

Evaluation of two In vitro Testing Methods for Determining Skin Irritation and Corrosivity for Johnson & Johnson.

Prepared by: Jenna *****
April 17, 2003

This report compares CORROSITEX[®] and EpiDerm[™] as replacement tests for skin irritation and corrosivity testing in Johnson & Johnson Laboratories. These two in vitro tests will be evaluated on accuracy, feasibility and cost.

Executive Summary

The Neutrogena Corporation, a health-care division of Johnson & Johnson, is one of the world's leaders in supplying quality health and beauty products. When developing new products, chemicals must be tested for potential skin irritation or possible skin corrosion. In the past, albino rabbits were used to do this testing, however with increased ethical pressures both within the toxicology industry as well as outside, in vitro tests must replace traditional in vivo testing.

Both CORROSITEX[®] and EpiDerm[™] were found to be accurate methods for determining skin corrosion, and both are recommended by regulatory agencies for that function. CORROSITEX[®] is purely for skin corrosion testing, while EpiDerm[™] can test for both corrosion and irritation, and CORROSITEX[®] is limited in the chemicals it is recommended to test for. However CORROSITEX[®] has had longer use in industry than EpiDerm[™] and is accepted by more regulatory agencies.

Both tests had high reproducibility between laboratories and within the same lab. EpiDerm[™] requires more maintenance by the laboratory to maintain living cells, while CORROSITEX[®] does not have a crucial shelf life until after the protein bio-barrier has been made. EpiDerm[™] is also more cost efficient, with 24-tissue samples, necessary media and well plates included for \$750, compared with only a 2-sample test kit at \$759 with CORROSITEX[®].

It was concluded that EpiDerm[™] human-skin model is the better choice initially. The laboratory can conduct the desired assays on the chemicals in question to determine their level of irritation or possible corrosion potential. If the substance is a suspect for possible skin corrosion, then the CORROSITEX[®] model can be purchased, assuming the chemical being tested is one of the recommended compounds to be tested with. Specific recommendations are given for the toxicology laboratories at Neutrogena Co. of Johnson & Johnson to follow.

Table of Contents

Executive Summary	ii
List of Visuals	iv
Introduction	pg. 1
Solution Criteria	pg. 2
<i>Accuracy</i>	pg. 2
<i>Feasibility</i>	pg. 2
<i>Cost</i>	pg. 3
Evaluation of CORROSITEX[®]	pg. 3
<i>Background</i>	pg. 3
<i>Accuracy</i>	pg. 4
<i>Feasibility</i>	pg. 5
<i>Cost</i>	pg. 6
Evaluation of EpiDerm[™]	pg. 7
<i>Background</i>	pg. 7
<i>Accuracy</i>	pg. 8
<i>Feasibility</i>	pg. 9
<i>Cost</i>	pg. 9
Conclusions	pg. 10
Recommendations	pg. 12
References	pg. 13
Glossary	pg. 14

List of Visuals

Figure 1: Color change observed when corrosive substance identified (5) - *pg. 4*

Table 1: Costs for in-lab testing or purchasing CORROSITEX[®] testing kit (5) – *pg. 6*

Figure 2: Histological slide of EpiDerm[™] human-skin model at 400X high magnification (6) – *pg. 8*

Table 2: General comparison of the Rat Skin TER Assay, EpiSkin[™], EpiDerm[™] and CORROSITEX[®] assays from ECVAM and ICCVAM (6) – *pg. 10*

Introduction

There is a growing need in the toxicology industry to reduce, refine and replace traditional animal-based testing methods with alternative tests that do not require the use of animals. As our world becomes more technologically advanced, it seems to many that there is no excuse to continue to use animals when we can synthesize these materials on our own. As humans we are continually trying new ways to have healthier looking skin, less wrinkles and a clear complexion. Companies like Neutrogena Corporation, a division of Johnson & Johnson, are constantly researching new compounds that will do just that. Neutrogena produces a variety of skin care items such as soaps and lotions, often medicated, as well as an entire line of cosmetics. Being one of the leaders in the health and beauty industry, they are under immense pressure to produce a quality product, while maintaining an ethically sound business.

Skin corrosion testing has typically been done using the skin of albino rabbits. However in recent years, several alternative tests have been developed and approved by both the US government and regulatory agencies overseas to replace animals. Two such models are CORROSITEX[®] and EpiDerm[™]. Both of these models offer a synthetic substrate to test potentially corrosive chemicals on, and are simply purchased from the manufacturer which eliminates the costs of having to maintain live animals. While both these methods pose an ethical solution to the problem, there are several differences between the two products. Is CORROSITEX[®] or EpiDerm[™] a more feasible solution for Johnson & Johnson to include in their chemical testing laboratories?

Using information from both products' websites, as well as comparison studies done by the European Centre for Validation of Alternative Methods (ECVAM) and the

Interagency Coordinating Committee on Validation of Alternative Methods (ICCVAM), this report will recommend a mode of action to the skin care division of Johnson and Johnson on which alternative skin corrosion should be used. This report will compare accuracy, feasibility and cost in making this recommendation, and provide specific steps to follow in solving this problem. Visual documentation will be provided to emphasize important points, and a glossary will also be included to clarify foreign terminology.

Solution Criteria

When choosing alternative testing methods to be used in a laboratory, a number of criteria must be taken into consideration. These criteria are outlined here in order of importance.

Accuracy

The most important factor to take into consideration when choosing an in vitro test is accuracy. Alternative testing is a relatively recent development in the toxicology industry, and the data acquired up until this point on various chemical compounds has been gathered through using animals. A chosen method to replace traditional animal tests must be consistent with previous data, as well as proven to be for use in future research. Without accuracy, there is no point in a major and well-respected corporation such as Johnson & Johnson considering the use of the test.

Feasibility

The second most important criterion for deciding on an in vitro toxicology test is feasibility. The test must be user friendly, fast and efficient. In today's competitive market for health and beauty products, the producer must be able to research new compounds and put the products on the market ahead of the competition. Testing methods must be accurate, but timesaving as well.

Cost

Cost is the third component when deciding on an alternative testing method. In no way am I implying that cost is unimportant when choosing amongst different alternative testing methods. However, with a company as large and profitable as Johnson and Johnson, cost is not as much of an issue as it would be for a smaller research company on a limited budget. Also, a company as prominent as Johnson & Johnson cannot justify cutting costs at the expense of an accurate test when it comes to human health.

Evaluation of CORROSITEX

This section will explain the methodology behind CORROSITEX[®], and apply each of the above criteria to the testing method.

Background

CORROSITEX[®] is an in vitro test that determines corrosivity of a chemical or mixture of chemicals and assigns it a packing group classification, or a category of corrosivity, for labeling purposes according to United Nations guidelines (5). It attempts to mimic the traditional in vivo testing method of using the skin of albino rabbits.

InVitro International produces CORROSITEX[®], and one can either send in the sample to their laboratories for analysis, or purchase the kits themselves. CORROSITEX[®] consists of a glass vial filled with a chemical detection fluid and a bio-barrier membrane caps the fluid (5). The bio-barrier membrane is designed to mimic the effects of corrosive chemicals on living skin. When the chemical destroys this bio-barrier, the chemical detection fluid below will change color, as seen in Figure 1.



Figure 1: Color change observed when corrosive substance identified (5).

The time that it took for the chemical to break the bio-barrier membrane is what the chemical's corrosivity classification is based upon.

Accuracy

CORROSITEX[®] has been validated and accepted by a number of European and US agencies, such as the US Department of Transportation, Consumer Product Safety Commission, European Coordinating Committee for the Validation of Alternative Methods, Food and Drug Administration and National Institute of Environmental Health Sciences. Canada's Department of Transportation in 1996 gave the earliest acceptance for the test (5).

Many studies have been conducted both by private organizations as well as regulatory agencies in validation procedures. In a study conducted by Cassidy and Stanton of Dow Corning Toxicology Laboratory, CORROSITEX[®] was tested on ten organosilicone products. Organosilicone products, in particularly liquid silicone products, are often used in skin care products due to the silky feeling they leave behind. Out of the ten chemicals tested, CORROSITEX[®] correctly identified five as corrosive. One compound was identified as corrosive when previous in vivo studies identified it as mildly irritating. CORROSITEX[®] did not qualify to test four out of the ten compounds (1). The study concluded that CORROSITEX[®] performed well in detecting both corrosive and noncorrosive chemicals. It performed well as far as sensitivity is concerned, in assigning the appropriate degree of corrosivity, and correctly identifying corrosive compounds, yet was somewhat lacking in overall performance and specificity, as in identifying noncorrosive compounds (1). This data correlated well with previous studies conducted on CORROSITEX[®] using both organic and inorganic compounds. CORROSITEX[®] identified corrosives with 96% accuracy, and noncorrosives with 81% accuracy (1).

Another factor that will influence accuracy is similarity to human skin. CORROSITEX[®] is based on a synthetic protein gel matrix. While this is designed to have certain characteristics of living tissue, it may not living epithelial tissue in all cases. Refer to Table 2 for a comparison of CORROSITEX[®] to EpiDerm[™] and some other in vitro skin toxicology testing methods.

Feasibility

In the ECVAM's validation studies of skin corrosivity tests, an advantage that CORROSITEX[®] had over other tests was its high reproducibility within the laboratory and between laboratories (3). This means that different laboratories and different users within the same laboratory could use CORROSITEX[®] and the results were consistent each time. This means that not only is CORROSITEX[®] user friendly, but it takes away the variability of human error in the testing. Besides being relatively easy to use, it is also fast. CORROSITEX[®] can provide a corrosivity level in as few as three minutes, and no longer than four hours. If the sample is sent to their laboratories, InVitro International will provide results in 7 working days, and require a small amount of sample, 3 grams of solid material and less than 10 ml of liquid. The sample kits come in 2 or 4 sample packages. The bio-barriers can be pre-made by the company prior to shipping, or the user can make them when needed. Once the bio-barrier is made, the kit must be used within 5 days for the results to be valid.

Costs

InVitro International sends out testing kits to laboratories, and will also run the tests in-house if a lab chooses to send in their samples. Lab services prices vary depending on the number of samples sent. Testing of one sample is \$795, and the price charged per sample drops as the number of samples sent increases. Testing kits come in quantities of 2 or 4 samples per kit. The 2-sample kit is \$795, and the 4-sample test kit is \$1195 (5). Refer to Table 1 for a complete price listing. Clearly, with the large volume of sampling that Johnson & Johnson will be conducting in skin corrosivity testing,

sending samples to InVitro International for testing is not cost efficient. Sending in four samples at \$595 each would total \$2380, yet a 4-sample testing kit costs \$1195.

Purchasing the test kits directly will be more cost efficient for Johnson and Johnson. Due to the user-friendly nature of the test, additional costs for labor will not be a factor, and the company will reduce costs by eliminating the costs of caring for laboratory animals.

In Lab Testing	Price	Testing Kits	Price
One Sample	\$795	2-sample kit	\$795
Samples 1,2,3, 4	\$595 ea.	4-sample kit	\$1,195
Samples 5-9	-10%	Pre-made biobarrier	\$50/ set of 2
Samples 10+	-15%		

Table 1: Costs for in lab testing or purchasing CORROSITEX[®] testing kit (5).

CORROSITEX[®] has been demonstrated to provide accurate results, however testing a limited number of compounds. The ICCVAM and the ECVAM both concluded that CORROSITEX[®] is valid for testing organic bases and inorganic acids (2).

CORROSITEX[®] is fast, efficient and requires little or no special accommodations to be included in a laboratory toxicology testing profile. However, the cost is high for the number of samples included in the kit. Submitting samples to the laboratory is out of the question as well as buying the pre-made bio-barriers. The research staff at Johnson & Johnson is capable of making the bio-barriers and with the high volume of testing being done, submitting samples to InVitro International is too costly.

Evaluation of EpiDerm™

EpiDerm™ is a testing kit designed for both skin irritation and corrosivity testing, and it will be subject to the same criteria as well as CORROSITEX® was.

Background

EpiDerm™, manufactured by MatTek Corporation in Ashland, MD, is a living model of human skin. The sample consists of normal, human-derived epidermal keratinocytes cultured to form a multi-layered, highly differential model of the human epidermis (6). It is metabolically and mitotically active, and exhibits morphological and growth characteristics seen in living human skin. Refer to Figure 2 for a histological depiction of the tissue. The human-derived epidermis allows for a number of testing assays to be used. The most common assays are MTT dye-metabolism assay of cell viability, measurement of enzymes released, and inflammatory cytokine expression. These are all indicators of cell damage and allow for quantifiable, less subjective endpoints, as opposed to basing results on observed skin changes (4). Based on ECVAM validation studies, in August 2002 the ICCVAM concluded that EpiDerm™ can be used to assess dermal corrosivity potential of chemicals and chemical mixtures. Any positive results will not require further testing, and the results can be used for classification of the compound (6).

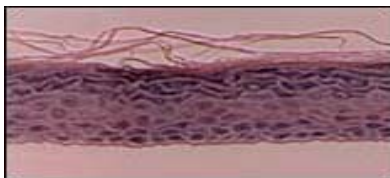


Figure 2. Histological slide of EpiDerm™ human-skin model at 400X magnification (6).

Accuracy

A study conducted by the ECVAM in 2000 evaluated EpiDerm™ and several other human skin models as potential replacement tests for skin corrosion tests in the future. EpiDerm™ demonstrated the best sensitivity (92%) and the lowest rate of false negatives (8%) of any of the models tested (6). Table 2 goes further in depth into the accuracy of EpiDerm™ and compares it to several other in vitro skin toxicology testing methods, including CORROSITEX®.

Several other studies have been conducted comparing EpiDerm™ skin irritation results to known results derived from testing on human skin ex vivo. One such study, conducted by Proctor & Gamble in 1999, evaluated the ability of EpiDerm™ to test common health and beauty products; surfactants, cosmetics, antiperspirants and deodorants. EpiDerm™ distinguished the surfactants irritancy rank and group similar to human skin-patch test results with the misranking of two compounds. The MTT dye-metabolism was used, in which a purple color means the cell is still viable, and a yellow color means the cell is no longer living. The antiperspirant and deodorant studies were based on quantifiable and objective observations. MTT cell viability assays as well as enzyme release tests distinguished between mild and moderately irritating compounds. The inflammatory cytokine expression assay was found to also correctly assess irritating cosmetics compounds as compared to human studies (4).

Feasibility

A prevalidation study conducted on EpiDerm™ along with other human skin models in 2000 found that EpiDerm™ had the best intralaboratory and interlaboratory reproducibilities (2). This speaks not only for the consistency of the results produced, but

also of the consistency of the product being produced by MatTek. However, being essentially living tissue, there is more work that is involved in maintaining these testing substrates than with other tests. The EpiDerm™ tissues must be stored at 4°C, and must be used within 48 hours of storage. MatTek also sells the MTT dye-metabolism assay kit, which must be stored at -20°C (6). Both the EpiDerm™ kit and the MTT assay kit provide all the necessary solutions and materials necessary to perform the test. Any other materials, such as acids or bases to adjust pH or different volume measuring devices, are all compounds that the lab will already have available. One major advantage to using EpiDerm™ is the wide variety of forms the materials to be tested can be in. Solids, semi-solids and liquids can be applied to the sample skin with out any negative effects on the results (4).

Costs

The entire EpiDerm™ test kit costs \$750, and the MTT dye-metabolism assay costs \$35. The EpiDerm™ test kit comes with 24-tissue cultures, along with several rinse solutions and maintenance media, and two 24-well testing plates. The tissues have also been screened for any diseases such as HIV or Hepatitis. The kits are sent on Mondays via FedEx priority mail. The cost of shipping is approximately \$12 for most locations in the US. While MatTek does to their own testing with EpiDerm™, it is done for smaller companies by special request (6).

EpiDerm™ is clearly and accurate method for testing chemicals on skin. The fact that is an accurate replica of living human tissue adds to the reliability of these results. EpiDerm™ can also be used to assess the skin irritation, which is not as easily observed

as skin corrosion. Because it is living tissue, there is more maintenance required to upkeep the samples, but the large amount of materials included in the testing kit make testing somewhat easier and worth the cost of the kit.

	Rat Skin Ter	EPISKIN™	EpiDerm™	CORROSITEX®
Number of Chemicals	122	60	24	163
Overall Sensitivity ID positives correctly	94% (51/54)	82% (23/28)	92% (11/12)	85% (76/89)
Overall Specificity ID negatives correctly	71% (48/68)	84% (27/32)	83% (10/12)	70% (52/74)
Overall Accuracy	81% (99/122)	83% (50/60)	92% (22/24)	79% (128/163)
False Positive Rate	29% (20/68)	16% (5/32)	17% (2/12)	30% (22/74)
False Negative Rate	6% (3/54)	18% (5/28)	8% (1/12)	15% (13/89)

Table 2: General comparison of the Rat Skin TER Assay, EpiSkin™, EpiDerm™ and CORROSITEX® assays from ECVAM and ICCVAM studies (6)

Conclusion

Based on the evaluation of accuracy, feasibility and costs associated with CORROSITEX® and EpiDerm™ in vitro skin cytotoxicity testing methods, the following conclusions have been made:

1. EpiDerm™ and associated assays is the best choice as far as accuracy is concerned. EpiDerm™ performs better in correctly identifying corrosive chemicals and has the least chance of falsely labeling compounds corrosive or noncorrosive. Due to the high similarity of EpiDerm™ to human tissue, the results given using EpiDerm™ can be better applied to human situations.

2. EpiDerm™ can also be used to assess skin irritation of many different health care compounds with fairly correct accuracy. CORROSITEX®, however, can only be used to assess skin corrosion.
3. Both in vitro tests have excellent reproducibility that speaks for the accuracy of the testing methods as well as the quality of the product. You can also administer test chemicals in a variety of forms with EpiDerm™, where as with CORROSITEX® you are limited to liquid compounds. However, EpiDerm™ requires more maintenance in the laboratory to keep the living tissue viable.
4. EpiDerm™ is also the cheaper option. At \$750 for 24 tissue samples, you are essentially getting more for your money than with \$795 for a 2-sample test kit.
5. CORROSITEX® has a longer history of use for detecting the corrosivity of compounds and has been accepted and approved by more regulatory agencies. The endpoint for the CORROSITEX® assay is complete tissue destruction, observed by the destruction of the bio-barrier and the color change. With EpiDerm™, while there are some quantifiable endpoints for irritation detection and corrosion, a considerable amount of data is also based on subjective observations of the extent of damage to the tissue.
6. Lastly, CORROSITEX® has only been recommended for use on acids, bases and their derivatives by the ICCVAM. At this point EpiDerm™ has no limitations on the types of compounds used.

Recommendations

EpiDerm™ is the better solution for Neutrogena to incorporate into their in vitro testing methods for skin irritation or corrosivity. If a compound is deemed corrosive through tests with EpiDerm™, further corrosivity tests must be conducted to assure this result. If the compound in question is an acid, base or a derivative, CORROSITEX® would be an appropriate test to choose. The following steps should be taken:

1. Purchase as many EpiDerm™ test kits from MatTek Corporation as necessary to assay the test chemicals in question.
2. Purchase the MTT cell viability assay testing kit as well.
3. Perform the MTT assay and any other desired tests using the EpiDerm™ human-skin model.
4. If the chemical in question is deemed to have possible corrosion potential and is either an acid, base or a derivative of either two, follow up testing with CORROSITEX®.
5. If the chemical in question is not one of the recommended types of chemicals for CORROSITEX® use, look into other skin corrosion testing methods.

References

- (1) Cassidy SL, Stanton ES. In Vitro Skin Irritation and Corrosivity Studies on Organosilicon Compounds. *J. Toxicol. Cut. & Ocular Toxicol* 1996; 15(4): 355-67.
- (2) Fentem JH, Botham PA. ECVAM's Activities in Validating Alternative Tests for Skin Corrosion and Irritation. *Altern. Lab Anim.* 2002 Dec. 30(Suppl): 61-7.
- (3) Knight DJ, Breheny D. Alternatives to Animal Testing in the Safety Evaluation of Products. *Altern. Lab Anim.* 2002 Dec. 30(1): 7-22.
- (4) Perkins MA, Osborne R, Fuz RR, Ghassemi A, Robinson MK. Comparison of *in Vitro* and *in Vivo* Human Skin Responses to Consumer Products and Ingredients With a Range of Irritancy Potential. *Tox Sci.* 1999 Apr. 48(2): 218-29.
- (5) InVitro International's homepage [Internet]. California: InVitro International; c 2002. [cited 2003 Apr. 16]. Available at: <http://www.invitrointl.com/>
- (6) MatTek Corporation's homepage [Internet]. Maryland: MatTex Corporation. [cited 2003 Apr. 16]. Available at: <http://www.mattek.com/>

Glossary

1. in vitro – a test performed in an artificial environment outside the living organism.
2. in vivo – a test performed on or within a living animal.
3. skin irritation – a local tissue reaction that is reversible; redness and swelling are observed.
4. skin corrosion – direct, irreversible damage to the tissue; ulceration and scar formation can be observed.
5. cytotoxicity – producing a toxic effect on the cells.
6. cytokine – proteins released by immune cells that function in stimulating and regulating an inflammatory response.