DB-ALM Protocol n° 116 : CORROSITEX® Continuous Time Monitor Assay

Skin Irritation and Corrosivity

The corrosivity potential of a chemical may be predicted by measurement of its penetration through a calibrated biobarrier into a chemical detection system. *(Validation study protocol)*

Objective & Application

TYPE OF TESTING	:	screening, replacement
LEVEL OF ASSESSMENT	:	toxic potential, toxic potency, hazard identification
PURPOSE OF TESTING	:	classification and labelling

The test method was granted regulatory approval as a replacement for the *in vivo* skin corrosivity test for specific classes of chemicals, such as acids, bases and their derivatives which meet the technical requirements of the assay, and to rank them with respect to their degree of corrosive effect as permitted in the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (OECD Test Guideline 435, OECD, 2006).

Corrositex® can be used to test liquids (aqueous and non-aqueous), solids (soluble and insoluble in water), emulsions, pure chemicals, dilutions formulations and waste (OECD, 2006). Many aqueous substances with a pH within the range of 4.5 - 8.5 are incompatible for testing with Corrositex®, therefore, prior to the test, compatibility test should be performed (OECD, 2006). However, it is estimated that 85% of solutions with a pH within this range are non-corrosive in animal tests (ICCVAM, 1999).

Résumé

Most international regulatory classification schemes define chemically induced dermal corrosion as full thickness destruction (necrosis) of the skin tissue, while some extend the definition of corrosion to include any irreversible alterations caused to the skin. The potential to induce skin corrosion is an important consideration in establishing procedures for the safe handling, packing and transport of chemicals.
The determination of the skin corrosion potential is therefore included in international regulatory requirements for the testing of chemicals, such as the U.S. Code of Federal Regulations (US DOT, 1991), the updated OECD Test Guideline No 404 (OECD, 2002) and the Method B.4 of the Annex to Commission Regulation 440/2008/EC (EU, 2008). Corrosivity is usually determined *in vivo* using the Draize rabbit skin test (Draize et al., 1944).

Corrositex® (*In Vitro* International, Irvine, CA, USA) was originally developed to predict the skin corrosivity potential of chemicals and to rank them with respect to their degree of corrosive effect (typically to assign chemicals to UN packing groups) (Botham *et al.,* 1995).
Corrositex® is a standardised, quantitative *in vitro* test for skin corrosivity, based upon determination of the time which is required for a test material to pass through a biobarrier membrane (a reconstituted collagen matrix, constructed to have physico-chemical properties similar to rat skin), and produce a visually detectable change.
The time required for this change to occur (the breakthrough time) is reported to be inversely proportional to the degree of corrosivity of the test material, i.e. the longer it takes to detect a change in the Chemical Detection System (CDS), the less corrosive is the substance.

Experimental Description

Endpoint and Endpoint Measurement:

PASSAGE THROUGH A BIOBARRIER: Penetration of the test material through a calibrated biobarrier, as reflected in its effects on the colour of a chemical detection system (CDS)

Endpoint Value:

BREAKTHROUGH TIME: time required for a test material to pass through a biobarrier membrane (a reconstituted collagen matrix, constructed to have physico-chemical properties similar to rat skin)

Experimental System(s):

CORROSITEX® BIOBARRIER MEMBRANE: reconstituted collagen matrix, constructed to have physico-chemical properties similar to rat skin

Basic Procedure

The compatibilities of the chemicals with the test kit have to be determined in a "qualification screen" (i.e. whether they possessed the chemical properties that enabled them to be detected by the CDS). Test material (150µl or 100 mg) was added to the "Qualify" test tube. If it failed to produce a colour or physical change within 5 min, it did not qualify for further testing with Corrositex ® and was recorded as "non-qualified"(NQ).

A "categorization screen" was used to enable the test chemical to be measured against the appropriate scoring scale. Chemicals are of high acidity/alkalinity (category 1) or low acidity/alkalinity (category 2). Membrane discs coated with the biobarrier matrix are placed into vials containing the CDS, which is located directly below the biobarrier.
Samples of the test material (solid or liquid) are placed onto the discs. The vials are observed for 3 minutes and the time of any colour change is noted. If no colour change has occurred, the vials are observed at regular intervals until a change does occur, to a maximum of 4 hours for category 1 and 1 hour for category 2 materials. The time of the change is recorded.

Data Analysis/Prediction Model

The classifications of the tested compounds, EU Risk Phrases (R35/R34/no label) and UN Packing Group classifications (I/II/III), are determined on the basis of the average time taken for the test material to produce a change in the CDS.
The respective classification tables [provided by In Vitro International with the approval of the U.S. Department of Transportation (Exemption DOT-E 10904 revision 1)] are reported in detail under section 7, "Evaluation of Test Results" of the present standard operating procedure.

Test Compounds and Results Summary

A total of 60 test compounds, consisting of 11 organic acids, 10 organic bases, 9 neutral organics, 5 phenols, 7 inorganic acids, 4 inorganic bases, 3 inorganic salts, 8 electrophiles, 3 soaps/surfactants were tested in the ECVAM validation study (Barratt *et al.*, 1998) .

Status

Participation in Validation Studies:

During 1999 the **US Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)** with support from the **National Toxicology Program (NTP) Interagency Centre for the Evaluation of Alternative Toxicological Methods (NICEATM)** sponsored a subsequent independent scientific review of Corrositex®. Consequently, an independent Peer Review Panel (PRP) was established. The results of the evaluation were that the test is limited in its utility because of the proportion of chemicals that are not compatible with the CDS and thus can not be evaluated. In contrast to the US DOT, the PRP excluded the use of Corrositex® for the detection of some chemical classes such as acyl halides, chlorosilanes, metal halides and oxyhalides. For other chemical classes the assay may be used as part of a tiered assessment strategy. In this case, negative responses must be followed by dermal irritation testing, and positive responses require no further testing.
ICCVAM issued a report endorsing the use of the Corrositex® for assessing the dermal corrosive potential of new chemicals and concluded that in specific testing circumstances Corrositex® is useful as a stand alone assay for evaluating the corrosivity or non corrosivity of acids, bases and acid derivatives (ICCVAM, 1999).

On the basis of these recommendations, the US Environmental Protection Agency, the Occupational Safety and Health Administration and the Consumer Product Safety Commission have recently agreed to accept the use of the Corrositex® skin model test as a replacement for the animal test for skin corrosivity (Anon., 2000).

After prevalidation (1993-1994) (Botham *et al.*, 1995), this method was also evaluated in the **ECVAM International Validation Study on *In Vitro* Tests for Skin Corrosivity**, conducted from 1996 to 1997 (Fentem *et al.*, 1998) and the test showed an acceptable intralaboratory and interlaboratory reproducibility.
Although the test did not meet all of the criteria set by the Management Team for it to be considered

acceptable as a replacement test, it was concluded that the test may be valid for testing specific classes of chemicals, such as organic bases and inorganic acids (Fentem *et al.,* 1998).

Furthermore, in 2000, the **ECVAM Scientific Advisory Committee (ESAC)** unanimously endorsed the statement that the Corrositex® assay is a scientifically validated test, but only for those acids, bases and their derivatives which meet the technical requirements of the assay (ESAC, 2001).

Regulatory Acceptance:

Corrositex® has been granted regulatory approval by the US DOT (Gordon *et al.*, 1994), in the form of an exemption for the detection of corrosives, such that a positive result in this non-animal test enables the chemical to be classified as a corrosive without this being confirmed in the standard animal procedure (Exemption DOT-E 10904 Revision 1). The US DOT limits the use of Corrositex® to specific classes of chemicals, including acids, acid derivatives, acyl halides, alkylamines and polyalkylamines, bases, chlorosilanes, metal halides and oxyhalides (ICCVAM, 1999).

In 2006, *In Vitro* Membrane Barrier Test Method for Skin Corrosion was adopted as the OECD Test Guideline No 435, which is applicable to Corrositex® (OECD, 2006).

Last update: December 2008

PROCEDURE DETAILS, 10 July 1996*

CORROSITEX® Continuous Time Monitor Assay

DB-ALM Protocol n° 116

this protocol presents the standard operating procedure used in the ECVAM International Validation Study on In Vitro Tests for Skin Corrosivity (Fentem et al., 1998).

* The accuracy of the SOP has been confirmed in October 2000.
During the preparation of the regulatory test guidelines some refinements have been introduced into the test method. Therefore, the proposed update of the SOP has been sent to the person responsible for the method for review and can be provided on request. As soon as new information will become available this version of the protocol will be updated.

Contact Details

Dr. Rodger Curren
Institute for In Vitro Sciences, Inc.
30 W. Watkins Mill Road Suite 100
Gaithersburg MD 20878
email: rcurren@iivs.org
telephone: +1 (301) 947 6523

1. PURPOSE

The potential corrosivity of a test material is assessed by measuring its penetration through a calibrated biobarrier into a chemical detection system.

2. SAFETY PRECAUTIONS

The materials to be tested are potentially corrosive. Handle every compound as if it were corrosive, according to the relevant safety regulations of your laboratory or national authority.

3. CONTROLS

Positive control	:	sodium hydroxide (solid/pellets)
Negative control	:	blank

4. TEST SYSTEM

The Corrositex® assay is a standardised and quantitative *in vitro* corrosivity test[1,2]. It is based on the time that is required for a test material to pass through a biobarrier membrane and produce a change in a Chemical Detection System (CDS). The Corrositex® Biobarrier Membrane consists of a reconstituted collagen matrix.

[1] The Corrositex® Instruction Manual. Directions for Use. InVitro International, Irvine, California.

[2]Application for Exemption, 49 CFR 173.136 & 173.137. InVitro International, Irvine, California. Submitted to US DoT, September 1992.

5. EXPERIMENTAL DESIGN AND METHODOLOGY

The experimental design consists of: (i) a qualification screen with the CDS; (ii) a categorisation screen to identify materials as having either a high or low acid/alkaline reserve; and (iii) two definitive Corrositex® assays.

The endpoint of the Corrositex® assay is a colour change in the CDS, which indicates that the test material has penetrated ("broken through") the biobarrier. The time at which a colour change is observed and recorded manually, and the average break-through time of the four replicates is used to determine whether the test material is corrosive and, if it is corrosive, the UN Packing Group and EU Risk Phrase designations.

5.1 Reagents and Materials

i) Corrositex® Biobarrier Matrix Powder: supplied by InVitro International

ii) Corrositex® Biobarrier Diluent: supplied by InVitro International

iii) Chemical Detection System (CDS): supplied by InVitro International

iv) membrane discs: supplied by InVitro International

v) deionised water

vi) Categorisation Screening Kit: buffered solutions containing acid or base indicator dyes. The Categorize A solution is composed of 0.1M (Na^+/ H^+)MOPSO and 10 mg/ml Methyl Red, pH 7.0. The Categorize B solution is composed of 0.05M (Na^+/ H^+)HEPES and 100 mg/ml *o*-cresolphthalein complexone, pH 7.0. Supplied by InVitro International.

5.2 Qualification Screen

For the qualification screen, 150µl or 100mg of the test material is added to the"Qualify" test tube. If the test material fails to produce a colour or physical change in the Qualify test within 5 minutes, it cannot be analysed with Corrositex®.

Recorded as "Qualified" (Q) or "Non-qualified" (NQ) on the Data Report Form (Appendix A).

5.3 Categorisation Screen

The categorisation screen is used to enable the test material to be measured against the appropriate scoring scale. Test materials having high acid/alkaline reserves are defined as Category 1 materials, while those with low acid/alkaline reserves are defined as Category 2 materials. The Category 1 test materials are scored by using the scheme in Table 1 (section 7 **"Evaluation of the test results"**), while Category 2 test materials are scored by using the scheme in Table 2.

The screen is performed by adding 150ml or 100mg of the test material to each tube (A and B). The tubes are then mixed and the resulting colours observed. The categorisation kit and colour chart provided by InVitro International are used to determine the category. If no colour change is observed in either tube, two drops of the "confirm" reagent are added to tube B; this is mixed and the resulting colour is used to confirm the category, recorded as Category 1 or Category 2 on the Data Report Form.

Any problems encountered with "borderline" cases or with coloured compounds should be noted in the "comments" section of the Data

Report Form (Appendix B). The vial may be centrifuged to remove the test material and thereby enable a colour assessment to be made, if required.

5.4 Preparation of Test Material

If the test material is a solid or a powder, four samples of approximately 500mg are pre-weighed. If the test material is a liquid, four 500µl aliquots are prepared immediately prior to its addition to the membrane disc. To avoid problems with low density test materials (where 500mg of the material is too much for the membrane disc), weighing vials are prepared which are marked at the approximate volume level at which the membrane disc is full. If the mark is reached before a weight of 500mg is attained, the amount in the vial is that which is used in the assay, and this weight is recorded on the Data Report Form.

5.5 Route of Administration

The test material is added directly to the membrane disc.

5.6 Controls

Each assay batch (group of test materials assayed at the same time by the same technician) includes a blank (colour) control and a positive control. Details of the lot number and kit information for each test kit used with each batch should be documented.

5.7 Biobarrier Preparation

The membrane discs are prepared the day prior to the assay and are refrigerated at 2-8°C overnight before use. The biobarrier is stable for 7 days if it is wrapped and stored at 2-8°C.

A scintillation vial containing the biobarrier matrix powder is placed in a water bath on a hot plate pad set at 68±1°C, with the stir switch set to maintain approximately 200rpm for the stir bar. The entire contents of the biobarrier diluent vial are added slowly to the matrix powder to ensure complete and uniform solubilisation. The solution is warmed to 64-68°C *[note: the mixture must always be kept below 70°]* to solubilise the biobarrier matrix. 200µl of the solubilised matrix is then pipetted onto the membrane discs.

5.8 Corrositex® Assay

For each assay, at least one vial for the positive control and one vial for the colour (blank) control is needed. Four replicate vials are used for each test material. A membrane disc coated with the biobarrier matrix is placed into a vial containing the CDS, and approximately 500mg or 500µl of the test material is added to the membrane disc.

Note:the vial is then observed for three minutes for any change in the CDS. The vial should always be observed against a solid white background (e.g. a large piece of white cardboard). The vial should be elevated, if necessary, so that it can be viewed straight on.

If no colour change is observed within three minutes, the application and observation is repeated, at one-minute intervals, until the remaining membranes (a total of four) have been treated with the test material. The vials are observed continuously for the first 10 minutes, and then at approximately 5-minute intervals for 4 hours (Category 1 test materials) or 60 minutes (Category 2 test materials), or until break-through of the

test material occurs. The first indication of the presence of the test material is detected as a change, either in colour or in physical appearance, in the CDS, compared to the blank control. The time of this change is recorded on the Data Report Form.

If test materials are run in a "batch", i.e. at the same time utilising the same positive control, this batch should be given a unique number which is recorded on the Data Report Form in association with each member of that batch.

The positive control vial is prepared as above, and receives one pellet (110±15mg) of sodium hydroxide. This vial is monitored continuously until break-through has occurred. The time is recorded in minutes and seconds (MM:SS).

Note: each test material should be tested twice; the qualification screen and the categorisation screen should also be undertaken on two separate occasions. The replicate test must be undertaken as part of a separate batch. Each test result should be recorded separately on the Data Report Form.

5.9 Data Analysis

The length of time it takes for the test material to produce a change in the CDS is recorded. The time should be recorded in minutes and seconds (MM:SS) up to 10 minutes, or to the nearest minute (MMM) if the break-through time is >10 minutes. The Packing Group and Risk Phrase designations are determined from the mean break-through times (see section 7 **"Evaluation of the test results")**.

6. CRITERIA FOR DETERMINATION OF A VALID TEST

The Corrositex® assay results are acceptable if the positive control time falls in the range 8-16 minutes. (This is generally within the range of the mean±2SD for the Lead Laboratory in the ECVAM Skin Corrosivity Validation Study, and encompasses the ranges found by the other participating laboratories during preliminary trials.)

If the positive control data are not within this range, the assay should be declared invalid and repeated. Data from invalid assays should be retained by the laboratory for potential future use, but should *not* be recorded on the Data Report Form.

7. EVALUATION OF TEST RESULTS

EU Risk Phrases (R35/R34/no label) and UN Packing Group classifications (I/II/III) are determined on the basis of the average time taken for the test material to produce a change in the CDS. The following classification tables [provided by InVitro International with the approval of the U.S. Department of Transportation (Exemption DOT-E 10904 revision 1)] are used:

Table 1 - Category 1 Test Materials

CORROSIVITY	PACKING GROUP	RISK PHRASE	MEAN TIME
Corrosive	I	R35	0 - 3 minutes

Corrosive	II	R34	>3 minutes -1hour
Corrosive	III	R34	>1 - 4 hours
Non-corrosive	Not applicable	No label	>4 hours

Table 2 - Category 2 Test Materials

CORROSIVITY	PACKING GROUP	RISK PHRASE	MEAN TIME
Corrosive	I	R35	0-3 minutes
Corrosive	II	R34	>3 minutes - 30 minutes
Corrosive	III	R34	>30 - 60 minutes
Non-corrosive	Not applicable	No label	>60 minutes

The mean break-through time, and Risk Phrase and Packing Group designations, are recorded on the Data Report Form.

APPENDIX A Data Report Form - ECVAM Skin Corrosivity Validation Study Data Sheet Number:_____

CORE DATA						SUPPLEMENTARY DATA		
Sample ID & Trial Number	Corrosive Rating (NC, R34, R35)	Physical Appearance	Qualified (Q/NQ)	Category (1 or 2)	Mean Breakthrough Time (MM:SS, <10min) (MMM, >10min)	Breakthrough Times of Replicate Vials (MM:SS,<10min) (MMM, >10min)	Batch #	Positive Control Time (MM:SS)

Performing Laboratory Code No.:

Study Director: _____ _____ _____

(print) (signature)

(date)

Assays Supervised by: _____ _____ _____

(print) (signature)

(date)

APPENDIX B Data Report Form - ECVAM Skin Corrosivity Validation Study Data Sheet Number: _____

SUPPLEMENTARY DATA	
Sample ID and Trial Number	Comments

Performing laboratory code N.:

Study Director: _____ _____ _____
(date) (print) (signature)

Assays supervised by: _____ _____ _____
(date) (print) (signature)

Bibliography

- Anon. (2000) News and Views. (2000) *Alternatives to Laboratory Animals (ATLA) 28, 363-364*
- Barratt M.D., Brantom P.G., Fentem J.H., Gerner I., Walker A.P. and Worth A.P. (1998) The ECVAM international validation study on *in vitro* tests for skin corrosivity.1. Selection and

distribution of the test chemicals.
Toxicology In Vitro 12, 471-482

- Botham P., Chamberlain M., Barret M. D., Curren R.D., Esdaile D.J., Gardner J.R., Gordon V.C., Hildebrand B., Lewis R.D., Liebsch M., Logemann P., Osborne R., Ponec M., Régnier J.-F., Steiling W., Walker A.P. and Balls M. (1995)
  A prevalidation study on in vitro skin corrosivity testing. The Report and Recommendations of ECVAM Workshop 6.
  Alternatives to Laboratory Animals (ATLA) 23, 219-255
- Draize J.H., Woodard G.K. and Calvery H.O. (1944)
  Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes.
  Journal of Pharmacology and Experimental Therapeutics 82, 377-390
- ESAC (2001)
  Statement on the Application of the CORROSITEX® Assay for Skin Corrosivity Testing, 15th meeting of ECVAM Scientific Advisory Committee, 5-6 December 2000, European Commission-Joint Research Centre, Institute for Health and Consumer Protection, European Centre for the Validation of Alternative Methods (ECVAM), Ispra, Italy. ECVAM News and Views.
  Alternatives to Laboratory Animals (ATLA) 29, 93-97
- EU (2008)
  Council Regulation (EC) No 440/2008 of 30 May 2008, laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). Annex, part B. Methods for the determination of toxicity and other health effects.
  Official Journal of the European Communities L 142, 141-443
- Fentem J.H., Archer G.E.B., Balls M., Botham P.A., Curren R.D., Earl L.K., Esdaile D.J., Holzhütter H.G. and Liebsch M. (1998)
  The ECVAM international validation study on *in vitro* tests for skin corrosivity. 2. Results and evaluation by the Management Team.
  Toxicology In Vitro 12, 483-524
- Gordon V.C., Harvell J.D. and Maibach H.I. (1994)
  Dermal corrosion, the Corrositex® system: a DOT accepted method to predict corrosivity potential of test materials.
  In Vitro Skin Toxicology; Irritation, Phototoxicity, Sensitization. Serialtitle: Alternative Methods in Toxicology 10, 37-45
- ICCVAM (1999)
  Corrositex®: An In Vitro Test Method for Assessing Dermal Corrosivity Potential of Chemicals.
  The Corrositex® Assay Peer Review Meeting Final Report, ICCVAM and NICEATM 99-4495
- OECD (2015)
  OECD Test Guideline No. 404: Acute Dermal Irritation/Corrosion. First adopted in 1981. Latest update adopted on the 28th of July 2015. **Link to document** (last access: 16.06.2016)
  OECD Guidelines for the Testing of Chemicals, Section 4, Health Effects
- OECD (2006)
  OECD Test Guideline No. 435: *In Vitro* Membrane Barrier Test Method for Skin Corrosion, adopted on the 19th of July 2006.
  OECD Guidelines for the Testing of Chemicals, Section 4, Health Effects
- US DOT (1991)
  U.S. Department of Transportation. Code of Federal Regulations. Method of Testing Corrosion to the Skin.
  Transportation Title 49, Part 173.136, Appendix A