— *International Trend* —

Current Status of Development of Alternative Test Methods in Europe

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Just in front of us is the deadline, 30 June 2000, stipulated in the Sixth Amendment (Sixth Amendment) of the European Cosmetic Directive (Cosmetic Directive)[1] and the following notice of extension[2] for totally banning animal testing on cosmetic ingredients and their combinations. We have been paying considerable attention to events in the European Union (EU), since its decisions greatly affect the United States and Japan, not only from scientific and regulatory standpoints, but also from the standpoint of world trade. This may be a good time to review the current status of the development of alternative test methods in the EU before the first regulatory action.

The characteristic feature of the EU's approach to the animal rights issue is that the regulatory action preceded the actual development of alternative methods. Further, the EU is attempting to cope with two conflicting issues at the same time, namely, safety assurance of cosmetic products and the welfare of animals, which have historically been indispensable for safety assurance testing. This regulatory action has promoted the development of alternative methods in many different ways, including pure science, validation studies, definition of appropriate regulatory requirements, harmonization, etc. The aim of this paper is to trace the course of these developments and to examine the philosophy that underlies them.

1. Conflicting issues in the Sixth Amendment

The Sixth Amendment, published on 14 June 1993,[1] proposed a total ban on animal testing of cosmetic ingredients or combinations of ingredients. Article 4 1 (i) said that 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."* This article also specified that, if there has been insufficient progress in developing satisfactory methods to replace animal testing, or where those alternative methods of testing have not been scientifically validated, that the Commission, by 1 January 1997, would *"submit in 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*

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Article 10 of the Directive." Further, it mentioned that the decision should be made with due consideration of developments at the Organization for Economic Cooperation and Development (OECD, Paris, France). The two deadlines stipulated in the Sixth Amendment were then postponed by the Commission Directive published on 17 April 1997[2] to 1 January 2000 and 30 June 2000, respectively because of lack of appropriate methodologies.

The other characteristic of the Sixth Amendment is formalized product safety requirements for the cosmetic industry. Although safety-related regulations have existed in most countries of the EU for many years, the Cosmetic Directive formally required every manufacturer or importer of cosmetic products to be responsible for the safety of the products they market. This means that the authorities can request detailed information on a product's safety, composition, method of manufacture and effects on human health, including the safety of ingredients that have mostly been examined by animal testing.

Many years ago, animal testing was the only way to assure the safety of cosmetic ingredients and finished products before humans were exposed to them. However, coordinated efforts by industry and government for more than a decade to develop non-animal safety evaluation methods have already reduced considerably the number of animals used for cosmetic safety assurance. Typical examples of such efforts are the establishment of the Steering Committee on Alternatives to Animal Testing (SCAAT) and its five task forces in the European Cosmetic Industry Association (COLIPA, Brussels, Belgium) and the European Center for Validation of Alternative Methods (ECVAM, Ispra, Italy). But it is still not possible to completely replace animal testing with validated alternative methods.

This situation creates a conflict even within the framework of the Sixth Amendment. There are real scientific difficulties in developing alternative methods and establishing the safety of cosmetic ingredients and finished products without any use of animals. Therefore the issue might be considered to be political, because the Sixth Amendment requires a total ban on animal testing without taking scientific feasibility into account. However, this may reflect the fact that the term "animal testing" was then seen synonymous with the Draize eye irritation test in rabbits. Even now, alternative test methods for systemic toxicity are insufficiently developed, in contrast to the test methods for skin-related toxicity that has been studied in detail by the cosmetics industry.

There are other potential problems in the Sixth Amendment, because it actually stipulated a marketing ban, not a testing ban, on cosmetic products containing ingredients or combinations of ingredients tested on animals. This means that the Cosmetic Directive indirectly affects business conduct and regulations in the rest of the world. Therefore, phrases such as "international harmonization", "regulatory acceptance", "regulatory science", etc. are in a sense more important than "scientific validation" and "pure science", which have generally been seen as key points in the development of alternative methods. In addition, clarification of the terminology is necessary. For example, there is no definition of finished products and the term, "combinations of ingredients", has some ambiguity, since it is not clear whether this refers to only pre-mixed ingredients, or also finished products. The phrase, "tested on animals", is also unclear, as it does not take time period into consideration and most cosmetic ingredients have been tested at least once on animals at some time in the past. It also does not specify endpoints of animal testing, and therefore imposes a total ban that is not presently considered to be scientifically justifiable if human safety is to be assured.

2. What will happen in the Seventh Amendment?

In order to cope with many issues raised by

the Sixth Amendment and to achieve the primary purpose of the Sixth Amendment, i.e., coexistence of consumer health protection and animal welfare, a further regulatory measure has been discussed as the Seventh Amendment of the Cosmetic Directive. This seems necessary because of a separation of the EU's approach from that of the rest of the world, despite the clear need for international harmonization of such regulatory issues. The easiest way, from the scientific viewpoint, might be to postpone further the deadline for a total ban, while taking the opportunity to make the proposed regime more clear and understandable. The first step that should be taken is to define clearly in regulatory terminology the things that have been accomplished and those that require additional efforts to be accomplished.

One step could be a ban on animal testing of finished cosmetic products, because most cosmetics companies have already eliminated animal testing procedures for finished products, and this is clearly a feasible step to take. However, the other side of the coin is the fact that safety assurance of ingredients still has to rely partially on animal testing, and that no guidelines are yet available for testing the safety of finished products without the use of animals. The main reason for this is probably because the safety testing of finished cosmetic products has been heavily dependent on human testing and even with the alternative methods so far developed, a huge data-base based upon long experience is needed, and this is not available to all cosmetic companies. It was reported that guidelines will be established for small and medium enterprises (SME), including independent testing laboratories, who have access to such a data-base, but are restricted in its utilization by considerations of commercial secrecy. Although there still remain many things to be done, a ban on animal testing of finished products would at least clarify the confusion between products and combinations of ingredients and give a strong focus on safety assurance of ingredients.

Another measure which could be taken is to specify and clarify endpoints of animal testing. While scientific validation has made some progress as regards endpoints of animal testing, such as phototoxicity, skin corrosivity, etc., there is no way at present to take account of such progress in the Cosmetic Directive. One problem is that a clear indication of endpoints of animal testing might pose a risk of misunderstanding by parties interested in the primary target of the Cosmetic Directive, i.e., total elimination of animal testing, especially since some endpoints really require a scientific breakthrough. In the real world, however, skin-related toxicity, including eye irritation, has been clearly separated from systemic toxicity involving skin absorption in terms of the industry's efforts and the scientific difficulties. Within the field of skin-related toxicity, the tests for phototoxicity, corrosivity, skin absorption and partial eye irritation can be classified as short-term issues, skin irritation as medium-term issue, and skin sensitization and full replacement of eye irritation as long-term issues.

As for regulatory and political issues, moves towards a testing ban from a marketing ban have been recognized and are thought to be quite likely to materialize, as such a ban would side-step major trade issues involving the World Trade Organization (WTO). Even though a testing ban would still separate the EU from the rest of the world, it would leave many issues still open to international scrutiny or to discussion from the viewpoint of international harmonization. A testing ban might create other areas of discussion as to when animal testing should be eliminated for particular products, product lines and/or companies, because such elimination would be useful for promotional and advertising purposes, even if it did not greatly impact on animal welfare.

3. Scientific validation and harmonization

The Seventh Amendment is still very likely to separate the EU from other parts of the

world in terms of regulation, but as far as test methods are concerned, they are still subject to international scrutiny including discussion at the OECD level. A key player in this area seems to be ECVAM which, of course, involves COLIPA. As described in the Sixth Amendment, the OECD has been playing an important role in validating and harmonizing alternative test methods, but essential developments have been limited in the last few years and there has been some frustration with the slow pace of progress. It was in 1998 when ECVAM started to validate methods independently of the OECD. The first example of such independent validation was an alternative method for phototoxicity testing,[3] which is the 3T3 NRU PT test. This was published in ATLA as statement on the scientific validity, in the form of an endorsement of the conclusions in certain validation studies. Conditions laid down in the endorsing statement are: firstly, that the ECVAM Scientific Advisory Committee (ESAC) has regularly been kept informed of the progress of the validation study and has had the opportunity to review and assess various related documents including the final report, and secondly, that the validation study was conducted in accordance with general principles laid down in several key validation guidelines. They are the report of the CAAT (Center for Alternatives to Animal Testing, Baltimore, USA) /ERGATT (European Research Group for Alternatives in Toxicity Testing, Utrecht, The Netherland) Workshop held in 1990,[4] the guidelines contained in the report of the ECVAM/ERGATT Workshop held in 1995,[5] criteria laid down by ECVAM and the ECB (European Chemicals Bureau, Ispra, Italy),[6] criteria recommended at the OECD Workshop held in 1996[7] and the US ICCVAM (ad hoc Interagency Coordinating Committee on the Validation of Alternative Methods, Research Triangle Park, USA) report on validation and regulatory accceptance.[8] Such endorsing statements continued to be published in 1998 for skin corrosivity tests, which were the EPISKIN test[9] and the rat skin Transcutaneous Electrical Resistance test[10] (TER).

The activities at ECVAM seem to be compatible with the possible development of the Seventh Amendment and are fully supported especially by the Scientific Committee on Cosmetic Products and Non-Food Products (SCCNFP) intended for consumers, another key player in establishing the Seventh Amendment, in the Directorate Generale (DG) XXIV of the EU Commission. The details are clearly indicated in several reports of the SCCNFP that recently became available through their internet home page. An example is the opinion adopted on 23 June 1999 concerning present development and validation of adequate alternative methodologies to the use of animals in safety testing of cosmetics, in which the SCCNFP identified three in vitro test methods for skin phototoxicity and corrosivity that can be used for the safety testing of cosmetics.

In addition, they are in support of an in vitro percutaneous absorption method under their own definition of "Basic Criteria for the in vitro Assessment of Percutaneous Absorption of Cosmetic Ingredients" to provide the industry with a set of recommendations for an adequate protocol for applying in vitro methods in studies of percutaneous absorption. This support was expressed without any published statement from ECVAM, probably because percutaneous absorption itself does not represent a specific endpoint in safety assurance. Further, they have produced a set of guidelines on "The Use of Human Volunteers in the Testing of Potentially Cutaneous Irritant Cosmetic Ingredients or Combinations of Ingredients", taking both scientific and ethical aspects into consideration. The actions taken for skin absorption and human testing of potentially irritant ingredients are considered to be another separational measure from the OECD, because both of them have long been discussed at the OECD, but no conclusion has been reached concerning the basic and ethical

issues.

All of such opinions and developments have already been reflected in the "Notes of Guidance for Testing of Cosmetic Ingredients for Their Safety Evaluation", which is the key document describing procedures to be taken for safety evaluation of cosmetic ingredients. Specifically, they are available as several annexes published on the EU home page as the third revision of the notes of guidance, with the adoption date of 23 June 1999. The relevant annexes are; "Annex 2: the use of methods alternative to animal studies in the safety evaluation of cosmetic ingredients and combinations of ingredients", "Annex 3: guidelines for in vitro assessment of the phototoxic potential of UV-filters", "Annex 7: guidelines for the safety assessment of the finished cosmetic product", "Annex 9: in vitro methods to assess skin corrosivity", "Annex 10: in vitro methods to assess percutaneous absorption", which includes "basic criteria for in vitro assessment", "Annex 11: guidelines on the use of human volunteers in the testing of potentially cutaneous irritant cosmetic ingredients" and "Annex 12: guidelines on the use of human volunteers in compatibility testing of finished cosmetic products".

Although all of the documents are ready-to-go, everything depends upon what the basic principle of the Seventh Amendment will be. It is not yet clear whether it might be simple postponement or a total modification; this remains behind the closed curtain of the restructured EU Commission. These issues were recently discussed at the Transatlantic Business Dialogue Conference established for trade dialogue between the EU and the US.[11] This particular topic was also identified as a candidate for "early warning", which is established to alert trade officials in both the EU and the US to any proposal that could lead to trade sanctions by either party. As previously described, a change from a "marketing ban" to a "testing ban" might be a solution that would allow the EU to maintain its stance of protection of animal health and life, but international regulatory acceptance, international regulatory science and international harmonization would still have to be fully discussed and agreed upon.

4. What is the program for the future?

Several endpoints of animal testing validated at the EU level have been described above, and progress and programs for the future directed at other endpoints are summarized below.

1) Eye irritation

The results of extensive collaborative studies in the EU, US and Japan have been reviewed and summarized in the paper entitled "The Way Forward".[12] The conclusion is that no single test can fully replace the Draize rabbit test. Therefore, both short- and long-term approaches have been proposed, firstly to optimize the current strategies and methods to reduce animal usage and secondly to allow gaps in knowledge to be filled in order to promote the development of complementary test methods to the current alternatives or to modify these with a view to improving their predictive capacity. A workshop in support of the latter aim was organized in 1997 and the outcome of the discussions was published.[13]

2) Skin irritation

In addition to two in vitro methods for skin corrosivity test, a notice of pre-validation study was published in 1999[14] for acute skin irritation. The study includes four methods, EPISKIN, PREDISKIN, EpiDerm and the non-perfused pig ear model, involving three laboratories and twenty chemicals (ten each of irritant and non-irritant). The study will be completed in April 2000 and it is assumed from past experience that the completion of the validation will be around the year 2005.

3) Skin sensitization

A recent report from SCCNFP indicated

that a method could be established by combining computerized expert systems with appropriate biological in vitro systems to identify chemicals able to induce the initial reaction. However, the initial stage of study is still under way and considerable research needs to be undertaken. Although refinement of test methods and reduction of animal usage are outside the scope of the Sixth Amendment, SCCNFP is taking such approach into consideration, especially for murine local lymph node assay.

4) Use of humans in safety evaluation

A basic principle laid down in the aforementioned guidelines is that tests on humans are considered to be confirmatory provided that the toxicological profile of the materials to be tested is available through either alternative or animal methods. Therefore, a test on humans cannot be regarded as an alternative method. This principle is applicable to skin corrosivity, indicating that tests on humans should not be preferred to animal testing and ethical concerns must be fully taken into account.

5) Other toxicological endpoints

The SCCNFP summarized progress in other fields of toxicology, saying that existing alternatives do not seem able at present to substitute for animal testing, except in the case of test methods for genotoxic carcinogens. The basic mechanisms of different types of toxic events, such as reproductive, neurological, teratogenic, sub-chronic toxicity, etc. still require long-term research to establish the molecular and cellular events underlying the toxicity.

5. What we can learn from the European situation

In summary, the recent European experience of developing alternative methods to animal testing under strong regulatory pressure can offer a number of lessons to us in Japan. The first point is the value of international information exchange. Although our society or individual members of it have been involved in such exchange, a more comprehensive and continuing exchange of information is needed. In addition, a more formal approach to such exchange is needed, possibly involving the setting up of an official validation center in this country. Though information exchange and international harmonization are taken seriously in Japan, we have no organization such as ECVAM or ICCVAM, other than a research project supported by the Ministry of Health and Welfare. The third aspect is the question of whether regulatory support is actually needed and whether it should precede research and development. The coexistence of safety assurance and animal welfare might be achieved without any regulatory assistance, with the aid of information disclosure and free flow of ideas, if our society reaches a consensus that this is desirable.

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