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 Cosmetics Directive (*76/768/EEC*)

The Report and Recommendations of an ECVAM/CPS Workshop (ECVAM Workshop 7)[1,2]

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[1]ECVAM — European Centre for the Validation of Alternative Methods. This workshop was organised jointly with the Consumer Policy Service (CPS) of the European Commission. As from 1 April 1995, the CPS became the Commission's "Directorate-General XXIV — Consumer Policy". [2]This document is not a text which was agreed word-by-word, but represents the consensus report of the participants as individual scientists.

Preface

This is the report of the seventh of a series of workshops organised by the European Centre for the Validation of Alternative Methods (ECVAM). ECVAM's main goal, as defined in 1993 by its Scientific Advisory Committee, is to promote the scientific and regulatory acceptance of alternative methods which are of importance to the biosciences and which reduce, refine or replace the use of laboratory animals. One of the first priorities set by ECVAM was the implementation of procedures which would enable it to become well-informed about the state-of-the-art of non-animal test development and validation, and the potential for the possible incorporation of alternative tests into regulatory procedures. It was decided that this would be best achieved by the organisation of ECVAM workshops on specific topics, at which small groups of invited experts would review the current status of various types of *in vitro* tests and their potential uses, and make recommendations about the best ways forward (1).

This particular workshop was a joint initiative of ECVAM and the Consumer Policy Service (CPS), and the two Commission services cooperated closely in its organisation. The workshop was held in Angera, Italy on 11–13 April 1994, under the co-chairmanship of Michael Balls (ECVAM) and Walter De Klerck (CPS). A number of relevant developments have taken place since the workshop was held. Some of these have been noted in this report, in order to make it as up-to-date as possible.

Introduction

Article 1 of *Council Directive 93/35/EEC* (2), which amends *Council Directive 76/768/EEC* (3) for the sixth time, defines a cosmetic product as *any substance or preparation intended to be placed in contact with the various external parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance and/or correcting body odours and/or protecting them or keeping them in good condition.*

Article 2 of the Sixth Amendment requires that *a cosmetic product put on the market within the Community must not cause damage to human health when applied under normal or reasonably foreseeable conditions of use, taking account, in particular, of the product's presentation, its labelling, and any instructions for its use and disposal, as well as any other indication or information provided by the manufacturer or his authorized agent or by any other person responsible for placing the product on the Community market.*

Article 4 states that, without prejudice to their general obligations deriving from Article 2, Member States shall prohibit the marketing of cosmetic products containing *ingredients or combinations of ingredients tested on animals after 1 January 1998 in order to meet the requirements of this Directive.* However:

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.

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.

At the joint ECVAM/CPS workshop, representatives of various services of the European Commission discussed the implications of the

Sixth Amendment with representatives of the cosmetic industry, centres involved in organising validation studies, databases on alternative methods, and the animal welfare movement, and with academic and industrial *in vitro* toxicologists.

The main objectives of the workshop were:

1. To assess the progress being made in the development of alternative methods.
2. To identify any obstacles to the successful validation and acceptance of these alternative methods.
3. To define the actions necessary for removing any such obstacles.
4. To identify the most promising areas for replacement of the current animal tests.
5. To assess the value of the existing databases on alternative methods.
6. To compare the predictive values of *in vivo* and *in vitro* tests.
7. To strengthen cooperation between those concerned in any way in the cosmetics testing issue.
8. To suggest criteria for developing and validating alternative methods to be used in the safety evaluation of cosmetics.

Responsibilities Within the Commission

The CPS (now DGXXIV) is responsible for the administration of *Council Directive 76/768/EEC*, including the implementation of the Sixth Amendment and the production of the annual report for the European Parliament and the Council. The performance of these duties requires liaison with other services of the Commission and with the cosmetic industry, notably with COLIPA (the European Cosmetic, Toiletry and Perfumery Association). The first annual report was published in December 1994 (4).

The CPS is advised by the Scientific Committee on Cosmetology (SCC), which consists of highly qualified scientists from the Member States of the European Union (EU). The SCC assists the CPS with all matters related to the safety evaluation of cosmetic ingredients (5). The SCC is consulted about every Commission proposal to adapt the Cosmetics Directive to technical progress. Furthermore, the Sixth Amendment requires that the European Commission consults the SCC before submitting draft measures to postpone the date of implementation of the ban on animal testing in the event that there is insufficient progress, by 1 January 1997, in developing satisfactory methods to replace animal testing.

Unit C/6 of DGXI, the Directorate-General for Environment, Nuclear Safety and Civil Protection, is responsible for the administration of *Council Directive 86/609/EEC* on the protection of animals used for experimental and other scientific purposes (6). Article 7(2) of this Directive requires that *an experiment shall not be performed if another scientifically satisfactory method of obtaining the result sought, not entailing the use of an animal, is reasonably and practicably available.* Article 26 of *Directive 86/609/EEC* requires that statistics on animal use should be submitted to the Commission by the Member States at regular intervals not exceeding three years. The first set of statistics was published in May 1994 (7), and it is proposed that publication will take place every two years in future. Discussions are taking place between the CPS, DGXI/C/6 and the Member States concerning the annual publication of statistics on animals used, in compliance with *Directive 76/768/EEC*.

Article 23 of *Directive 86/609/EEC* states 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.* This led to the announcement by the Commission in 1991 that a European Centre for the Validation of Alternative Methods (ECVAM) was to be established, as a *relatively independent* unit of the Environment Institute of the Commission's Joint Research Centre, at Ispra, in Italy (8). The duties of ECVAM were spelled out as follows:

1. To coordinate the validation of alternative test methods at EU level. This will involve the specification of test protocols, the organisation of ring-test exercises, the choice of chemicals to be used in these tests, and the analysis and evaluation of the results, etc.
2. To act as a focal point for the exchange of information on the development of altern-

ative test methods.

3. To set up, maintain and manage a database on alternative procedures, with associated user services.
4. To promote dialogue among legislators, industrial companies, biomedical scientists, consumer organisations and animal welfare groups, with a view to the development, validation and international recognition of alternative test methods.

ECVAM's principal aim is to promote the scientific and regulatory acceptance of alternative methods which are of importance to the biosciences and which reduce, refine or replace the use of laboratory animals. ECVAM's attention will not be focused solely on regulatory toxicity testing, but also, for example, on the use of alternatives in assessing the efficacy of medicines and the potency of vaccines. ECVAM has a Scientific Advisory Committee composed of individuals from the Member States, industry, the academic world and animal welfare organisations.

Also in response to Article 23 of *Directive 86/609/EEC*, support for the development of non-animal tests is included in the BIOTECH and BIOMED 2 programmes of *Directorate E - R & TD: Life Sciences and Technologies* of DGXII, the Directorate-General for Science, Research and Development. *Directorate C - R & TD: Industrial and Materials Technologies* of DGXII is also interested in the development of new methods for routine use by industrial companies, including *in vitro* toxicity tests.

The Scientific Committee on Cosmetology

The SCC was set up in 1978 to advise the Commission on all matters related to the safety of cosmetics (5). SCC guidelines for the safety evaluation of cosmetic ingredients were first published in 1982 and were last revised in 1990 (9). The SCC must be consulted on any problem of a scientific or a technical nature in the field of cosmetic products and, in particular, on substances used in the preparation of cosmetic products and on the composition and conditions of use of such products (5). Therefore, under the terms of *Directive 76/768/EEC*, and acting on the advice of the SCC, the Commission published lists of chemicals which may not be used in cosmetic products manufactured or marketed in the EU, and positive lists of ingredients which can be used as UV-filters, colouring agents and preservatives. The main goal of the SCC/CPS is the safety evaluation of cosmetic ingredients for human use and application. In fulfilment of this task, the SCC has also to advise on which alternative methods are applicable in this safety evaluation. Moreover, the SCC and its responsible subcommittees will update the appropriate guidelines.

To date, the SCC has evaluated about 300 cosmetic ingredients for their safety. The Committee takes into account physicochemical data, *in vivo* and *in vitro* test results, and likely human exposure route, pattern and level. For toxicological characterisations, data on the following are required: acute toxicity, dermal absorption, dermal irritation, mucous membrane irritation, skin sensitisation, sub-chronic toxicity, mutagenicity, phototoxicity, and human responses (if available). When considerable oral intake is possible, or when the dermal absorption data indicate the possibility of considerable skin penetration, further *in vivo* data may be required on toxicokinetics, teratogenicity, reproductive toxicity, additional genotoxicity, and carcinogenicity.

The SCC's approach assumes that the safety evaluation of products can usually be based on the toxicological potentials of their ingredients. However, in certain cases, the testing of finished products may be advisable, for example, where the formulations of the finished products incorporate solvents which are different from those used in the toxicity testing of the ingredients, since this may affect dermal absorption considerably. Other examples are where the potentiation of toxic effects might occur because of interactions between the ingredients, and when manufacturers claim that inclusion in the formulation reduces the potential hazard of one or more of its ingredients.

The SCC is keen to encourage the use of alternative methods as soon as their relevance and reliability have been established and has, therefore, established a subgroup on alternatives with the following remit:

— to follow up the validation studies coordinated by ECVAM and by other organisations;

— to evaluate the applicability of validated alternative methods in the context of cosmetics safety evaluation;

— to recommend updating of the SCC test guidelines; and

— to produce an annual report.

The members of the SCC who participated in the workshop emphasised that, since the three categories for which positive lists of ingredients have been produced (i.e. UV-filters, colouring agents and preservatives) constitute chemicals of many different classes, validation studies on the testing of chemicals likely to be used for these purposes should be given a high priority.

The Animal Welfare Viewpoint

The participants from animal welfare organisations emphasised that the Sixth Amendment to *Directive 76/768/EEC* meant that the ban on the use of ingredients tested in animals after 1 January 1998 would come into force, unless it could be shown that the necessary scientifically validated alternatives were not available. Thus, significant progress must be achieved by 1 January 1997, or there would be great pressure for a complete ban on animal tests, regardless of the status of the alternatives to replace them. It was therefore essential that there should be ways of quantitatively assessing the progress being made toward the elimination of the need for animal testing. This, in turn, meant that the precise data specified by the Sixth Amendment must be provided. These data should include the purpose of testing, the animal species used, the types of experiment conducted, and the categories of ingredients and products tested.

Moreover, although it could be argued that the Commission need only produce statistics on animal use directly in compliance with *Directive 76/768/EEC*, information should also be provided on the testing of cosmetic ingredients conducted within the EU in compliance with other Directives and/or in accordance with the regulatory or other requirements of other countries and regions. In addition, information should be provided on the regulation and licensing of the testing of cosmetic ingredients in animals in each of the Member States of the EU.

The conclusions reached at a meeting organised jointly by the International Fund for Animal Welfare and Eurogroup for Animal Welfare (10), held in Munich in March 1994, were discussed. The participants in that discussion had identified three categories of testing in terms of the prospects of meeting the 1998 deadline:

— those for which the prospects of meeting the deadline could be considered to be high, i.e. finished product testing, and mutagenicity, dermal absorption, phototoxicity, dermal irritation and ocular irritation testing;

— those in which progress was promising, so the deadline could possibly be met, i.e. carcinogenicity and systemic acute toxicity testing; and

— those where sufficient progress was unlikely to be made, because of the need for greater basic understanding of the phenomena involved or for technical breakthroughs, i.e. investigations of toxicokinetics, and sub-chronic toxicity, dermal sensitisation (including photosensitisation), teratogenicity and reproductive toxicity testing.

The Cosmetic Industry Perspective

The workshop was informed of a number of COLIPA initiatives, including:

— the creation of a COLIPA Steering Committee on Alternatives to Animal Testing (SCAAT);

— the establishment of a COLIPA Task Force on dermal absorption;

— the continued operation of the COLIPA Task Force on photoirritation and the progress of the European Commission/COLIPA validation study on photoirritation (11);

— a forthcoming COLIPA validation study on alternatives to the Draize eye irritation test for cosmetic ingredients and formulations; and

— international cooperation with the cosmetic industry in Japan and the USA.

Participants from the cosmetic companies represented at the workshop outlined their in-house strategies for developing non-animal tests and incorporating them into the decision-making process.

One approach is to compare the results obtained with a range of *in vitro* methods, as a means of evaluating their respective advantages and disadvantages when used alone

or in combination. For example, in the case of alternatives to the Draize eye irritation test, the performances of physicochemical, cell culture, organotypic and eye-part methods have been evaluated by deriving Pearson and Spearman correlation coefficients and by multivariate analysis. It was found that different tests and combinations of tests can be useful for surfactant-based and finished products. Therefore, the selected alternative methods have been incorporated in a stepwise approach into the decision-making process for finished products, and are part of the evaluation of ingredients.

Another approach involves a sequential consideration of:

— (quantitative) structure-activity relationships ([Q]SAR), molecular modelling, and the prediction of possible toxic effects from the chemical structure;

— the development of a rapid and reliable biological or chemical method for confirming the predicted properties; and

— the definition of an *in vitro* test protocol.

This approach is being used to distinguish between corrosive and non-corrosive materials and to predict skin sensitisation for new chemicals.

An example was given of a stepwise approach for making decisions with respect to new chemicals. It involves three steps. Step 1 is to study the physicochemical properties of a molecule and ask whether it can be classified on this basis alone. If not, Step 2 involves the use of an *in vitro* test or the conduct of a limited animal study. If necessary, a full OECD/Annex V study is undertaken in Step 3. In the case of particularly harmful materials, decisions can sometimes be taken on the basis of the results of Step 1 alone, or those from Step 1 and Step 2. For example, some chemicals can be classified as eye irritants on the basis of their pH values alone, while others can be classified as irritants on the basis of results obtained in cytotoxicity tests, eye-part tests (for example, the isolated rabbit eye test) and/or organotypic tests (for example, the HET-CAM method).

The strategy used for the eye and skin irritation testing of chemicals by the regulatory authorities in Germany was outlined. The stepwise scheme is as follows:

1. If the pH of a material is higher than 11 or less than 2, it is labelled as being corrosive (R34).
2. If the pH is between 2 and 11, it is tested for skin corrosivity.
3. If the result is positive, the material is labelled as being corrosive (R34 or R35).
4. If the result is negative, SAR analysis is performed.
5. If the SAR analysis clearly indicates that the material is likely to be a severe irritant, the material is labelled as such (R41).
6. If the result is negative, the material is tested by *in vitro* methods (for example, the HET-CAM test).
7. If the *in vitro* result is positive, the material is labelled as being a severe irritant (R41).
8. If the result is negative, the material is tested on one animal.
9. If the *in vivo* result is positive, the material is labelled as a severe irritant (R41).
10. If the result is negative, the material is tested on two more animals; according to the result obtained, the material is labelled as being irritant (R36) or does not require labelling (no risk phrase).

Validation

The proper scientific validation of alternative methods is the key to their acceptance into the regulatory process to complement or replace animal tests. The key elements in a successful validation study are (12):

1. A clear and unequivocal statement of what the validation study is designed to accomplish.
2. A well-defined plan for the study.
3. A sufficiently large set of test substances covering the relevant chemical classes, the different categories of cosmetic ingredients, and the range of toxic endpoints to be evaluated.
4. *In vivo* data of high quality on all the test substances to be used.
5. Evidence that the methods to be evaluated are scientifically sound, relevant, reproducible, and have the potential to replace the animal test in question.

6. An optimised protocol for each test, with any necessary standard operating procedures.
7. A clear description of how each alternative method can be used to predict an *in vivo* endpoint.
8. Agreed statistical procedures for testing whether the methods can predict the *in vivo* endpoints defined by their developers.
9. Agreed criteria to be met in order to show that an alternative method could successfully and safely replace an animal test.
10. Results which meet these criteria.

Major problems arise when sufficient *in vivo* data are not available, or when the results of animal tests are highly variable.

Toxicology Databases

For the safety evaluation of cosmetic ingredients required by *Directive 76/768/EEC*, information is needed on both *in vivo* and *in vitro* toxicity tests conducted in the past, before any decision is taken about the need for further *in vivo* or *in vitro* testing. Bawden (13) has suggested that a comprehensive information system can assist in reducing the number of animal experiments conducted by:

— reducing the necessity for further tests, by showing that the information required already exists;

— providing information which can be used in the design of any further tests;

— permitting the application of new statistical techniques to extract information which may not be immediately obvious, for example, by combining apparently disparate pieces of information;

— providing evidence of the usefulness, or otherwise, of particular experimental procedures, so that the performance of unproductive tests can be avoided; and

— supporting the development of systems for modelling, simulation, hazard prediction and risk assessment.

In the EU, the potential users of an information system focusing on cosmetic ingredients would include the following:

— small cosmetic companies not having ready access to research laboratories and which need information on the safety evaluation of cosmetic ingredients for insertion into their product information dossiers, as requested by the Sixth Amendment (*Directive 93/35/EEC*);

— animal welfare organisations, to assist in the development of their actions on animal testing and on the use of alternatives in the cosmetics sector;

— the European Commission (CPS), for establishing a database containing the toxicological profiles of old and new cosmetic ingredients of concern;

— ECVAM, for use in the organisation of validation studies and for the development of new alternative tests and testing strategies; and

— various groups of scientists wanting to compare their own data with those contained in a larger database.

The data required are not readily available from established databases, such as MEDLINE and TOX-LINE, but the following sources are making, or could make, valuable contributions to such an information system:

— the *INVITTOX* Data Bank (14), set up by FRAME (Fund for the Replacement of Animals in Medical Experiments) with the assistance of ERGATT (European Research Group for Alternatives in Toxicity Testing), and which is shortly to be transferred to ECVAM. The aim of *INVITTOX* is to provide detailed methodological protocols on *in vitro* methods of interest to toxicologists. The service is already well-known among *in vitro* toxicologists and has distributed approximately 5000 copies of protocols since it became fully operational in April 1990. Each protocol aims to be a self-sufficient document that will allow a scientist to set up the system described without having to refer to other literature, apart from basic manuals of laboratory procedures. The level of methodological detail provided in an *INVITTOX* protocol should ideally correspond to that required in a protocol submitted to the managers of a validation study. *INVITTOX* provides its services free of charge, and any information submitted is fully accessible. The decision that protocols used in

validation studies supported or initiated by ECVAM should be deposited with *INVITTOX* is a logical step forward in facilitating access to this information, and in providing the means for agreement on which variants of a method should be accepted as standardised protocols for various needs. It is to be hoped that other funders and initiators of validation and evaluation studies will similarly make their protocols available through *INVITTOX*;

— the GALILEO Data Bank, a factual database which contains detailed information on results obtained from *in vitro* and *in vivo* toxicity tests. The sources of the information are publicly available literature, EU scientific projects, international validation studies (for example, the US Cosmetic Toiletry and Fragrance Association's evaluation study), and individual contributions. Each study is scientifically analysed and all raw data (chemicals, cell lines, treatment times, etc.) are stored in more than 120 fields, as are complete descriptions of the experimental procedures employed. The information collected so far relates to 2211 chemicals, 317 formulations, 98 different methodologies, 319 biosystems, and 20,686 individual test results (15, 16);

— ZEBET, the German Federal Government's Centre for Documentation and Evaluation of Alternative Methods to Animal Experiments, which has established a data bank to facilitate the common use of all information available on *in vitro* methods in German-speaking countries. ZEBET collects and documents information on alternative methods to animal experiments taken from the international published literature (17);

— cosmetic companies themselves and contract testing laboratories, which have developed their own databases containing data from both animal tests and alternative tests which they have undertaken; and

— academic institutions, federal agencies and animal welfare groups, dealing with educational or certain research aspects, which have developed small databases in support of their activities, as well as information centres related to alternatives in general.

Conclusions and Recommendations

General

1. The approach to replacing animal testing should be subdivided, and attention should be focused separately on types of toxic endpoints, degrees of toxicity, and types of test materials (especially finished products *versus* ingredients). The results of current validation studies can be expected to emphasise the need for such an approach.

2. Effective safety evaluation should be recognised as the ultimate goal, and it should be recognised that animal tests, *in vitro* tests and human volunteer studies, etc., are all merely means of providing information for use in that evaluation.

3. It would be wrong to insist that non-animal tests or testing strategies must be significantly more relevant and more reliable than the animal test procedures they will replace; this could delay their acceptance, and the Sixth Amendment requires only that they provide an *equivalent level of protection for the consumer*.

4. The validation process will be much easier if the non-animal tests which are being developed provide less variable results than the currently accepted animal tests, and if they have a clear mechanistic relationship with the type of toxicity to be assessed.

5. Consideration should be given to ways of incorporating non-animal test data into safety assessment dossiers. Both industry and regulators must share information regarding the use of alternative methods, if they are to be ultimately accepted as replacements for the currently used *in vivo* toxicity tests.

6. The CPS and DGXI/C/6 should seek to obtain the most comprehensive annual statistical information possible on cosmetics testing on animals within the Member States. This should include information on the tests that were performed, the animals which were used, and the purpose of the testing.

7. A wider survey should also be conducted, in order to provide information on what animal testing for cosmetics safety purposes, including tests on finished products, is required, both in each Member State and in other countries which represent major markets, for example, Japan and the USA.

Product testing

8. There are no major barriers to the cessation of finished product testing on animals; however, there might be a slightly increased risk of certain types of adverse reactions occurring in some consumers. For example, problems might arise with respect to skin sensitisation.

Non-animal tests and testing strategies

9. The importance of conducting validation studies of the highest possible standard cannot be overemphasised; excellence of study design is essential, as is the selection of appropriate methods and test materials.

10. Attention should be focused on whether non-animal methods could be used to identify materials which were so unlikely to cause any adverse effects (for example, eye irritancy) that no animal testing would be necessary, rather than considering only whether such methods could be used to avoid the animal testing of materials likely to cause severe effects. The ultimate objective is the validation and acceptance by regulatory authorities of non-animal tests and testing strategies capable of providing information on all levels of toxic responses.

11. For a variety of reasons and purposes, more effort should be invested in developing computer models and appropriate *in vitro* systems for assessing biokinetics (absorption, distribution, metabolism and excretion), not least to assist in determining the appropriate range of chemical concentrations to be used in *in vitro* toxicity tests.

12. Similarly, the introduction of QSAR approaches into the safety assessment of cosmetic ingredients, either alone or in conjunction with other approaches, such as the use of *in vitro* tests, should be encouraged in every possible way.

13. Since it has become clear that it will often not be possible to replace an animal test with a single *in vitro* test, attention should be focused on the principles of devising, optimising and using tier-testing strategies.

14. Many companies are already using *in vitro* methods for internal risk assessment purposes, using their own "islands" of knowledge; they should be encouraged to pool this information in order to provide larger islands, but it must be recognised that extrapolation from such in-house approaches to the wider sphere is not necessarily straightforward. Particular attention should therefore be paid to the accuracy of analysis of the original experimental data on which the conclusions drawn by the various industrial companies are based.

Eye irritation

15. Organotypic models (for example, isolated eye, isolated cornea and HET-CAM methods) are already used as screens for chemicals from certain classes, and as a means of identifying severe eye irritants.

16. More research is needed on modelling recovery from ocular damage and on the effects of repeated chemical insult.

17. The results of the European Commission/Home Office-sponsored international study on alternatives to the Draize eye irritancy test are eagerly awaited, as are the conclusions of the US Inter-agency Regulatory Alternatives Group (IRAG) workshop held in November 1993.

18. The current COLIPA-sponsored study on alternative tests for cosmetic ingredients and products is crucial, as it may be the last major eye irritation study to be completed before the implementation of the proposed January 1998 ban on animal testing is considered (i.e. during 1996).

19. A better mechanistic understanding of eye irritation, particularly in the human eye, would facilitate the development and validation of non-animal testing strategies directly relevant to human safety assessment.

Skin irritation

20. There is debate within the cosmetic industry about whether *in vitro* tests for skin irritancy are needed, or whether it

would be possible to screen new ingredients for genotoxicity, systemic toxicity, corrosivity and sensitisation, and then proceed directly to human volunteer studies without undertaking animal or non-animal tests for dermal irritancy *per se*.

21. Attention should therefore be paid, as a matter of urgency, to what information is essential before *in vivo* human skin testing can be considered to be ethically acceptable.

22. A standard guideline for human skin testing for irritancy should be devised, agreed and approved, also as a matter of urgency.

23. Promising *in vitro* methods for detecting corrosive chemicals are currently being subjected to an international validation study (18); however, it should be noted that such chemicals are not normally used in cosmetic products.

Skin penetration

24. Encouraging developments are taking place with regard to the development of *in vitro* and (Q)SAR systems for evaluating the possibility of percutaneous absorption of chemicals; this is a key step toward improving safety evaluation.

25. When it can be shown satisfactorily that a chemical will not penetrate the skin, certain other toxicity tests, for example, for reproductive toxicity, should not be required.

26. Human skin should be used in *in vitro* systems in preference to rat and pig skin, and effort should be put into solving current ethical, safety and logistical problems connected with the supply of human skin for *in vitro* studies. However, studies on the usefulness of pig skin as a possible alternative to human skin for *in vitro* studies should be encouraged.

27. Reconstructed human skin equivalents should be improved with respect to their barrier/absorption properties.

Skin sensitisation

28. Skin sensitisation is another key problem. Effort should be put into finding reliable and relevant *in vitro* testing strategies for identifying the sensitising potential of chemicals. A better fundamental understanding of the immunobiology of the skin will be essential.

29. The question of the significance of metabolic activation in the skin must also be addressed.

30. It must be recognised that low levels of impurities in commercial-grade chemicals will remain a problem to be taken into account.

Photosensitisation

31. Although there is pressure from some quarters for an OECD guideline for an animal test or tests for phototoxicity to be produced, at present there is no widely accepted animal procedure to be replaced by non-animal methods. However, there are a number of promising non-animal tests for photoirritancy (19), which are currently being evaluated in a European Commission/COLIPA international validation study.

32. Photoallergy is no less of a problem than other types of (contact) allergy (sensitisation).

Genotoxicity

33. Generally speaking, negative results obtained from *in vitro* genotoxicity tests, such as the *Salmonella typhimurium* bacterial point mutation test and the chromosomal aberration test in mammalian cells *in vitro*, are indicative of the non-genotoxicity of a given chemical, with some exceptions, for example, those chemicals which present one or more "structural alerts" in their molecules.

34. Positive results obtained in one or both of these types of *in vitro* genotoxicity tests are indicative of the mutagenic activity of the chemical. However, for an assessment of the mutagenic risk involved upon exposure to such a chemical, *in vivo* genotoxicity testing (for example, the micronucleus test) is necessary.

35. At present, several attempts are being made to improve the ability of *in vitro* genotoxicity tests to identify chemicals able to produce genotoxic effects by mechanisms other than gene mutation, chromosomal aberration and DNA damage (such as aneuploidy).

Carcinogenicity

36. The specificities of *in vitro* tests for predicting carcinogenic potential, for example, the *in vitro* mammalian cell transformation assay, are being improved by incorporating specific endpoints for carcinogenicity, such as the activation of oncogenes and the inactivation of tumour suppressor genes.

Difficult areas

37. For certain types of toxicity, it is unlikely that the replacement of animal tests will be possible in the short-to-medium term. These include acute systemic and subacute toxicity testing, teratogenicity and reproductive toxicity testing, and toxicokinetic studies. However, research in these areas is in progress and breakthroughs are possible.

Databases and information centres

38. There is now a need to improve the collection, storage, analysis and availability of the various types of information, so that a better service can be provided for interested parties.

39. Criteria for selecting the data to be included in databases need to be defined and agreed.

40. Some coordination of the ways of processing data and comparing various methodologies and *in vivo* and *in vitro* data needs to be encouraged.

41. A network should be established, to provide the fastest possible supply of information to those needing to use it.

42. Where questions of confidentiality of the source or exact details of the data may be involved, an independent organisation or organisations should be identified, to be responsible for the safe storage and appropriate use of such data, in order to encourage owners to make the data available.

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