TRADE SECRET

Study Title

H-24616: *In Vitro* Dermal Penetration Through Rat Skin

Laboratory Project ID: DuPont-8837

TEST GUIDELINES: None were applicable

AUTHOR: William J. Fasano, Sr., B.S.

STUDY COMPLETED ON: July 26, 2002

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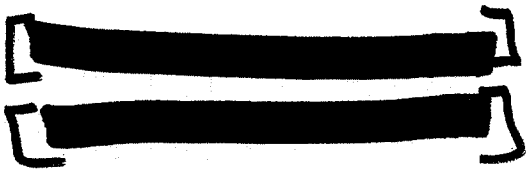

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with U.S. EPA TSCA (40 CFR part 792) Good Laboratory Practice Standards, which are consistent with the OECD Principles of Good Laboratory Practice (as revised in 1997) published in ENV/MC/CHEM(98)17 and MAFF Japan Good Laboratory Practice Standards (59 NohSan No. 3850) except for the item documented below. The item listed does not impact the validity of the study.

The test substance was characterized by the sponsor prior to the initiation of this study. Although the characterization was not performed under Good Laboratory Practice Standards, the accuracy of the data is considered sufficient for the purposes of this study.

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

Study Director: _____ 26-Jul-2002
William J. Fasano, Sr., B.S. Date
Research Scientist

Applicant / Sponsor: _____ _____
DuPont Representative Date

Company Sanitized. Does not contain TSCA CBI

QUALITY ASSURANCE STATEMENT

Haskell Sample Number(s):

24616

Dates of Inspections:

Protocol: March 11, 2002

Conduct: March 11,12 ,21, 2002

Records, Reports: July 10-12,15-18, 2002

Dates Findings Reported to:

Study Director: July 22, 2002

Management: July 25, 2002

Reported by: Wonda K. Kelly 26-Jul-2002

Wonda K Kelly
Quality Assurance Auditor

Date

CERTIFICATION

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

Issued by Study Director: _____ 26-Jul-2002
William J. Fasano, Sr., B.S.
Research Scientist

Approved by: _____ 26-July-2002
Matthew S. Bogdanffy, Ph.D., D.A.B.T. Date
Management

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STUDY INFORMATION

Substance Tested: [redacted]

Synonyms/Codes: • H-24616
[redacted]

Haskell Number: 24616

Composition: [redacted]

Known Impurities in Total Solids: [redacted]

[redacted]

Physical Characteristics: Opaque tan liquid

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

Study Initiated/Completed: March 6, 2002 / (see report cover page)

In-Life Initiated/Completed: March 11, 2002 / March 12, 2002

STUDY PERSONNEL

Study Director:	William J. Fasano, Sr., B.S.
Management:	Matthew S. Bogdanffy, Ph.D., D.A.B.T.
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Management:	Nancy S. Selzer, M.S.

SUMMARY

The dermal penetration potential of H-24616 has been evaluated using rat skin mounted in an *in vitro* static diffusion cell system. H-24616 is an aqueous suspension containing an [redacted] which was applied undiluted to rat epidermal membranes at a rate of 20 $\mu L/cm^2$. The test material, a total of 1233.6 μg F or 82.4 ppm, remained in contact with the skin for 6 hours. At the end of the exposure period, the receptor fluid was removed from the static cell system and combusted using a Wickbold Torch followed by quantitative analysis for total fluorine using a fluoride ion selective electrode.

The results presented in this study show that following a 6-hour dermal exposure to H-24616 applied to rat epidermal membranes mounted in an *in vitro* static diffusion cell system, receptor fluid total fluorine levels were found to be below the limit of detection (<0.052 ppm). Under the conditions of this study, H-24616 did not penetrate rat epidermal membranes.

INTRODUCTION

H-24616 is an aqueous dispersion product containing a fluorinated active ingredient currently under investigation by the sponsor. The objective of this study was to determine the dermal penetration of H-24616, based on total fluorine, following a 6-hour exposure to a single, finite dermal application of H-24616 to rat epidermal membranes using an *in vitro* static diffusion cell test system.

MATERIALS AND METHODS

A. Regulatory Test Guidelines

This study was not designed to meet any specific regulatory guideline requirement. There are, however, guidelines on percutaneous absorption from the Organization for Economic Co-operation and Development (OECD) that provided information on the practical use of the *in vitro* dermal absorption method.[1]

B. Test Substance

The test material, H-24616, was an opaque liquid containing [redacted] was the fluorinated active ingredient. Characterization of the test substance was the responsibility of Regional Analytical Services (RAS), Jackson Laboratories, Deepwater, New Jersey.

C. Test Species

Female rats of the Sprague-Dawley strain, Crl:CD®(SD)IGS BR, were obtained from Charles River Laboratories, Inc., Raleigh, North Carolina. Rats were approximately 28 days old at the time of sacrifice for the collection of skin.

Dermal contamination is a potential route of human exposure. The rat was the species of choice for use in the *in vitro* test system as this species has been used in toxicological evaluations of H-24616. *In vitro* dermal techniques employing glass (static) diffusion cells (Figure 1) have been shown to predict percutaneous absorption of various chemicals *in vivo*. [2,3,4]

D. Animal Husbandry

Upon arrival at Haskell Laboratory, animals were removed from shipping cartons and housed in appropriate cages, according to Standard Operating Procedures [redacted] unless specified in the study records. Animals were maintained under quarantine for at least 6 days unless approved

otherwise by the site veterinarian, had 3 recorded weight gains, and no abnormalities detected. After the quarantine period, animals were selected for study.

Animal rooms were targeted at a temperature of 23 ±1°C and a relative humidity of 40-60%. Animal rooms were artificially illuminated (fluorescent light) on an approximate 12-hour light/dark cycle.

E. Animal Health Monitoring

As specified in the Haskell Laboratory animal health and environmental monitoring program, the following procedures are performed periodically to ensure that contaminant levels are below those that would be expected to impact the scientific integrity of the study:

- Water samples are analyzed for total bacterial counts, and the presence of coliforms, lead, and other contaminants.
- Feed samples are analyzed for total bacterial, spore and fungal counts.
- Samples from freshly washed cages and cage racks are analyzed to ensure adequate sanitation by the cagewashers.

Certified animal feed is used, guaranteed by the manufacturer to meet specified nutritional requirements and not to exceed stated maximum concentrations of key contaminants, including specified heavy metals, aflatoxin, chlorinated hydrocarbons, and organophosphates. The presence of these contaminants below the maximum concentration stated by the manufacturer would not be expected to impact the integrity of the study.

The animal health and environmental monitoring program is administered by the attending laboratory animal veterinarian. Data are maintained separately from study records and may be included in the final report at the discretion of the study director.

F. Preparation of Skin Membranes

Following quarantine, rats were euthanized by carbon dioxide asphyxiation, and the fur from the dorsal region was carefully shaved using clippers. Any animals showing obvious abrasion within the region of the test skin area were considered unsuitable and discarded. The shaved area was excised, held briefly on wet ice, and frozen at ≤-10°C until processed.

Frozen samples of skin were allowed to thaw at room temperature before treatment with sodium bromide. Full thickness skin was soaked for approximately 18 hours in 1.5M sodium bromide. The skin sample was then removed, rinsed in distilled water, the epidermis carefully peeled from the dermis, floated onto an aluminum pan, and allowed to air-dry. Each epidermal membrane was identified using the Haskell animal number and stored refrigerated at 0-10°C until readied for use.

G. *In Vitro* Diffusion Cells

Glass [static] diffusion cells (Figure 1) were used in this study. The *in vitro* cells had an exposure area of 0.64 cm^2.

H. Assessment of Membrane Integrity and Membrane Equilibration

The integrity of each membrane was assessed by measurement of electrical resistance prior to application of test substance.

Membranes were removed from refrigeration storage and hydrated in 0.9% saline for approximately 15 minutes. Following hydration, the membrane was mounted onto the top of the receptor chamber, which was filled with 0.9% saline. The donor chamber was then clamped in place and filled with 0.9% saline. The membrane was then allowed to equilibrate for approximately 30 minutes. During equilibration, the water-jacketed cells were maintained at approximately 32°C using a recirculating water bath system. Following equilibration, a resistance measurement of each skin membrane was taken.

Membranes with a resistance of ≥5.8 kΩ were considered intact and retained for use on study. Membranes not meeting this criteria were replaced, and electrical resistance confirmed following equilibration. This procedure was followed until a minimum of 16 skin preparations represented by at least 3 individual rats was achieved.

Following electrical resistance measurement, saline in the donor chamber was removed and discarded. Saline in the receptor chamber was reduced to approximately half of the total volume, and cells with acceptable membranes maintained at approximately 32°C overnight, without occlusion of the donor chamber, prior to dose application. The receptor chamber sampling arm remained occluded with Parafilm®.

I. *In Vitro* Penetration of H-24616

1. *Experimental Groups*

This study was composed of the following groups.

Group	Designation	Number of replicates	Exposed to
A	Exposure group	8	H-24616
B	Negative controls	8	Deionized water

2. *Pretreatment Procedures*

Following overnight equilibration, the contents of the receptor chamber were removed and discarded and refilled with 0.9% saline.

3. ***Application of the Test Substance***

The test substance was applied to the skin surface of group A, via the donor chamber, as a single application at a rate of approximately 20 $\mu L/cm^2$ (i.e., 12.8 μL). Group B received a dose of deionized water at 20 $\mu L/cm^2$ (i.e., 12.8 μL). The donor chambers remained unoccluded for the duration of the exposure period.

4. ***Exposure Period***

The exposure period for groups A and B was 6 hours.

5. ***Terminal Procedures***

At the end of the 6-hour exposure phase, the contents of the receptor chamber for each replicate in Groups A and B was removed and each placed separately into a glass container.

No other samples were collected and skin samples were discarded.

Samples of receptor fluid not immediately processed for analysis were stored refrigerated at 0-10°C.

6. ***Positive Controls***

A set of 6 replicates was prepared by spiking deionized water with 12.8 μL of H-24616. These samples were used to determine average fluorine applied to the test skins (Group A).

J. Total Fluorine Determination

Receptor fluid samples (Groups A and B) and positive controls were combusted using a Wickbold Torch followed by analysis for total fluorine using a fluoride ion selective electrode.

K. Data Presentation

Calculated values in tables were computer generated and rounded appropriately for inclusion in the report. As a consequence, calculation of mean data will, in some instances, yield a minor variation from an aesthetic calculation.

RESULTS AND DISCUSSION

A. Positive Control Data (Table 1)

The mean weight of H-24616 for the six replicates of 12.8 µL each was established to be 12.3 mg (%CV = 3.71). The mean amount of total fluorine (F) contained in each 12.8 µL of H-24616, as determined by Wickbold Torch combustion, was 1233.6 µg F (82.4 ppm).

B. Negative Control Group Data (Table 2)

Total fluorine in receptor fluid from the negative control group, dosed with 12.8 µL of deionized water to establish background fluorine levels associated with the test system, ranged from not detected (<LOD of 0.052 ppm) to 0.105 ppm.

C. Exposure Group Data (Table 3)

Following a 6-hour dermal exposure to H-24616, applied at a rate of 20 µL/cm^2 (12.8 µL), total fluorine levels in receptor fluid samples were found to be below the limit of detection (<0.052 ppm).

CONCLUSIONS

The results presented in this study show that following a 6-hour dermal exposure to H-24616 applied to rat epidermal membranes mounted in an *in vitro* static diffusion cell system, receptor fluid fluorine levels were found to be below the limit of detection (<0.052 ppm). Under the conditions of this study, H-24616 did not penetrate rat epidermal membranes.

RECORDS AND SAMPLE STORAGE

Laboratory-specific or site-specific raw data, such as personnel files and equipment records will be retained by the facility where the work was done.

Raw data and the final report will be retained at Haskell Laboratory, Newark, Delaware, or at Iron Mountain Records Management, Wilmington, Delaware. Characterization data (percent purity, composition, and known impurities) will be stored at Regional Analytical Services, Jackson Laboratories, Deepwater, New Jersey.

REFERENCES

1. OECD Guideline for the Testing of Chemicals (Draft Guideline 428), Skin Absorption: *in vitro* Method (December 2000).

2. Scott R.C., Batten P.L., Clowes H.M., Jones B.K. and Ramsey J.D. (1992). Further Validation of an *In Vitro* Method to Reduce the Need for *In Vivo* Studies for Measuring the Absorption of Chemicals through Rat Skin. *Fundamental and Applied Toxicology* **19**, 484-492.

3. Ramsey J.D., Woollen B.H., Auton T.R. and Scott R.C. (1994). The Predictive Accuracy of *In Vitro* Measurements for Dermal Absorption of a Lipophilic Penetrant (Fluazifop-Butyl) through Rat and Human Skin. *Fundamental and Applied Toxicology* **23**, 230-236.

4. Scott R.C., Walter M. and Dugard .P.H. (1986). A comparison of the *in vitro* permeability properties of human and some laboratory animal skins. *International Journal of Cosmetic Science* **8**, 189-194.

TABLES

Table 1: Positive Control Data

Replicate	Weight of H-24616 (mg)	Weight of H-24616 plus water (A) (g)	Aliquot weight of a 1:10 dilution of A for combustion (g)	ppm fluorine, A (µg F/g)	Total fluorine, A (µg F)	H-24616 Percent fluorine (%F)
1	12.2	14.7909	0.5006	91.9	1359.3	11.1
2	12.3	14.9951	0.5005	73.7	1104.5	8.98
3	12.0	15.0190	0.5002	75.4	1132.1	9.44
4	13.0	15.0207	0.5013	87.4	1313.2	10.1
5	12.6	15.0192	0.5013	84.4	1267.4	10.1
6	11.7	15.0204	0.5007	81.5	1224.3	10.5
Mean	12.3	14.9776	0.5008	82.4	1233.6	10.0
SD	0.46	0.0920	0.0004	7.00	100.1	0.76
%CV	3.71	0.61	0.09	8.49	8.11	7.56

Table 2: Negative Control Group

Replicate	Weight of receptor fluid (g)	Aliquot weight for combustion (g)	ppm, fluorine receptor fluid (µg F/g)[a]
B1	5.0054	3.0009	ND[b]
B2	4.8118	3.0005	ND
B3	4.7837	3.0010	ND
B4	4.7907	3.0004	0.105
B5	5.1710	3.0003	<0.103
B6	5.1669	3.0012	<0.103
B7	5.1647	3.0006	<0.103
B8	4.8344	3.0014	ND

[a]LOD = 0.052 µg F/g (ppm); LOQ = 0.103 µg F/g (ppm)
[b]Not detected; below the LOD

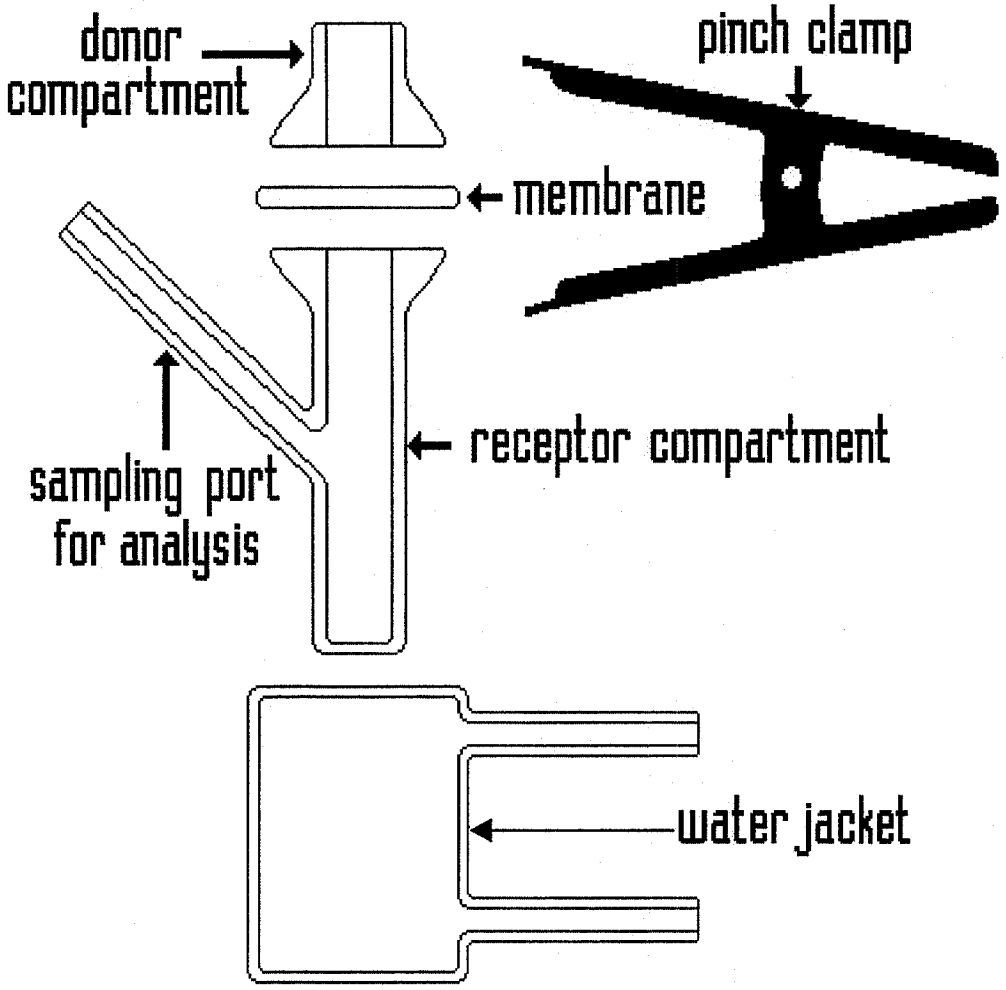
Table 3: Exposure Group Data

Replicate	Weight of receptor fluid (g)	Aliquot weight for combustion (g)	ppm, fluorine receptor fluid (μg F/g)[a]
A1	4.8725	3.0013	ND[b]
A2	4.7954	3.0013	ND
A3	5.1263	3.0006	ND
A4	5.0250	3.0009	ND
A5	5.0181	3.0007	ND
A6	4.9800	3.0014	ND
A7	5.1295	3.0005	ND
A8	4.8289	3.0009	ND

[a]LOD = 0.052 μg F/g (ppm); LOQ = 0.103 μg F/g (ppm)
[b]Not detected; below the LOD

FIGURES

FIGURE 1 – STATIC DIFFUSION CELL



Company Sanitized. Does not contain TSCA CBI