" has been resolved as far as the Commission is concerned, which considers that the ban also covers finished cosmetic products.

The implementation of this ban is linked to the development of *"satisfactory methods to replace animal testing ... scientifically validated as offering an equivalent level of protection for the consumer"*.

With a view to providing regular information on how the situation is developing it is specified that *"[t]he Commission shall present an annual report to the European Parliament and the Council on progress in the development, validation and legal acceptance of alternative methods to those involving experiments on animals. That report shall contain precise data on the number and type of experiments related to cosmetic products carried out on animals"*.

1994 Report

A first report was presented in 1994. It described interesting but limited results, concluding that "animal models cannot be replaced, though they can contribute to reducing the number of animals used...". However, the outlook was reasonably optimistic as regards the validation of alternative methods for eye irritation, percutaneous absorption, phototoxicity/photoirritancy and basic mutagenicity tests. It was also underlined that given the current stage of the art it was unlikely that animal tests could be totally replaced in toxicity studies for evaluating the systemic risk, i.e. action via the circulatory system on the organism in its entirety after percutaneous absorption.

II 1995 REPORT

The objectives of the report and the notion of the potential risk for human health are the same as in the 1994 report.

The players involved in implementing this instrument are:

- the European Commission: DG XI, DG-JRC, DG XXIV, DG XII
- the European industry, represented by COLIPA (the European Cosmetic, Toiletry and Perfume Association)
- the American administration and the American Cosmetology Federation, CFTA
- the Japanese administration and the Japanese Cosmetology Federation, JCIA
- the OECD.

1 Clarification of the validation stages

Validation is the procedure via which the reliability and relevance of a procedure are established to a specific end. This process turned out to be more complex than envisaged and will take more time than foreseen. It has been necessary to define more precisely the validation stages of the new tests (development of a test and production of a protocol; prevalidation; validation proper; objective and independent evaluation of the study, progress towards legal acceptance).

2 The initiatives

The various players have taken numerous initiatives with a view to achieving the prescribed objectives.

- DG XI

  DG XI manages Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes and, to this end, is responsible for collecting statistics on the animals used. It also manages Directive 67/548/EEC on the classification and labelling of dangerous chemical substances, whose annexes define the test methods recognised by the EU.

- DG-JRC:
  - ECVAM (European Centre for the Validation of Alternative Methods) has already organised 14 seminars on the development and validation of alternative methods. Seven of these seminars concerned the safety of cosmetic products and the reports have been published. Two other reports on the prevalidation and validation procedures are also available.
  - ECB (European Chemicals Bureau) provides the technical and scientific support needed by DG XI.

- DG XXIV/CSC (Scientific Committee on Cosmetology):
  - DG XXIV plays a motor role in implementation, being responsible for the Cosmetic Products Directive, and must prepare for the Commission the draft measures postponing the deadline for the ban on animal tests, should this be necessary.
  - The CSC and its subcommittee more specifically responsible for alternative methods have evaluated various documents and dossiers presented by COLIPA. They have also adopted a document titled "The information required for the scientific evaluation of validation studies of

alternative tests with a view to their utilisation in evaluating the safety of cosmetic products"

- DG XII
  DG XII has funded various research programmes on the development and validation of alternative methods.

- COLIPA/SCAAT (Steering Committee on Alternatives to Animal Testing)
  Besides preparing dossiers and participating in numerous studies COLIPA organised an international scientific symposium on alternatives to animal tests in Brussels on 29 and 30 November 1995.

- USA
  - The American government is actively seeking international cooperation with a view to issuing recommendations.
  - The FDA (Food and Drug Administration), which is the agency responsible for the safety of cosmetic products, has expressed its misgivings as regards the ban on animal experiments in the implementation of Directive 93/35/EEC.

- OECD
  The OECD publishes guidelines on toxicity tests, approved by the Member States. A guideline on the in vitro control of percutaneous absorption is currently under discussion.

3 Statistics on animal experiments

The compilation of data specifically relating to cosmetic ingredients and products is difficult because the collection of data on the number of animals used for experimental and other scientific purposes in application of Directive 86/609/EEC is not foreseen on an annual basis.

- Eight Member States have declared that animal tests for finished cosmetic products have not been conducted on their territory (Italy, Greece, Belgium, Ireland, Sweden, Finland, Luxembourg, Germany).

- Six Member States have declared that animal tests for cosmetic ingredients have not been conducted on their territory (Greece, Netherlands, Ireland, Sweden, Finland, Luxembourg).

- Three Member States (Austria, France, United Kingdom) have communicated figures while specifying in some cases that these figures are unreliable and not interpretable.

It should also be noted that certain Member States that do not produce ingredients may use ingredients tested in other Member States or in third countries and that certain ingredients may have been tested for other purposes.

4 **Conclusions and perspectives** relating to the development of alternative methods for the various toxicity tests used in the evaluation of the safety of cosmetic ingredients and cosmetic products.

4-1 *Ingredients*

Phototoxicity/photoirritancy (skin reaction after epicutaneous application of a chemical substance in the presence of UV radiation). This concerns in particular UV filters.
The results of Phase II of the EU/COLIPA international validation study on in vitro photoirritancy tests have already allowed considerable progress to be made. A Phase III is envisaged. A supplementary study on UV filters will be necessary. Validation should be possible in the near future.

Percutaneous absorption
A new guideline on in vitro tests for percutaneous absorption could be approved by the Member States and the OECD in the near future.

Skin irritation
It is proposed to evaluate skin irritation of cosmetic ingredients on human volunteers. This could be done provided prior studies concerning the toxic risk furnish adequate guarantees.

Skin sensitisation
In vitro evaluation of skin sensitisation requires more in-depth research into the mechanistic basis of sensitisation before a validation study can be considered.

Eye irritation
The results of the validation studies of alternative methods to the Draize eye irritation tests have been disappointing (EU/Home Office study and COLIPA) and none of the tests used met the studies' objective. A more flexible approach to experimentation could be encouraged by accepting a combination of scientifically-validated methods and evaluating the toxicity of the substances under consideration by comparison with appropriate benchmark substances.

Photomutagenicity (all modifications of the information content of the genetic material arising in the presence of UV radiation)
Recommendations have been proposed on the criteria to be adopted with a view to defining the in vitro test protocols.

Skin corrosivity
Corrosive ingredients are not used in cosmetic products. However, the ECVAM validation study of in vitro tests on skin corrosivity could be used at the screening phase and provide essential information, before considering studies on human volunteers.

Mutagenicity

In vitro tests to evaluate the mutagenic potential of cosmetic ingredients are in current use. In vivo tests are carried out only when the in vitro tests results are unsatisfactory.

It will probably be impossible to totally replace in vivo tests to evaluate the systemic risk, such as tests of acute toxicity, subchronic toxicity, carcinogenicity, toxicity of reproduction and development, in the foreseeable future. However by refining the methods used it has already been possible to reduce substantially the number of animals used.

4-2 *Finished cosmetic products*

Eye tolerance and skin tolerance of finished cosmetic products can normally be evaluated in vitro provided the data relating to the ingredients' toxicity and their physico-chemical properties are known and provide the necessary guarantees.

Skin compatibility (absence of skin irritation) of finished cosmetic products can be evaluated in man provided this is done in the context of strictly controlled clinical studies.

It is important to emphasise that the use of human volunteers as a way to replace animal tests must be considered with the greatest prudence. Such studies, whose ethical dimension is obvious, should only be authorised after in vitro and in vivo tests have demonstrated that there is no risk of serious consequences.

In summary:

- The validation of alternative methods for evaluating percutaneous absorption and photoirritancy in the case of ingredients, and for evaluating eye tolerance and skin tolerance in the case of finished products, can be envisaged in the near future.
- Progress in alternative methods for evaluating eye irritation, skin irritation and skin sensitisation in relation to ingredients requires that additional studies first be carried out;
- The development of in vitro methods in the domains involving a systemic risk is not likely in the foreseeable future, even if it may be possible to reduce the number of animals used.

GLOSSARY

CAPT:	Committee of Adaptation to Technical Progress
CAAT:	Centre for Alternatives to Animal Testing (John Hopkins University. USA)
CFTA:	Cosmetic, Toiletry and Fragrance Association (USA)
COLIPA:	European Cosmetic, Toiletry and Perfumery Association
DEREK:	Deductive Estimation of Risk from Existing Knowledge
ECB:	European Chemicals Bureau
ECVAM:	European Centre for the Validation of Alternative Methods
ERGATT:	European Research Group for Alternatives in Toxicity Testing
ESAC:	ECVAM Scientific Advisory Committee
EU:	European Union
EU/HO international validation study:	European Union/Home Office international validation study
FDA:	Food and Drug Administration (USA)
FDCA:	Food, Drug and Cosmetics Act (USA)
FLT:	Fluorescein Leakage Test
FRAME:	Fund for the Replacement of Animals in Medical Experiments
GLP:	Good Laboratory Practice
ICCVAM:	US Inter-Agency Coordinating Committee for the Validation of Alternative Methods
IFAW:	International Fund for Animal Welfare
IRAG:	Interagency Regulatory Alternatives Group
IVTIP:	In Vitro Testing Industrial Platform
JCIA:	Japanese Cosmetics Industry Association
JRC:	Joint Research Centre
MMAS:	Modified Maximum Average Score
MHW:	Ministry for Health and Welfare (Japan)
NIEHS:	National Institute of Environmental Health Sciences (USA)
NRU:	Neutral Red Uptake Assay
NTP:	National Toxicology Program (USA)
PM:	Prediction Model
OECD:	Organisation for Economic Cooperation and Development
QSAR:	Quantitative Structure-Activity Relationships
RBC:	Red Blood Cell Haemolysis Test
SCAAT:	Steering Committee on Alternatives to Animal Testing
SCC:	Scientific Committee on Cosmetology
SIAT:	Sweizerisches Institut für Alternativen zu Tierversuchen
TEA:	Tissue Equivalent Assay
UV:	Ultra Violet
ZEBET:	Zentralstelle zur Erfassung und Bewertung von Ersatz und Ergänzungsmethoden zum Tierversuch im Bundesgesundheitsamt

CONTENTS

A. INTRODUCTION

This is the second of the reports which the Commission must present annually to the European Parliament and Council on progress in the development, validation and legal acceptance of methods which could replace animal experiments for cosmetics testing.

> *'The Commission shall present an annual report to the European Parliament and the Council on progress in the development, validation and legal acceptance of alternative methods to those involving experiments on animals. That 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 Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes. The Commission shall in particular ensure the development, validation and legal acceptance of experimental methods which do not use live animals.' (Article 4(i) of Directive 76/768/EEC, as modified by Directive 93/35/EEC).*

With the exception of the objectives, players and notion of potential risk for human health, which remain unchanged, the points developed in the first annual report (COM(94) 606) are reviewed:

Clarification of the stages in validation (B)
Initiatives in 1995 (C)
The state of play (D)
Statistics on animal experiments (E)
Conclusions (F)
The outlook (G)

B. CLARIFICATION OF THE STAGES IN VALIDATION

The Commission's main objective remains that of encouraging the development, validation and legal adoption of alternative methods which can offer the consumer a level of protection at least equivalent to that achieved by using animal studies.

Validation is the process by which the reliability and relevance of a procedure are established for a specific purpose.

The stages involved in achieving this objective are more numerous, and the process a more complex one, than initially foreseen.

The first Amden workshop (held in Switzerland in 1990) laid down the theoretical basis which is essential for the validation process, viz. intra-laboratory validation, inter-laboratory evaluation, the development of databases, and evaluation of the results.

Experience with validation studies conducted since 1990 has shown that it is necessary to

better define the various objectives of the validation studies and to integrate new stages into the process of evaluating new tests.

ECVAM (European Centre for the Validation of Alternative Methods), a unit of the Environment Institute of the Joint Research Centre (ISPRA), is at the hub of the discussions on validation, and major contributions have been made in the following fields:

* practical aspects of validation
* prevalidation scheme
* international discussions with ICCVAM (US Inter-Agency Coordinating Committee for the Validation of Alternative Methods) and the OECD (Organisation for Economic Cooperation and Development) on the harmonisation of validation and acceptance criteria.

Recommendations concerning the practical and logistical aspects of validating alternative tests were made at the second Amden Workshop (24-28 January 1994), organised jointly by ECVAM and ERGATT (the European Research Group for Alternatives in Toxicity Testing), and are set out in ECVAM workshop report No 5.

Validation of alternative methods used for different purposes

Validation of alternative tests can be conducted with four main types of objectives in mind. A distinction is made between:

1. Validation of alternative procedures for the use in non-regulatory studies.

2. Validation of alternative tests for inclusion as part of regulatory guidelines.

3. Validation of alternative tests to replace existing guidelines.

4. Validation of alternative tests which are designed to provide part of the information required by a regulatory guideline.

Stages in the evaluation of new tests

1. Development of a test and production of a protocol

The criteria taken into consideration in developing a test mainly comprise a description of the basis of the method, the definition of its scientific objective, the specification of its biological endpoint, and the expression and interpretation of the results, via a prediction model (PM).

The **prediction model** must enable the result obtained with an alternative method to be converted into a correct prediction of in vivo toxicity.

The prediction model is critical for the success of a validation programme. It enables a clearly defined hypothesis to be tested and an objective evaluation of test performance, and it serves as a guide for planning the validation study.

2. Prevalidation

Experience has shown that the outcome of large and expensive validation studies can be compromised if their managers do not insist that optimised test protocols and proof of their performance are submitted before the start of the formal validation study. The objectives of prevalidation, and a scheme of the prevalidation process, are described in the first report of the ECVAM Prevalidation Task Force.

The aims of prevalidation are:

to optimise and standardise the protocol
to evaluate the method's transferability.

The prevalidation exercise comprises three phases:

refinement of the protocol, involving collaboration between the laboratory which has developed the method and the laboratory designated to optimise the method
transfer of the protocol, involving collaboration between the first two laboratories and the laboratory designated for the transfer
a blind study, involving, as a minimum, participation of the three laboratories responsible for studying the protocol's performance.

3. Validation

The validation stage comprises a formal inter-laboratory study.

The main stages are:

study design
selection of the tests
selection of the laboratories
selection and distribution of the test materials
collection and analysis of the results
evaluation of the study.

4. Independent assessment of the conduct and outcome of the study.

This should include an objective evaluation of the value of the scientifically validated tests by comparison with other tests, taking into account the validation objectives.

5. Progression towards legal acceptance

It is essential that any new method that is considered to be adequately validated as a replacement for an existing method receives as widespread international recognition as possible. For example, the OECD test guidelines are particularly important in this respect, since they are used for tests conducted in member countries in Europe and North America, and in Japan, Australia and New Zealand. Furthermore, under the OECD Mutual Acceptance of Data Agreement, member countries have agreed to accept data from tests performed according to OECD test guidelines, provided that the principles of Good Laboratory Practice (GLP) are

observed. The OECD has established a procedure for updating test guidelines and for the introduction of new test methods.

C. INITIATIVES IN 1995

THE PLAYERS
For the record, the players involved in encouraging the research, development and validation of alternative methods are:

1. EUROPEAN UNION

The European Commission

Several Commission services are involved in issues relating to animal experimentation and the development and evaluation of alternative methods.

DG XI, Environment, Nuclear Safety and Civil Protection manages and is responsible for Directive 86/609/EEC, on the protection of animals used for experimental or other scientific purposes, and for Directive 67/548/EEC, on the classification, packaging and labelling of dangerous substances.

DG- *JRC, Joint Research Centre*

ECVAM, European Centre for the Validation of Alternative Methods
ESAC, ECVAM Scientific Advisory Committee
ECB, European Chemicals Bureau

DG XXIV: Consumer Policy

Unit A3 - Products, manages Directive 76/768/EEC (the Cosmetics Directive)
SCC: the Scientific Committee on Cosmetology, a Commission advisory committee

DG XII, Science, Research and Development funds research programmes:

European industry

COLIPA - Comité de Liaison Européen des Industrie Cosmétiques, des Produits de Toilette et de la Parfumerie, has set up SCAAT.

SCAAT - Steering Committee on Alternatives to Animal Testing; supervises COLIPA's validation studies and coordinates the validation efforts of the cosmetics industry on a worldwide basis.

2. USA

CTFA - Cosmetic, Toiletry and Fragrance Association

3. JAPAN

JCIA - Japanese Cosmetics Industry Association

4. ORGANISATION FOR ECONOMIC COOPERATION AND DEVELOPMENT (OECD)

The OECD publishes test guidelines, as approved by consensus among its member countries. These guidelines are almost identical to the Annex V test methods of the EU, and, in association with the principle of Mutual Acceptance of Data, provide an opportunity for international harmonisation and for a rational approach to the application of the Three Rs.

THE INITIATIVES

1. EUROPEAN UNION

DG XI

DG XI, Environment, Nuclear Safety and Civil Protection has reached agreement with the Member States on the best approach to be adopted in gathering, on a bi-annual basis, the statistical data required under Articles 13 and 26 of Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes. The table which the Member States must complete includes a figure for the total number of animals used for cosmetic testing. DG XI is also responsible for Directive 67/548/EEC and for the EU test methods, as detailed in Commission Directive 87/302/EEC and in the Annex to Commission Directive 92/69/EEC (Part B - Methods for the Determination of Toxicity).

DG XI supported the EU/HO international validation study on alternatives to the Draize eye irritation test, and also the EU/COLIPA international validation study on in vitro phototoxicity.

DG JRC

ECVAM (the European Centre for the Validation of Alternative Methods) is at the service of all the DGs concerned with the issues associated with alternative methods.

ECVAM was established by the European Commission in accordance with a Communication from the Commission to the Council and the European Parliament in October 1991, and in response to Article 23 of Directive 86/609/EEC, which stated that:

"The Commission and Member States should encourage research into the development and validation of alternative techniques which could provide the same level of information as that obtained in experiments using animals, but which involve fewer animals or which entail less painful procedures, and shall take such other steps as they consider appropriate to encourage research in this field".

ECVAM has been set up to:

1. Coordinate the validation of alternative test methods at the European Union level.
2. Act as a focal point for the exchange of information on the development of alternative test methods.
3. Establish, maintain and manage a database on alternative procedures.
4. Promote dialogue among legislators, industries, biomedical scientists, consumer organisations and animal welfare groups, with a view to the development, validation and international recognition of alternative test methods.

The ECVAM opening symposium organised at ISPRA on 18 October 1994 was devoted to discussion of the validation of replacement alternative methods. At this symposium, ECVAM's role in the development, validation and acceptance of alternative tests and test strategies, including those for cosmetics testing, was considered, and may be summarised as follows:

contribute to the development of guidelines via the validation process itself, since this is linked to the development and acceptance of pertinent and reliable tests for specific objectives;
identify priorities, taking into account the state of the art in specific fields;
ensure that the alternative tests relate to simple, distinct and well-defined biological outcomes whose mechanistic basis is sufficiently well known;
promote a multi-disciplinary approach with a view to developing integrated test strategies, incorporating quantitative structure-activity relationships (QSAR), biokinetics and cell and tissue culture methods;
address the difficulties resulting from the lack of test materials with associated high-quality in vivo data;
encourage the reevaluation of the existing guidelines and regulatory procedures;
achieve consensus by working with the appropriate government, industrial, academic and other bodies;
draw up criteria for validation, for independent evaluation of the results of validation studies, and for the legal acceptance of alternative tests and tests strategies, through active cooperation with such bodies as the OECD and ICCVAM (Inter-Agency Coordinating Committee for the Validation of Alternative Methods, USA).

To date, ECVAM has organised several workshops on the development and validation of alternative tests and has published the reports of 14 of these workshops. **ECVAM has given priority to alternatives for cosmetics testing.** The reports of direct relevance to evaluating the safety of cosmetics are:

report 2	In vitro phototoxicity testing (1994)
report 5	Practical aspects of the validation of toxicity test procedures (1995)
report 6	A prevalidation study on in vitro skin corrosivity testing (1995)

report 7	Development and validation of non-animal tests and testing strategies: the identification of a coordinated response to the challenge and the opportunity presented by the Sixth Amendment to the Cosmetic Directive (1995)
report 8	The integrated use of alternative approaches for predicting toxic hazard (1995)
report 11	The Three Rs: the way forward (1995)
report 13	Methods for assessing percutaneous absorption (published in 1996)

Two other reports were published in 1995 on:

the validation of alternative test methods (a joint statement by ECVAM and the ECB)
the first ECVAM Prevalidation Task Force Report.

ECVAM's priorities in the field of cosmetics testing for 1995-1998 include:

developmental toxicity
metabolism
corrosivity
phototoxicity
dermal penetration
ocular and dermal irritation
sensitisation

ECB (the European Chemicals Bureau) provides technical and scientific support for DGXI on the classification and labelling of dangerous substances, on notification of new substances, on existing chemicals, on export/import control of dangerous substances, and on testing methods (according to Annex V of Directive 67/548/EEC). An effective liaison is being developed between the ECB and ECVAM with respect to replacement alternative test methods, and modifications to animal procedures which reduce the numbers of animals required or lessen the suffering of any animals necessarily used in complying with regulations and guidelines.

DG XXIV / SCC

DG XXIV Consumer Policy, which manages the Cosmetic Directive (Directive 76/768/EEC), remains the driving force and has expanded its activities with a view to the fullest possible compliance with the provisions of Directive 93/35/EEC within the agreed time limits.

If alternative methods have not been scientifically validated and legally accepted, DG XXIV has been instructed to prepare for the Commission draft measures designed to extend the time limit for the ban on animal tests in accordance with the Committee for Adaptation to Technical Progress Procedure (Article 10, Directive 76/768/EEC).

This Committee (CATP) is made up of representatives of the Member States and is chaired by a Commission representative. The Chairman submits to the Committee the draft measures to be adopted.

The opinion is delivered by qualified majority.

In compliance with the new co-decision procedure, the European Parliament will be informed at the same time and under the same conditions of all draft measures submitted to the CATP.

> *If there has been insufficient progress in developing satisfactory methods to replace animal testing, and in particular in those cases where alternative methods of testing, despite all reasonable endeavours, have not been scientifically validated as offering an equivalent level of protection for the consumer, taking into account OECD toxicity test guidelines, the Commission shall, by 1 January 1997, submit draft measures to postpone the date of implementation of this provision, for a sufficient period, and in any case for no less than two years, in accordance with the procedure laid down in Article 10. Before submitting such measures, the Commission will consult the Scientific Committee on Cosmetology. (Article 4(i) of Directive 76/768/EEC, as modified by Directive 93/35/EEC).*

On several occasions DG XXIV has urged the Member States to meet their obligations regarding the collection of precise data on animal experiments concerning ingredients and finished cosmetic products. The statistical data to be collected under Directive 93/35/EEC with regard to cosmetic products go beyond the data which Member States must supply to comply with the requirements of Directive 86/609/EEC. The Member States have been requested to submit these more detailed data on an annual basis.

DG XXIV has liaised regularly with DG XI and with ECVAM, and has facilitated communication between all players.

SCC: the Scientific Committee on Cosmetology, a Commission advisory committee, has been requested to deliver a scientific opinion to DG XXIV on the applicability of validated alternative methods for evaluating the safety of cosmetic products for human use.

The SCC's subcommittee on alternative methods and guidelines organised two working meetings (2 February, 11 May) and two joint meetings with SCAAT/COLIPA (19 September, 1 December) during which the following actions were developed.

1. Evaluation of documents and dossiers submitted by COLIPA

In vitro photoirritation (EU/COLIPA)

An analysis of the data communicated by COLIPA - viz. the partial results published in the literature, in regard to the prevalidation phase, and partial information on phase 2 has not allowed the SCC to deliver a scientific opinion on the draft *as a whole.*

However, the SCC emphasises that the animal models are inadequate for predicting the phototoxic effects in man and **considers that a study of "in vitro" phototoxic**

potential is an important step in the process of evaluating the safety of cosmetic products containing UV filters.

In vitro percutaneous absorption (COLIPA)

After analysis of the documents presented by COLIPA, viz. guidelines for in vitro percutaneous absorption tests, standard protocols and a general overview of in vitro/in vivo correlations, the SCC recommends presenting the "in vitro" percutaneous absorption methodology in a standard manner and supplementing the methodology with intra- and inter-laboratory results obtained on suitably chosen test materials and vehicles.

Photomutagenicity (COLIPA)

The COLIPA recommendations in the final report transmitted to the SCC indicate the criteria to be used in defining the test protocols.

The SCC regrets that it cannot yet base the evaluation of the photomutagenic potential of UV filters on validated tests, despite the fact that it developed general criteria for realising photomutagenicity tests in 1990 (SPC/803/90).

2. Information required for the scientific evaluation of the validation studies of the alternative tests with a view to using them in evaluating the safety of cosmetic products

Taking into consideration the problems arising in connection with validation of the alternative methodologies, the SCC considered there was a need to prepare a document that would be of direct use for future validation studies.

The qualitative and quantitative aspects of the validation process are taken into account in this report. The criteria selected:

concern the *scientific validation* of the tests
make it possible to measure a degree of toxicity
are applicable to cosmetic ingredients
involve definition of the statistical analysis procedures

The need to provide evidence of protocol optimisation and to formalise the link between in vitro and in vivo data in the form of a mathematical relationship are highlighted.

DG XII

DG XII, Science, Research and Development funds research programmes, notably including the development and validation of alternative methods according to Decision N° 1110/94/EC of the European Parliament and the Council of 26 April 1994 concerning the Fourth Framework Programme for Research and Technology Development, which clearly set out instructions as follows: "Whenever possible, experimentation and testing on

animals should be replaced by in vitro or other methods...

Several programmes on the development and validation of in vitro tests have been supported by DG XII or are in progress. Some of the projects could be of use for the cosmetic sector:

BRIDGE - Final report (EUR 15777, published in 1995)
Development of a predictive in vitro test for the detection of sensitising compounds.

BIOTECH (1992-1994)
Some projects concern in vitro developmental toxicology.

BIOTECH (1994-1998)
The work programme foresees prenormative research on in vitro alternatives to animal experiments in pharmacotoxicology.

IVTIP (In Vitro Testing Industrial Platform): forum organised to improve contacts between technology producers and users. Two meetings were organised in 1995, on progress with the Fourth Framework Programme and on an overview of progress made with validation.

COLIPA/SCAAT

For each field of investigation SCAAT has established specific working parties made up of various experts to implement the validation work.

An international scientific symposium on alternatives to animal experiments was organised by COLIPA in Brussels on 29 and 30 November 1995, with representatives of industry, the scientific community, the Commission and the European Parliament, and animal protection societies.

The first session, devoted to VALIDATION, emphasised the critical role of prediction models and surveyed ongoing validation studies on photoirritation and eye irritation. The session ended with a round table on the perspectives of a regulatory approach.

The second session, devoted to DEVELOPMENT, included presentations on percutaneous absorption, skin compatibility and skin sensitisation.

The third session, devoted to SAFETY EVALUATION, addressed the initiatives taken by the SCC as well as the approaches of the British and German regulatory authorities. Emphasis was placed on the importance of providing scientific data in support of the cosmetics industry's "in-house" studies and test strategies for evaluating the safety of finished products.

The presentations were supplemented by a poster exhibition. In the context of this exhibition, a brochure setting out the approach of IFAW (the International Fund for Animal Welfare) was provided to the participants. As there is clearly no question of

replacing animal tests by a simple in vitro test, IFAW presents a phase-based strategy based essentially on alternatives which focus on the use of cells and tissues of human origin, excluding all in vivo animal experiments and recommending in vivo tests on volunteers.

The symposium highlighted the important role of COLIPA (European cosmetics industry) in undertaking research into the development and validation of alternative methods. The work being done by the major European cosmetic companies is very substantial when one considers the number of animals used in cosmetic experiments and the knowledge acquired greatly benefits other industries.

Moreover, the symposium provided the different players involved with precise scientific information and gave rise to an interesting exchange of views. A joint approach of industry and the Commission was called for, so that animal tests in the fields of skin compatibility, photoirritation and percutaneous absorption can rapidly be replaced.

2. USA

The US government is keen on **international cooperation** to recommend an approach and to define the criteria for the regulatory adoption of new alternative methods, clearly establishing the conditions under which these methods are used (biological outcomes, relevance for classes of substances, etc.). ICCVAM organised a workshop on the validation and regulatory acceptance of alternative toxicological testing methods, sponsored by the National Institute of Environmental Health Sciences (NIEHS) and the National Toxicology Program (NTP), in December 1995 in Arlington, Virginia. The purpose of the workshop was to discuss the draft ICCVAM report containing recommendations about criteria and processes for the validation and regulatory acceptance of new and revised toxicological testing methods.

The Food and Drug Administration (FDA) is the US agency responsible for cosmetics safety. The FDA has interactive relations with the public and private agencies involved in the development and validation of alternative tests, and focuses on methods which generate reliable scientific data.

The FDA has misgivings about the prohibition of animal experiments in the implementation of Directive 93/35/EEC. Certain cosmetics manufactured in the EU might not satisfy the safety requirements (for health) laid down by the US FDCA (Food, Drug and Cosmetics Act).

3. JAPAN

The Japanese Ministry for Health and Welfare (MHW) is responsible for testing and test guidelines in Japan. Japanese research groups are particularly involved in the validation of alternatives to the Draize eye test. The acceptance of the alternatives depends on their specific role in the evaluation of eye irritation (screening or replacement; individual test or batteries of tests). JCIA considers that it would be difficult to recommend for regulatory testing purposes for evaluating the safety of cosmetic products, a test or test battery whose predictivity was less than that of in vivo methods.

4. OECD

In October 1994, the 5th meeting of the National Coordinators of the OECD Test Guidelines Programme agreed that an attempt should be made to internationally harmonise the various published and advocated concepts for the validation of alternative test methods. In view of the international debate on the issue, it was considered timely for the OECD to step in and provide a platform for all parties involved, through which it might be possible to reach international consensus on validation and acceptance criteria. The National Coordinators emphasised that existing proposals should be used as the basis for an internationally acceptable approach, rather than the development of yet another concept. In this respect, the work of centres such as CAAT (Johns Hopkins University Center for Alternatives to Animal Testing) in the US, ECVAM in the EU, ERGATT, and various national centres and committees (e.g. FRAME in the UK, IRAG and ICCVAM in the US, NCA in the Netherlands, SIAT in Switzerland, and ZEBET in Germany) was well-recognised.

The National Coordinators agreed that an OECD Workshop would be the best approach, since such a meeting would offer ample opportunity for all parties having an interest in the subject to discuss the issue and to seek consensus. Furthermore, it was considered of crucial importance that member countries would include in their nominations individuals having responsibilities in the regulatory area. Sweden offered to host the workshop.

A Steering Committee was established in January 1995 to advise the OECD Secretariat on the scope and structure of the Workshop, and to assist in the development of the programme for the Workshop, which was scheduled for 22-24 January 1996, in Solna, Sweden. ECVAM and ZEBET were represented on this Steering Committee.

D. THE STATE OF PLAY

METHODS UNDER DEVELOPMENT AND VALIDATION

Skin sensitisation

Sensitisation is an area of considerable research and is extremely relevant to cosmetics safety. An interesting approach to the in vitro evaluation of skin sensitisation was presented at the symposium organised by COLIPA (29-30 November 1995). An ECVAM workshop on skin sensitisation was held during 1995, the report of which will be published in 1996.

The chemical structures and sensitising potentials of various compounds have been extensively examined and expert computer systems, such as DEREK (Deductive Estimation of Risk from Existing Knowledge), are being developed to identify structural alerts associated with skin sensitisation potential. A possible predictive approach could be based on measurement of the molecular signals induced in cell cultures by sensitising substances.

Notably in this domain it should be emphasised that prediction of sensitisation potential in humans remains a major problem because of the inter-individual variability in response.

Photomutagenicity Validation (COLIPA)

Since 1990, COLIPA has submitted dossiers on UV filters containing photomutagenicity data obtained from different types of tests.

COLIPA organised a ring test to define a suitable protocol by using two substances whose photomutagenic potentials were known, and which were activated by UV-A radiation and different bacterial strains. The results of this ring test have made it possible to draft recommendations on the criteria for selecting bacterial strains or cell cultures, the solar simulator, the technique of sample irradiation, UV radiation doses, doses of substances to be tested, and the use of positive controls. It is difficult to plan a validation study in the absence of *in vivo* reference data.

Percutaneous absorption

ECVAM, COLIPA and the OECD have collaborated to facilitate the international adoption of a testing guideline on in vitro percutaneous absorption.

Photoirritation/phototoxicity: Validation - phase II (EU/COLIPA)

ECVAM and DG XI are represented on the Management Team for the study, which is being coordinated by ZEBET, and is examining the possibility of a supplementary validation study designed to predict the photoirritant potential of UV filters.

In the first (prevalidation) phase of the study, analysis of the results obtained in a cell viability test made it possible to determine a photoirritancy factor for distinguishing photoirritant substances from non-photoirritant substances. The objective of the second (validation) phase, planned as a blind trial, was to determine whether a cell toxicity test and a test measuring cell damage (the NRU[1] and the combined test RBC[2] [photohaemolysis + haemoglobin oxidation]) could correctly predict the photoirritant potential of 32 chemical substances, which have been administered systemically or topically in man.

The preliminary results of phase II, which ended in 1995, are confined to the NRU test used in all the laboratories. The RBC protocol was used only in three laboratories. Supplementary statistical evaluations were carried out by an independent biostatistician and will be available during the first half of 1996.

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[1]NRU: neutral red uptake assay; cells are used as the target and cell toxicity is the biological endpoint

[2]RBC: red blood cell haemolysis test; used to evaluate damage to the cell membranes

Eye irritation: final report on the EC/HO study on alternatives to the Draize eye irritation test

The objective of this study was to determine whether nine alternative tests, alone or in combination, could replace the Draize test for severely irritant test materials or evaluate the irritant potential of chemical substances, with or without regard to the chemical class and over the entire range of measurable potentials.

A total of 60 chemical substances were analysed independently in 37 laboratories. The results were compared with the MMAS (modified maximum average score) obtained in the Draize test and submitted for statistical evaluation.

With the exception of predicting the irritant potential of surfactants, none of the nine tests achieved any of the envisaged objectives.

Many valuable lessons were learned during this study, which were taken into account in the planning of the COLIPA study. They also led to the ECVAM workshop on practical aspects of validation, and to the development of the ECVAM prevalidation scheme. The variablility of the in vivo data was identified as a major obstacle for the establishment of the relevance and reliability of the in vivo tests.

Eye irritation: Validation (COLIPA)

The validation exercise was conducted on 23 cosmetic ingredients (surfactants, alcohols, preservatives), of which 20 were the same as those tested in the EC/HO study, and on 32 cosmetic formulations (with a large range of physico-chemical forms). The test materials were tested in vivo in compliance with OECD guideline 405.

The specific objectives of this study were to determine whether the data obtained on the basis of 10 in vitro protocols currently used in the cosmetic industry (of which five had also been evaluated in the EC/HO study):

correlate acceptably with the MMAS (modified maximum average score);
correlate acceptably with the individual scores and recovery times, such as those described in OECD guideline 405;
correctly predict the eye irritation potential in the rabbit on the basis of algorithms for the alternative method.

For the first time in a validation study, prediction models (PMs) were laid down before the start of the study. They define precisely the biological outcomes obtained for each in vitro model and how to convert each in vitro biological outcome into a prediction of eye irritation potential. The PMs used in this study are based on historical data available for the ingredients and formulations of interest to the cosmetics industry, notably for substances with a low to moderate irritation potential.

The programme did not permit complete validation because of the small number of laboratories that participated in assays specific to the programme. None of the methods tested can yet validly replace the Draize eye irritation test.

The reliability of the PMs was evaluated by determining whether the inter-laboratory results were reproducible and whether the data were distributed within the prediction intervals of the predefined PMs.

The RBC test showed good inter-laboratory reproducibility and also satisfied the reliability criteria.

Three of the protocols tested conformed reasonably with their prediction models (FLT[3], RBC, TEA[4]), but supplementary studies are necessary to resolve several technical problems associated with the PMs before final conclusions about their performances can be drawn.

This validation study highlights the importance of the availability of good-quality in vivo data.

The in vivo data available are adequate for evaluating the risk. However, in order to compare the in vivo and in vitro data, the two sets of data should theoretically have as small a variability as possible, to permit precise statistical evaluation. Experience has shown that this is not always the case.

Examination of the results indicate that certain PMs could be refined and tested in a future study.

Skin corrosivity: Prevalidation (ECVAM)

Four skin corrosivity tests are being evaluated and optimised (TER - rat skin transcutaneous electrical resistance assay, CORROSITEX, Skin[2] and EPISKIN) with a view to planning a formal validation study for 1996.

Skin compatibility (COLIPA)

Skin compatibility is defined **as the absence of skin irritation in normal conditions** of use and in reasonably foreseeable conditions of improper use.

Seven COLIPA member companies evaluate the skin compatibility of their finished products in man under controlled conditions, because the irritation potentials of most cosmetic products are very low.

COLIPA has developed guidelines for evaluating the compatibility of finished cosmetic products on human skin:

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[3]FLT: fluorescein leakage test: measures the damage caused to a cell barrier.

[4]TEA: tissue equivalent assay; measures the time required to cause a 50% reduction in the viability of cells in reconstituted human skin

taking ethical requirements into account, studies on man must be conducted in accordance with the Helsinki Declaration (1964, 1989);
a prudent approach to tests, stage by stage, is essential;
prior knowledge of the composition and stability of the products tested, as well as prior evaluation of toxicity data on the product ingredients, is necessary.

Guidelines on the skin compatibility of cosmetic ingredients are currently being developed.

Human volunteer studies (ECVAM)

Human volunteer studies are often listed along with other replacement alternatives for animal tests. The ethical, legal, safety, logistic and scientific problems associated with such studies are being investigated by ECVAM, in collaboration with the University of Pavia, the University of Nottingham, and a number of cosmetic companies.

E. STATISTICS ON ANIMAL EXPERIMENTS

The collection of data on the number of animals used for experimental or other scientific purposes pursuant to Articles 13 and 26 of Directive 86/609/EEC is not planned on an annual basis.

This partly explains the difficulties encountered in collecting annual data specifically concerning cosmetic ingredients and products.

However, the Commission has on numerous occasions urged the Member States to obtain the statistics required, as is recalled in relation to the activities of DG XXIV and DG XI.

(1) Eight Member States have declared that animal tests for finished cosmetic products have not been carried out on their territory (Italy, Greece, Belgium, Ireland, Sweden, Finland, Luxembourg, Germany).

(2) Six Member States have declared that animal tests for cosmetic ingredients have not been carried out on their territory (Greece, Netherlands, Ireland, Sweden, Finland, Luxembourg).

(3) Only three Member States (Austria, France, United Kingdom) have communicated figures relating to the number of animals used, while pointing out however that these figures cannot be interpreted and that uncertainties remain as to their correspondence to reality. Under these circumstances this report cannot present comparative tables consisting of numerical data because such a table would give a false view of reality.

(4) As regards the declarations that tests have not been carried out, it should also be noted that certain Member States that do not produce ingredients may of course use ingredients tested in other Member States or in third countries and that certain ingredients used for cosmetic products may have been tested for other purposes.

The Commission strongly regrets that it does not have more precise data at this moment, but it depends on the Member States for the collection of these data.

F. CONCLUSIONS

Progress during 1995 with regard to alternative test methods and their validation can be summarised as follows:

1. The report on Phase II of the EU/COLIPA international validation study on in vitro tests for **phototoxicity** (photoirritancy) will be made available in 1996, but it is already clear that the validation of the NRU cell culture test has been very successful. Those involved in the study are confident that an acceptable OECD guideline can be drafted within the next two years, i.e. after the final report on the study has been published and minor refinements to protocols have been made.

2. Experience in European industry on the critical issue of **percutaneous absorption** has led to a concrete proposal for an OECD guideline for an in vitro test. ECVAM and COLIPA are working with the OECD and others, to facilitate the adoption of an in vitro test guideline by the OECD Member Countries.

3. The results of the EC/HO international validation study on alternatives for the **Draize eye irritancy test** were disappointing, in that none of the tests met the goals of the study. However, much of value was learned during this study, as a result of which the quality of future validation studies will be markedly improved.

4. The first phase of the COLIPA evaluation **of alternatives to the Draize eye test,** conducted on a more-limited range of materials than the EC/HO study, produced more-promising results, and showed the value of incorporating prediction models into test protocols.

5. As a result of a COLIPA study, recommendations have been drafted on criteria to be adopted in defining in vitro test protocols for **photomutagenicity.**

6. As a result of another COLIPA initiative, guidelines on the **skin compatibility** testing of cosmetic products and their ingredients in man have been developed.

7. The use of human volunteers as a replacement alternative deserves further scrutiny, but it must be emphasised that such studies should only be conducted after results from previous in vivo and/or in vitro studies permit avoidance of the risk of any serious consequences.

8. Validation has turned out to be even more difficult, more time-consuming and more costly than had been expected. Indeed, discussions are still going on about

what validation actually means and how the relevance and reliability of alternative methods can best be established. ECVAM is at the heart of these discussions, and particularly important contributions have been made in a number of ways.

9. The ECVAM prevalidation scheme, which is currently under international evaluation, is likely to improve the success rate of validation studies, and to reduce the time needed to conduct them, as well as their cost.

10. International discussions have taken place within Europe, and also with the USA and Japan, on the harmonisation of criteria for the validation and acceptance of replacement alternative test methods.

G. THE OUTLOOK

1. The emergence of more-realistic expectations of the validation process, and the success of international discussions on criteria for the validation and acceptance of replacement alternative methods can be expected to result in substantial progress in the future.

2. After the conclusion of a further small study on chemicals of particular concern to the SCC, it can be expected that a draft regulatory guideline for **in vitro phototoxicity** testing will be produced and proposed for acceptance by the regulatory authorities.

3. It is hoped that a new guideline on in vitro tests for **percutaneous absorption** will shortly be accepted by the EU Member States and the OECD Member Countries.

4. An acceleration of progress toward the development of standardised and appropriate in vitro methods for detecting **sensitisation potential** can be expected.

5. A reconsideration of what is expected of **eye irritancy tests** (in vivo and in vitro) will be essential. Meanwhile, a more flexible approach to testing could be encouraged, for example, by accepting the evaluation of the toxicities of new products and ingredients by comparison with knowledge of appropriate benchmark substances.

6. Greater use could be made of the fact that the **eye tolerance and skin tolerance of finished cosmetic products** can be evaluated in vitro, provided one crucial condition is met, viz. prior knowledge of the toxicity data pertaining to the ingredients and their physico-chemical properties. In addition, the skin **compatibility** of finished cosmetic products can be evaluated in the context of strictly controlled clinical studies.

7. A successful outcome can be expected for the ECVAM validation study on tests for **skin corrosivity,** which could then be used, for example, to provide part of the information considered essential before human volunteer studies can be considered permissible.

8. There will be greater emphasis on the integrated use of various different approaches (e.g. computer-based predictions, in vitro tests), and on hierarchical testing strategies, to reduce, refine and replace the use of animals for testing purposes.

The Sixth Amendment to Directive 76/768/EEC provides a unique opportunity for sensible progress toward the replacement of animal testing in the field of cosmetics. Such progress is being made, but its continuation will depend upon the maintenance of good relationships and effective collaborations between all the parties involved, i.e. the Commission and its advisory committees, the Member States, the cosmetics industry, and the regulatory authorities.