MR# 309034

American® Chemistry Council

January 9, 2008

Document Control Office (7407M)
U.S. Environmental Protection Agency
ATTN: TSCA Section 8(e) Coordinator
Office of Pollution Prevention and Toxics
1200 Pennsylvania Avenue, N.W.
Washington, D.C. 20460

Re: Section 8(e) Submission for Propanol, oxybis-Dipropylene Glycol, mixed isomers (CAS No. 25265-71-8) 8EHQ-07-16930

Dear Sir or Madam:

As follow-up to its letter dated May 24, 2007 (copy enclosed), the American Chemistry Council's Propylene Oxide/Propylene Glycol Panel submits a final report on propanol, oxybis-dipropylene glycol, mixed isomers entitled, "Dipropylene Glycol: In Vitro Dermal Absorption Rate Testing." This information is submitted on behalf of the following members of the Panel: The Dow Chemical Company, Huntsman Corporation and Lyondell Chemical Company.

If you have any questions, please contact me at (703) 741-5612. Thank you.

8EHQ-07-16930

Sincerely yours,

Kate Schroen

Kate Schroen
Manager,
Chemical Products and Technology Division

Enclosures

8908000078

American® Chemistry Council

May 24, 2007

Via: Messenger Delivery

Document Processing Center (7407M)
Office of Pollution Prevention and Toxic Substances
U.S. Environmental Agency
1200 Pennsylvania Avenue, NW
Washington, DC 20460-001

Re: Section 8(e) Submission for Propanol, oxybis-
Dipropylene Glycol, mixed isomers
CASRN 25265-71-8

Dear Sir or Madam:

The following information is being submitted in accordance with EPA's interpretation of Section 8(e) of the Toxic Substances Control Act by the American Chemistry Council's Propylene Oxide/Propylene Glycol Panel (Panel)[1] . The Panel has made no determination as to whether a significant risk of injury to health or environment is presented by the findings, which are preliminary and un-audited.

An *In Vitro* Dermal Absorption Rate Testing study on dipropylene glycol is being conducted by CEFIC's Propylene Oxide and Derivatives Sector Group on behalf of producer member companies. The study is being conducted according to OECD 428 guidelines at E.I. DuPont de Nemours and Company's Haskell Laboratory in Newark, Delaware. Preliminary results from the study, based on 7 samples from 4 donors, are as follows:

Steady-State Penetration Rate ($\mu g/cm^2/hr$): 39.3 ± 10.7
Kp (cm/hr): $3.85 \times 10^{-5} \pm 1.05 \times 10^{-5}$
Lag time (h): 1.05 ± 0.33

As noted above, this information should be considered preliminary. The results are not audited and no written report of the results is yet available. The final report will be submitted to the Agency as soon as it is available. If you have any questions regarding the information contained in this letter, please contact me at (703) 741-5609 or by email at Barbara_Francis@americanchemistry.com.

Sincerely,

Barbara Francis

Barbara Francis
Managing Director,
Chemical Products and Technology Division

[1] Members of the ACC Propylene Oxide/Propylene Glycol Panel are: The Dow Chemical Company, Huntsman Corporation, and Lyondell Chemical Company.

Study Title

Dipropylene Glycol:
In Vitro Dermal Absorption Rate Testing

TEST GUIDELINES: OECD Guideline for the Testing of Chemicals. Guideline 428: Skin Absorption: *in vitro* Method (2004)

Guidance Document for the Conduct of Skin Absorption Studies. OECD Series on Testing and Assessment Number 28. (2004)

European Commission Guidance Document on Dermal Absorption. Sanco/222/2000 rev 7 (2004)

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

STUDY COMPLETED ON: September 28, 2007

PERFORMING LABORATORY: E.I. du Pont de Nemours and Company
DuPont Haskell Global Centers for
Health & Environmental Sciences
P.O. Box 50
Newark, Delaware 19714
U.S.A.

LABORATORY PROJECT ID: DuPont-22212

WORK REQUEST NUMBER: 16155

SERVICE CODE NUMBER: 1623

SPONSOR: The Propylene Oxide and Glycols Sector Group of CEFIC
Avenue E. Van Nieuwenhuyse, 4 box 1, B-1160 Brussels
Belgium

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 compatible with current OECD and MAFF (Japan) Good Laboratory Practices.

Study Director: _____ 28-Sept-2007

William J. Pasano, Sr., B.S. Date
Senior Research Toxicologist

QUALITY ASSURANCE STATEMENT

Work Request Number: 16155
Service Code Number: 1623

Key inspections for DuPont work request 16155, service code 1623 were performed for the tasks completed by the Quality Assurance Unit of DuPont and the findings were submitted on the following dates.

Phase Audited	*Audit Dates*	*Date Reported to Study Director*	*Date Reported to Management*
Protocol:	March 27, 2007	March 27, 2007	March 27, 2007
Conduct:	April 11, 2007	April 11, 2007	April 11, 2007
Report/Records:	June 6-8, 2007	June 8, 2007	July 10, 2007
Sponsor Edits:	September 5, 2007	September 6, 2007	September 6, 2007

Reported by: Kenneth Gruelle for Molly A. Butler 27, Sept 2007
Molly A. Butler Date
Quality Assurance Auditor

CERTIFICATION

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

Reviewed and Approved by: _____ 28-Sept-2007
Steven R. Frame, D.V.M., Ph.D., Diplomate A.C.V.P. Date
Research Fellow and Manager

Issued by Study Director: _____ 28-Sept-2007
William J. Fasano, Sr., B.S. Date
Senior Research Toxicologist

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STUDY INFORMATION

Substance Tested:
- Dipropylene Glycol
- CAS RN 25265-71-8, a mixture of 3 structural isomers (CAS RNs 108-61-2, 110-98-5, 106-62-7)

Haskell Number: 27231

Composition: Dipropylene Glycol

Purity: 99.9%

Physical Characteristics: Colorless liquid

Stability: The test substance appeared to be stable under the conditions of the study; no evidence of instability was observed.

Study Initiated/Completed: March 26, 2007 / (see report cover page)

Experimental Start/Termination: April 11, 2007 / April 18, 2007

SUMMARY – INTERNATIONAL UNIFORM CHEMICAL INFORMATION DATABASE (IUCLID) FORMAT

Species: Human cadaver skin
Sex: Male and female
Race: Caucasian
Route of administration: Topical
Exposure period: 24 hours
Frequency of treatment: Single application
Duration of test: 24 hours
Dose: 1200 μL/cm^2; 768 μL
Control group: Not applicable
Method: *In vitro* flow-through diffusion cell model
GUIDELINES FOLLOWED
- OECD Guideline for the Testing of Chemicals. Guideline 428: Skin Absorption: *in vitro* Method (2004)
- Guidance Document for the Conduct of Skin Absorption Studies. OECD Series on Testing and Assessment Number 28. (2004)
- European Commission Guidance Document on Dermal Absorption. Sanco/222/2000 rev 7 (2004)

Year: 2007
GLP: Yes
Test substance: Dipropylene glycol (DPG)
SOURCE: The Dow Chemical Company
PURITY: 99.9%
Test conditions TEST SYSTEM:
- *In vitro* flow-through diffusion cells

MEMBRANE:
- Human cadaver skin, dermatomed
- Four donors
- Seven replicates
- Receptor fluid, deionized water

ADMINISTRATION-EXPOSURE:
- Neat DPG
- Vehicle, none
- 768 μL DPG topical dose with occlusion; infinite dose
- Exposure duration, 24 hours

PARAMETERS ASSESSED DURING STUDY:
- Skin thickness
- Stratum corneum barrier integrity, pre- and post-exposure by electrical impedance (skin damage ratio, post/pre)
- Concentration of DPG in receptor with time; analysis of receptor fluid samples by Gas Chromatography Flame Ionization Detection (GC-FID)

- Lag time (hours), penetration rate at steady-state (flux) and extent (absolute amount and percent of applied dose) of DPG
- Permeability coefficient (Kp); slope of cumulative penetration of DPG per area vs. time (in hours) normalized to concentration of DPG applied (1.02 mg/mL)

Results (Mean ±SD): SKIN THICKNESS

- 389 ±54 μm

SKIN DAMAGE RATIO

- 0.97 ±0.23 (unit less)

LAG TIME

- 1.05 ±0.33 hours

STEADY-STATE PENETRATION

- 39.3 ±10.7 $\mu g/m^2/h$

CUMULATIVE AMOUNT PENETRATED AT 24 HOURS

- 912.6 ±219.0 $\mu g/cm^2$

CUMULATIVE PERCENT PENETRATED AT 24 HOURS

- 0.075 ±0.018%

PERMEABILITY COEFFICIENT (Kp)

- $3.85 \times 10^{-5} \pm 1.05 \times 10^{-5}$ cm/hr

Conclusions

- DPG penetrated through human skin, under infinite dose and occlusion conditions, at 39.3 ±10.7 $\mu g/m^2/h$.
- DPG's Kp was calculated to be $3.85 \times 10^{-5} \pm 1.05 \times 10^{-5}$ cm/hr.
- DPG could be categorized as a slow penetrant.

SUMMARY - OVERVIEW

A permeability coefficient (Kp) has been determined for dipropylene glycol (DPG) using human skin mounted in an *in vitro* flow-through diffusion cell model. Human cadaver abdominal skin was dermatomed to approximately 400 μm and the section mounted onto an *in vitro* flow-through diffusion cell, stratum corneum uppermost, with an exposure area of 0.64 cm^2. Initially, the underside of each skin replicate was perfused with 0.9% saline at a rate of approximately 1.5 mL/h. Following system equilibration at 32°C, skin integrity was confirmed by electrical impedance. Following skin integrity confirmation, the saline in the donor chamber was removed and discarded, and the skin surface was dried prior to application of DPG. With the receptor chamber perfused with water, an infinite dose of neat DPG (approximately 1200 $\mu L/cm^2$) was applied to the epidermal surface, via the donor chamber, to 7 skin replicates representing 4 human subjects, and the donor chamber opening was occluded with tape. Serial receptor fluid samples were collected hourly during the first 8 hours post-application, and then every other hour until 24 hours post-application. The receptor fluid samples were analyzed for DPG by gas chromatography-flame ioization detection (GC-FID), and the results used to construct a cumulative penetration per area vs. time curve. The slope of the line at steady-state penetration, represented by no less than 10 data points, was normalized to the concentration of the applied DPG to yield a Kp.

A. Skin Integrity

The integrity of human skin, as determined by electrical impedance, was unaffected by continuous exposure to DPG under occlusive conditions. The ratio of the post-EI values to pre-EI values was 0.97 confirming that the barrier properties of the stratum corneum were unaltered by DPG.

B. Cumulative Percent Penetrated

At the conclusion of the 24-hour exposure interval, only a negligible portion of the applied dose of neat DPG (0.075%) had penetrated through human skin into the receptor fluid.

C. Steady-State Penetration, Kp

Based on the slope at steady-state (39.3 $\mu g/cm^2/h$), and the concentration of DPG in the applied solution, taken at its density (1,020,000 $\mu g/cm^3$, DPG's permeability coefficient was calculated to be 3.85×10^{-5} cm/h.

INTRODUCTION

Dipropylene glycol (DPG), a component of many commercial products such as antifreeze, air fresheners, cosmetic products, solvents, and plastics, is produced as a byproduct of the manufacture of propylene glycol. The United States production capacity of dipropylene glycol was 131 million pounds (60 thousand tonnes) in 1998.[1]

Although DPG possess low acute toxicity by the oral, dermal, and inhalation exposure routes, its high production volume and worldwide exposure potential to humans, either in the workplace or via various consumer products, warrants evaluation of its dermal penetration potential using conservative *in vitro* testing methods.[2-6]

The objective of this study was to determine a permeability coefficient (Kp) for dipropylene glycol (DPG) using human cadaver skin mounted in an *in vitro* diffusion cell model.

MATERIALS AND METHODS

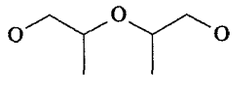
A. Test Guidelines

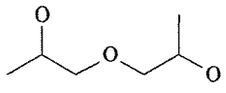
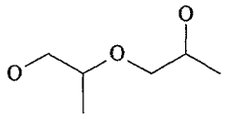
The study design complied with the following test guidelines:

- OECD Guideline for the Testing of Chemicals. Guideline 428: Skin Absorption: *in vitro* Method (2004)
- Guidance Document for the Conduct of Skin Absorption Studies. OECD Series on Testing and Assessment Number 28. (2004)
- European Commission Guidance Document on Dermal Absorption. Sanco/222/2000 rev 7 (2004)

B. Test Substance

The DPG test sample (CASN 25265-71-8) was supplied by the sponsor and assigned Haskell Laboratory Number 27231 upon receipt. The certificate of analysis (COA) is presented in Appendix A. Based on correspondence with the sponsor, the test substance has a shelf life of 12 months. DPG, with a molecular weight of 134.18 and a logP of -0.819, is a non-volatile, water-miscible liquid that is composed of the following three structural isomers:

1-Propanol, 2,2'-oxybis-	CASN 108-61-2 

2-Propanol, 1,1'-oxybis-	CASN 110-98-5 
1-Propanol, 2-(2-hydroxypropoxy)-	CASN 106-62-7 

C. Test System

1. System Reliability

The reliability of Haskell's *in vitro* dermal diffusion cell systems has been reported.[7] Overall, the results for penetration rate and total absorption for the OECD recommended chemicals benzoic acid, caffeine and testosterone were found to be comparable for Haskell's *in vitro* static and flow through diffusion cell systems and consistent with the published literature for both human and rat skin.[8,9]

2. Human Skin

Samples of human cadaver skin from the National Disease Research Interchange (NDRI), Philadelphia, Pennsylvania, U.S.A., were received frozen and stored at approximately -20°C until prepared for use.

The source and identity of the skin samples (sex, anatomical locale, and age of donor) was documented in the study records and is presented here in this report. Skin specimens selected for use were identified using a unique code (e.g., HCFA-XXX =Human, Caucasian, Female, Abdomen XXX). The following specimens were used in this study.

ID	Sex	Anatomical locale	Age of Donor	Death date	Skin harvest date	Date Used for testing
HCMA 282	Male	Abdomen	47	May 16, 2006	May 17, 2006	April 11, 2007
HCFA 275	Female	Abdomen	75	May 4, 2006	May 4, 2006	April 11, 2007
HCMA 281	Male	Abdomen	71	May 16, 2006	May 17, 2006	April 11, 2007
HCMA 284	Male	Abdomen	67	May 11, 2006	May 12, 2006	April 11, 2007

3. Justification for Selection of Test System

Dermal contamination is a potential route of human exposure. In vitro dermal techniques have been shown to be a conservative model for predicting percutaneous absorption of various chemicals in vivo.[10-12]

4. *In Vitro* Diffusion Cell Model

A flow-through diffusion cell system, model ILC14 Automated System (Permegear Inc., Bethlehem, Pennsylvania, U.S.A.), was used for this study (Figure 1). The *in vitro* cells have an exposure area of 0.64 cm^2 and a receptor fluid chamber volume of approximately 250 μL.

D. Dose Formulation and Concentration

The test substance, which was a liquid at room temperature, was applied neat. The concentration of the applied chemical was taken as its density (1.02 g/mL; g/cm^3).

E. Preparation of Skin Membranes

Samples of human cadaver skin, which were stored frozen, were thawed at room temperature. Full thickness skin was dermatomed using a Padgett Electro Dermatome® (Padgett Instruments, Inc., Kansas City, Missouri, U.S.A.). Each skin sample was placed onto an aluminum pan, with its identity written on the pan, and stored refrigerated at 1-10°C until readied for use.

F. Membrane Equilibration and Assessment of Membrane Integrity

Skin membranes were removed from refrigeration storage. After allowing the skin to come to ambient temperature, thickness measurements were taken in approximately 1.5 cm wide increments along its length using a digital micrometer (Mahr Federal Inc., Providence, Rhode Island, U.S.A., Type 40 EX). The sections along the measured edge, ~1.5 cm x 1.5 cm, were cut away and placed in 0.9% saline to hydrate for approximately 15 minutes.

Following hydration, the skin section was mounted onto the top of the receptor chamber, stratum corneum uppermost, which was maintained with 0.9% saline. The donor chamber was then clamped in place and the system perfused at a rate of 1.5 mL/h for approximately 30 minutes. During equilibration, the *in vitro* cells were heated using a re-circulating water bath system to yield a receptor fluid temperature of 32 ±1°C. Following equilibration, the integrity of each skin section was assessed by measurement of electrical impedance (EI), in kilo-ohms (k-ohms), prior to application of the test substance using a Tinsley Databridge Model 6401 (Tinsley Precision Instruments, Croydon, England) set in the resistance and parallel equivalent mode operated with AC wavelength of 1000 hertz.[13-14]

Equilibrated skin sections with impedance values of ≥17 k-ohms were considered intact and retained for use on study. Skin sections not meeting the minimal threshold for acceptance were replaced by cutting additional sections from the primary skin membrane, hydrating them in saline followed by integrity confirmation by EI until a minimum of 7 sections representing at least 3 donors was achieved. Once the number of replicates and donors were obtained, saline in the donor and receptor chambers was removed and the receptor chamber perfused with fresh receptor fluid (deionized water).

The following 7 skin sections, representing 4 donors, which ranged in thickness from 335 μm to 498 μm (389 ±54 μm), were used in this study.

Cell ID	Donor ID	Thickness (μm)
1	HCMA 282	376
2	HCMA 282	335
3	HCFA 275	343
4	HCMA 281	498
5	HCFA 275	403
6	HCMA 282	378
7	HCMA 284	391

G. Receptor Fluid

The flow-through diffusion cell system was maintained with deionized water perfused at a rate of approximately 1.5 mL/h and was allowed to equilibrate for at least 15 minutes prior to application of the test substance.

DPG was considered to be infinitely soluble in the receptor fluid using a flow-through diffusion cell system. The use of deionized water as a receptor fluid was justified in order to (1) offer sink conditions to DPG, and (2) facilitate trouble-free analysis of serial samples for total DPG by gas chromatography flame-ionization detection (GC-FID).

H. Determining the Permeability Coefficient (Kp)

Neat DPG was applied at a rate of approximately 1200 $\mu L/cm^2$ to the surface of 7 dermatomed skin replicates representing 4 donors. Following application, the donor chamber opening was occluded with tape. Serial receptor fluid samples were collected hourly during the first 8 hours post-application, and then every other hour until 24 hours post-application.

I. Dose Determination

The amount of DPG applied to each skin replicate (783,360 μg) was based on the volume of the applied dose (1200 $\mu L/cm^2$ or 768 μL to a 0.64 cm^2 *in vitro* cell exposure area), which completely filled to donor chamber compartment and so was considered infinite, and the density of the test substance (1.02 g/mL; 1,020,000 $\mu g/cm^3$).

J. Receptor Fluid Analysis – Gas Chromatography Flame Ionization Detection

The concentration of DPG in the serial receptor fluid samples was determined using the following GC-FID analytical equipment and method.

System:	Agilent Model 6890 Gas Chromatograph with FID (Agilent Technologies, Palo Alto, California, U.S.A.)
Column:	Rtx-5, 30m x 320 μm i.d. x 0.25 μm film thickness (Restek, Bellefonte, Pennsylvania, U.S.A.)
Carrier gas:	Helium
Constant flow:	2 mL/min
Oven temperature:	150°C, isothermal
Run time:	3 min

Split injection with single-taper liner:	250°C
Injection volume:	1 μL, split 10:1
Flame ionization detector:	300°C
	H_2 =40 mL/min
	Air =450 mL/min
	He (makeup) =10 mL/min

Calibration standards were prepared from a stock solution (approximately 1000 μg/mL) of the test substance (H-27231) in Omnisolve water (EM Science, Gibbstown, New Jersey, U.S.A.) at 5, 10, 20, 50, and 100 μg/mL. GC-FID response for total DPG, based on peak area for each standard, was plotted against the nominal concentration and a best-fit linear regression of the data was performed. The resultant line equation ($y = mx \pm b$, where y is the peak area, x is the concentration, m is the slope and b is the y-intercept) was used to determine the concentration of DPG in the serial receptor fluid samples.

K. Data Presentation

Steady-state penetration was determined by plotting the cumulative amount of DPG detected in the receptor compartment at each serial collection timepoint (normalized to an exposure area of 0.64 cm^2) against time (in hours) to produce an absorption profile. A permeability coefficient (Kp, in cm/h) was calculated by dividing the penetration rate or slope of the line at steady-state (μg/cm^2/h; correlation coefficient (r^2)≥0.9), represented by a minimum of 10 data points, by the concentration of applied chemical (1,020,000 μg/cm^3).

Group data is presented as a mean ±the standard deviation (SD) in the tables. Key observations of mean data are presented in the results section.

L. Protocol Deviations

1. Human Skin Specimens

Although the human skin specimens used for this investigation were stored for longer than three months, this had no affect on the data or the interpretation of the results since the integrity of all skin replicates were qualified by electrical impedance (≥17 k-ohms) prior to application of DPG.

2. Post-Exposure Electrical Impedance

A final EI was taken for each skin replicate. This was accomplished by removing excess DPG from the donor chamber, washing the skin surface with a 2% Ivory soap solution (3X), and rinsing with deionized water (1X). The donor chamber was then filled with saline and an EI measurement was then taken using the Tinsley Databridge as previously described in section F. Although this was not planned nor described in the protocol, it in no way affected the results of this experiment. The final EI, in comparison to the initial EI, provides valuable information on the condition of the stratum corneum (i.e., a damage ratio) following continuous exposure to DPG under occluded conditions.

RESULTS AND DISCUSSION

A. GC-FID Methodology

(Figures 2-3)

The correlation coefficient (r^2) of the GC-FID method for DPG, a measure of linearity for DPG concentrations of 5 to 100 μg/mL was >0.99. The GC-FID method taken with the typical concentration of DPG detected in the receptor fluid samples (~10-25 μg/mL) was sufficient to achieve the primary endpoint of the study.

B. *In Vitro* Dermal Penetration of DPG

(Tables 1-3, Figure 4, Appendix B)

Key observations of mean data:

- The integrity of human skin, as determined by EI, was unaffected by continuous exposure to DPG under occlusive conditions. The ratio of the post-EI values to pre-EI values was 0.97 confirming that the barrier properties of the stratum corneum were unaltered by DPG.
- By the conclusion of the 24-hour exposure interval, only a negligible portion of the applied dose of neat DPG (0.075%) had penetrated through the skin into the receptor fluid.
- In general, DPG was detected in receptor fluid within about an hour of application (lag time =1 hour and 3 minutes; 1.05 hours); steady-state penetration, which was represented by no less than 10 data points, was determined to be 39.3 $\mu g/cm^2/h$ ($r^2 \geq 0.999$).
- Based on the slope at steady-state (39.3 $\mu g/cm^2/h$) and the concentration of DPG in the applied solution, taken as its density (1,020,000 $\mu g/cm^3$), the permeability coefficient was calculated to be 3.85×10^{-5} cm/h.

CONCLUSIONS

A. Skin Integrity

The integrity of human skin, as determined by electrical impedance, was unaffected by continuous exposure to DPG under occlusive conditions. The ratio of the post-EI values to pre-EI values was 0.97 confirming that the barrier properties of the stratum corneum were unaltered by DPG.

B. Cumulative Percent Penetrated

At the conclusion of the 24-hour exposure interval, only a negligible portion of the applied dose of neat DPG (0.075%) had penetrated through human skin into the receptor fluid.

C. Steady-State Penetration, Kp

Based on the slope at steady-state (39.3 μg/cm²/h), and the concentration of DPG in the applied solution, taken at its density (1,020,000 μg/cm³, DPG's permeability coefficient was calculated to be 3.85 $\times 10^{-5}$ cm/h.

RECORDS AND SAMPLE STORAGE

Specimens (if applicable), raw data, the protocol, amendments (if any), and the final report will be retained at Haskell Laboratory, Newark, Delaware, and will be returned to the sponsor after 10 years, unless alternative terms are arranged for by the sponsor.

Data recorded and archived electronically, and laboratory-specific raw data such as personnel files, instrument, equipment, refrigerator and/or freezer raw data will be retained at the facility where the work was done.

REFERENCES

1. ChemExpo Chemical Profile of Dipropylene Glycol (1998).

2. The Dow Chemical Company (1999). Evaluation of dipropylene glycol in the mouse bone marrow micronucleus test. Unpublished report, The Dow Chemical Company, Midland, Michigan.

3. Oettel, H., Hofmann, H.T. (1961). Dipropylene Glycol. Report of Acute Inhalation of Dipropylene Glycol n Rats and Guinea Pigs. BASF internal report, 2 pages.

4. BIBRA Toxicology International (1991). Dipropylene glycol - Toxicity profile. BIBRA Toxicology International - British Industrial Biological Research Association, 1-4.

5. Opdyke, D.J. (1978). Dipropylene glycol. Food and Cosmetics Toxicology 16, Suppl. In: Monographs on Fragrance of Raw Materials, 729-730.

6. Deichmann, W.B., Gerarde, H.W. (1969). Acute toxicity of glycols. Toxicology of Drugs and Chemicals , 4. AFL., Academic Press, New York, 731.

7. DuPont Haskell (2005). Benzoic Acid, Caffeine, and Testosterone: In Vitro Predictions of Skin Absorption. Unpublished report DuPont-15362.

8. European Centre for Ecotoxicology and Toxicology of Chemicals (1993). Monograph Number 20, Percutaneous Absorption, ECETOC, Brussels.

9. van de Sandt, J.J.M., van Burgsteden, J.A., Cagei, S., Carmichael, P.L., Dick, I., Kenyon, S., Korinth, G., Larese, F., Limasset, J.C., Maas, W.J.M., Montomoli, L., Nielsen, J.B., Payan, J.P., Robinson, E., Sartorelli, P., Schaller, K.H., Wilkinson, S.C., and Williams, F.M. (2004). *In vitro* predictions of skin absorption of caffeine, testosterone, and benzoic acid: a multi-centre comparison study. Regulatory Toxicology and Pharmacology 39, 271-281.

10. 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.

11. 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.

12. Scott, R.C., Walker, 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.

13. Fasano, W.J., Manning, L.A., and Green, J.W. (2002). Rapid Integrity Assessment of Rat and Human Epidermal Membranes for *In Vitro* Dermal Regulatory Testing: Correlation of Electrical Resistance with Tritiated Water Permeability. Toxicology *In Vitro* 16, 731-740.

14. Fasano, W.J., Hinderliter, P.M. (2004). The Tinsley LCR Databridge Model 6401 and electrical impedance measurements to evaluate skin integrity *in vitro*. Toxicology *In Vitro* 18, 725-729.

TABLES

TABLES

EXPLANATORY NOTES

ABBREVIATIONS:

SD standard deviation
EI electrical impedance
h hour(s)

Table 1
EI values, pre- and post-exposure

Pre EI (k-ohms)		Post EI (k-ohms)		Ratio: Post/Pre	
Mean[a]	SD	Mean[a]	SD	Mean[a]	SD
26.0	6.9	24.1	3.2	0.97	0.23

a Mean and SD, n=7 replicates

Table 2
Cumulative penetration

Time (hours)	Cumulative Penetration ($\mu g/cm^2$) Mean[a]	SD
1	19.3	4.76
2	44.0	11.4
3	77.7	20.9
4	115.0	31.1
5	153.0	40.8
6	195.6	52.1
7	238.0	64.9
8	279.8	76.2
10	357.8	98.2
12	436.3	119.9
14	516.0	142.2
16	594.1	162.9
18	672.8	179.8
20	751.0	194.0
22	831.0	207.7
24	912.6	219.0

a Mean and SD, n=7 replicates

Table 3
Summary of results

	Mean[a]	SD
Steady-state penetration rate ($\mu g/m^2/h$)	39.3	10.7
Lag time (hours)	1.05	0.33
Cumulative penetration, at 24 hours (%)	0.075	0.018
Permeability coefficient, Kp (cm/h)	3.85×10^{-5}	1.05×10^{-5}

a Mean and SD, n=7 replicates

FIGURES

FIGURES

EXPLANATORY NOTES

ABBREVIATIONS:

ppm parts per million; μg/mL
GC-FID gas chromatography-flame ionization detection

Figure 1
Flow-through diffusion cell model - ILC14 Automated System
(Permegear Inc., U.S.A.)

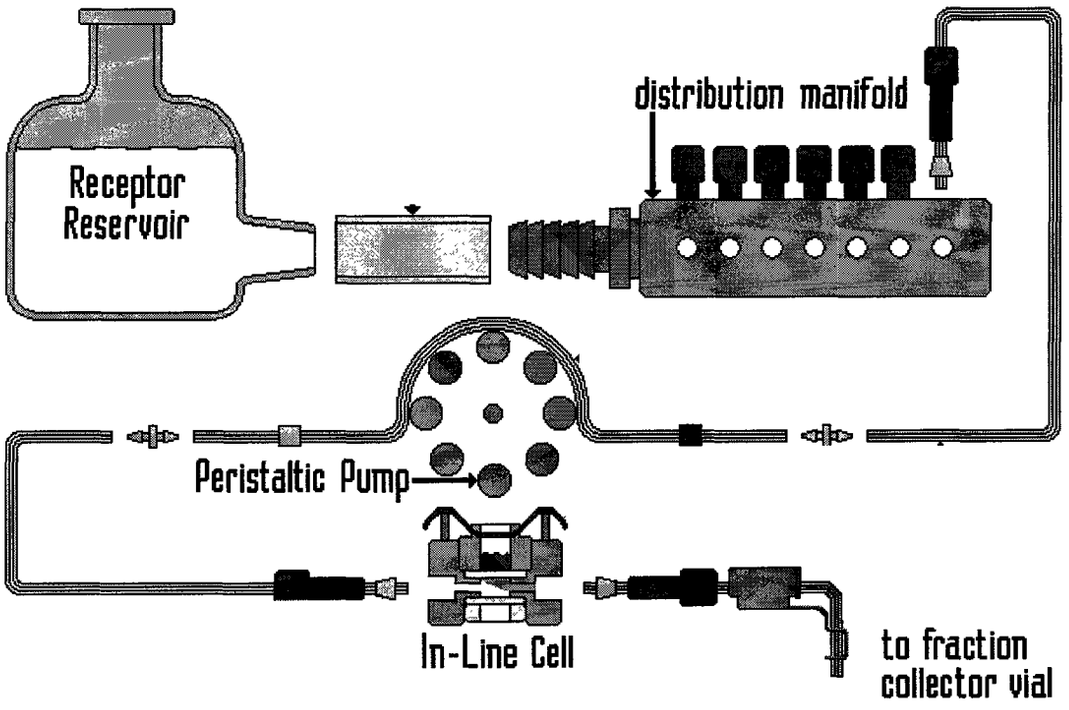


Figure 2
GC-FID, representative calibration curve

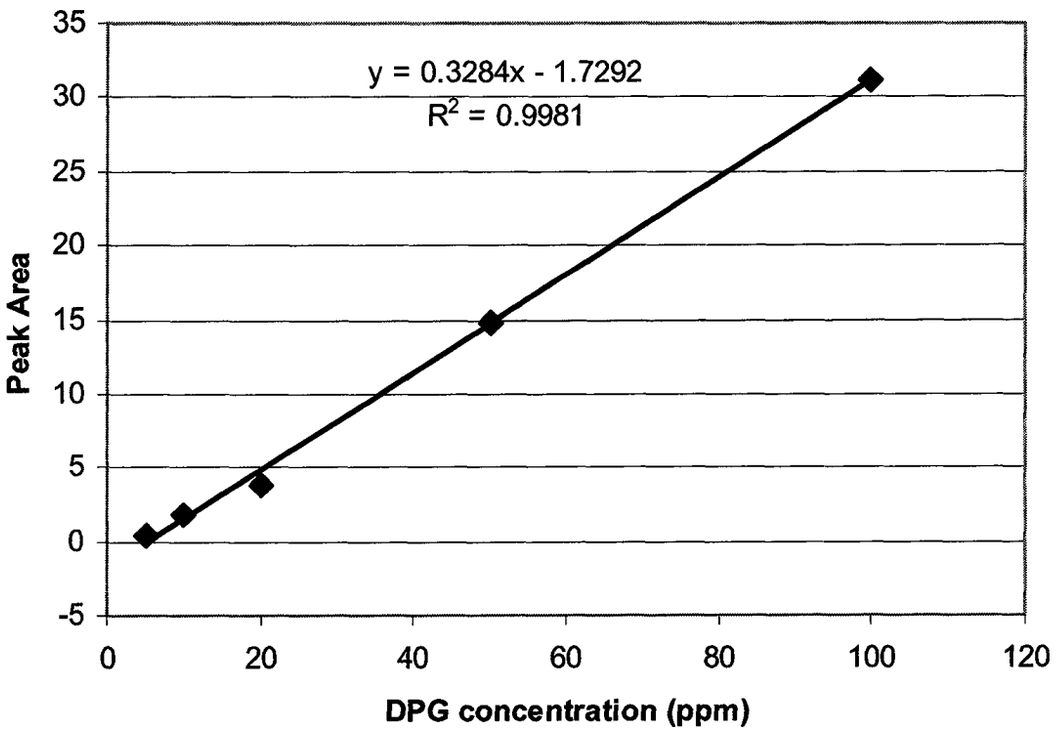
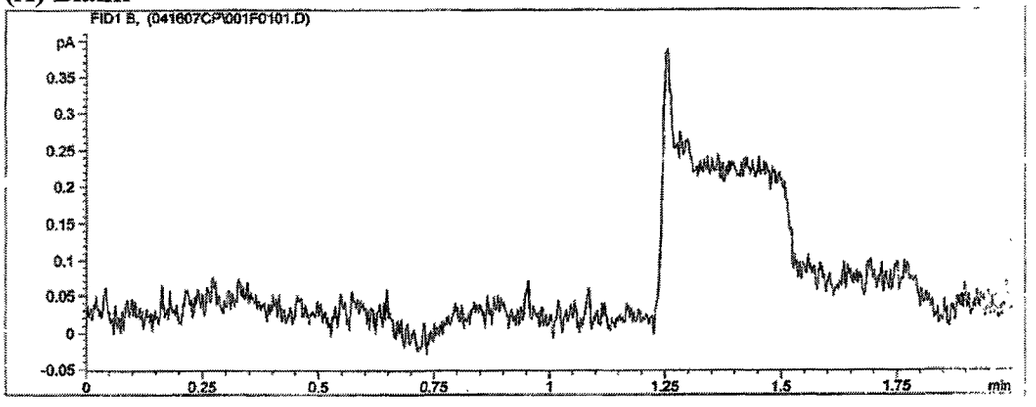
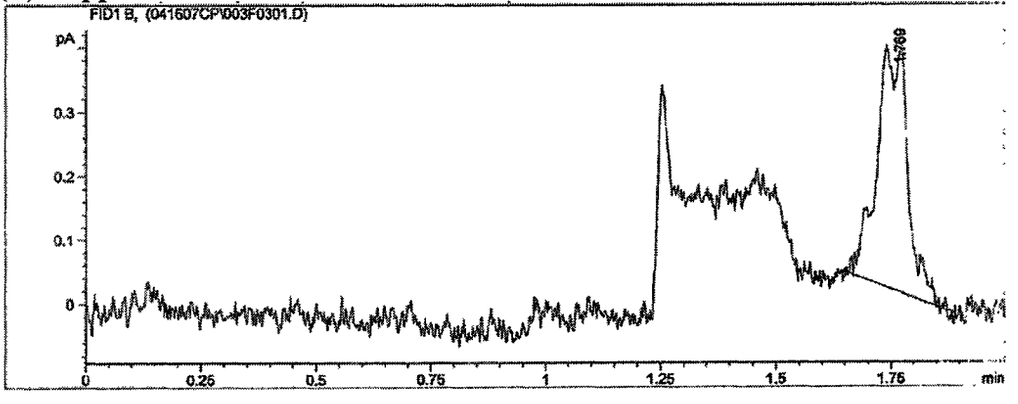


Figure 3
GC-FID, representative chromatograms

(A) Blank



(B) 10 ppm standard



(C) Cell 1, 3 hours

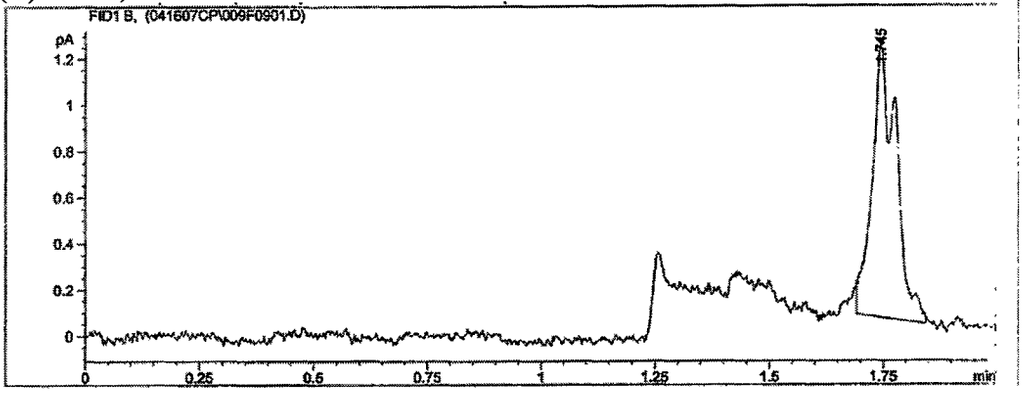
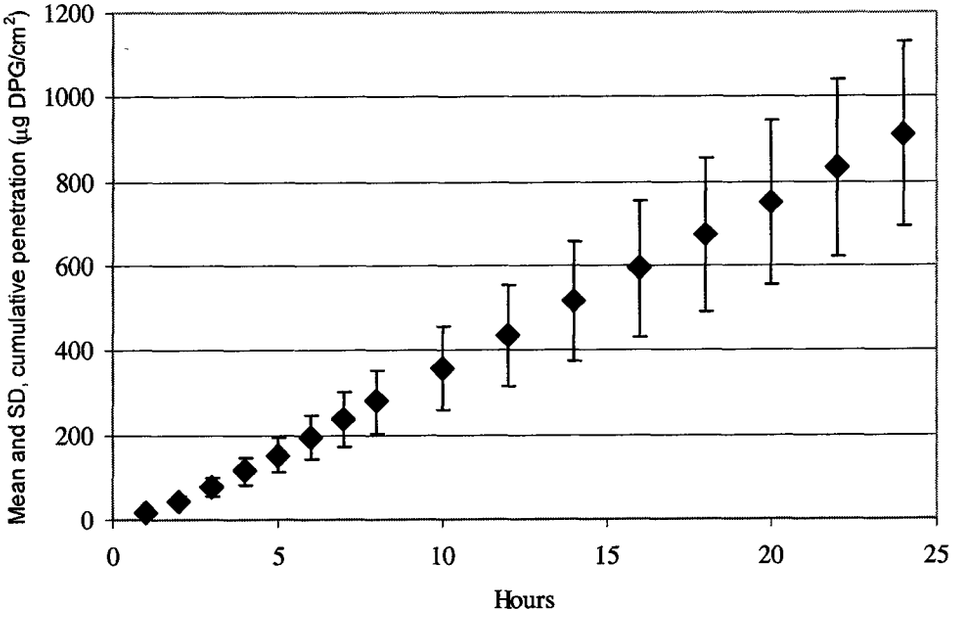


Figure 4
Cumulative penetration of DPG using human skin mounted in an *in vitro* flow-through diffusion cell model (n =7 replicates)



APPENDICES

APPENDICES

EXPLANATORY NOTES

ABBREVIATIONS:

conc	concentration
EI	electrical impedance
ID	identification
SD	standard deviation
Kp	permeability coefficient
k-ohms	kilo-ohms

Appendix A
Certificate of Analysis

Certificate 2942227 The Dow Chemical Company Page 1

Date: 19.12.2006 Certificate of Analysis

File Copy
[redacted] Fax: COA ARCHIVE
[redacted] 15 06179 GERMANY

Cust P.O.: FAX 18.12.06 AK/WO Dlvy Note: 23100042 10

Material: Dipropylene Glycol LO+
Cust Mtl:

Batch: UL19192041

Vehicle: BIDU 499257-8

Ship from: DOW EUROPE GMBH STADE 03 GERMANY

It is hereby certified, that the material indicated above has been inspected and tested in accordance with the conditions and requirements of the contract or purchase order and, unless agreed otherwise, conforms in all respects to the specification relevant thereto.

Feature	Units		Results UL19192041	Limits Minimum	Maximum	Method	
Assay calculated	% wt		99,9	99,5	----	DOWM 100761	
Acidity As Acetic Acid	% wt		0,0004	----	0,0010	DOWM 101370	
Appearance clear/matter free			Passes	----	----	Visual	
Chlorides	ppm	<	0,5	----	1	DOWM 101867	
Color, Pt-Co	-		2,3	----	10	ASTM D5386	
Iron	ppm		0,01	----	0,100	ASTM E202	
Odor characteristic, slight			Passes	----	----	Olfactory	
Propylene Glycol	%		0,01	----	0,40	DOWM 100761	
Tripropylene Glycol	%		0,052	----	0,500	DOWM 100761	
Water Content	% wt		0,001	----	0,100	ASTM E203	
DPG Isomer #1	%		38	27	43	DOWM 101518	D
DPG Isomers #2 + #3	%		49	42	56	DOWM 101518	D
DPG Isomer #4	%		6	5	10	DOWM 101518	D
DPG Isomer #5	%		6	3	14	DOWM 101518	D
Residue on Ignition	% wt		0,001	----	0,0050	ASTM D1119	D

SOURCE OF ANALYSIS
M Quarterly Sample
O Annual Sample

Plant Quality Coordinator

For inquiries please contact Customer Service or local sales

The certificate of analysis for the DPG stated the following breakdown:

DPG isomer 1: 38% (27-43)
DPG isomers 2 & 3: 49% (42-56)
DPG isomer 4: 6% (5-10)
DPG isomer 5: 6% (3-14)

These correspond with the following identities:

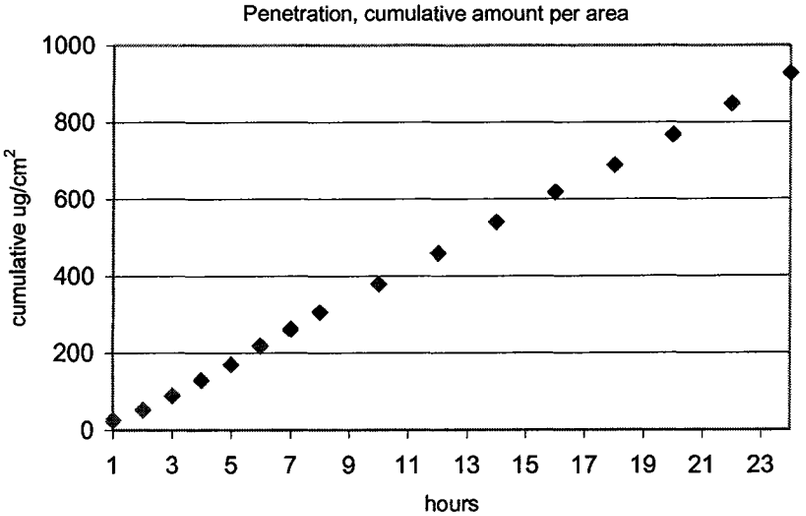
1: 2,2'-dihydroxydipropylether, CAS 110-98-5
1,1'-oxybis-2-propanol
2 & 3: 2-hydroxypropyl-2'-hydroxyisopropyl-ether, CAS 106-62-7
2-(2-hydroxypropoxy)-1-propanol
4 & 5: 2,2'-dihydroxydiisopropylether, CAS 108-61-2
2,2'-oxybis-1-propanol

Appendix B
Individual Data

Work Request	16155	
Service Code	1623	
Protocol group	STUDY	
Species	Human, Dermatomed	
Test Material	H# 27231	
Specimen ID	HCMA-282	
Cell ID	Cell 1	
Cell area	0.64	cm^2
Dose volume	0.768	mL
Dose concentration	1020000	ug/cm^3
Total DPG in dose	783360	ug
Initial EI	25.0	k-ohms
Final EI	29.8	k-ohms

NB E-99681-DQ

sample ID	Time (hours)	Sample conc. (ug/mL)	Total volume (mL)	DPG in sample (ug)	Cumulative DPG (ug)	Cumulative Percent Absorbed	Cumulative amount/area (ug/cm^2)
1	1	12.0	1.31	15.7	16	0.002	25
2	2	13.2	1.31	17.3	33	0.004	52
3	3	18.4	1.31	24.1	57	0.007	89
4	4	19.3	1.31	25.3	82	0.011	129
5	5	20.2	1.31	26.5	109	0.014	170
6	6	22.9	1.31	30.0	139	0.018	217
7	7	21.1	1.31	27.6	167	0.021	260
8	8	21.4	1.31	28.0	195	0.025	304
9	10	18.4	2.62	48.2	243	0.031	379
10	12	19.3	2.62	50.6	293	0.037	458
11	14	19.6	2.62	51.4	345	0.044	539
12	16	19.3	2.62	50.6	395	0.050	618
13	18	17.1	2.62	44.8	440	0.056	688
14	20	19.3	2.62	50.6	491	0.063	767
15	22	19.9	2.62	52.1	543	0.069	848
16	24	19.6	2.62	51.4	594	0.076	928

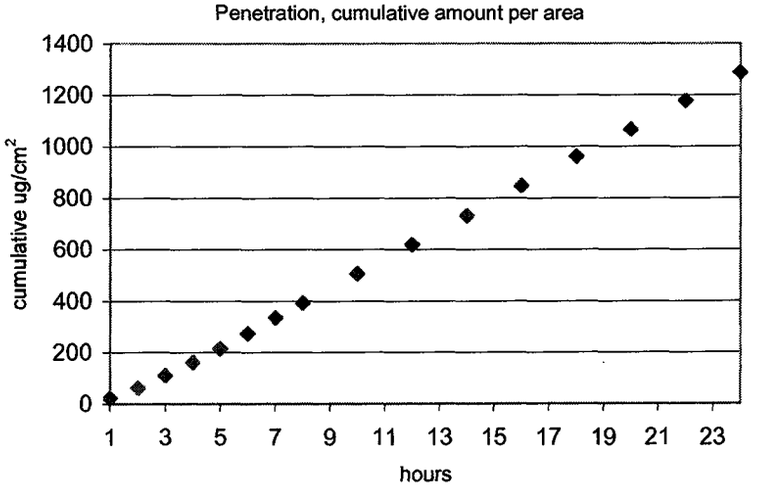


Penetration rate	39.7	ug/cm2/h
Kp	3.89E-05	cm/h
Lag time	0.57	h
Kp interval	3 to 24	h
Correlation	1.000	

Work Request	16155	
Service Code	1623	
Protocol group	STUDY	
Species	Human, Dermatomed	
Test Material	H# 27231	
Specimen ID	HCMA-282	
Cell ID	Cell 2	
Cell area	0.64	cm^2
Dose volume	0.768	mL
Dose concentration	1020000	ug/cm^3
Total DPG in dose	783360	ug
Initial EI	17.3	k-ohms
Final EI	21.6	k-ohms

NB E-99681-DQ

sample ID	Time (hours)	Sample conc. (ug/mL)	Total volume (mL)	DPG in sample (ug)	Cumulative DPG (ug)	Cumulative Percent Absorbed	Cumulative amount/area (ug/cm^2)
1	1	10.6	1.31	13.9	14	0.002	22
2	2	19.8	1.31	25.9	40	0.005	62
3	3	23.4	1.31	30.7	70	0.009	110
4	4	24.9	1.31	32.6	103	0.013	161
5	5	25.5	1.31	33.4	137	0.017	213
6	6	29.4	1.31	38.5	175	0.022	273
7	7	30.0	1.31	39.3	214	0.027	335
8	8	28.5	1.31	37.3	252	0.032	393
9	10	27.3	2.62	71.5	323	0.041	505
10	12	27.6	2.62	72.3	395	0.050	618
11	14	27.6	2.62	72.3	468	0.060	731
12	16	28.5	2.62	74.7	542	0.069	848
13	18	27.6	2.62	72.3	615	0.078	961
14	20	25.8	2.62	67.6	682	0.087	1066
15	22	27.0	2.62	70.7	753	0.096	1177
16	24	27.0	2.62	70.7	824	0.105	1287

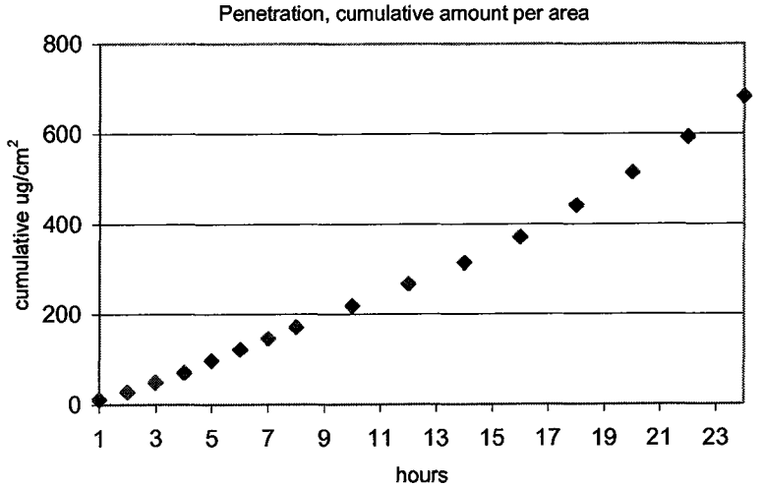


Penetration rate	56.5	ug/cm2/h
Kp	5.54E-05	cm/h
Lag time	1.10	h
Kp interval	4 to 24	h
Correlation	1.000	

Work Request	16155	
Service Code	1623	
Protocol group	STUDY	
Species	Human, Dermatomed	
Test Material	H# 27231	
Specimen ID	HCFA-275	
Cell ID	Cell 3	
Cell area	0.64	cm^2
Dose volume	0.768	mL
Dose concentration	1020000	ug/cm^3
Total DPG in dose	783360	ug
Initial EI	36.1	k-ohms
Final EI	23.8	k-ohms

NB E-99681-DQ

sample ID	Time (hours)	Sample conc. (ug/mL)	Total volume (mL)	DPG in sample (ug)	Cumulative DPG (ug)	Cumulative Percent Absorbed	Cumulative amount/area (ug/cm^2)
1	1	5.72	1.31	7.49	7	0.001	12
2	2	7.83	1.31	10.3	18	0.002	28
3	3	10.5	1.31	13.8	32	0.004	49
4	4	10.5	1.31	13.8	45	0.006	71
5	5	12.3	1.31	16.1	61	0.008	96
6	6	12.6	1.31	16.5	78	0.010	122
7	7	11.7	1.31	15.3	93	0.012	146
8	8	12.3	1.31	16.1	109	0.014	171
9	10	11.4	2.62	29.9	139	0.018	217
10	12	12.0	2.62	31.4	171	0.022	267
11	14	11.4	2.62	29.9	200	0.026	313
12	16	14.1	2.62	36.9	237	0.030	371
13	18	16.8	2.62	44.0	281	0.036	440
14	20	18.0	2.62	47.2	329	0.042	513
15	22	19.2	2.62	50.3	379	0.048	592
16	24	22.0	2.62	57.6	437	0.056	682

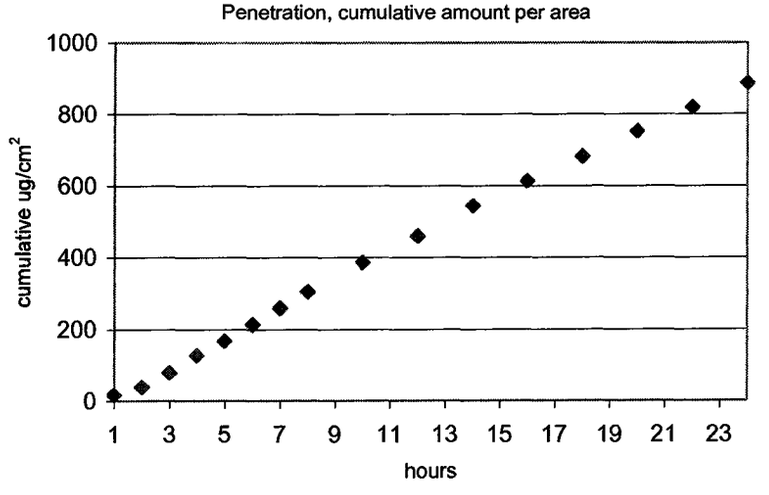


Penetration rate	25.5	ug/cm2/h
Kp	2.50E-05	cm/h
Lag time	1.36	h
Kp interval	4 to 18	h
Correlation	0.999	

NB E-99681-DQ

Work Request	16155	
Service Code	1623	
Protocol group	STUDY	
Species	Human, Dermatomed	
Test Material	H# 27231	
Specimen ID	HCMA-281	
Cell ID	Cell 4	
Cell area	0.64	cm2
Dose volume	0.768	mL
Dose concentration	1020000	ug/cm3
Total DPG in dose	783360	ug
Initial EI	27.7	k-ohms
Final EI	25.8	k-ohms

sample ID	Time (hours)	Sample conc. (ug/mL)	Total volume (mL)	DPG in sample (ug)	Cumulative DPG (ug)	Cumulative Percent Absorbed	Cumulative amount/area (ug/cm2)
1	1	8.28	1.31	10.8	11	0.001	17
2	2	10.7	1.31	14.0	25	0.003	39
3	3	19.8	1.31	25.9	51	0.006	79
4	4	23.5	1.31	30.8	82	0.010	127
5	5	20.2	1.31	26.5	108	0.014	169
6	6	21.5	1.31	28.2	136	0.017	213
7	7	22.5	1.31	29.5	166	0.021	259
8	8	21.9	1.31	28.7	194	0.025	304
9	10	20.1	2.62	52.7	247	0.032	386
10	12	18.2	2.62	47.7	295	0.038	461
11	14	20.4	2.62	53.4	348	0.044	544
12	16	17.0	2.62	44.5	393	0.050	614
13	18	16.7	2.62	43.8	436	0.056	682
14	20	17.3	2.62	45.3	482	0.062	753
15	22	16.0	2.62	41.9	524	0.067	818
16	24	16.7	2.62	43.8	567	0.072	887

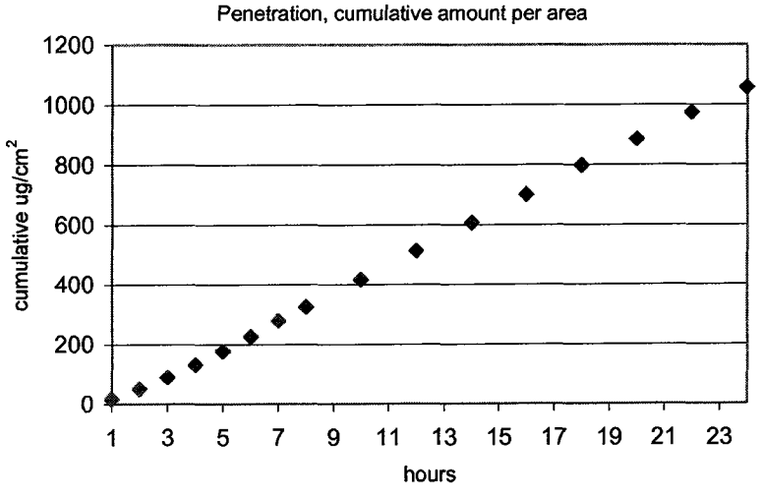


Penetration rate	38.7	ug/cm2/h
Kp	3.80E-05	cm/h
Lag time	0.92	h
Kp interval	2 to 24	h
Correlation	0.999	

Work Request	16155	
Service Code	1623	
Protocol group	STUDY	
Species	Human, Dermatomed	
Test Material	H# 27231	
Specimen ID	HCFA-275	
Cell ID	Cell 5	
Cell area	0.64	cm2
Dose volume	0.768	mL
Dose concentration	1020000	ug/cm3
Total DPG in dose	783360	ug
Initial EI	17.4	k-ohms
Final EI	20.2	k-ohms

NB E-99681-DQ

sample ID	Time (hours)	Sample conc. (ug/mL)	Total volume (mL)	DPG in sample (ug)	Cumulative DPG (ug)	Cumulative Percent Absorbed	Cumulative amount/area (ug/cm2)
1	1	8.92	1.31	11.7	12	0.001	18
2	2	15.8	1.31	20.7	32	0.004	51
3	3	19.6	1.31	25.7	58	0.007	91
4	4	20.5	1.31	26.9	85	0.011	133
5	5	21.6	1.31	28.3	113	0.014	177
6	6	23.9	1.31	31.3	145	0.018	226
7	7	25.1	1.31	32.9	177	0.023	277
8	8	23.4	1.31	30.7	208	0.027	325
9	10	22.1	2.62	57.9	266	0.034	416
10	12	23.5	2.62	61.6	328	0.042	512
11	14	23.1	2.62	60.5	388	0.050	606
12	16	23.1	2.62	60.5	449	0.057	701
13	18	23.6	2.62	61.8	510	0.065	798
14	20	21.4	2.62	56.1	566	0.072	885
15	22	21.5	2.62	56.3	623	0.080	973
16	24	20.6	2.62	54.0	677	0.086	1057

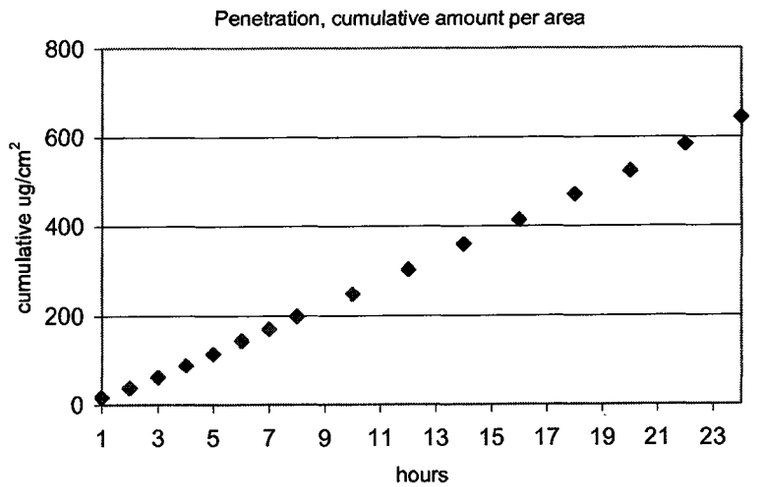


Penetration rate	46.5	ug/cm2/h
Kp	4.56E-05	cm/h
Lag time	1.05	h
Kp interval	2 to 24	h
Correlation	1.000	

Work Request	16155	
Service Code	1623	
Protocol group	STUDY	
Species	Human, Dermatomed	
Test Material	H# 27231	
Specimen ID	HCMA-282	
Cell ID	Cell 6	
Cell area	0.64	cm2
Dose volume	0.768	mL
Dose concentration	1020000	ug/cm3
Total DPG in dose	783360	ug
Initial EI	31.8	k-ohms
Final EI	25.4	k-ohms

NB E-99681-DQ

sample ID	Time (hours)	Sample conc. (ug/mL)	Total volume (mL)	DPG in sample (ug)	Cumulative DPG (ug)	Cumulative Percent Absorbed	Cumulative amount/area (ug/cm2)
1	1	8.23	1.31	10.8	11	0.001	17
2	2	10.1	1.31	13.2	24	0.003	38
3	3	11.8	1.31	15.5	39	0.005	62
4	4	13.2	1.31	17.3	57	0.007	89
5	5	12.5	1.31	16.4	73	0.009	114
6	6	14.8	1.31	19.4	93	0.012	145
7	7	12.9	1.31	16.9	109	0.014	171
8	8	13.5	1.31	17.7	127	0.016	199
9	10	12.3	2.62	32.2	159	0.020	249
10	12	13.3	2.62	34.8	194	0.025	303
11	14	13.6	2.62	35.6	230	0.029	359
12	16	13.2	2.62	34.6	264	0.034	413
13	18	14.0	2.62	36.7	301	0.038	470
14	20	12.9	2.62	33.8	335	0.043	523
15	22	14.6	2.62	38.3	373	0.048	583
16	24	14.8	2.62	38.8	412	0.053	644

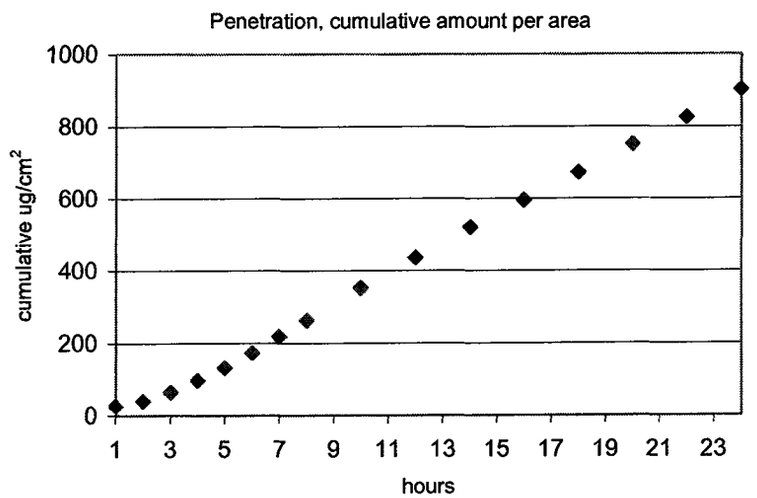


Penetration rate	27.4	ug/cm2/h
Kp	2.69E-05	cm/h
Lag time	0.79	h
Kp interval	2 to 24	h
Correlation	1.000	

NB E-99681-DQ

Work Request	16155	
Service Code	1623	
Protocol group	STUDY	
Species	Human, Dermatomed	
Test Material	H# 27231	
Specimen ID	HCMA-284	
Cell ID	Cell 7	
Cell area	0.64	cm2
Dose volume	0.768	mL
Dose concentration	1020000	ug/cm3
Total DPG in dose	783360	ug
Initial EI	26.5	k-ohms
Final EI	22.0	k-ohms

sample ID	Time (hours)	Sample conc. (ug/mL)	Total volume (mL)	DPG in sample (ug)	Cumulative DPG (ug)	Cumulative Percent Absorbed	Cumulative amount/area (ug/cm2)
1	1	12.2	1.31	16.0	16	0.002	25
2	2	7.21	1.31	9.45	25	0.003	40
3	3	11.6	1.31	15.2	41	0.005	63
4	4	15.8	1.31	20.7	61	0.008	96
5	5	17.4	1.31	22.8	84	0.011	131
6	6	20.6	1.31	27.0	111	0.014	174
7	7	21.8	1.31	28.6	140	0.018	218
8	8	21.9	1.31	28.7	168	0.021	263
9	10	21.8	2.62	57.1	225	0.029	352
10	12	20.4	2.62	53.4	279	0.036	436
11	14	20.5	2.62	53.7	333	0.042	520
12	16	18.3	2.62	47.9	381	0.049	595
13	18	18.8	2.62	49.3	430	0.055	672
14	20	19.0	2.62	49.8	480	0.061	749
15	22	18.6	2.62	48.7	528	0.067	826
16	24	18.9	2.62	49.5	578	0.074	903



Penetration rate	40.6	ug/cm2/h
Kp	3.98E-05	cm/h
Lag time	1.53	h
Kp interval	3 to 24	h
Correlation	1.000	

Pre- and Post-EI values (k-ohms)

Cell ID	Donor ID	Pre-EI (k-ohms)	Post-EI (k-ohms)	Ratio: Post/Pre
1	HCMA 282	25.0	29.8	1.19
2	HCMA 282	17.3	21.6	1.25
3	HCFA 275	36.1	23.8	0.66
4	HCMA 281	27.7	25.8	0.93
5	HCFA 275	17.4	20.2	1.16
6	HCMA 282	31.8	25.4	0.80
7	HCMA 284	26.5	22.0	0.83
	Mean	26.0	24.1	0.97
	SD	6.9	3.2	0.23

Cumulative penetration (µg/cm²)

		Hours							
Cell ID	Donor ID	1	2	3	4	5	6	7	8
1	HCMA 282	25	52	89	129	170	217	260	304
2	HCMA 282	22	62	110	161	213	273	335	393
3	HCFA 275	12	28	49	71	96	122	146	171
4	HCMA 281	17	39	79	127	169	213	259	304
5	HCFA 275	18	51	91	133	177	226	277	325
6	HCMA 282	17	38	62	89	114	145	171	199
7	HCMA 284	25	40	63	96	131	174	218	263
	Mean	19.3	44.0	77.7	115.0	153.0	195.6	238.0	279.8
	SD	4.76	11.4	20.9	31.1	40.8	52.1	64.9	76.2

		Hours							
Cell ID	Donor ID	10	12	14	16	18	20	22	24
1	HCMA 282	379	458	539	618	688	767	848	928
2	HCMA 282	505	618	731	848	961	1066	1177	1287
3	HCFA 275	217	267	313	371	440	513	592	682
4	HCMA 281	386	461	544	614	682	753	818	887
5	HCFA 275	416	512	606	701	798	885	973	1057
6	HCMA 282	249	303	359	413	470	523	583	644
7	HCMA 284	352	436	520	595	672	749	826	903
	Mean	357.8	436.3	516.0	594.1	672.8	751.0	831.0	912.6
	SD	98.2	119.9	142.2	162.9	179.8	194.0	207.7	219.0

Summary of Results

Cell ID	Donor ID	Steady-state Penetration rate (µg/cm²/h)	Cumulative Percent Penetrated	Kp (cm/h)	Lag time (h)
1	HCMA 282	39.7	0.076	3.89×10^{-5}	0.57
2	HCMA 282	56.5	0.105	5.54×10^{-5}	1.10
3	HCFA 275	25.5	0.056	2.50×10^{-5}	1.36
4	HCMA 281	38.7	0.072	3.80×10^{-5}	0.92
5	HCFA 275	46.5	0.086	4.56×10^{-5}	1.05
6	HCMA 282	27.4	0.053	2.69×10^{-5}	0.79
7	HCMA 284	40.6	0.074	3.98×10^{-5}	1.53
	Mean	39.3	0.075	3.85×10^{-5}	1.05
	SD	10.7	0.018	1.05×10^{-5}	0.33

Appendix C
Protocol

DuPont-22212

Dipropylene Glycol:
In Vitro Dermal Absorption Rate Testing

Work Request Number 16155

Service Code 1623

Protocol

Performing Laboratory: E.I. du Pont de Nemours and Company
Haskell[SM] Laboratory for Health and Environmental Sciences
P.O. Box 50
Newark, Delaware 19714
U.S.A.

Dipropylene Glycol.
In Vitro Dermal Absorption Rate Testing DuPont-22212

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Dipropylene Glycol:
In Vitro Dermal Absorption Rate Testing DuPont-22212

INTRODUCTION

Dipropylene glycol (DPG), a component of many commercial products such as antifreeze, air fresheners, cosmetic products, solvents, and plastics, is produced as a byproduct of the manufacture of propylene glycol. The United States production capacity of dipropylene glycol was 131 million pounds (60 thousand tonnes) in 1998.[1]

Although DPG possess low acute toxicity by the oral, dermal, and inhalation exposure routes, its high production volume and worldwide exposure potential to humans, either in the workplace or via various consumer products, warrants evaluation of its dermal penetration potential using conservative in vitro testing methods.[2-6]

OBJECTIVE

The objective of this study is to determine a permeability coefficient (Kp) for dipropylene glycol (DPG) using human cadaver skin mounted in an *in vitro* diffusion cell model.

SPONSOR AND CONTACT INFORMATION

Sponsor:	The Propylene Oxide and Glycols Sector Group of CEFIC Avenue E. van Nieuwenhuyse, 4 box 1, B-1160 Brussels Belgium	
Sponsor Contact:	Administration:	Christian Guilliard cgi@cefic.be
	Scientific:	Chantal Smulders +31 70 377 2176 chantal.smulders@shell.com
Testing Facility Contact:	William J. Fasano, Sr. 302-366-5216 william.j.fasano@usa.dupont.com	
Sponsor Approval:	found on the Work Authorization Form	

REGULATORY COMPLIANCE AND TEST GUIDELINES

This study will be conducted in compliance with the following good laboratory practice(s), which are compatible with current OECD and MAFF (Japan) Good Laboratory Practices:

- U.S. EPA TSCA (40 CFR part 792) Good Laboratory Practice Standards

This study has been designed to meet the following testing requirements:

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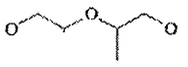
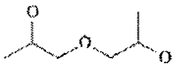
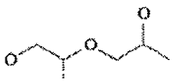
- OECD Guideline for the Testing of Chemicals. Guideline 428: Skin Absorption: *in vitro* Method (2004)
- Guidance Document for the Conduct of Skin Absorption Studies. OECD Series on Testing and Assessment No. 28. (2004)
- European Commission Guidance Document on Dermal Absorption. Sanco/222/2000 rev 7 (2004)

MATERIALS AND METHODS

A. Test Substance

DPG (*logP* = -0.819), a mixture of three structural isomers (CASN 25265-71-8), was supplied by the sponsor and assigned Haskell Laboratory Number 27231 upon receipt. Available information on the purity, composition, contaminants, basic physical properties, hazards, and hazardous material classification(s) will be documented in the study records and/or report.

Isomers:	CASN 108-61-2 
	CASN 110-98-5 
	CASN 106-62-7 

B. Test System

1. Human Skin

Samples of human cadaver skin from the National Disease Research Interchange (NDRI) will be stored frozen at approximately -20°C until prepared for use. The elapsed time between death of the donor and harvesting of the skin sample, if available from the supplier, will be documented in the study records. Samples should be stored for less than three months prior to use.

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The source and identity of the skin sample (sex, anatomical locale (abdomen), and age of donor) will be documented in the study records. Skin specimens selected for use will be identified using a unique code (e.g., HCFA-26A = Human, Caucasian, Female, Abdomen sample 26-A).

2. Justification for Selection of Test System

Dermal contamination is a potential route of human exposure. *In vitro* dermal techniques have been shown to be a conservative model for predicting percutaneous absorption of various chemicals *in vivo*.[7-9]

3. *In Vitro* Diffusion Cell Model

A flow-through diffusion cell model will be used for this study (Figure 1). The *in vitro* cells have an exposure area of 0.64 cm^2 and a receptor fluid chamber volume of approximately 250 µL.

C. Dose Formulation and Concentration

The test substance, which is a liquid at room temperature, will be applied neat. The concentration of the applied chemical will be taken as its density (1.02 g/mL; g/cm^3).

D. Preparation of Skin Membranes

Samples of human cadaver skin obtained from the abdominal region, which are stored frozen, will be thawed at room temperature. Full thickness skin will be dermatomed to approximately 200-500 µm using a Padgett Electro Dermatome® (Padgett Instruments, Inc., Kansas City, MO). The thickness of each dermatomed skin section will be confirmed using a micrometer. The skin sample will then be placed onto an aluminum pan, with its identity written or embossed on the pan, and stored refrigerated at 0-10°C until readied for use.

E. Membrane Equilibration and Assessment of Membrane Integrity

Membranes will be removed from refrigeration storage and hydrated in 0.9% saline for approximately 15 minutes. Following hydration, the membrane will be mounted onto the top of the receptor chamber, *stratum corneum* uppermost, which is maintained with 0.9% saline. The donor chamber will then be clamped in place and the system perfused at a rate of 1.5 mL/h for approximately 30 minutes. During equilibration, the *in vitro* cells will be heated using a re-circulating water bath system to yield a receptor fluid temperature of approximately 32°C. Following equilibration, the integrity of each membrane will be assessed by measurement of electrical impedance prior to application of the test substance.[10-11]

Membranes with an impedance of ≥17 kΩ will be considered intact and retained for use on study. Membranes not meeting these criteria may be replaced, and electrical impedance confirmed following equilibration. Saline in the donor and receptor chambers will be removed prior to dosing, and the receptor chamber filled with fresh receptor fluid.

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F. Receptor Fluid

The flow-through diffusion cell system will be maintained with deionized water perfused at a rate of approximately 1.5 mL/h and allowed to equilibrate for at least 15 minutes prior to dosing.

For the purposes of offering sink conditions, DPG will be considered to be infinitely soluble in the water receptor fluid.

G. Determining the Permeability Coefficient (Kp)

DPG will be applied to the skin surface at a rate of approximately 1200 $\mu L/cm^2$ to at least 6 replicates represented by at least 3 donors. Following dosing, the donor chamber opening will be occluded and serial receptor fluid samples will be collected at recorded intervals throughout the exposure period until steady-state penetration is achieved, which should be represented by a minimum of four data points.

H. Dose Determination

The amount of DPG applied to each skin replicate will be based on the volume of the applied dose and the density of the test substance (1.02 g/mL; g/cm^3).

I. Sample Handling and Processing

The concentration of DPG in the receptor fluid samples will be determined using a suitable analytical method (e.g., APCI-MS), which will be documented in the study records and presented in the final report.

J. Data Presentation

Steady-state penetration will be determined by plotting the cumulative amount of DPG detected in the receptor compartment at each serial collection timepoint against time (in hours) to produce an absorption profile. The chemical's Kp (i.e., cm/h) will be calculated by dividing the penetration rate or slope of the line at steady-state (e.g., $\mu g/cm^2/h$) by the concentration of applied chemical (e.g., $\mu g/cm^3$).

K. Pilot Experiments

Pilot experiments may be conducted to establish definitive methods and procedures. Results of the pilot experiments will not be included in the final report but will be maintained with the study records.

SAFETY AND HOUSEKEEPING

All chemicals used during this study will be handled according to the procedures specified in the MSDS and disposed of according to the Stine-Haskell Waste Disposal Guidelines and the area Safety, Health and Environmental (SHE) manual.

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RECORDS AND SAMPLE STORAGE

All raw data, the protocol, amendments (if any), and the final report will be retained.

PROPOSED STUDY DATES

Experimental Start:	March 2007
Experimental Termination:	March 2007

REFERENCES

1. ChemExpo Chemical Profile of Dipropylene Glycol (1998).
2. The Dow Chemical Company (1999). Evaluation of dipropylene glycol in the mouse bone marrow micronucleus test. Unpublished report, The Dow Chemical Company, Midland, Michigan.
3. Oettel, H., Hofmann, H.T. (1961). Dipropylene Glycol. Report of Acute Inhalation of Dipropylene Glycol n Rats and Guinea Pigs. BASF internal report, 2 pages.
4. BIBRA Toxicology International (1991). Dipropylene glycol - Toxicity profile. BIBRA Toxicology International - British Industrial Biological Research Association, 1-4.
5. Opdyke, D.J. (1978). Dipropylene glycol. Food and Cosmetics Toxicology 16, Suppl. In: Monographs on Fragrance of Raw Materials, 729-730.
6. Deichmann, W.B., Gerarde, H.W. (1969). Acute toxicity of glycols. Toxicology of Drugs and Chemicals , 4. AFL., Academic Press, New York, 731.
7. 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.
8. 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.
9. Scott, R.C., Walker, 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.
10. Fasano, W.J., Manning, L.A., and Green, J.W. (2002). Rapid Integrity Assessment of Rat and Human Epidermal Membranes for In Vitro Dermal Regulatory Testing: Correlation of Electrical Resistance with Tritiated Permeability. Toxicology In Vitro 16, 731-740.

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In Vitro Dermal Absorption Rate Testing DuPont-22212

11. Fasano. W.J., Hinderliter, P.M. (2004). The Tinsley LCR Databridge Model 6401 and electrical impedance mensurements to evaluate skin integrity in vitro. Toxicology In Vitro 18, 725-729.

SIGNATURES

Approved by: _____ 26-MAR-2007
William J. Fasano, Sr., B.S. Date
Study Director

Dipropylene Glycol:
In Vitro Dermal Absorption Rate Testing DuPont-22212
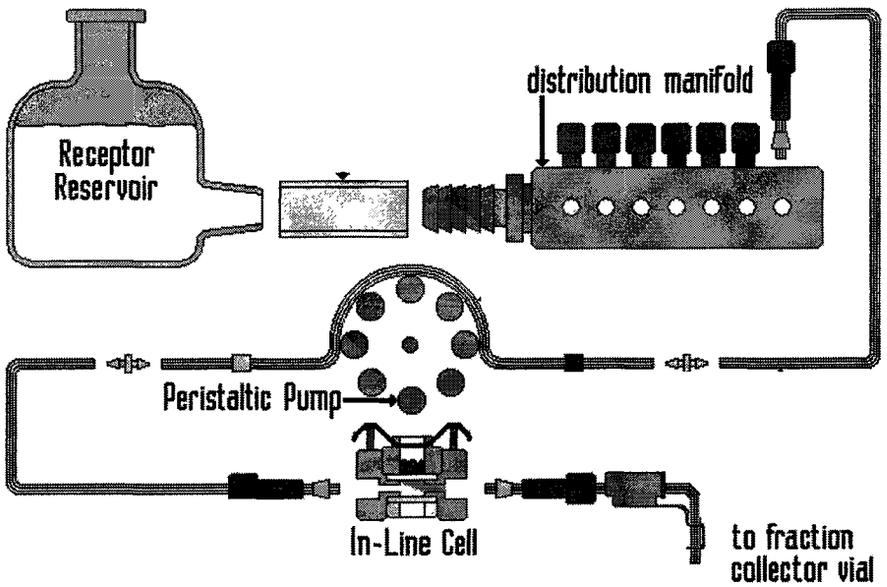
Figure 1: Flow-Through Diffusion Cell