

3M MEDICAL DEPARTMENT, CORPORATE TOXICOLOGY

Protocol for Study Nos. T-6295.23; ST-46

Exploratory In-Vitro Percutaneous Absorption Study of Theophylline, Salicylic Acid, Perfluorooctylsulfonate, and Ammonium Perfluorooctanoate in SkinEthic Reconstituted Epidermis Model

Study Objective:

This study is designed to gain familiarity with the SkinEthic Reconstituted Epidermis Model by conducting a simple absorption study on two previously characterized compounds (theophylline and salicylic acid in a paper by Doucet, et. al.), as well as one fluorochemical compound (perfluorooctylsulfonate, K⁺). Supporting analytical work for this study will be done in the new Corporate Toxicology Laboratory in Building 236 as part of method and instrument qualification. A MTT assay will be conducted at the end of the absorption study to determine if SkinEthic viability was affected by application of the test compounds.

Research Client: 3M Specialty Chemicals Division
3M Center, Building 236
Saint Paul, MN 55144

Sponsor: 3M Specialty Chemicals Division
3M Center, Building 236
Saint Paul, MN 55144

Study Location: 3M Strategic Toxicology Laboratory
3M Center, Building 270-3S-06 room SB314
Saint Paul, MN 55144

Study Director: John Butenhoff, Ph.D.
Senior Laboratory Manager
3M Medical Dept. / Corporate Toxicology
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Study Toxicologist: Kathy Thompson, M.P.H.
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Proposed Study Timeline

Start Date: Wednesday, April 12th, 2000

End Date: Friday, April 14th, 2000

Analytical Completion Date: TBD

Final Report Completion Date: TBD

Regulatory Compliance:

This study will be performed in the 3M Strategic Toxicology Laboratory under a defined protocol and classified as a "Class C Study" as explained in TOX SOP 0950, Strategic Toxicology Lab GLP Program Procedure.

Test Material:

The fluorochemical test material will be provided by Dan Hakes, Product Responsibility Liaison, 3M Chemicals Division. The drug test material will be supplied by Sigma.

Identification:**Names:**

Perfluorooctylsulfonate, K⁺ (PFOS, K⁺ salt)

Theophylline (THE)

Salicylic Acid (SA)

Molecular Formulas:

C₈F₁₇SO₃·K⁺ (PFOS)

C₇-H₈-N₄-O₂ (THE)

C₇-H₆-O₃ (SA)

Lot Number: Documentation will be kept on file by the Sponsor for PFOS (Lot 217) and by the Corporate Toxicology Analytical Laboratory for THE and SA.

Purity:

Documentation will be kept on file by the Sponsor for PFOS, and by the Corporate Toxicology Analytical Laboratory for THE and SA.

Stability:

Documentation will be kept on file with the Sponsor for PFOS, and by the Corporate Toxicology Analytical Laboratory for THE and SA.

Storage Conditions:

Upon receipt, test material will be stored tightly sealed at room temperature.

Characteristics:

Information on synthesis methods, composition or other characteristics that define the test material will be kept on file with the Sponsor for PFOS, and by the Corporate Toxicology Analytical Laboratory for THE and SA.

SkinEthic Preparation Procedure:

The SkinEthic Reconstituted Epidermis will be prepared immediately upon arrival as per the manufacturers instructions (see Attachment One). Absorption testing will begin on April 13, 2000. All handling of SkinEthic material will be done using aseptic techniques.

004436

Materials:

- ❑ Twelve SkinEthic Human Reconstituted Epidermis, Size 0.63 cm², and twelve blank filters
- ❑ SkinEthic Maintenance Medium (supplied by manufacturer)
- ❑ Additional empty 12 well culture plates (Becton Dickinson)
- ❑ Heating Block (37°C)
- ❑ Incubator (37°C, 5%CO₂, saturated humidity)

Reagents:

- ❑ Receptor Medium - Phosphate Buffered Saline (GibcoBRL Cat #14200-075, Lot # 1027781), 1.5% Bovine Serum Albumin (Sigma, A-8022 Lot#39H0691) added for FC compound
- ❑ Compound application vehicle – Milli-Q Water
- ❑ 1% Soap Solution (Tween 20, Mallinckrodt, lot #H285 N13H32)
- ❑ Trypsin (Sigma, lot #39H0952, cat#T-7418)
- ❑ MTT (Sigma, lot #39H5076, cat #M-2128)

Procedure:

- ❑ Unpack and maintain Skinethic Reconstituted Epidermis (REp) skin cell cultures and blank filter wells according to manufacturer's instructions in controlled temp and humidity incubator.
- ❑ Before product application, expose REp and blank filter wells to standard room environmental conditions for 2 hours to equilibrate with ambient atmosphere.
- ❑ Place 0.6 ml receptor medium in each well of 12-well culture plates.
- ❑ Transfer REp and blank filters gently with forceps to each well and place on heating block.
- ❑ Evenly apply 200 µl of THE and SA compound solutions to REp and blank filter surface in triplicate, 200 µl of PFOS compound solution in quadruplicate, and 200 µl of blank application vehicle (water) to the remainder and start timer:
 1. THE applied as saturated water solution of 6 mg/ml
 2. SA applied as saturated water solution of 2 mg/ml
 3. PFOS applied as a solution of 0.025 mg/ml (50 µM)
- ❑ Transfer REp and blank filter gently with forceps to new well containing receptor medium after 0.25 hr, 0.5 hr, 1 hr, 2 hr, 4 hr, and 6 hr.
- ❑ At end of experiment, place the receptor medium of each plate well into a polypropylene vial for later analysis.

004437

- Wash the REp and blank filter surface 3 times with 200 μ L of 1% soap solution, and rinse 3 times with same volume of water. Gently wipe cleaned surface with a cotton tip. Save washing fluids and tips in a polypropylene vial for later analysis.
- Conduct MTT Reduction assay on one sample for each test compound and two samples for negative control (water application only) to determine SE viability.
- Cut remaining skin membranes and blank filters into small pieces with scissors and digest for 48 hours with Trypsin at 40°C. After homogenization and centrifugation (1000 g) for 10 min, filter supernatant through a 0.45 μ m filter and save in a polypropylene vial for later analysis.

Number of analytical samples generated : total =182:

Surface Washing Fluids:

- A. PFOS: 8 at t = 6 hrs
- B. THE: 6 at t = 6 hrs
- C. SA: 6 at t = 6 hrs
- D. Water: 4 at t = 6 hrs

Receptor Medium:

- E. PFOS: 8 each at t = 0.25 hrs, 0.5 hrs, 1.0 hrs, 2.0 hrs, 4.0 hrs, 6.0 hrs
- F. THE: 6 each at t = 0.25 hrs, 0.5 hrs, 1.0 hrs, 2.0 hrs, 4.0 hrs, 6.0 hrs
- G. SA: 6 each at t = 0.25 hrs, 0.5 hrs, 1.0 hrs, 2.0 hrs, 4.0 hrs, 6.0 hrs
- H. Water: 4 each at t = 0.25 hrs, 0.5 hrs, 1.0 hrs, 2.0 hrs, 4.0 hrs, 6.0 hrs

Digest Filtered Supernatant:

- I. PFOS: 6 at t = 6 hrs
- J. THE: 4 at t = 6 hrs
- K. SA: 4 at t = 6 hrs

Ship samples on dry ice to:

Dave Ehresman, BS
Toxicology Specialist
3M Medical Dept. / Corporate Toxicology
3M Center, **Building 236-C146**
Saint Paul, MN 55144
Ph: 651-736-8410

for analysis of the test compound and the presumed metabolites (3-methylxanthine, 1,3,7-trimethyluric acid, and 1,3-dimethyluric acid for THE, gentisic acid for SA, none for PFOS).

004438

Data Analysis:

Analytical results will be used to derive maximal flux rate in $\mu\text{g}/\text{hour}/\text{cm}^2$. The flux rate obtained for THE and SA will be compared to that reported in the paper by Doucet, et. al. Mass balance recovery should be $90 \pm 10\%$. MTT results will be used to determine if skin viability was affected (as compared to negative control) by application of the test compounds.

Responsibilities:

- Kathy Thompson will be responsible for performing the experiment and sending specimens for analysis.
- Dave Ehresman will be responsible for analytical evaluation of the samples.
- Kathy Thompson will draft a final report and ensure the report receives appropriate 3M review before a final report is issued, to be signed by Kathy Thompson and Dave Ehresman.

References:

Doucet, O., N. Garcia and L. Zastrow. Skin Culture Model: a Possible Alternative to the Use of Excised Human Skin for Assessing *In Vitro* Percutaneous Absorption. *Toxicology in Vitro* 12 (1998) 423-430.

004439

Signatures:

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Date

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004440