HAP Task Force
P.O. Box 331, Millwood, VA 22646 (540) 837-1602

June 24, 2005

BY HAND

Document Control Office (7407)
Office of Pollution Prevention and Toxics
Room G-099
U.S. Environment Protection Agency
1200 Pennsylvania Ave., N.W.
Washington, DC 20460

Attn: TSCA § 4

Re: OPPT-2003-0006; ***In Vitro*** Dermal Absorption Rate Testing for Ethylene Dichloride (CAS No. 107-06-2)

Dear Sirs:

Enclosed in sextuplicate is the final report required under the TSCA § 4 Test Rule "*In **Vitro*** Dermal Absorption Rate Testing of Certain Chemicals of Interest to the Occupational Health and Safety Administration," 40 C.F.R. § 799.5115, 60 Fed. Reg. 22402-441 (April 26, 2004) (the "Test Rule"), entitled "Ethylene Dichloride: ***In Vitro*** Dermal Absorption Rate Testing."

Please do not hesitate to contact me if there is any question about this report.

Sincerely,

Peter E. Voytek /ADS

Peter E. Voytek, Ph.D.
Manager

Enclosures

cc: W. Caffey Norman, Esq.

287277

Study Title

Ethylene Dichloride:
In Vitro Dermal Absorption Rate Testing

TEST GUIDELINES: In Vitro Dermal Absorption Rate Testing of Certain Chemicals of Interest to the Occupational Safety and Health Administration. Federal Register: April 26, 2004 (Volume 69, Number 80)

OECD Guideline for the Testing of Chemicals. Draft New Guideline 428: Skin Absorption: in vitro Method. (2002)

OECD Draft Guidance Document for the Conduct of Skin Absorption Studies. OECD Environmental Health and Safety Publications Series on Testing and Assessment No. 28. (2002)

European Commission Guidance Document on Dermal Absorption. Sanco/222/2000 rev 6 (2002)

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

STUDY COMPLETED ON: June 14,2005

PERFORMING LABORATORY: E.I. du Pont de Nemours and Company
Haskell[SM] Laboratory for Health and Environmental Sciences
Elkton Road, P.O. Box 50
Newark, Delaware 19714-0050
U.S.A.

LABORATORY PROJECT ID: DuPont-16275

WORK REQUEST NUMBER: 15525

SERVICE CODE NUMBER: 1623

SPONSOR: HAP **Task** Force
1680 Bishop Meade Highway
Millwood, Virginia 22646
U.S.A.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with U.S. EPA TSCA (40 CFR part 792) Good Laboratory Practice Standards (1989), which are compatible with the OECD Principles of Good Laboratory Practice (as revised 1997), ENV/MC/CHEM(98)17, OECD, Paris, 1998, and MAFF Japan Good Laboratory Practice Standards (11 NohSan Number 6283), except for the item documented below. The item listed did not impact the validity of the study.

- The test substances used for this study were not characterized according to Good Laboratory Practices. However, both the high-grade technical ethylene dichloride (EDC), which was supplied by the sponsor, and the radiolabeled EDC provided by Perkin-Elmer Life Sciences, provided documentation indicating the purities to be >98%.

Study Director: 14-JUN-2005

William J. Fasano, Sr., B.S. **Date**
Research Toxicologist
Haskell Laboratory for Health and Environmental Sciences

QUALITY ASSURANCE DOCUMENTATION

Work Request Number: 15525
Study Code Number: 1623

The conduct of this study has been subjected to periodic Quality Assurance inspections. The dates of inspection are indicated below.

Phase Audited	***Audit Dates***	***Date Reported to Study Director***	***Date Reported to Management***
Protocol:	January 7,2005	January 7,2005	January 7,2005
Conduct:	March 3,2005	March 3,2005	March 3,2005
Report/Records:	April **8,** 10-11,2005	April 11,2005	April 15,2005

Reported by: Annet L Kugel for JCH 14 Jun 2005
Joseph C. Hamil **Date**
Quality Assurance Auditor

CERTIFICATION

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

Approved by: _____ 13-JUN-2005
Gary W. Jepson, Ph.D. Date
Research Manager

Issued by Study Director: _____ 14-JUN-2005
William J. Fasano, Sr., B.S. Date
Research Toxicologist

This report is approved by the sponsor

Approved by: _____ 03-JUN-2005
Peter Vovtek, Ph.D. Date
Sponsor Representative

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STUDY INFORMATION

Substance Tested:
- 1,2-Dichloroethane (CAS Name)
- Ethylene Dichloride (Chemical Name)
- 107-06-2 (CAS Number)

Haskell Number: 26709

Composition: 1,2-Dichloroethane

Purity: 99.94%

Physical Characteristics: Clear 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: January 6,2005 / (see report cover page)

Experimental Start/Termination: March 3,2005 / March **8,** 2005

SUMMARY

The permeability coefficient (Kp) and the short-term absorption rates at 10 and 60 minutes have been determined for ethylene dichloride (EDC) using human abdominal skin from cadavers mounted in an ***in vitro*** static diffusion cell model. Human cadaver skin was dermatomed and the section mounted onto an ***in vitro*** static diffusion cell, ***stratum corneum*** uppermost, with an exposure area of 0.64 cm^2. Using a recirculating water bath system, the receptor fluid (0.9% saline) was maintained at 32°C. Following system equilibration, skin integrity was confirmed by electrical impedance (EI). The saline in the donor and receptor chambers was removed and discarded and the receptor chamber was filled with 0.9% saline fortified with ***6%*** polyethoxyoleate (polyethylene glycol (PEG) 20 oleyl ether).

For the permeability coefficient experiment, an infinite dose of EDC was applied to the epidermal surface, via the donor chamber, at a target rate of 1200 µL/cm^2, to 6 skin replicates representing 3 human subjects, and the donor chamber opening was occluded with a rubber stopper. Serial receptor fluid samples were taken at 0.5, 1, 2, 3, 4, 5, 6, 7, and 8 hours post-application and analyzed for radioactivity by liquid scintillation counting. At the end of the 8-hour exposure, excess EDC was removed and the skin washed with a 2% soap solution followed by rinsing with water. The receptor fluid was removed and discarded, and the receptor and donor chambers were filled with 0.9% saline and an end of experiment integrity asssessment was determined using EI.

For the short-term exposure experiments, a finite dose of EDC (10 µL/cm^2) was applied to the epidermal surface, via the donor chamber, to 12 skin replicates representing 3 human subjects, and the donor chamber opening was covered with a volatile organic trap. At the end of the required exposure interval (10 minute and 60 minutes), 6 replicates each were terminated. At termination, the volatile trap was removed and extracted with ethanol. The skin surface was washed with a 2% soap solution, rinsed with water, and the receptor fluid was removed and retained for analysis. The receptor and donor chambers were filled with 0.9% saline and end of experiment integrity asssessment was taken using EI. The saline in both chambers was removed and discarded and the skin membrane removed and placed into a glass vial for digestion. The receptor fluid and the skin were analyzed by liquid scintillation counting.

Based on the slope at steady-state (1 197.I µg equiv/cm^2/h) and the concentration of the applied dose of EDC taken as its density (1,235,100 µg/cm^3), the permeability coefficient was calculated to be 9.69 x 10^{-4} cm/h.

Following a 10-minute exposure to a finite application of EDC, a total of 138.7 µg equivalents of EDC was detected in the receptor fluid, with 41.4 µg equivalents in the skin. Based on the amount of EDC in the receptor fluid and skin, an exposure area of 0.64 cm^2, and an exposure time of 10 minutes (0.17 hours), the short-term absorption rate was calculated to be 1655.2 µg equiv/cm^2/h

Following a 60-minute exposure to a finite application of EDC, a total of 193.4 µg equivalents of EDC were detected in the receptor fluid and 17.6 µg equivalents in the skin. Based on the

amount of EDC in the receptor fluid and skin, an exposure area of **0.64** cm^2, and an exposure time of one hour, the short-term absorption rate was calculated to be **329.6** μg equiv/cm^2/h.

INTRODUCTION

EPA has promulgated a final rule under the Toxic Substances Control Act (TSCA) that requires manufacturers, importers, and processors of certain chemicals to conduct in *vitro* dermal absorption rate testing. The data obtained under this test rule entitled "*In Vitro* Dermal Absorption Rate Testing of Certain Chemicals of Interest to the Occupational Safety and Health Administration," published in the Federal Register April 26, 2004 (Volume 69, Number 80), will be used by OSHA to evaluate the need for skin designations for the selected chemicals. Skin designations are used to alert industrial hygienists, employers, and workers to the potential contribution of dermal exposure to overall systemic toxicity.

The objective of this study was to determine a permeability coefficient (Kp) and short-term absorption rate for ethylene dichloride (EDC) using human cadaver skin mounted in an *in vitro* diffusion cell model. A Kp is determined using an infinite dose. which is an excess of test material applied to the skin where the maximum absorption rate is achieved and maintained and the concentration of the test substance in the donor compartment is not significantly diminished during the experiment. The short-term absorption rate requires application of a finite dose, or a dose volume or amount necessary to cover the skin exposure area and mimic in-use conditions.

MATERIALS AND METHODS

A. Test Guidelines

The study design complied with the following guidelines:

- In Vitro Dermal Absorption Rate Testing of Certain Chemicals of Interest to the Occupational Safety and Health Administration. Federal Register: April 26, 2004 (Volume 69, Number 80)
- OECD Guideline for the Testing of Chemicals. Draft New Guideline 428: Skin Absorption: *in vitro* Method. (2002)
- OECD Draft Guidance Document for the Conduct of Skin Absorption Studies. OECD Environmental Health and Safety Publications Series on Testing and Assessment No. 28. (2002)
- European Commission Guidance Document on Dermal Absorption. Sanco/222/2000 rev 6 (2002).

B. Test Substance(s)

1. Non-Radiolabeled Test Substance (CASN 107-06-2)

The non-radiolabeled EDC (*logP* = 1.48) was supplied by Occidental Chemical Corporation (Dallas, Texas) and assigned Haskell Laboratory Number 26709. The certificate of analysis (Appendix A) stated that the purity was 99.94%.

2. Radiolabeled Test Substance

The radiolabeled test substance, [1,2-^{14}C]EDC, was obtained by the sponsor from Perkin-Elmer Life Sciences (Boston, Massachusetts) and assigned Haskell Laboratory Number 22705-93. The test substance had a radiochemical purity of 98.1% and a specific activity of 44.0 mCi/mmoL. The certificate of analysis is presented in Appendix A.

Structure:

*denotes position of radiolabel

C. Test System

1. Human Skin

Samples of human cadaver skin from the National Disease Research Interchange (NDRI) were stored frozen at approximately -20°C until prepared for use. Samples were removed from donors within 24 hours of death and used within three months. Skin specimens selected for use were 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, which are required by the test rule described in the Federal Register dated April 26,2004 (Volume 69, Number 80), have been shown to be a conservative model for predicting percutaneous absorption of various chemicals ***in vivo***.[1-3]

3. *In **Vitro*** Diffusion Cell Model

A static diffusion cell model was used for this study (Figure 1). The ***in vitro*** cells had an exposure area of 0.64 cm^2 and a receptor fluid chamber volume of approximately 5 mL.

D. Dose Formulation, Homogeneity, Concentration, and Stability

The non-radiolabeled test substance, which was liquid at room temperature, was spiked with radiolabeled test substance.

The homogeneity and the amount of radiolabeled EDC in the formulation was determined by subjecting aliquots of the prepared formulation to radioanalysis by liquid scintillation counting (LSC).

The concentration of EDC in the prepared solution was taken as its density, 1.2351 g/mL (1,235,100 µg/cm^3).

The results of the homogeneity analysis were used to calculate the specific activity of radiolabeled EDC in the formulation (µCi/mg).

The purity of the radiolabeled EDC in the technical EDC was determined using the following analytical method.

System: Hewlett-Packard 1100 Series Equipment (Agilent Technologies, Palo, Alto, CA, USA)
Column: Zorbax SB-C18 4.6 mm x 250 mm, 5 µm particles
Column temperature: Ambient
Mobile phases: A: Water

Gradient:

Time (min)	%A	%B
0.00	80	20
15.00	0	100
15.01	**80**	20

Flow rate: 1.0 mL/min
Radiodetection: Fraction collection (Foxy 200™, Isco, Inc., Lincoln, Nebraska) followed by liquid scintillation counting

E. Preparation of Skin Membranes

Samples of human cadaver skin obtained from the abdominal region, which were maintained frozen, were thawed at room temperature. Full thickness skin was immersed in 60°C water for 45 seconds to 2 minutes and the epidermis was peeled away from the dermis. The human epidermal membrane was then placed onto an aluminum pan, with its identification written on the pan, and stored refrigerated at 0-10°C until readied for use. The thickness of the prepared skins, as measured with a Mahr micrometer (Mahr Federal Inc., Providence, Rhode Island), ranged from 50 to 98 µm.

F. Membrane Equilibration and Assessment of Membrane Integrity

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, *stratum corneum* uppermost, which was maintained 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 *in vitro* cells were heated using a recirculating water bath system to yield a receptor fluid temperature of 32°C. Following equilibration, the integrity of each membrane was assessed by measurement of electrical impedance (EI) prior to application of the test substance.[4-5]

Membranes with an EI of ≥17 kΩ were considered intact and retained for use on study. Saline in the donor and receptor chambers was removed prior to dosing, and the receptor chamber filled with fresh receptor fluid.

G. Receptor Fluid

The receptor chamber was filled with 0.9% saline fortified with 6% polyethoxyoleate (polyethylene glycol (PEG) 20 oleyl ether), and allowed to equilibrate for at least 15 minutes prior to dosing.

Solubility of EDC in the selected receptor fluid was confirmed prior to study start to ensure that the maximum possible concentration of the EDC in the receptor fluid, based on the total amount of EDC applied to the skin surface, did not exceed 10% of its solubility.

H. Exposure Groups, Target Parameters

1. Determining the Permeability Coefficient (Kp)

Protocol Group: A

Number of skin replicates: 6, representing 3 donors
Dose volume: 1200 µL/cm^2.
Termination time: following steady-state determination

Following dose application, the donor chamber opening was occluded with a rubber stopper. Serial receptor fluid samples, duplicate 50 µL aliquot, were taken at 0.5, 1, 2, 3, 4, 5, 6, **7,** and **8** hours post-dose. The volume of receptor fluid in the receptor chamber was maintained by the replacement of a volume of fresh receptor fluid, equal to the sample volume. The receptor chamber arm remained occluded with Parafilm® at all times other than at sampling. At the end of the exposure period, the receptor fluid was removed and discarded.

2. Determining the Short-Term Absorption Rate, 10 and 60 minutes

Protocol Group: B

Number of skin samples: **4,** representing a single unique donor
Dose volume: 10 µL/cm^2
Termination times: 2 replicates at 10 minutes, 2 replicates at 60 minutes

Protocol Group: C

Number of skin samples: **4,** representing a single unique donor
Dose volume: 10 µL/cm^2
Termination times: 2 replicates at 10 minutes, 2 replicates at 60 minutes

Protocol Group: D

Number of skin samples: **4,** representing a single unique donor
Dose volume: 10 µL/cm^2
Termination times: 2 replicates at 10 minutes, 2 replicates at 60 minutes

Following dose application, the donor chamber opening was occluded with an organic volatile trap containing Anasorb 747 (SKC Inc., Eighty Four, Pennsylvania). At the end of the exposure period, the receptor fluid was removed and placed into a suitable container for analysis.

I. Dose Determination

The actual dose applied to each skin replicate was determined by subjecting aliquots of the prepared solution to liquid scintillation counting. The total amount of EDC applied to the skin was determined by the total radioactivity applied and the verified specific activity.

J. Terminal Procedures

For the Kp exposure group, the stopper was removed and extracted with ethanol and excess dose solution was removed from the donor chamber and retained for analysis. For the short-term exposure groups, the charcoal trap was removed and extracted with ethanol at the conclusion of the exposure period.

For all groups, the surface of each skin replicate was washed with a 2% soap solution (e.g., Ivory® Soap) followed by rinsing with deionized (DI) water. The wash/rinse was collected into a liquid scintillation vial. The donor chamber remained clamped in-place during washing.

The saline in the receptor chamber was replaced with fresh saline, The donor chamber was filled with saline and an end of experiment EI measurement taken. Following the impedance measurement, the saline in both the donor and receptor chambers was removed and discarded. The donor chamber was removed and rinsed with ethanol directly into a liquid scintillation vial. The skin membrane was removed from the receptor chamber and placed into a glass liquid scintillation vial for digestion.

K. Determination of Radioactivity

1. Sample Handling and Processing

Aliquots of the serial receptor fluid samples and excess dose removed from the Kp exposure group, along with the stopper and charcoal trap extraction fluid, the skin wash/rinse, and the donor chamber rinse samples were mixed with Ultima Gold™ XR liquid scintillation cocktail (Perkin-Elmer Inc., Boston, Massachusetts) and analyzed for total radioactivity

Each skin piece was digested using Soluene®-350 (Perkin-Elmer Inc., Boston, Massachusetts). Heating at approximately 60°C accompanied by constant shaking was used to facilitate sample digestion. Hionic-Fluor™ liquid scintillation cocktail (Perkin-Elmer Inc., Boston, Massachusetts) was added directly to each vial and the samples analyzed for total radioactivity.

2. Liquid Scintillation Counting

Samples were analyzed in a Packard liquid scintillation counter. Receptor fluid samples from group D were counted for 10 minutes or until 160,000 disintegrations were accumulated (0.5%, 2σ), whichever came first. Samples from groups A, B, and C (receptor fluid and mock dose)

were counted for 5 minutes, and although this deviated from the protocol, this had no impact on the interpretation of the results.

The limit of detection (LOD) for the analysis of each sample was taken as twice the background disintegration rate obtained from analysis of appropriate blank samples.

L. Data Presentation

EDC's permeability coefficient (Kp) was determined by plotting the cumulative amount of µg equivalents detected in the receptor compartment at each serial collection time-point, adjusted for total receptor fluid volume, against time (in hours) to produce an absorption profile. Kp (cm/h) was calculated by dividing the penetration rate or slope of the line at steady-state (µg equiv/cm^2/h), represented by at least **4** data points, by the concentration of applied chemical (µg/cm^3).

The short-term absorption rate (µg equiv/cm^2/h) for each exposure interval (10 and 60 minutes) was calculated by dividing the sum of the µg equivalents in the receptor fluid and skin by the skin exposure area **(0.64** cm^2) and exposure time.

Total recovery of the applied formulation for each exposure group was the sum of the amount detected in the charcoal trap (Groups B-D only), the amount detected in the receptor fluid, the amount of excess dose removed from the skin and extracted from the rubber stopper (Group A only), the amount washed/rinsed from the skin and the donor chamber, and the amount in/on the skin not removed by washing.

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.

The values in the tables and appendices were generated by computer and rounded appropriately for inclusion in the report. **As** a consequence, calculations made using individual data in the appendices and tables will, in some instances, yield a value that is not aesthetically the same.

RESULTS AND DISCUSSION

A. Radiochemical Purity of [1,2-^{14}C]EDC

(Figure 2, Appendix A)

The radiochemical purity of the stock [1,2-^{14}C]EDC was **98.1%.** The certificate of analysis (COA) is presented in Appendix **A.** When mixed with technical EDC, the radiochemical purity of [1,2-^{14}C]EDC was >98%. A radiochromatogram is presented in Figure 2.

B. Chemical and Radiochemical Concentration of EDC

(Appendix A)

The chemical concentration of EDC in the prepared solution was taken as its density, 1.2351 g/mL. The verified radiochemical concentration (specific activity) for the prepared solution was 0.02686 µCi/mg.

The COA for the technical EDC is presented in Appendix A. The purity was 99.94%

C. Solubility of EDC in Receptor Fluid

EDC was determined to have a maximum solubility of 9,690 µg/mL in 0.9% saline fortified with 6% polyethoxyoleate (polyethylene glycol (PEG) 20 oleyl ether), which was ~1.2-fold greater than water alone (~8400 µg/mL).

D. EDC, Permeability Coefficient

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

Key observations of mean data:

- The integrity of human skin, as determined by EI, was lower following continuous exposures to EDC under occlusive conditions. The ratio of the post-E1 values to the pre-EI values was 0.59. The decrease in EI did not affect the results of the experiment.
- EDC was detectable in the receptor fluid at the 0.5-hour serial sampling timepoint (481.6 µg equiv/cm^2); the final receptor fluid sample (8 hours) was 9,406.5 µg equiv/cm^2.
- Steady state penetration of EDC, which was represented by a minimum of **4** data points, had a slope of 1197.1 µg equiv/cm^2/h.
- At the end of the 8-hour exposure interval, <0.7% of the applied EDC was detected in the receptor chamber. This was well below the level of saturation that would have affected the penetration of EDC.
- The permeability coefficient was calculated to be 9.69 x 10^{-4} cm/h, based on the slope at steady-state (1 197.1 µg equiv/cm^2/h) and the concentration of EDC in the applied formulation taken as its density (1,235,100 µg/cm^3).
- Recovery of the applied radioactive dose was 85.9%.

E. EDC, 10- and 60-Minute Short-Term Absorption Rates

(Tables 5-7, Appendix C)

Key observations of mean data:

- The integrity of human skin, as determined by EI, was unaffected by either short-term exposure interval of 10 and 60 minutes under occlusive conditions to EDC. The ratio of the post-E1 values to pre-EI values for the IO-minute and 60-minute exposure groups were 0.73 and 0.92, respectively.

- Following a 10-minute exposure to a finite application of EDC, a total of 138.7 µg equivalents of EDC was detected in the receptor fluid, with 41.4 µg equivalents in the skin. Based on the amount of EDC in the receptor fluid and skin, an exposure area of 0.64 cm^2, and an exposure time of 10 minutes (0.17 hours), the short-term absorption rate was calculated to be 1655.2 µg equiv/cm^2/h.

- Following a 60-minute exposure to a finite application of EDC, a total of 193.4 µg equivalents of EDC was detected in the receptor fluid and 17.6 µg equivalents in the skin. Based on the amount of EDC in the receptor fluid and skin, an exposure area of 0.64 cm^2, and an exposure time of one hour, the short-term absorption rate was calculated to be 329.6 µg equiv/cm^2/h.

- Recovery of the applied radioactive dose was 80.3% and 86.4% for the 10- and 60-minute exposure groups, respectively.

CONCLUSIONS

Based on the slope at steady-state (1 197.1 µg equiv/cm^2/h) and the concentration of the applied dose of EDC taken as its density (1,235,100 µg/cm^3), the permeability coefficient was calculated to be 9.69 x 10^{-4} cm/h.

Following a 10-minute exposure to a finite application of EDC, a total of 138.7 µg equivalents of EDC were detected in the receptor fluid, with 41.4 µg equivalents in the skin. Based on the amount of EDC in the receptor fluid and skin, an exposure area of 0.64 cm^2, and an exposure time of 10 minutes (0.17 hours), the short-term absorption rate was calculated to be 1655.2 µg equiv/cm^2/h

Following a 60-minute exposure to a finite application of EDC, a total of 193.4 µg equivalents of EDC were detected in the receptor fluid and 17.6 µg equivalents in the skin. Based on the amount of EDC in the receptor fluid and skin, an exposure area of 0.64 cm^2, and an exposure time of one hour, the short-term absorption rate was calculated to be 329.6 µg equiv/cm^2/h.

RECORDS AND SAMPLE STORAGE

All data and analytical characterization records conducted by or for the sponsor will be retained by the sponsor. Raw data, and the final report will be retained at Haskell Laboratory, Newark, Delaware, or at Iron Mountain Records Management, Wilmington, Delaware, and will be returned to the sponsor within 6 months after the final report issues.

REFERENCES

1. 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.

2. 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.

3. 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.

4. 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.

5. 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:

EI	electrical impedance
h	hour(s)
k-ohms	kilo-ohms
RF	receptor fluid
SD	standard deviation

Table 1: Permeability coefficient, EI values, pre- and post-exposure

Pre EI (k-ohms)		Post EI (k-ohms)		Ratio: Post/Pre	
Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
45.6	13.4	26.9	9.97	0.59	0.13

Table 2: Permeability coefficient, cumulative amount penetrated (µg equiv/cm^2)

Time (hours)	Mean	SD
0.5	481.6	238.3
1	1124.4	519.9
2	2458.2	1161.6
3	3494.5	1642.7
4	4734.0	2128.5
5	5679.0	2266.0
6	7035.6	2590.5
7	8268.5	2936.9
8	9406.5	3071.1

Table 3: Permeability coefficient, percent absorbed, steady-state penetration, Kp

	Mean	**SD**
Steady-state penetration rate (µg equiv/cm^2/h)	1197.1	351.1
Percent absorbed at **8** hours (%)	0.67	0.24
Permeability coefficient (Kp;cm/h)	9.69×10^{-4}	2.84×10^{-4}

Table 4: Permeability coefficient, recovery data (percent of applied dose)

	Mean	**SD**
Receptor **Fluid**	0.60	0.21
Skin Wash	2.27	1.21
Skin	<0.01	<0.01
Donor Chamber	<0.01	<0.01
Stopper	13.4	3.26
Excess Dose	69.6	3.83
Total Recovery	85.9	6.20

Table 5: Short-term absorption rates, EI values, pre-and post-exposure

Exposure Time (minutes)	Pre EI (k-ohms)		Post EI (k-ohms)		Ratio: Post/Pre	
	Mean	SD	Mean	SD	Mean	SD
10	47.0	13.2	34.8	20.6	0.73	0.35
60	47.6	15.1	42.1	15.9	0.92	0.29

Table 6: Short-term absorption rates, receptor levels, skin levels, total absorbed, penetration rates

Exposure Time (minutes)	EDC in RF (µg equiv)		EDC in Skin (µg equiv)		Total Absorbed RF + Skin (µg equiv)		Absorption Rate (µg equiv/cm^2/h)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
10	138.7	174.0	41.4	42.3	180.1	213.4	1655.2	1961.2
60	193.4	151.2	17.6	10.5	210.9	159.8	329.6	249.7

Table 7: Short-term absorption rates, recovery data (percent of applied dose)

	10 Minutes		60 Minutes	
	Mean	**SD**	**Mean**	**SD**
Receptor **Fluid**	1.89	2.37	2.64	2.06
Skin Wash	3.63	3.58	0.24	0.18
Skin	0.56	0.58	0.24	0.14
Donor Chamber	0.04	0.02	<0.02	<0.02
Charcoal Trap	74.0	9.40	83.2	10.2
Total Recovery	80.3	6.32	86.4	8.50

FIGURES

FIGURES

EXPLANATORY NOTES

ABBREVIATIONS:

dpm	disintegrations per minute
h	hour(s)

Figure 1: Static diffusion cell

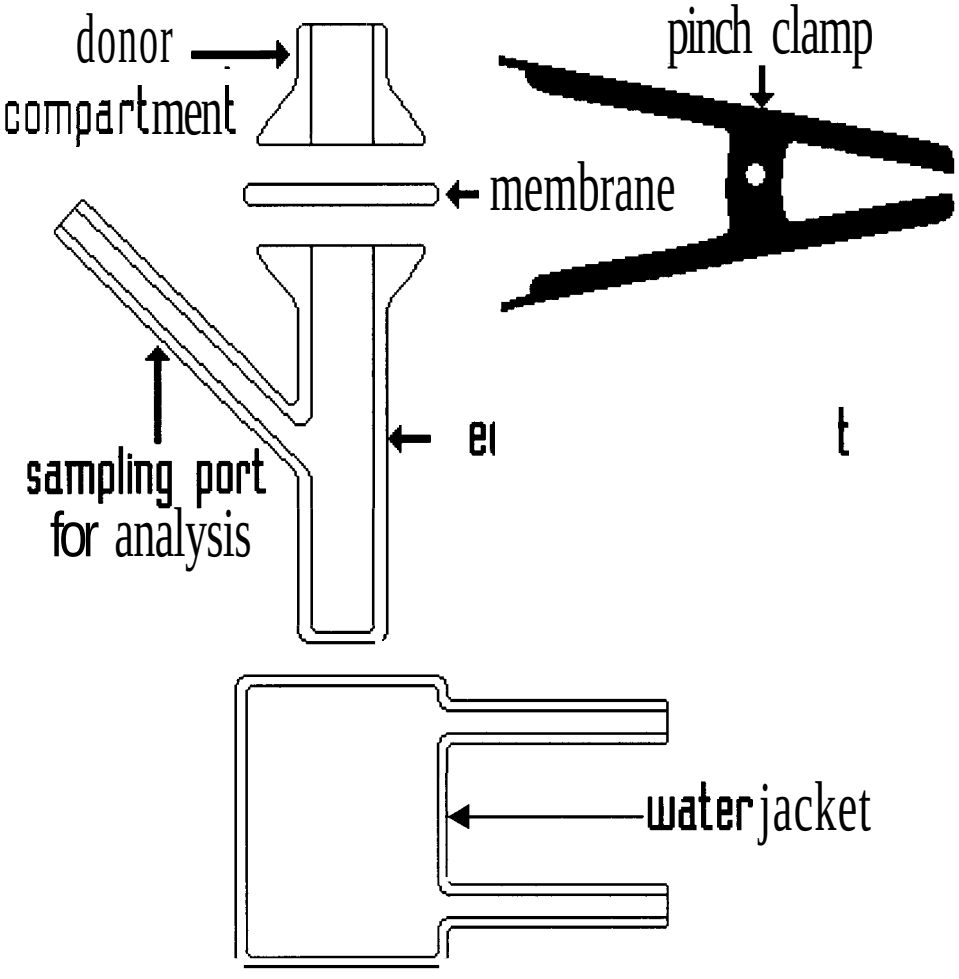


Figure 2: Radiochromatogram of [1,2-^{14}C]EDC in technical EDC

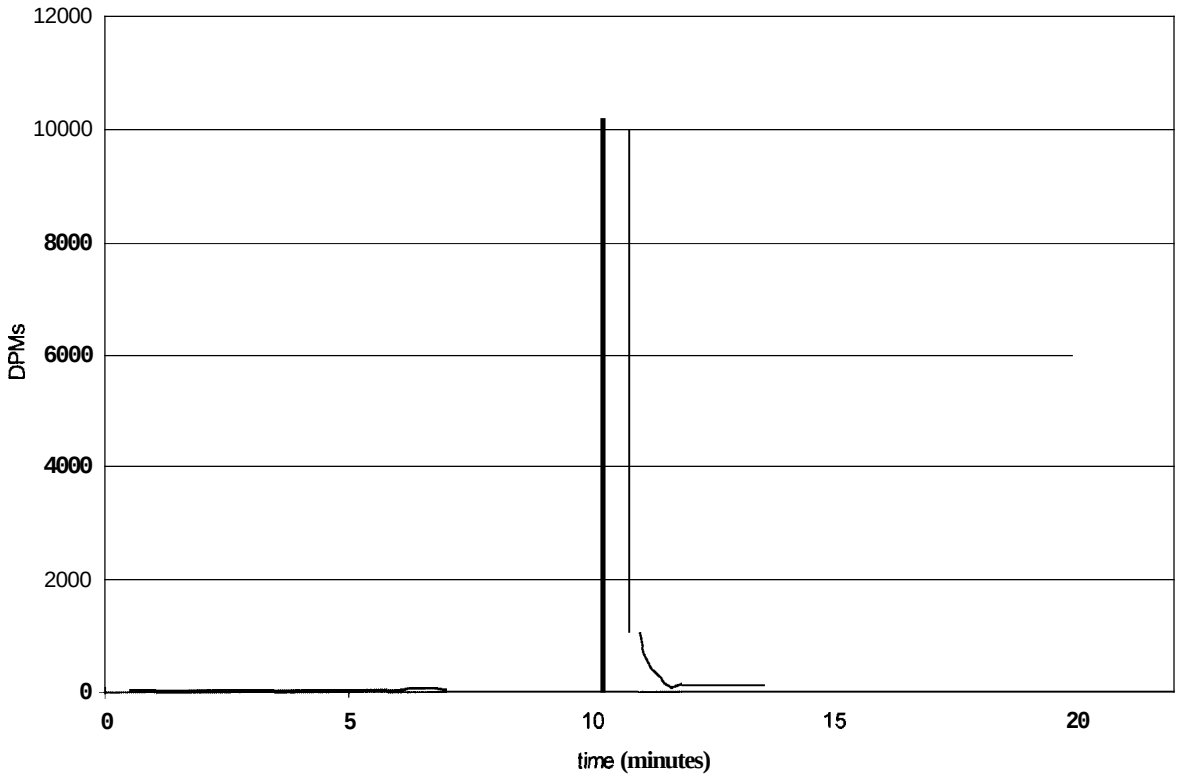
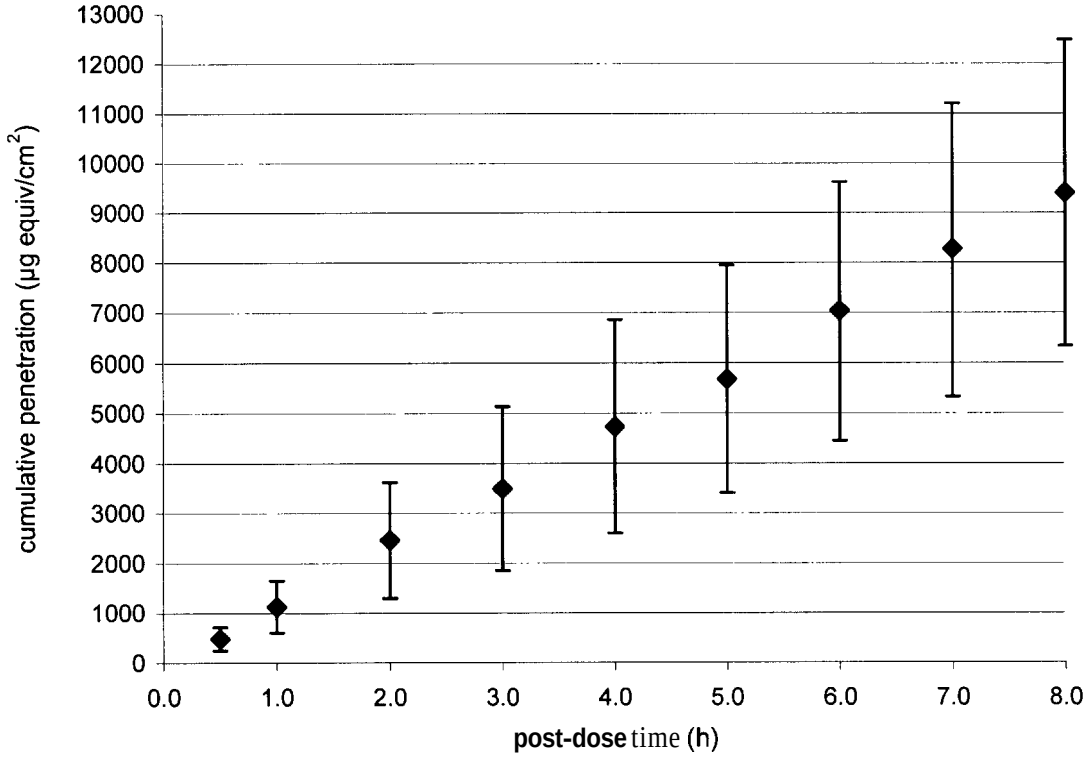


Figure 3: Permeability coefficient, cumulative amount penetrated (µg equiv/cm^2)



APPENDICES

APPENDICES

EXPLANATORY NOTES

ABBREVIATIONS:

EI	electrical impedance
h	hour(s)
HCMA-80B	Human, Caucasian, Male, Abdomen, sample SOB
Kp	permeability coefficient
RF	receptor fluid
SD	standard deviation
EDC	Ethylene Dichloride

Appendix A:

Certificates of Analysis

PerkinElmer Life and Analytical Sciences
549 Albany Street
Boston, MA 02118, USA
www.perkinelmer.com

CUSTOM SERVICES

Caution: For Laboratory Use. A research chemical for research purposes only.

Dr. Steve Hansen
Tha Dow Chemical Company
Analytical Chemistry
Midland MI 48674

September 28, 2004

WR 15525 SC1623
NBE-99681-CC

Dear Dr. Hansen

Below and enclosed is technical data pertaining to your order of **1,2-Dichloroethane,[1,2-14C]-**. If you have any further questions regarding this product, please forward them to our Custom Synthesis Administration Group or contact me directly at 617-574-9766.

Thank you for your order. We will be happy to provide any assistance you may need.

Sincerely,

Antonino D'Urso
PerkinElmer Life and Analytical Sciences

TECHNICAL DATA

Compound Name:	1,2-Dichloroethane,[1,2-14C]-
Lot Number:	2800-414
Assay Number:	2800-414
Physical Form:	Liquid
Solvent:	Ethanol
Concentration:	5.0 mCi/ml
Packaging Information:	1 x 5mCi in sealed glass ampoule
Radiochemical Purity:	98.1% by HPLC
Specific Activity:	44.0 mCi/mmole
Total Activity:	5 mCi
Special Information:	Product Identity verified by GC

HAZARD INFORMATION:
WARNING: This product contains a chemical known to the state of California to cause cancer.

OxyChem.

Certificate of Analysis

ETHYLENE DICHLORIDE - BULK

Standard Order Information

Date
06/24/2004
Purchase order item/date

Delivery item/date

Order item/date

Customer Number Container Number

Attention: Quality Department

Batch 303MICHIGA

	Characteristic	Unit	Value	Specifications Min.	Max.
	Purity	Wt. %	99.94	99.94	
	Low Boiling Impurities	Wt.ppm	132		450
1	High Boiling Impurities	Wt.ppm	46	-	300
	C3 and Higher Nonchlorinated	Wt.ppm	24		250
	Oxygenated Impurities	Wt.ppm	< 50		50
2	Color	APHA	2		10
3	Acidity as HCl	Wt.ppm	1.00	-	4.00
4	Water	Wt.ppm	25		100
5	Iron	Wt.ppm	0.02		0.75
6	Non Volatile Residue		0		50
7	Free Halogens		0.0	-	0.0
	Suspended Matter		Free of suspended matter		- -

Instrument Used

ISSUED BY: D. M. FOREMAN

1. GC
2. Visual
3. Titration
4. Moisture Meter
5. UV
6. Gravimetric
7. Visual / Colorimetric
8. Visual

analyst : Lynette Brand

THIS IS A TRUE COPY
OF THE ORIGINAL DATA.
Initial/Date: MSM 11/8/04

Occidental Chemical Corporation
Convent EDC
7377 Hwy 3214

Appendix B:

Permeability Coefficient (Kp) Data

Mock Dose

Replicate aliquots for liquid formulations

NB	E99681-CC	input cell
WR	15525	
SC	1623	
Formulation	H# 26709,Grp A	

Replicate	Aliquot (µL)	Dilution (mL)	Aliquot (mL)	DPMs in aliquot	Background Subtract (DPM)
					19
1	64	10	0.1	42168	42149
				44626	44607
				43990	43971
2	64	10	0.1	44922	44903
				45500	45481
				45150	45131
3	64	10	0.1	45916	45897
				44803	44784
				44660	44641
				Sum (dprn)	401564
				Average (dpm)	44618
				Amount in mock (dpm)	4461822

A - Radioactivity applied (dpm)	4461822	
B - Radioactivity applied (µCi)	2.01	(=A/2.22 x 10^6 dpm/µCi)
C - Verified specific activity (µCi/mg)	0.02686	
D - Specific activity (dpm/µg)	59.6	(=[C*2.22 x 10^6 dpm/µCi]/1000 µg/mg)
E - Total compound applied (µg)	74826.1	(=A/D)
F - Application rate (µg/cm^2)	116915.8	(=E/0.64 cm^2)

Pre-and post-E1 values

Cell ID	Skin ID	Pre El (k-ohms)	Post El (k-ohms)	Ratio: Post/Pre
A	HCMA-121	34.5	21.3	0.62
B	HCMA-121	34.6	22.1	0.64
D	HCMA-122	35.0	24.6	0.70
E	HCMA-122	45.5	17.5	0.38
H	HCFA-128	61.7	45.2	0.73
I	HCFA-128	62.3	30.6	0.49
	Mean	45.6	26.9	0.59
	SD	13.4	9.97	0.13

Cumulative amount penetrated (μg equiv/cm^2)

	Time after dosing (hr)								
Cell ID	0.5	1	2	3	4	5	6	7	8
A	278.6	634.8	1284.5	1925.4	2939.6	3827.4	5253.3	6195.8	7516.3
B	423.9	1188.6	3099.9	4471.8	6239.6	7727.8	8914.9	10256.8	11794.1
D	311.9	703.3	1360.5	1984.6	2904.1	3658.3	4732.7	6000.2	7236.8
E	598.7	1535.2	3746.1	5292.7	7169.0	8430.6	10088.4	12299.5	13449.1
H	910.1	1919.5	3638.0	5148.9	6555.7	6941.8	9047.6	9886.5	10869.5
I	366.2	764.9	1620.4	2143.5	2596.1	3488.4	4176.7	4972.2	5573.2
MEAN	481.6	1124.4	2458.2	3494.5	4734.0	5679.0	7035.6	8268.5	9406.5
SD	238.3	519.9	1161.6	1642.7	2128.5	2266.0	2590.5	2936.9	3071.1

Percent absorbed, steady-state penetration, Kp

Cell ID		Steady State Penetration rate (μg equiv/cm^2/h)	Percent absorbed at 8 hours	Kp (cm/h)
A		1118.5	0.55	9.06×10^{-4}
B		1424.3	0.86	1.15×10^{-3}
D		1046.4	0.53	8.47×10^{-4}
E		1652.3	0.98	1.34×10^{-3}
H		1604.7	0.79	1.30×10^{-3}
I		743.8	0.41	6.02×10^{-4}
	Mean	1197.1	0.67	9.69×10^{-4}
	SD	351.1	0.24	2.84×10^{-4}

Recovery data, percent of applied dose

Cell ID	Receptor fluid	Skin Wash	Skin	Donor Chamber	Stopper	Excess Dose	Total Recovery
A	0.45	3.71	0.0036	0.0004	15.9	70.9	91.0
B	0.75	1.20	0.0051	0.0004	11.0	72.7	85.6
D	0.44	3.74	0.0064	0.0003	12.3	72.1	88.5
E	0.90	0.93	0.0046	0.0002	18.6	71.6	92.0
H	0.72	1.95	0.0138	0.0009	9.86	62.6	75.2
I	0.36	2.12	0.0089	0.0019	13.1	67.7	83.2
MEAN	0.60	2.27	0.0071	0.0007	13.4	69.6	85.9
SD	0.21	1.21	0.0038	0.0006	3.26	3.83	6.20

Appendix C:

Short-Term Absorption Rate Data – 10 and 60 Minutes

Mock Dose

Replicate aliquots for liquid formulations

NB	E99681-CC
WR	15525

input cell

Formulation H# 26709, Gr s B, C & D

Replicate	Aliquot (µL)	Dilution (mL)	Aliquot (mL)	DPMs in aliquot	Background Subtract (DPM)
					25
1	6.4	10	0.1	4239	4214
				4518	4493
				4383	4358
2	6.4	10	0.1	4292	4267
				4495	4470
				4508	4483
3	6.4	10	0.1	4233	4208
				4455	4430
				4476	4451

Sum (dpm)	39374
Average (dpm)	4375
Amount applied (dprn)	437489

A - Radioactivity applied (dpm)	437489	
B - Radioactivity applied (µCi)	0.20	(=A/2.22 x 10^6 dpm/µCi)
C - Verified specific activity (µCi/mg)	0.02686	
D - Specific activity (dpm/µg)	59.6	(=[C*2.22 x 10^6 dpm/µCi]/1000 µg/mg)
E - Total compound applied (µg)	7336.8	(=A/D)
F - Application rate (µg/cm^2)	11463.8	(=E/0.64 cm^2)

Pre-and post-EI values

10 Minutes

Exposure Time	Cell ID	Skin ID	Pre EI (k-ohms)	Post EI (k-ohms)	Ratio: Post/Pre
10 minutes	C	HCMA-121	28.6	12.3	0.43
	D	HCMA-121	35.4	38.9	1.10
	I	HCMA-122	50.4	12.7	0.25
	J	HCMA-122	50.0	52.4	1.05
	N	HCFA-128	66.1	62.6	0.95
	O	HCFA-128	51.2	29.8	0.58
		Mean	47.0	34.8	0.73
		SD	13.2	20.6	0.35

60 Minutes

Exposure Time	Cell ID	Skin ID	Pre EI (k-ohms)	Post EI (k-ohms)	Ratio: Post/Pre
60 minutes	A	HCMA-121	28.0	34.8	1.24
	B	HCMA-121	33.2	33.2	1.00
	F	HCMA-122	46.4	46.9	1.01
	G	HCMA-122	50.3	20.0	0.40
	K	HCFA-128	62.4	64.5	1.03
	M	HCFA-128	65.3	53.1	0.81
		Mean	47.6	42.1	0.92
		SD	15.1	15.9	0.29

Penetration rate data

10 Minutes

Cell ID	Skin ID	EDC in RF (µg equiv)	EDC in Skin (µg equiv)	Total absorbed RF+Skin (µg equiv)	Absorption rate (µg equiv/cm^2/h)
C	HCMA-121	93.4	11.0	104.4	960.0
D	HCMA-121	48.9	12.8	61.6	566.4
I	HCMA-122	36.3	24.5	60.8	558.8
J	HCMA-122	56.8	52.7	109.5	1006.2
N	HCFA-128	107.1	25.2	132.3	1216.2
O	HCFA-128	489.6	122.2	611.8	5623.3
	Mean	138.7	41.4	180.1	1655.2
	SD	174.0	42.3	213.4	1961.2

60 Minutes

Cell ID	Skin ID	EDC in RF (µg equiv)	EDC in Skin (µg equiv)	Total absorbed RF+Skin (µg equiv)	Absorption rate (µg equiv/cm^2/h)
A	HCMA-121	171.3	27.3	198.5	310.2
B	HCMA-121	233.8	11.7	245.5	383.6
F	HCMA-122	35.3	7.90	43.2	67.5
G	HCMA-122	96.5	11.1	107.6	168.2
K	HCFA-128	154.0	13.3	167.3	261.4
M	HCFA-128	469.6	34.0	503.5	786.7
	Mean	193.4	17.6	210.9	329.6
	SD	151.2	10.5	159.8	249.7

Recovery data, percent of applied dose

10 Minutes

Cell ID	Receptor fluid	Skin Wash	Skin	Donor Chamber	Charcoal Trap	Total Recovery
C	1.27	1.68	0.15	0.034	84.7	89.1
D	0.67	0.85	0.17	0.039	77.0	78.7
I	0.49	10.4	0.33	0.035	63.7	75.0
J	0.77	1.56	0.72	0.075	84.4	87.5
N	1.46	4.59	0.34	0.005	69.4	75.8
O	6.67	2.62	1.67	0.031	64.8	75.8
MEAN	1.89	3.63	0.56	0.036	74.0	80.3
SD	2.37	3.58	0.58	0.023	9.40	6.32

60 Minutes

Cell ID	Receptor fluid	Skin Wash	Skin	Donor Chamber	Charcoal Trap	Total Recovery
A	2.33	0.13	0.37	0.022	88.8	91.6
B	3.19	0.12	0.16	0.016	87.6	91.1
F	0.48	0.13	0.11	0.005	89.8	90.5
G	1.32	0.18	0.15	0.032	92.2	93.9
K	2.10	0.31	0.18	0.003	74.3	76.9
M	6.40	0.58	0.46	0.006	66.7	74.2
MEAN	2.64	0.24	0.24	0.014	83.2	86.4
SD	2.06	0.18	0.14	0.012	10.2	8.50