Federal Institute for Risk Assessment

Bundesinstitut für Risikobewertung

BfR

zebet

ZEBET at the BfR, Diedersdorfer Weg 1, 12277 Berlin, Germany

Zentralstelle zur Erfassung und Bewertung von Ersatz- und Ergänzungsmethoden zum Tierversuch

Centre for Documentation and Evaluation of Alternatives to Animal Experiments

Diedersdorfer Weg 1
D-12277 Berlin
Germany
☎ +49-1888-412-2270
Fax: +49-1888-412-2958
E-mail: zebet@bfr.bund.de

P.O. Box 33 00 13
D-14191 Berlin
Germany

☎: (0)1888-412-0
Fax: (0)1888-412-4741
Internet:
http://www.bfr.bund.de

Dr. William Stokes
Director, NICEATM
NIEHS, MD EC-17
P.O. Box 12233
Research Triangle Park, NC, 27709
USA

your message date & initials	our document number & initials	Phone	Berlin,
	ZEBET-2 / B4122	+49-1888-412- 2275	15 August 2003

Comments on ICCVAM Minimum Performance Standards on three types of *In Vitro* Tests for Skin Corrosion (Federal Register Notice Vol. 68, No. 126 / Tuesday, July 1, 2003, page 39104)

Dear Dr. Stokes

The institutions ZEBET and ECVAM have in 1997 already worked on the concept of a general use of skin models for regulatory toxicology. We have developed test protocols and prediction models that were generally applicable to different commercial skin models. For example, our skin model phototoxicity test developed with the full thickness skin model Skin_ [*Liebsch et al. Toxic. in Vitro* 9, 557 – 562, 1994] could later be applied without any change to the epidermis model EpiDerm [*Liebsch et al. Altex 14: 165 – 174, 1997*], and was just recently successfully applied to the epidermis model SkinEthic [*Jones et al. Toxic. In Vitro 17, 471-480, 2003*]. Taking into account that experience and a comparable experience in the field of skin corrosion tests Michael Balls wrote in 1997 an ATLA editorial about definition of structural and performance criteria (copy enclosed) to facilitate the use of equivalent biological test systems in validated robust test methods. Finally, as you will recall, in the year 2002 we have internationally agreed on that concept in the OECD Workshop on Validation and Acceptance in Stockholm.

With this detailed introduction we want to emphasise that ZEBET very much welcomes the general concept and the definition of Minimum Performance Standards for the future use of "me too" test systems that claim to be equivalent to validated systems. In November 2001 this concept has been intensively discussed in the two OECD Extended Nominated Expert Consultations for the revision of Draft Test Guideline proposals on new Guidelines for Skin Corrosion and Phototoxicity, that finally resulted in accepted new OECD TG 430 and 431 on *Skin Corrosion*, and TG 432 on *Phototoxicity*. The Experts (incl. an ICCVAM representative) defined, for example, in TG 431 functional and performance criteria for new skin models in paragraphs 9, 10 and 11. In addition, 12 Reference Chemicals were defined that should be correctly classified if a new skin model was used or the test protocol modified. The Experts agreed that meeting these criteria is a sufficient proof of equivalency for a new skin model, and this was later confirmed by the National Co-ordinators of the OECD Member Countries. For TG 430 (TER Test), the same Reference Chemicals were defined to address the problem that the TER is sensitive to the rat strain used and the dimensions of the apparatus used. Here the twelve chemicals function as re-calibration chemicals rather than as a confirmation of the usability of the biological test system.

Because international consensus has been reached on OECD Test Guidelines 430 and 431, we welcome that the wording of these Guidelines has been used unchanged also in the ICCVAM MPS documents. **However, ZEBET is opposing the additional mandatory requirement to test a**

larger set of chemicals with the TER and Skin Model Corrosion Test, since it results in mandatory re-validation of validated methods.

If testing a new skin model or a modified TER technology provides correct and reproducible results for the 12 OECD Reference Chemicals, then there is no need for testing additional chemicals, if we accept the robustness and general applicability of the new corrosion methods.

However, if not all of the 12 OECD Reference Chemicals are correctly classified additional refinement work and additional data is needed (depending on whether it looks promising). In that case, a list of well selected and easily available chemicals like the ones defined in the MPS documents can be very helpful. **We therefore ask ICCVAM to accept the 12 OECD Reference Chemicals* and make it a mandatory requirement. The second set of 12 Test Chemicals should be recommended for test refinement when the 12 OECD Reference Chemicals have not 100% correctly been classified**.

(* ICCVAM has deleted one of the twelve OECD Reference Chemicals (Acrylic Acid) from the list, because this was not included in the ECVAM Validation studies. However, the OECD experts had intentionally selected this chemical as a challenge for the skin model test, because it has a clear *in vivo* database as a strong corrosive.)

To emphasise our statement I can inform you that ZEBET and L'ORÉAL are currently very successfully co-operating on the generation of a common skin model test for *Skin Irritation Testing* that can be applied both to EPISKIN and EpiDerm models and that provides the same results in both models.

We do not comment in detail on the MPS document of the third Skin Corrosion Test (Barrier Test), since the situation is totally different: Because no OECD Test Guideline has been adopted, the ICCVAM MPS on the Barrier Test is not in conflict with international consensus. Moreover, to date the Barrier Method is still more a "black box" than the well validated and characterised skin models. Therefore, we support the definition of a sufficient number of reference chemicals, as suggested by the MPS document.

We do hope ICCVAM re-considers the TER and Skin Model MPS documents accordingly

On behalf of ZEBET

Sincerely yours

Dr. Manfred Liebsch

PS: We would like to put your attention to a few minor points (typos etc.):

Skin Model MPS:

Page 3, 3rd para: Although historically EpiDerm has been validated as an alternative to EPISKIN because it was not available any more, it was the catch up validation concept, only to show that EpiDerm was equivalent to EPISKIN. Delete that sentence, as EPISKIN is available again.

Page 4, 3rd para: Change reference (221) into (22)

Page 6, 4th para: Delete "cell"

Page 10, Table 2: As a strong MTT reducer that accumulates in the tissues n-Heptylamine is now correctly classified in all skin models (including SkiEthic), if the killed tissue control procedure is applied (see paragraph 15 of TG 431 and Liebsch et al ATLA 28, 371-401, 2000)

ENCLOSURE

ATLA **25**, 483–484, 1997

Editorial

Defined Structural and Performance Criteria would Facilitate the Validation and Acceptance of Alternative Test Procedures

The developers of new test procedures tend to want them to be tightly defined, so that they can gain their specific acceptance in the face of real or imagined competition, either for commercial reasons or to ensure that they gain the personal recognition they may deserve. However, it has become clear that this attitude is not in the interests of *in vitro* toxicology in general and may delay, or even prevent, the acceptance and application of scientifically relevant and reliable new approaches.

Three examples will illustrate the point. Firstly, Advanced Tissue Sciences withdrew their reconstituted human skin product, Skin^{2TM}, from the market, *after* it had been accepted by the US Department of Transport as a basis for classifying chemicals in terms of their skin corrosivity. Secondly, the withdrawal of Skin^2 and of EPISKIN^{TM}, a similar product made by Imedex, took place *during* a formal international study on *in vitro* tests for skin corrosivity, funded by ECVAM. Thirdly, Skin^2 was also in the process of being evaluated in the EU/COLIPA international validation study on *in vitro* tests for photoirritancy. As in the case of the withdrawal of a human skin product by Organogenesis a few years earlier, these developments led to annoyance and frustration since, whatever the manufacturers themselves had invested, and while one must sympathise with them, many other companies and laboratories had themselves invested considerable time and effort in evaluating the use of these systems for their own particular purposes. The results they had obtained had been most encouraging, which added to their sense of frustration.

This kind of problem could be avoided if, rather than validating and accepting particular kinds of commercial products, or methods involving particular cell lines, endpoints or endpoint assays, clearly laid down structural and performance criteria were to be defined and agreed for test systems to be used for particular purposes, then themselves subjected to prevalidation and formal validation. Any new test system which could meet these criteria would then be considered to be scientifically valid and acceptable, albeit after a small and independent confirmatory study in some circumstances.

It is for this reason that ECVAM and ZEBET are supporting studies on the applicability for *in vitro* corrosivity and photoirritancy testing of another human reconstituted human skin equivalent, EpiDerm^{TM}, made by MatTek, which, happily, promises to survive longer than its competitors. We are using our experience with Skin^2 and EPISKIN to speed up the acceptance of EpiDerm, not because we have any particular interest in MatTek or its products, but because we do not want much valuable experience to be wasted or the undoubted promise of this kind of test system to be lost.

At the same time, in order to provide one possible route of escape from the current impasse in the case of the acceptance of *in vitro* systems for percutaneous absorption, ECVAM has commissioned a study to define the structural and performance criteria which would be needed in such systems. Clearly, the structural characteristics required would include an effective barrier sufficiently similar to that found in the skin *in vivo*, and the performance criteria would include an ability to prevent the passage of certain standard test materials, while permitting the passage of others. Ideally, some of the *in vitro* systems should have the capacity to metabolise those kinds of test materials which would be likely to be metabolised by the human skin *in vivo*.

This having been done, ECVAM would be willing to support a prevalidation/validation study on *in vitro* systems which might meet the structural and performance criteria defined for percutaneous absorption testing.

This approach could be linked to the benchmarking concept as a possible route of escape from another impasse, namely, the absence of sufficient chemicals representative of the spectrum of chemicals to be tested in terms of type and scale of toxicity, backed by knowledge of sufficiently high quality. For example, an appropriate, and relatively small, set of standard materials which met *these* criteria, could be used to provide a standard curve, not only to establish the performance of the system on a particular occasion, but also as a means of expressing the result of the test on a novel material under investigation.

The structural and performance criterion approach could be taken further, since new tests could be developed to provide knowledge which is needed (i.e. to provide what Björn Ekwall has called "missing tests"). For example, it would be much more intelligent to devise realistic new tests for identifying *human* carcinogens, rather than merely speeding up the rodent bioassay or finding alternative methods for identifying chemicals which might be carcinogenic at high doses in *rodents*.

Michael Balls