

TRADE SECRET

Study Title

H-25538: Corrositex® *In Vitro* Test

Laboratory Project ID: DuPont-12737

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PERFORMING LABORATORY: E.I. du Pont de Nemours and Company
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WORK REQUEST NUMBER:



SERVICE CODE NUMBER:



CERTIFICATION

We, the undersigned, declare that this report provides an accurate evaluation of data obtained from this study.

Reviewed by James Mackay II 1-April-2003
James C. Mackay II Date
Associate Scientist

Issued by Study Director: Carol Finlay 3-April-2003
Carol Finlay, B.A. Date
Staff Toxicologist

STUDY INFORMATION

Substance Tested: [REDACTED]

Synonyms/Codes: [REDACTED]
• H-25538

Haskell Number: 25538

Composition:

[REDACTED]

Sponsor: E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

Study Initiated/Completed: February 27, 2003 / (see report cover page)

STUDY PERSONNEL

Study Director: Carol Finlay, B.A.

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SUMMARY

H-25538 was evaluated for skin corrosion potential using the *In Vitro* International Corrositex® assay. Corrositex® is a standardized and quantitative *in vitro* corrosivity test. The model is based on the time required for the test substance to pass through a biobarrier membrane and produce a change in a chemical detection system.

An aliquot of 500 µL of H-25538 was applied to each of 4 membrane discs. The test substance did not pass through any membranes.

Under the conditions of this test, H-25538 was not a corrosive substance.

INTRODUCTION

The purpose of this study was to determine skin corrosion potential of H-25538 using the *In Vitro* International Corrositex® assay kit.

MATERIALS AND METHODS

A. Test Substance

The test substance, H-25538, was supplied by the sponsor as a pink liquid. Container 2 was used. The test substance appeared to be stable under the conditions of the study. No evidence of instability, such as a change in color or physical state, was observed.

B. Principles of the Method

Corrositex® is an *in vitro* assay used to determine the skin corrosion potential of a test substance. The assay is based on the ability of a corrosive test substance to pass through a biobarrier membrane and to cause a color change in the liquid Chemical Detection System (CDS). The proprietary biobarrier membrane consists of a reconstituted collagen mixture. The CDS is composed of water and pH indicator dyes. Corrositex® is conducted in three steps:

1. Qualification Step

The test substance is pre-qualified to establish compatibility with the assay. The test substance is placed directly into a vial containing the CDS. If a change in color or consistency occurs, the test substance can be tested by using this assay.

2. Categorization Step

This step establishes the category of the test substance for use in the classification step. The test substance is placed into Vial A (containing an acid buffer) and Vial B (containing a base buffer), and the vials are observed for a color change. A color change observed in either vial is matched to a color chart. The test substance is assigned to either Category 1 or Category 2 based on the color change in each vial. Category 1 and Category 2 are indicative of the time scales used in the classification step.

3. Classification Step

This step determines if the test substance is a skin corrosive according to UN and DOT regulations and classifications. The test substance is applied on top of the proprietary biobarrier membrane. A color change or consistency change in the CDS beneath the membrane indicates that the test substance has passed through the membrane. The determination of corrosive/non-

corrosive and the Packing Group assigned are based on the amount of time it takes the test substance to pass through the membrane. The time scales differ for Category 1 and 2.

The category assigned and the mean value of the breakthrough time for all 4 sample replicate vials determine the packing group. The following table is used to assign the appropriate Packing Group by category and breakthrough time.

PACKING GROUP DESIGNATION

Corrositex® Times

Category 1	0 to 3 minutes	> 3 to 60 minutes	> 60 to 240 minutes	> 240 minutes
Category 2	0 to 3 minutes	> 3 to 30 minutes	> 30 to 60 minutes	> 60 minutes
	Packing Group I	Packing Group II	Packing Group III	Non-Corrosive

> = greater than

C. Protocol

The biobarrier membrane discs provided by the manufacturer were stored in a refrigerator (2-8°C) until the biobarrier membranes were prepared. A thermometer was inserted into a water bath container that was placed on a hot plate/magnetic stirrer. The water bath container was filled with approximately 1 inch of water and heated to 68-70°C. While the water bath was warming, the membrane discs were removed from the refrigerator. The tray lid was removed to prevent condensation. The entire contents of the biobarrier diluent were added to the vial of biobarrier matrix powder. The vial was then placed inside the water bath. The mixture was stirred at medium speed until the biobarrier matrix powder was completely dissolved (approximately 20 minutes). The stir knob and heat were turned off. The solution remained in the water bath for 5 minutes to allow any air bubbles to rise to the surface.

The biobarrier solution was refrigerated since the biobarrier membranes were not immediately made. The biobarrier membranes were prepared the day prior to conducting the assay. Prior to biobarrier membrane preparation, the biobarrier solution was melted in a 60°C water bath for 3-4 minutes. Two hundred microliters was pipetted into each membrane disc, ensuring the entire membrane was covered and no air bubbles had formed. The filled tray containing the biobarriers was wrapped with plastic wrap and immediately stored in a refrigerator (2-8°C).

1. Qualification

The test substance was first tested to determine if the sample was compatible with the Corrositex® system. A 150 µL aliquot of the test substance was added to the Qualify test tube, shaken, and allowed to stand for 1 minute. A change in color or consistency was observed qualifying the test substance for use in the Corrositex® system.

2. Categorization

A 150 μ L aliquot of H-25538 was added to Vial A (containing an acid buffer) and Vial B (containing a base buffer). The vials were capped and shaken. No color change was observed. Two drops of Confirm Reagent were added to Vial B. The vial was capped and shaken until mixed. The color was matched to the colors on the color charts. The color change placed the sample in Category 2.

3. Classification

The filled tray containing the biobarriers was put onto crushed ice. A membrane disc containing the biobarrier matrix was placed into a chemical detection system (CDS) vial. The CDS vials were at room temperature (17-25°C). Within 2 minutes, an aliquot of 500 μ L of H-25538 was evenly applied on top of the disc and a timer was started. This procedure was followed for each of the 4 test vials (vials 1-4). One vial was similarly treated with 500 μ L of a positive control [(vial labeled "+") containing sulfuric acid] and another was similarly treated with 500 μ L of a negative control [(vial labeled "-") containing citric acid]. A vial labeled C served as a CDS color control. The vials were then observed for a change in the CDS. The time it took for the positive control to break through the membrane was documented.

RESULTS

Breakthrough of the biobarrier occurred as follows (hour:minute:second):

- Vial 1 (Test Substance) - No breakthrough
- Vial 2 (Test Substance) - No breakthrough
- Vial 3 (Test Substance) - No breakthrough
- Vial 4 (Test Substance) - No breakthrough

- Vial 5 (Positive Control) - 00:00:47
- Vial 6 (Negative Control) - No breakthrough

The positive control and negative control performed as anticipated.

CONCLUSION

Under the conditions of this test, H-25538 was not a corrosive substance.

RECORDS AND SAMPLE STORAGE

Specimens (if applicable), raw data, and the final report will be retained at Haskell Laboratory, Newark, Delaware, or at Iron Mountain Records Management, Wilmington, Delaware.