SAMPLE PROTOCOL

CORROSITEX®

A VALIDATED AND ACCEPTED DERMAL CORROSION TEST METHOD FOR CLASSIFYING SUBSTANCES ACCORDING TO UN PACKING GROUPS

OCTOBER 2003

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SCOPE AND APPLICATION

1. Corrositex® is a validated and accepted (1)(2)(3)(4) *in vitro* test method that assesses if a substance can produce skin corrosion. The test method may be used for solids, liquids, and emulsions. The liquids may be aqueous or non-aqueous. Solids can be water-soluble or non-soluble. The samples may be pure chemicals, dilutions, formulations, or waste. No prior treatment of the sample is required. This method may be used to meet certain regulatory uses (e.g., United Nations [UN] Packing Group classifications)(5) and has been accepted by regulatory authorities (1)(2)(3)(6)(7). The protocol described below is designed to be used with the Corrositex® Assay Kit (InVitro International, 16632 Millikan Avenue, Irvine, CA 92606, USA) and has been adapted from the protocol provided with the kit (1).

SUMMARY OF METHOD

2. The *in vitro* assay system is composed of two components, a synthetic macromolecular biobarrier and a Chemical Detection System (CDS). Test substances are applied to the upper surface of the macromolecular biobarrier. Corrosive substances are able to disrupt the integrity of the biobarrier, leading to the penetration of the test substance through the biobarrier into the CDS located beneath (6)(8). The presence of the test substance in the CDS results in a color change that is detected visually. The time it takes a test substance to penetrate (or breakthrough) the biobarrier into the CDS is inversely proportional to its corrosivity; the more corrosive a test substance, the shorter the time required to affect a color change. Non-corrosive samples do not disrupt the biobarrier, or disrupt the biobarrier too slowly to be identified as corrosive.

3. Corrosive substances may be placed into one hazard class for most authorities, or into three different classes of corrosivity for transportation hazard classification. Corrositex® is able to distinguish the three hazard classes for transportation based on the time required for the substance to breakthrough the biobarrier. These three classes are called Packing Groups by the UN Committee of Experts on the Transport of Dangerous Goods.

4. Packing Groups are assigned according to the degree of danger presented by the corrosive substance. Packing Group I, II, and III indicate great, medium, and minor danger, respectively. For consistency, these same definitions are used for this test method and are referred to as Group I, Group II, and Group III.

5. Prior to performing Corrositex®, a test substance is evaluated as to its compatibility with the CDS. A sample of the test substance is placed in a small amount of CDS fluid. If a detectable change in color occurs, the test substance can be tested for corrosivity using Corrositex® (i.e., the test substance qualifies). If the test substance does not cause a color change, it must be tested by another method. (Note: a review of validated study data found that 85% of non-qualified substances were non-corrosive [6]).

6. Qualified test substances are classified into one of four categories by a screening test that is supplied with the assay kit. The category that a test substance is assigned to will determine how the breakthrough time (if one occurs) will be interpreted. A test substance is assigned to a category based on its ability to induce a pH change in one of two defined buffers. One buffer is designed for detecting acids, the other for detecting bases.

7. Four different categories of test substances are defined:

- Category A1 substances produce a large change in pH when they are added to the acid buffer. This change in pH is indicated by a strong color change.

- Category A2 substances produce little or no pH changes when added to the acid buffer and, therefore, little or no color change in the buffer solution is observed.

- Category B1 substances produce a large change in pH when they are added to the base buffer. This change in pH is indicated by a strong color change.

- Category B2 substances produce little or no pH changes when added to the base buffer and, therefore, little or no color change in the buffer solution is observed.

8. In Corrositex®, a test substance is assigned to an UN transportation Packing Group by considering the pH category that is assigned based on the result obtained in the screening test and by the time it takes for the test substance to penetrate the biobarrier.

9. Category A1 and B1 test substances are assigned to Group I if, after application to the biobarrier, a color change is observed between zero and three minutes, to Group II if a color change is observed after three minutes and up to one hour, and to Group III if a color change is observed after one hour and up to four hours. If no color change occurs in four hours after application to the biobarrier, the test substance is classified as non-corrosive.

10. Category A2 and B2 samples are assigned to Group I if, after application to the biobarrier, a color change is observed between zero and three minutes, to Group II if a color change is observed after three minutes and up to 30 minutes, and to Group III if a color change is observed after 30 minutes and up to 60 minutes. If no color change occurs in 60 minutes after application to the biobarrier, the test substance is classified as non-corrosive.

11. The test method is not subject to interference from color, turbidity, colloidal matter, or high salinity.

12. The qualification test, the screening test, and the assay should be performed at room temperature (17-25°C). The samples should be at room temperature when applied.

PROCEDURE (see Figure 1)

Qualification Test

13. One hundred milligrams or 150 µL of the test substance is added to the qualification tube in the kit. The sample qualifies (can be tested using Corrositex®) if there is a color reaction within five minutes. If no reaction is observed within five minutes, the sample is non-qualified and a different method must be used to assess the corrosivity of the test substance.

Screening Test

Liquid Samples

14. One hundred and fifty microliters of the test substance are added to Test Tubes A and B. The tubes are capped and shaken vigorously for 10 seconds. After one minute, the color change of the mixture is read. If the test substance is immiscible in the solution, the color change is read at the interface after another minute.

15. Based on the color change obtained, the test substance is assigned to a category. If an intense color change (similar to the Category 1 color chart) is observed in Tube A or Tube B, the test substance is assigned to Category 1. If a less intense color change (similar to the Category 2 color chart) is observed in either Tube A or Tube B, the test substance is assigned to Category 2. If no color change is observed in either Tube A or Tube B, a confirmation test is conducted.

16. Confirmation Procedure. Two drops of the Confirm reagent are added to Tube B. The tube is capped and shaken vigorously for five seconds. The color of the solution will match one of the colors shown in the accompanying color chart, confirming that the test substance is a Category 2 substance.

Solid Samples

17. One hundred milligrams of the test substance are added to Test Tubes A and B. The tubes are capped and shaken vigorously for one minute. After another minute, the color change of the mixture is read. If the sample is insoluble in the solution, the mixture is allowed to settle and the color change is read at the interface of the solution and the solid.

18. Based on the results obtained, the test substance is assigned to a category. If an intense color change (similar to the Category 1 color chart) is observed in either Tube A or Tube B, the test substance is assigned to Category 1. If a less intense color change (similar to the Category 2 color chart) is observed in either Tube A or Tube B, the test substance is assigned to Category 2. If no color change is observed in either Tube A or Tube B, a confirmation test is conducted.

19. Confirmation procedure. Two drops of the Confirm reagent are added to Tube B. The tube is capped and shaken vigorously for five seconds. The color of the solution will match one of the colors shown in the color chart, confirming that the sample is a Category 2 substance.

BIOBARRIER PREPARATION

20. Preparation of the biobarrier matrix must be completed at least one day prior to performing the assay, but not more than seven days (if properly stored) prior to assay performance.

21. The scintillation vial containing the biobarrier matrix powder is placed on a hot plate (usually in a glass small pan of water to maintain temperature stability). The entire contents of the biobarrier diluent vial are added slowly and at a constant rate to the vial of biobarrier matrix powder. The stir bar must be turning while the diluent is added, while avoiding high speeds to eliminate foaming of the solution.

22. To solubilize the matrix, the temperature of the solution is increased slowly to 68°C (±1°C). This may take approximately 20 minutes. The temperature must not be allowed to exceed 70°C.

23. The tray of 24 membrane discs is removed from the refrigerator and allowed to come to room temperature (17-25°C).

24. After the biobarrier matrix solution has been completely solubilized, the hot plate is turned off and the vial is moved toward the edge of the hot plate to keep the matrix solution warm while it is being dispensed onto the membrane discs. Two hundred microliters of the solubilized biobarrier solution is dispensed onto each membrane disc, while ensuring that the entire membrane is covered and no air bubbles have formed. Air bubbles in the gel will affect the permeability of the test substance; a disc with air bubbles cannot be used.

25. The filled tray is wrapped evenly with plastic wrap, and stored at 2-8°C on a level surface overnight before beginning any test. The biobarrier is stable for up to seven days if wrapped and stored between 2-8°C.

ASSAY PROCEDURE

26. One tray of seven pre-filled black-capped scintillation vials is removed from the kit box. Vials one through four are to be utilized for sample replicate testing. The vial labeled (+) is to be utilized for a positive control, the vial labeled (-) is for a negative control sample, and the vial labeled C serves as a CDS color control. The tray of 24 membrane discs (from step 25) is removed from the refrigerator, and placed on ice.

27. A disc is placed into a scintillation vial. Five hundred microliters or 500 mg of the test substance is applied to the disc. The discs must not be in contact with the CDS for longer than two minutes before applying the test substance. A timer is started the instant the test substance is added. Three more discs and samples of the test substance are added to vials, with each start time staggered so that the most accurate reaction times are recorded. The vials are not capped while the test is in progress due to potential buildup of pressure.

28. Each vial is observed for three minutes, ensuring that the color reaction is observed and recorded to determine if it is a Group I test substance. Changes in the CDS may include various color changes, flaking, or precipitation.

29. The assay is allowed to run until a color or physical reaction occurs. As the first indication of the presence of a chemical reaction in the CDS, there will be a color change produced beneath the bottom-center of each biobarrier disc. Each vial should be observed continuously for the first three minutes after application of the test substance. Ideally, the vials are observed at regular intervals (e.g., five minutes) throughout the assay to detect the initiation of the color change. This type of close monitoring allows for the detection of unusual membrane performance and is required to determine the breakthrough times for positive control vials (6)(8). Category 1 samples must be checked for reactions at three minutes, one hour, and four hours. Category 2 samples must be checked for reactions at three, 30, and 60 minutes. Except as indicated in step 28, observations should be made five minutes before and after each time point in order to assign Packing Group numbers (e.g., a Category 1 test substance is assigned to Packing Group II if the Corrositex® time is > three minutes and ≤ 60 minutes).

30. A positive (e.g., sodium hydroxide solid or other Packing Group II corrosive)(6)(8) and negative control (e.g., citric acid at 10 wt. %; propionic acid at 6 wt. %) is evaluated in the appropriately labeled vial(s). Additional positive control substances (e.g., weak corrosives) may be used to assess the accuracy of the test system for substances close to the cut off point between corrosive and non-corrosive.

ASSIGNMENT OF GROUPS

31. Category 1 samples are assigned to Packing Group I if, after addition of the test substance, a color change is observed between zero and three minutes, to Packing Group II if a color change is observed after three minutes and up to one hour, and to Packing Group III if a color change is observed after one hour and up to four hours. If no color change occurs in four hours, the test substance is classified as non-corrosive.

32. Category 2 samples are assigned to Packing Group I if, after administration of the test substance, a color change is observed between zero and three minutes, to Packing Group II if a color change is observed after three minutes and up to 30 minutes, and to Packing Group III if a color change is observed after 30 minutes and up to 60 minutes. If no color change occurs in 60 minutes, the test substance is classified as non-corrosive.

PACKING GROUP DESIGNATION

Category 1

CORROSIVITY	PACKING GROUP	MEAN TIME
Corrosive	I	0-3 Minutes
Corrosive	II	>3 Minutes to 1 Hour
Corrosive	III	>1 to 4 Hours
Non-Corrosive	Not Applicable	>4 Hours

Category 2

CORROSIVITY	PACKING GROUP	MEAN TIME
Corrosive	I	0 - 3 Minutes
Corrosive	II	>3 Minutes to 30 minutes
Corrosive	III	>30 to 60 minutes
Non-Corrosive	Not Applicable	>60 minutes

QUALITY CONTROL

33. It is recommended that each test substance be tested in quadruplicate. The test may be performed in duplicate if a simple screening of corrosives and non-corrosives is all that is required. Positive and negative controls should be included in each test.

CRITERIA FOR DETERMINATION OF AN ACCEPTABLE TEST

The Corrositex assay will be accepted if the positive control time falls within ± two standard deviations of the positive control historical mean breakthrough time.

REFERENCES

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Figure 1. Flow Chart for the Use of the Corrositex® Assay