research and advise the European Commission on industrial requirements in the specific sector of interest.

Interactions with ECVAM have been ensured. Currently, two tests resulting from EU Biotechnology programme-funded projects have been transferred to ECVAM for further evaluation. These are:

- Predictive test for excitotoxicity
- *In vitro* nephrotoxicity testing and epithelial barrier functions

BIOMED

The following *in vitro* tests are currently funded in the area of "Pharmaceutical research" for a total of 3,820,000 ECUs:

- Molecular and cellular mechanisms of photoxicity: multidisciplinary strategy for predicting *in vitro* the phototoxic risk of new drugs
- Evaluation of oculotoxicity of drugs *in vitro*
- Validation of an *in vitro* assay to evaluate drug effects on synaptic functioning and plasticity
- Integration of *in vitro* approaches to predict drug metabolism and interactions in man in the development of pharmaceuticals

Lastly, funding for three RTD projects of the Standards, Measurements and Testing programme was maintained during 1997:

SMT4-CT96-2070	Development of standardised *in vitro* methodology for hepatic and renal toxicity testing
SMT4-CT97-2152	Measurements to assess the efficacy of sunscreens in industrial research
SMT4-CT97-2174	Testing and improvement of reconstituted skin kits in order to elaborate European standards

In 1997, DG XII prepared the proposal for the 5th Framework Programme for a decision in 1998 by the European Parliament and the Council. The first call for tender could be issued around the end of the year. Provision for the funding of research into alternative test methods will be examined in the drafting of the work programme of the theme "Quality of life and management of living resources" – Key action: "Cell factory."

DG XXIV

The management and work program of the scientific committees that advise the European Commission fall under the responsibility of DG XXIV. Consequently, DG XXIV plays a pivotal role in the provision of scientific advice to the Commission services. Most notably, in the field of cosmetics, DG XXIV manages the SCCNFP (formerly the SCC), which advises the Commission on the safety of cosmetic materials.

Since the publication of the previous Annual Report, the SCC adopted the second revision (XXIV/1878/97) of their "Notes of guidance for testing of cosmetic ingredients for their safety evaluation." Annex 8 of these guidelines, "The use of methods alternative to animal studies in the safety evaluation of cosmetic ingredients or combinations of ingredients," discusses the status of alternative methods. The SCC notes reviewed the

data obtained from the various validation studies that had recently been completed. The SCC review concluded that there were good prospects for alternative methods in the fields of skin irritation, percutaneous absorption and phototoxicity.

Through another initiative, the SCC discussed the ethical considerations of tests on humans.

OECD

In September 1996, the 7th National Co-ordinators meeting endorsed the recommended validation and acceptance criteria that followed the OECD Solna workshop, and agreed that a guidance document should be drafted on the validation of new test methods. Further, a document on the validation of test methods considered for adoption as OECD test guidelines has been prepared and will be discussed in an OECD meeting of the Chemicals Group and Management Committee in early 1998.

The OECD continued its lead in the discussions over percutaneous absorption and the development of test guidelines. The latest summary of the test guidelines was discussed at the 8th National Co-ordinators meeting and later at a meeting of the Steering Committee during 1997.

COLIPA

During 1997, COLIPA worked on guidelines for the training of SMEs in the use of alternative methods for safety assessment and in interpretation of their results. These guidelines and training programmes will be essential to the dissemination of information on alternative methods and to the acceptance of their use.

COLIPA have contributed to the exhaustive reviews of the databases on skin penetration and eye irritation that have been carried out in the past year, including data reviews from the respective validation studies. In the area of skin sensitisation, COLIPA have reviewed the current database on mechanism of action and will propose studies to begin in 1998.

Following the disappointing results of the studies to evaluate methods for eye irritation, COLIPA held an expert symposium in October 1997 to discuss the results obtained to date, analyse mechanistic theories and propose next steps. The report of the meeting will be available in early 1998 and will form the basis of the next round of studies on eye irritation.

During 1997, the SCAAT team of COLIPA reviewed and refocused its strategy on the development of alternative test methods, in order to ensure that appropriate resources were allocated to the priorities of the cosmetics industry. The SCAAT strategy is to make the cosmetics sector the "lead" industry in the development of alternative tests in those end-points that are relevant to cosmetics, namely:

- skin and eye irritation
- skin absorption
- skin sensitisation
- phototoxicity

Over the short to medium term, skin irritation, skin penetration, phototoxicity and skin corrosivity are priority topics and will be allocated 60 % of the organisation's resources. Eye irritation, skin sensitisation and photoallergy will developed over the medium to long term since they involve research into the mechanisms of action. SCAAT will not actively work on the development of tests for systemic, teratogenic or carcinogenic end-points, although initiatives in this field will be monitored in order to assess any application to the testing of cosmetic ingredients.

F. CURRENT STATUS OF DEVELOPMENT OF ALTERNATIVES

Phototoxicity

This year has seen the completion of the data analysis for the Phase II study on the validation of the 3T3 NRU PT test. This is a cytotoxicity test in which UV sensitivity of Balb/c 3T3 cells is determined by their capacity to take up the vital dye, neutral red. If the toxicity of a chemical increases significantly in the presence of UVA, the chemical can be considered as having a phototoxic potential.

The data analyses confirmed the reliability and relevance of the test for predicting phototoxic effects and identifying phototoxic chemicals. Two forms of the statistical model were applied to the data in test on 30 chemicals in 9 laboratories, namely the PIF (PhotoIrritation Factor and the MPE (Mean Photo Effect). On comparison of the *in vitro* classifications with the *in vivo* classifications assigned to the chemicals before the trial began, the following statistical parameters were found:

	PIF	MPE
Specificity	90 %	93%
Sensitivity	82 %	84 %
Positive predictivity	96 %	96 %
Negative predictivity	64 %	73 %
Accuracy	88 %	92 %

Consequently, the ECVAM Scientific Advisory Committee unanimously endorsed the following statement , agreeing with the conclusion of the Management Team, on the scientific validity of the 3T3 NRU PT test, at its 9th meeting on 1-2 October 1997:

> The results obtained with the 3T3 NRU PT test in the blind trial phase of the EU/COLIPA international validation study on *in vitro* tests for phototoxic potential were highly reproducible in all the nine laboratories that performed the test, and the correlations between the *in vitro* data and the *in vivo* data were very good. The Committee therefore agrees with the conclusion from this formal validation study that the 3T3 NRU PT test is a scientifically validated test which is ready to be considered for regulatory acceptance.

The study was conducted in accordance with general scientific principles laid down in various ECVAM/ERGATT workshops and the criteria recommended at an OECD workshop held in 1996.

Other methodologies included in this validation trial were:

- the SOLATEX PT test
- the histidine oxidation test
- a protein binding test
- the Skin^2 ZK 1350 PT test
- a complement PT test
- a human keratinocyte test
- the Red Blood Cell (RBC) phototoxicity test

Some of these methods showed potential as tools for mechanistic testing and may be investigated further. These results will be published in a later report, which will discuss the use of the methods for distinguishing between different types of phototoxicity, estimating phototoxic potencies and differentiating photoirritant and photosensitising chemicals. In addition, data from the application of the NRU PT test to human keratinocytes has been used in the development of a method to compare dose response curves.

The SCC requested a study to confirm whether the 3T3-NRU PT test is suitable to test UV-filters, as regulated in Annex VII of the EU Cosmetics Directive 76/768/EEC. This was considered an additional study rather than constituting a further part of the formal validation programme.

The biometrical analyses referred to in the 1996 Commission Report suggested that the predictivity of the model could be improved by using more detail from the area under the concentration response curves than by determination of the PIF (PhotoIrritation Factor). Therefore, both the MPE (Mean Photo Effect) and the PIF were measured in this study.

The study was funded by ECVAM and managed by the contractor, ZEBET. The experimental procedures were either performed by ZEBET or contracted out to third parties. The study involved the testing of 20 chemicals (10 photoirritants, 10 non-photoirritants of which 8 were UV filters), each of which was tested in two independent runs.

The study was completed in August 1997, at which time the data on the 20 coded chemicals were statistically analysed.

Preliminary review of the results of the study demonstrate that most chemicals were correctly classified by the 4 participating laboratories, whether PIF or MPE were used to assess phototoxic potential. This special study further supported the finding that the 3T3 NRU PT test accurately predicts the phototoxic potential of chemicals, as demonstrated in Phases I and II of the validation trial. A full report will be available in summer 1998.

Currently, there are no OECD test guidelines for *in vivo* photoirritancy tests as the Secretariat halted proposals for such guidelines, pending the results of the ECVAM validation study. A draft guideline incorporating the standard protocol for the 3T3 NRU PT test will be prepared during summer 1998, according to OECD guidance on the preparation of test guidelines.

Eye irritation

Over the last year the data analyses from a number of validation studies have become available. However, none of the protocols have demonstrated reliable prediction, under the conditions of the respective validation programme. A review of the data obtained from all available studies is currently being carried out by experts, for discussion at an ECVAM workshop in June 1998. A meeting was held in London in October 1997, in which a working group was established to review the databases of the following studies:

- the EU/HO study
- the COLIPA study
- the BgVV study
- the CTFA study
- the JCIA study
- the IRAG study

A report from this study will be available by June 1998.

Whilst no single method demonstrated accurate prediction of eye irritation, several cosmetics companies employ a hierarchical approach to *in vitro* testing for eye irritation, utilising a battery of tests. To investigate the efficacy of this approach, a thorough review of hierarchical testing strategies is being carried out under the auspices of ECVAM; a report will be available in May 1998, for discussion at the ECVAM workshop.

ECVAM are also leading a discussion on the concept of benchmarking ; comparison of the results obtained for new materials with a small number of test materials bearing high quality data on the toxic end-points of interest. The discussion is aimed at defining the concept, identification of its use, selection of materials and availability of data generated. A report will be available in June 1998.

COLIPA sponsored a workshop on the mechanisms of eye irritation in Brighton in October 1997. The workshop brought together thought-leading researchers in eye biology, ophthalmology and toxicology in order to identify a research programme aimed at developing mechanistically-based tests. This workshop was very successful and will enable COLIPA to define a medium to long term research programme. A report will be available in the summer of 1998.

Percutaneous absorption

As summarised in the last annual report, the regulatory acceptance of *in vitro* methods for the measurement of percutaneous absorption has been the topic of great discussion. The fact that an *in vivo* guideline has been concurrently proposed has further exacerbated the situation. Following the OECD/ECVAM meeting in Brussels in early 1996, an expert sub-group redrafted the *in vitro* test guideline for percutaneous absorption. This guideline was forwarded to the OECD co-ordinators in June 1996, accompanied by in-house data provided by COLIPA members, supporting the reproducibility and predictivity of the *in vitro* method.

The new guidelines were reviewed by OECD Member Countries, as well as the Commission services and industry. All Member Countries supported the guidelines with the exception of the US and Canada. The 7th National Co-ordinators meeting in

September 1996 agreed to a workshop to discuss data supporting the validity of the *in vitro* method.

From the discussions of the 8th National Co-ordinators meeting in April 1997, the OECD drafted a summary of the status of the test guidelines. The summary proposed an OECD workshop on the acceptability of the *in vitro* test guideline to be held in the US in October 1997. The aim of the workshop was to discuss the database supporting the validity of the *in vitro* method and to agree upon the approach to be taken for the further work considered to be necessary to enable the draft *in vitro* guideline to be acceptable to all parties.

The workshop did not take place, but instead a Steering Committee meeting was held in the US from 15-17 October 1997. Agreement was reached on the need for a guidance document to accompany the two test guidelines (*in vivo* and *in vitro*). Also, comparable "acceptance criteria" must be applied to both the *in vitro* and the *in vivo* methodologies.

Finally, it was proposed that the draft *in vitro* guideline should be rewritten, to include more details on:

a) the rationale for the methodology
b) the endpoints used, and their relevance and limitations
c) the protocol
d) sources of variability
e) identification of reference chemicals/standards (selected on the basis of appropriate physico-chemical properties).

The data submitted by COLIPA were reviewed and it was agreed that these data were valuable, although some clarifications and revisions were needed. Other possible data sources (chemicals and pesticides industries) were identified.

The OECD Secretariat have proposed a schedule which will enable a decision to be taken about the acceptabilities of both the *in vitro* and *in vivo* guidelines by September 1998. The OECD Secretariat is currently collating a huge literature database of references and abstracts on percutaneous absorption giving comparisons of the *in vitro* and *in vivo* methodologies. The draft *in vitro* guideline is currently under revision, and a draft of the guidance document is in preparation.

Skin irritancy

An ECVAM skin irritation task force was established in November 1996. The group was asked to prepare an ECVAM report on the current status of alternative test development and validation in the field of skin irritation/corrosion and to identify any appropriate non-animal tests for predicting human skin irritation that could be proposed as candidate methods for pre-validation/validation studies. Meetings were held in December 1996 and February 1997, a third meeting is planned for January 1998. The task force has discussed the testing strategy that was proposed at an OECD workshop on validation and regulatory acceptance (Solna, 1996) and has reviewed the use of structure-activity relationship models, pH and acid/alkali reserve measurements, *in vitro* test protocols and human patch testing.

An ECVAM workshop on skin irritation was held in November 1997 and discussed the use of human keratinocytes and human skin models in the prediction of skin irritation.

The group discussed the testing strategy that was proposed by an earlier OECD workshop on skin irritation and reviewed the use of structural-activity relationship models, pH and acid/alkali reserve measurements, *in vitro* test protocol and human patch testing. The recommendations of this workshop will be forwarded to the ECVAM taskforce.

The meeting of the ECVAM taskforce on skin irritation is planned for January 1998 and will involve consideration of proposals for prevalidation and validation studies to be conducted during 1998/1999.

As a result of the above-mentioned OECD workshop on skin irritation, a protocol for an ethically approved 4-hour human patch test is currently under consideration as an OECD guideline.

COLIPA published sets of guidelines on the assessment of skin compatibility of cosmetic finished products in man (during 1996) and on the assessment of skin tolerance of potentially irritant cosmetic ingredients in man (during 1997).

Skin corrosivity

This is an important first step in any safety testing programme as the results of such studies will often determine the design of the safety test battery. The results of a prevalidation study on three *in vitro* tests for skin corrosivity were published as an ECVAM workshop report in 1995. Subsequently, an ECVAM validation study was planned, which was completed during 1997.

The main objectives of the validation study on *in vitro* tests for skin corrosivity were to identify tests capable of discriminating corrosives from non-corrosives for selected types of chemicals and/or all chemicals, and to determine whether these tests could correctly identify known R35 (UN packing group I) and R34 (UN packing groups II & III) chemicals. Four methods were evaluated in this study:

- EPISKIN (a human skin model)
- Skin^2 ZK1350 corrosivity test
- Transcutaneous electrical resistance in rat skin
- CORROSITEX (a physicochemical method)

Each test was conducted in three independent laboratories and a total of 60 coded chemicals were tested (27 corrosive, 33 non-corrosive). The test chemicals fell under the following classification:

11 organic acids
10 organic bases
9 neutral organics
5 phenols
7 inorganic acids
4 inorganic bases
3 inorganic salts
8 electrophiles
3 surfactants

The data demonstrated that the EPISKIN and rat skin TER protocols met the criteria concerning acceptable prediction rates. These two tests were considered to be accurate in distinguishing between corrosive and non-corrosive chemicals. All tests showed

acceptable intralaboratory and interlaboratory reproducibility. Importantly, the EPISKIN test proved effective in identification of known R35/I and R34/II and III chemicals (EU risk phrases and UN packing groups).

However, the Skin^2 test kit was withdrawn from the market during the validation study. To avoid recommending the use for regulatory testing of a specific commercial human skin model (EPISKIN), a special further prevalidation study is being conducted using EpiDerm, with ECVAM support.

Two manuscripts on this study have been submitted for publication

Sensitisation

An ECVAM/COLIPA study investigating the induction of IL-1ß expression in cultures of human skin dendritic cells as an endpoint for screening for potential was initiated in late 1997 (Interleukin-1ß is a mediator of the induction phase of contact sensitisation and has been shown to be increased within minutes of application of allergens in some pre-clinical experiments).

In addition, an ex vivo explant method is under evaluation and a prevalidation study may be conducted during 1998 under contract with ECVAM.

Clinical testing of cosmetic products

In early 1997, the second revision of the SCC "Notes of guidance for testing of cosmetic ingredients for their safety evaluation" became available. In these guidance notes the SCC stated that for analysis of potential adverse effects of a cosmetic product or ingredient (in this sense adverse effects relate to skin irritation) observations in human subjects should be used if available.

In this respect, the SCC has worked to produce a document on the ethical considerations for clinical studies on cosmetic products in human subjects. The final version of these guidelines will be available in 1998. The SCC expressed the opinion that, for ethical and scientific reasons in general, human testing should only be carried out after careful scientific consideration. The two key factors in the use of clinical trials is that:

- Experiments on man cannot replace those on animals
- The purpose of experiments on humans is to confirm findings on safety and to verify the acceptability and efficacy of cosmetic products

However, the SCC document does outline the value of clinical tests on cosmetics in the evaluation of safety in use and performance of such products.

In a separate initiative, ECVAM produced a working paper on, "The use of human volunteers in cosmetics efficacy and safety testing." The paper described research projects that would study the procedures used in human volunteer testing, the measurements made and the equipment involved.

At the Department of Dermatology, University of Pavia (Italy), an ECVAM-funded study on the quantification of allergic and irritant reactions induced by cosmetics in human

volunteers has taken place, which has analysed the use of both visual and instrumental techniques. The results from this study will be available in a report to be published in early 1998. AN ECVAM workshop on the utilisation of non-invasive bioengineering techniques in human volunteer studies is planned for March 1998.

An ECVAM-supported study on the integrated use of human data with results obtained from other alternative methods was made at the University of Nottingham (UK), in the FRAME Alternatives Laboratory. The study is now complete and a report is expected in early 1998.

G. STATISTICS

According to Article 13 of Council Directive 86/609/EEC (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), Member States have the obligation to collect, and periodically make publicly available, the statistical information on the use of animals in respect of:

(a) the number and kinds of animals used in experiments;
(b) the number of animals, in selected categories, used in experiments
(c) the number of animals, in selected categories, used in experiments required by legislation.

These data shall be obtained on the basis of requests for authorisation and notifications received, and on the basis of the reports made, to the authorities of the Member State.

Further to the requirements laid down in this Directive, the basic Cosmetics Directive 76/768/EC, Article 4 (i), as amended by the 6th amendment Council Directive 93/35/EEC, requires the Member States to collect additional information on the number and type of experiments relating to cosmetic products carried out on animals.

The Commission has encountered difficulties in obtaining statistics relating to animal testing in cosmetics. In spite of communications from the Commission, reminding Member States of their obligations and urging them to supply such statistics, not all Member States have been in a position to disclose data. Those data that have been made available are given below:

1996

- Ireland and Finland and Portugal confirmed that cosmetic products had not been tested on animals in their territories during 1996.

- Germany confirmed that no finished cosmetic products had been tested on animals but could not comment on the testing of cosmetic ingredients.

Since publication of the 1996 Annual report, the following statistics have been made available:

- Greece reported that no animals had been used for testing of substances used or to be used mainly in cosmetics.

- Ireland indicated that no animals were used for the testing of cosmetics.

- Denmark submitted detailed statistics demonstrating that a total of 692 animals had been used to test products/substances used or intended to be used mainly as cosmetics or toiletries.

- Belgium reported that a total of 58 animals were used for testing materials used or destined to be used in cosmetic products during 1996.

- The UK reported animal procedures involving 101 cosmetics, of which 85 were tests upon ingredients and 16 were tests on finished products. A total of 2803 animals were used for testing purposes. Of these 2551 (91 %) were for ingredients and 252 (9 %) were for finished products.

At the end of 1997, a final letter was sent to the Permanent Representations of all Member States who had not supplied data on animal usage in cosmetic testing for the previous year. The communication stressed the importance of these data and urged the Member States to comply with the provisions of the EU Cosmetics Directive. Following this final communication, the Commission will initiate infringement measures against those Member States which failed to report data on animal usage in the testing of cosmetic products.

Despite both verbal and written requests to Member States in early 1998, reminding Member States of their obligations in this respect, no data were available pertaining to animal experimentation for cosmetics during 1997. The Commission will take appropriate measures to ensure that such data are made available at the earliest opportunity.

Some Member States have taken unilateral, national legislative measures regarding animal testing on cosmetics.

In the Netherlands, the Experiments on Animals Act entered into force on 5th February 1997. This measure prohibits animal experimentation in the development of new cosmetics or the testing of existing cosmetics, as per the provisions of the Commodities Act.

In November 1997, the UK announced an end to cosmetic product testing on animals. The context of this announcement was that the three UK-based contract facilities that previously held licences to test cosmetic formulations on animals, agreed to give up these licences. They voluntarily returned the licences to the Home Office. Therefore, there are no longer any facilities in the UK that are authorised to carry out animal testing on finished cosmetic products.

Germany also took measures to prohibit animal testing of cosmetics. In November 1997 the German Parliament passed a bill that placed a ban on animal testing for the purposes of development of cosmetics. This bill entered into force on 1st January 1998 and it effectively prohibits the testing of finished products or ingredients. However, it is possible for derogations to be granted.

For cases in which finished product testing has been prohibited, the distinction between a finished product and a combination of ingredients has not been defined.

It is important to note that whilst national bans prohibit animal testing on a Member State's territory, this may not actually represent a true reduction in animal usage. Rather, the tests could be carried out in an alternative Member State or in a Third Country. This could also be the case in those examples in which Member States have reported that no tests were conducted on their territory. Further, it is pertinent to note that these prohibitions are placed on the actual animal test rather than on a marketing ban of the cosmetic product.

H. CONCLUSIONS

1997 saw the completion of several experimental programmes aimed at the development of novel alternative methods to animal testing. Unfortunately, efforts to date did not yield the results that were hoped for. Whilst some promising innovations have come from some of these programmes, the validation of new methods across the range of toxic end-points did not prove to be possible. Following an exhaustive scientific review of the status of alternative methods to animal testing in 1997, the Commission put forward a postponement of the ban in the absence of scientifically validated alternative methods.

Since then, experts in this field have continued to analyse the existing data and work towards a better mechanistic understanding of the science underlying these toxic end-points. New programmes have been and are being developed to build on the progress made thus far.

Previously, there has been a demand for a timetable for the development and validation of alternatives in the Commission's annual reports. However, the prediction of a realistic timetable for basic research and method development of this kind is simply not feasible. Research into biological systems is subject to variability and practical problems that must be resolved in a step-wise manner if research is to be conducted in a scientifically valid and appropriate fashion.

This report demonstrates that the submission of statistics on animal usage in the field of cosmetics remains problematic for some Member States. Obviously, the Commission remains at the disposal of all parties to help resolve any practical issues that arise as a result of this obligation, and will continue to press Member States to fulfil these obligations. In cases where Member States remain unable to meet the requirements of the Cosmetics Directive in terms of submission of these data, the Commission is preparing legislative action in the form of infringement procedures.

Clearly, the cosmetics industry has taken a lead role in the development of alternative methods to animal testing, committing significant funds and scientific resources. The resources that the cosmetics industry have given to this issue are disproportionate to its use of animals for experimentation. However, the industry continues to support the concepts of reduction, refinement and replacement of animal use in the safety testing of its products and ingredients. Following the completion of the experimental phase of several validation studies, experts from the cosmetics and chemical sectors are working to develop a second round of research programmes.

The Commission services have allocated significant resources to the development, validation and acceptance of alternative methods to animal testing. Most notably, in the past year ECVAM has dedicated a large proportion of its attention to this industry sector. In the future, the Commission services will collaborate and pool resources in these efforts, particularly in the work programmes of its scientific committees. Lastly, it is proposed that the 5th Framework Programme will provide the potential for the funding of new and innovative proposals in the development of novel methods.

Following the recent re-organisation of some of the Commission services, new working practices have been established and the collaboration between scientists from the Commission, Member States and Industry continue to strengthen. Through the development of short, medium and long-term research proposals it will be possible to make significant progress in this challenging scientific issue and meet the common objective of a meaningful reduction in animal experimentation.

I. GLOSSARY OF ABBREVIATIONS

BgVV	Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin
COLIPA	Comité de Liaison Européen des Industrie Cosmétiques, des Produits de Toilette et de la Parfumerie (The European Cosmetic Toiletry and Perfumery Association)
CTFA	Cosmetic, Toiletry and Fragrance Association (USA)
ECVAM	European Centre for the Validation of Alternative Methods
EU/HO	European Union/Home Office
ERGATT	European Research Group for Alternatives in Toxicity Testing
ESAC	ECVAM Scientific Advisory Committee
EU	European Union
GM-CFU	Granulocyte-macrophage-colony forming unit
IRAG	Interagency Regulatory Alternatives Group
IVTIP	*In vitro* Testing Industrial Platform
JCIA	Japanese Cosmetic Industry Association
NRU	Neutral Red Uptake
OECD	Organisation for Economic Co-operation and Development
SCAAT	Steering Committee on Alternatives to Animal Testing
SCC	Scientific Committee on Cosmetology
SCCNFP	Scientific Committee on Cosmetics and Non-food products
SIS	Scientific Information Service
SME	Small and Medium Sized Enterprises
UV	Ultra Violet
ZEBET	Zentralstelle zur Erfassung und Bewertung von Ersatz und Ergänzungsmethoden zum Tierversuch im Bundesgesundheitsamt

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Test Guidelines Programme

HUMAN SKIN PATCH TESTING: A CONTENTIOUS ISSUE

Tenth Meeting of the National Co-ordinators of the Test Guidelines Programme, 16th - 17th September, 1998, to be held at the Chardon-Lagache Annex, Paris, beginning at 9.30 a.m. on 16th September, 1998.

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APPENDIX

DEVELOPMENT OF OECD TEST GUIDELINES FOR USE WITH HUMAN VOLUNTEERS

Document prepared for:

27th Joint Meeting of the Chemicals Group and Management Committee, 11th-13th February 1998, to be held at the Château de la Muette, Paris, beginning at 9H30 on 11th February.

This document outlines the current and proposed use of human data in hazard classification and describes the lack of consensus among National Co-ordinators on the need to develop OECD Test Guidelines for studies using human volunteers.

ACTION REQUIRED: *The Joint Meeting is invited to:*

i) take note of the discussions on human testing by the National Co-ordinators of the Test Guidelines Programme and the Advisory Group Members of the Programme on Harmonization of Classification and Labelling Systems, and

ii) reach consensus on the need to develop an OECD Test Guideline for local skin effects in human volunteers.

i) to supplement non-clinical information,

ii) to confirm that exposure will not cause significant harm, and or,

iii) in a controlled fashion that minimises subject risk.

5. Member countries appear to have different ways of looking at testing in humans: i) some discourage human testing in general on ethical grounds; ii) many others make use of ethically-obtained human test data; iii) some countries require for each case an ethical consideration of whether human testing should be undertaken; and iv) for some chemical use categories, e.g., pharmaceuticals, testing of some end-points in humans is mandatory.

THE ISSUE

6. Although the Advisory Group of the Programme on Harmonization of Classification and Labelling Systems was of the opinion that testing in humans should certainly not be encouraged, the Group accepted the use of human data for the purpose of classification provided data were obtained under well-defined conditions.

7. The proposed Guideline for the "Acute Dermal Irritation Study in Human Volunteers" includes the following paragraphs dealing with the safety protection of the volunteers, ethical standards and other initial considerations:

In the interests of human safety the following criteria must be met before the study is initiated:

i) the study must meet any local legal requirements and conform fully to the "Helsinki Guidelines" (reference added);

ii) the study should follow the principles of Good Clinical Practice (reference added);

iii) the protocol is reviewed before initiation of the study and considered acceptable by an independent ethical review committee; and

iv) the experiment should be performed in compliance with the national regulations on ethical concerns of the testing facility.

Adequate information on the toxicity profile, including percutaneous absorption data, should be available to indicate that the study does not present any significant health risk. Substances should not be tested in humans when:

- *they have been shown to be irritant in a predictive assay, either in vitro or in vivo;*
- *they have been shown to be corrosive in a predictive assay, either in vitro (reference added) or in vivo;*
- *a corrosive potential for human skin can be predicted on the basis of structure-activity relationships and/or physico-chemical properties such as strong acid or alkaline reserve (reference added);*

添付資料-9

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Test Guidelines Programme

PERCUTANEOUS ABSORPTION GUIDELINE : STATUS REPORT

Tenth Meeting of the National Co-ordinators of the Test Guidelines Programme, 16th-17th September, 1998, to be held at the Chardon-Lagache Annex, Paris, beginning at 9:30 a.m. on 16th September, 1998

68127

Or. Eng.

PERCUTANEOUS ABSORPTION GUIDELINE: STATUS REPORT

INTRODUCTION

1. At the 8th National Co-ordinators Meeting in April 1997 it was agreed to hold a Workshop to resolve existing differences in appreciation between Member countries of the status of validation of an *in vitro(ex vivo)* methods for percutaneous absorption.

2. Several telephone conference calls of the Steering Committee of the Workshop failed to resolve basic differences and it was agreed to arrange for a Steering Committee Meeting, with some additional experts, on the dates initially set for the Workshop. The meeting was held in Research Triangle Park, North Carolina, USA on 15th-16th October 1997.

3. The draft Meeting Report was sent to the Extended Steering Committee in April, 1998 and was subsequently revised based on comments and suggestions received from the participants. The Final Meeting report was circulated to the Extended Steering Committee and National Co-ordinators on 12th June, 1998 (see Appendix).

CURRENT ACTIVITIES

Revision Of Test Guidelines

4. Two Steering Committee members (from government and industry respectively) charged with the task to revise the *in vitro* Guideline proposal are in the last stages of finalizing this work product. In addition to the revision of the *in vitro* method, they have also drafted a proposal for a Guidance Document on the subject. It is expected that the revised proposal and the draft Guidance Document will be available shortly for circulation to the Extended Steering Committee for discussion, review and comment. The European Crop Protection Association (ECPA) has provided valuable input.

Selection Of Reference Chemicals

5. At the Extended Steering Committee Meeting it was suggested that a small number of reference substances should be incuded in the revised Guideline proposals as positive/negative control substances. Following the Meeting, members of the Extended Steering Committee made suggestions for substances to be considered. These include:

- water (tritiated water),
- caffeine,
- inulin,
- nicotine,
- hydrocortisone,
- benzoic acid,
- salicylic acid
- testosterone.

13. The report of the External Review Committee will contain the criteria used to review and evaluate the published literature and other data. It will recommend the inclusion or exclusion of any study in the final assesment of the validation status of the proposed Test Guidelines. It is anticipated that the report of the Panel will allow a conclusion on the validity or lack thereof of the *in vitro* and *in vivo* methodology as described in the percutaneous absorption Test Guideline proposals . It should further provide the areas (personal care, household and consumer products, pharmaceuticals, among others) for potential utilization of the proposed Test Guidelines if the data support the utilization of these methods.

14. A conference call to initiate the activities of the External Review Committee is scheduled for 7th August, 1998. It is expected that the External Review Panel will be able to provide a final report at a meeting of the Extended Steering Committee in March/April 1999. Following this presentation the Extended Steering Committee will hopefully be able to come to a conclusion on the validity of the methods described in the proposed Test Guidelines and make final recommendations to the NCs.

Time Schedule

15. The External Review Committee should complete their evaluation by the February 1999 and be able to prepare a written report in advance of a meeting of the Extended Steering Committee to be scheduled in March/April 1999. The Colipa Data (already submitted) and the literature search will be provided to them by the 1st Week in September. Additional industry data has been requested and will be forwarded as soon as received by the Secretariat.

16. The draft Guidelines and Guidance Document will be distributed to the Extended Steering Committee and External Review Panel as soon as possible, but not later than the end of September, 1998.

17. The Extended Steering Committee will meet in March/April 1999 to complete their discussion and provide their recommendations to the NCs.

emphasize. The Test Guidelines do not approach the Level of detail found in standard operating procedures or similar documents. This is intentional because toxicology is a developing experimental science, and excessive rigidity or over-detailed specification of methods could inhibit scientific inniative and be counter-productive. There must be provision for the exercise of toxicological skill and judgement during the course of the study, even where this forms part of a prescribed set of test requirements, and so guidelines or similara defined procedures should allow for this; obviously, the rationale for changes in procedure must be explained and supported scientifically. The emphasis on a flexible approach should not be construed as a recommendation for a lack of order, it should be seen as creating a situation in which the examination of the toxicity of a chemical substance is conducted as a scientific exercise rather than as a set of stereotyped tests to be conducted in a routine.

THE ISSUES

6. The discussion continued with short explanations by all participants of their preferred approach which could lead to the acceptance of one or more Test Guidelines for percutaneous absorption. Although there were considerable differences of opinion it was also clear that there was substantial common ground: the Canadian/US proposal for " a resolution process" -which had been proposed to the Steering Committee earlier and discussed during previous teleconferences- could be used as a tool to determine the quality and extent of data available and, subsequently, to decide on what more (if anything) needs to be done to validate the proposed *in vitro* Guideline.

7. During the initial discussions participants indicated that issues which have complicated the discussions, and where concensus has not been fully achieved, include the following:

- the *in vitro* Guideline proposal should be referred to as an *ex vivo* proposal;
- the *ex vivo* Guideline proposal comprises a series of options for testing rather than a straight forward test protocol; the proposal could be considered as guidance rather than a Test Guideline;
- although the proposal is not a toxicity test but a technique to measure whether or not a substance passes the skin, it does have toxicological implications;
- various *ex vivo* methods for percutaneous absorption have been used and accepted for many years and laboratories have confidence in their specific protocols;
- all currently available techniques, both *in vivo* and *ex vivo*, are broad estimations, rather than precise measurements of skin absorption;
- to date validation studies have been conducted with strict protocols which make results from different laboratories (relatively) easy to compare; however, the current proposals allow various methods to be used, and each of them should be reliable and relevant; consequently, validation should be considered for each of the options separately;
- currently available *ex vivo* methods are not designed to replace *in vivo* methods, therefore, validation should not be based on the *in vivo* test as the reference standard;
- the current proposals do not clearly indicate the purpose of the test: type and extent of validation would be quite different for tests conducted as a (pre)screen or adjunct than for those used as definitive measure of absorption.

8. The Meeting also considered the comments received from Member countries on the current proposals. It appeared that most Member countries who had responded had only minor technical comments Apart from Canada and the US who were not ready to accept the Guidelines, France indicated that the Guideline would be acceptable in a tier, viz., an *in vivo* method should always be included as a final tier. This limitation, implying that the *in vivo* method would be more reliable than an *in vitro* (preferably referred to as *ex vivo*) method, triggered an extensive discussion of the purpose and place of both the *in vivo* and *ex vivo* tests in risk assessment. Some participants indicated that it is common practice today to use the *in vivo* method for active ingredients and the *ex*