Study Title

p-Dichlorobenzene:
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OECD Draft Guidance Document for the Conduct of Skin Absorption Studies.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)

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STUDY COMPLETED ON: May 24, 2005

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LABORATORY PROJECT ID: DuPont-17161

WORK REQUEST NUMBER: 15690

SERVICE CODE NUMBER: 1623

SPONSOR: Chlorobenzene Producers Association of the
Synthetic Organic Manufacturers Association (SOCMA)
1850 M Street, NW – Suite 700
Washington, DC 20036
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, which are compatible with current OECD and MAFF (Japan) Good Laboratory Practices

Study Director: John A. Tedesco 24-May-2005

John. A. Tedesco, B.S.
Staff Toxicologist
Haskell Laboratory for Health and
Environmental Sciences

Date

QUALITY ASSURANCE DOCUMENTATION

Work Request Number: 15690
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:	March 4, 2005	March 4, 2005	March 4, 2005
Conduct:	March 21, 2005	March 21, 2005	March 21, 2005
Report/Records:	April 26, 27, 2005	April 27, 2005	May 4, 2005

Reported by: Joseph C Hamill 24-MAY-2005

Joseph C. Hamill
Quality Assurance Auditor

Date

CERTIFICATION

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

Approved by: _____ 23-MAY-2005

Gary W. Jepson, Ph.D.
Research Manager
Date

_____ 23-MAY-2005

William J. Fasano, Sr., B.S.
Research Toxicologist
Date

Issued by Study Director: _____ 24-May-2005

John A. Tedesco, B.S.
Staff Toxicologist
Date

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STUDY INFORMATION

Substance Tested:	• p-Dichlorobenzene (Chemical Name) • 106-46-7 (CAS Number)
Haskell Number:	26781
Composition:	p-Dichlorobenzene
Purity	99.7%
Physical Characteristics:	Colorless crystals
Stability:	The test substance appeared to be stable under the conditions of the study; no evidence of instability was observed.
Study Initiated/Completed:	March 2, 2005 / (see report cover page)
Experimental Start/Termination:	March 21, 2005 / April 5, 2005

SUMMARY

The permeability coefficient (Kp) and the short-term absorption rates at 10 and 60 minutes have been determined for p-dichlorobenzene using human abdominal skin from cadavers mounted in an *in vitro* static diffusion cell model. Human cadaver skin was heat-treated at approximately 60°C and the epidermis was peeled from the dermis 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 donor chamber filled with 0.9% saline fortified with 6% polyethoxyoleate (polyethylene glycol (PEG) 20 oleyl ether).

For the permeability coefficient experiment, an infinite dose of p-dichlorobenzene in isopropyl myristate vehicle (100 µL/cm^2) was applied to the epidermal surface, via the donor chamber, to 6 skin replicates representing 3 human subjects, and the donor chamber opening was occluded with Parafilm®. Serial receptor fluid samples were taken at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 18, 24, and 30 hours post-application and analyzed for radioactivity by liquid scintillation counting. At the end of the 30-hour exposure, excess p-dichlorobenzene was removed by washing 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 p-dichlorobenzene in isopropyl myristate vehicle (30 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 occluded with Parafilm®. At the end of the required exposure interval (10 minute and 60 minutes), 6 replicates each were terminated. At termination, 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 the donor chamber was retained for analysis. The skin membrane removed and placed into a glass vial containing Soluene®-350. The receptor fluid and the skin were analyzed by liquid scintillation counting.

Based on the slope at steady-state (12.8 µg equiv/cm^2/h), and the concentration of the applied dose of p-dichlorobenzene (98,418.2 µg/cm^3), the permeability coefficient (Kp) was calculated to be 1.31 x 10^{-4} cm/h.

Following a 10-minute exposure to a finite application of p-dichlorobenzene, a total of 1.66 µg equivalents of p-dichlorobenzene was detected in the receptor fluid, with 2.36 µg equivalents in the skin. Based on the amount of p-dichlorobenzene 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 exposure rate was calculated to be 36.9 µg equiv/cm^2/h.

Following a 60-minute exposure to a finite application of p-dichlorobenzene, a total of 7.06 µg equivalents of p-dichlorobenzene was detected in the receptor fluid and 2.74 µg equivalents in the skin. Based on the amount of p-dichlorobenzene in the receptor fluid and skin, an exposure area of 0.64 cm^2 and an exposure time of one hour, the short-term exposure rate was calculated to be 15.3 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 p-dichlorobenzene 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 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)

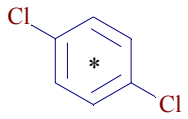
1. Non-Radiolabeled Test Substance (CASN 106-46-7)

The non-radiolabeled p-dichlorobenzene (*logP* = 3.336) was supplied by Sigma-Aldrich (Milwaukee, Wisconsin) and assigned Haskell Laboratory Number 26781. The certificate of analysis (Appendix A) stated that the purity was 99.7%.

2. Radiolabeled Test Substance

The radiolabeled test substance, [^{14}C]p-dichlorobenzene, was obtained from Sigma-Aldrich (St. Louis, Missouri) and assigned Haskell Laboratory Number 22705-98. The test substance had a radiochemical purity of 95.7% and a specific activity of 19.9 mCi/mmoL. The certificate of analysis is presented in Appendix A.

Structure:



*(U) uniformly labeled

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 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 (glass) 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

Non-radiolabeled and radiolabeled test substance was mixed with isopropyl myristate to achieve a target concentration of 100 mg p-dichlorobenzene/mL.

The homogeneity and the amount of radiolabeled p-dichlorobenzene (µCi/g) was determined by subjecting aliquots of the prepared formulation to radioanalysis by liquid scintillation counting (LSC).

The concentration of p-dichlorobenzene in the prepared formulation was determined chromatographically using the following analytical method.

System:	Hewlett-Packard 1100 Series Equipment (Agilent Technologies, Palo Alto, CA, USA)
Column:	Waters symmetry C-18, 2.1 x 50 mm, 3.5 µm
Column temperature:	Ambient
Mobile phases:	A: Water, pH 2.8 (H_3PO_4) B: Acetonitrile
Elution:	Isocratic, 55% (A), 45% (B)
Injection volume:	5 µL
Flow rate:	0.5 mL/min
UV Wavelength:	230 nm

The results of the homogeneity and concentration analysis were used to calculate the specific activity of radiolabeled p-dichlorobenzene in the formulation (µCi/mg).

The purity of the radiolabeled p-dichlorobenzene in the prepared formulation 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, 5.0 µm particles
Column temperature:	Ambient
Mobile phases:	A: Water, pH 2.8 (H_3PO_4) B: Acetonitrile

Gradient:

Time (min)	%A	%B
0.00	50	50
10.00	50	50
15.00	0	100
25.00	0	100
25.01	50	50

Flow rate:	1.0 mL/min
Radiodetection:	Fraction collection (Foxy 200™, Isco, Inc., Lincoln, NE) 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 representative membranes, as measured with a Mahr micrometer (Mahr Federal Inc., Providence, Rhode Island), ranged from 39 to 71 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 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% (w/v) polyethoxyoleate (polyethylene glycol (PEG) 20 oleyl ether), and allowed to equilibrate for at least 15 minutes prior to dosing.

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

H. Exposure Groups, Target Parameters

1. Determining the Permeability Coefficient (Kp)

Protocol Group: A

Number of skin replicates: 6, representing 3 donors
Dose volume: 100 µL/cm^2
Termination time: following steady-state determination

Following dose application, the donor chamber opening was occluded with Parafilm®, and serial receptor fluid samples were taken at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 18, 24, and 30 hours. 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 replicates: 4, representing a single unique donor
Dose volume: 30 µL/cm^2
Termination times: 2 replicates at 10 minutes, 2 replicates at 60 minutes

Protocol Group: C

Number of skin replicates: 4, representing a single unique donor
Dose volume: 30 µL/cm^2
Termination times: 2 replicates at 10 minutes, 2 replicates at 60 minutes

Protocol Group: D

Number of skin replicates: 4, representing a single unique donor
Dose volume: 30 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 Parafilm®. 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 formulation to liquid scintillation counting. The total amount of p-dichlorobenzene applied to the skin was determined by the total radioactivity applied and the verified specific activity.

J. Terminal Procedures

At the conclusion of each exposure interval, the surface of each skin replicate was washed with 2% Ivory Soap followed by rinsing with deionized (DI) water and retained for analysis. The receptor and donors chambers were filled with 0.9% saline and an end of experiment EI measurement taken. The donor chamber was removed and rinsed with acetonitrile directly into a liquid scintillation vial. The skin membranes were removed and placed into separate glass vials for digestion.

K. Determination of Radioactivity

1. Sample Handling and Processing

Aliquots of the serial receptor fluid samples, along with the skin wash/rinse and donor chamber rinse samples, were mixed with Ultima Gold™ XR liquid scintillation cocktail and analyzed for total radioactivity

Each skin piece was digested using Soluene®-350. Heating at approximately 60°C accompanied by constant shaking was used to facilitate sample digestion. Hionic-Fluor™ liquid scintillation cocktail 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. Samples were counted for 10 minutes or until 160,000 disintegrations were accumulated (0.5%, 2σ), whichever came first.

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

p-Dichlorobenzene's permeability coefficient (Kp) was determined by plotting the 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 a sum of the amount of radioactivity detected in the receptor fluid, the amount washed/rinsed from the skin and 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.

The larger volume receptor fluid aliquot (0.5 mL) taken for LSC counting from the 10 and 60 minute exposure groups, offered greater sensitivity (i.e., detectable levels of radioactivity) when compared to the smaller volume serial aliquots (0.05 mL) taken for LSC counting from the permeability coefficient experiment, where radioactivity was not detected until 1 hour post-dose in four of six replicates. This difference in sensitivity did not affect the objectives of either experiment.

RESULTS AND DISCUSSION

A. Radiochemical Purity of [^{14}C]p-Dichlorobenzene

(Figure 2, Appendix A)

The radiochemical purity of the stock [^{14}C]p-dichlorobenzene was 95.7%. The certificate of analysis (COA) is presented in Appendix A. When incorporated into the isopropyl myristate vehicle, the radiochemical purity of [^{14}C]p-dichlorobenzene was 95.3%. A radiochromatogram is presented in Figure 2.

B. Concentration of p-Dichlorobenzene in Isopropyl Myristate

(Figure 3, Appendix A)

The verified chemical (mg/mL) and radiochemical (µCi/mg) concentration for the prepared solution was 98.4 mg/mL and 0.0.3118 µCi/mg, respectively. A representative HPLC-UV chromatogram of p-dichlorobenzene analytical standard and of p-dichlorobenzene in the isopropyl myristate vehicle is presented in Figure 3. The COA for the technical p-dichlorobenzene is presented in Appendix A. The purity was 99.7%

C. Solubility of p-Dichlorobenzene in Receptor Fluid

p-Dichlorobenzene was determined to have a maximum solubility of 2352 µg/mL in 0.9% saline fortified with 6% (w/v) polyethoxyoleate (polyethylene glycol (PEG) 20 oleyl ether) which is 29-fold the maximum solubility in water of 80 µg/mL.

D. p-Dichlorobenzene, Permeability Coefficient

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

Key observations of mean data:

- The integrity of human skin, as determined by EI, was unaffected by continuous exposure under occlusive conditions to p-dichlorobenzene in isopropyl myristate. The ratio of the post-EI values to pre-EI values was 1.20. This increase in EI, which occurred over the exposure phase, may have been due to a rise in skin hydration and occlusion of the skin surface by residual p-dichlorobenzene not removed by washing.

- p-Dichlorobenzene was detectable in the receptor fluid at the 1-hour serial sampling timepoint (5.31 µg equiv/cm^2); the final receptor fluid sample (30 hours) was 429.8 µg equiv/cm^2.

- Steady state penetration of p-dichlorobenzene was reported by a minimum of 4 data points with a slope of 12.8 µg equiv/cm^2/h.

- At the end of the 30-hour interval, <5% of the applied radioactivity was detected in the receptor chamber ensuring sink condition for passive diffusion of p-dichlorobenzene.

- The permeability coefficient was calculated to be 1.31 x 10^{-4} cm/h, based on the slope at steady-state (12.8 µg equiv/cm^2/h), and the concentration of p-dichlorobenzene in the applied formulation (98,418µg/cm^3).

- Recovery of the applied radioactive dose was >76%.

E. p-Dichlorobenzene, 10- and 60-Minute Short-Term Penetration 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 p-dichlorobenzene in isopropyl myristate. The ratio of the post-EI values to pre-EI values for the 10-minute and 60-minute exposure groups were 1.02 and 0.82, respectively.

- Following a 10-minute exposure to a finite application of p-dichlorobenzene in isopropyl myristate, a total of 1.66 µg equivalents of p-dichlorobenzene was detected in the receptor fluid, with 2.36 µg equivalents in the skin. Based on the amount of p-dichlorobenzene 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 penetration rate was calculated to be 36.9 µg equiv/cm^2/h.

- Following a 60-minute exposure to a finite application of p-dichlorobenzene in isopropyl myristate, a total of 7.06 µg equivalents of p-dichlorobenzene was detected in the receptor fluid and 2.74 µg equivalents in the skin. Based on the amount of p-dichlorobenzene in the receptor fluid and skin, an exposure area of 0.64 cm^2 and an exposure time of one hour, the short-term penetration rate was calculated to be 15.3 µg equiv/cm^2/h.

- Recovery of the applied radioactive dose was 81.2 and 73.7% for the 10- and 60-minute exposure groups, respectively.

CONCLUSIONS

Based on the slope at steady-state (12.8 µg equiv/cm^2/h) and the concentration of the applied dose of p-dichlorobenzene (98,418 µg/cm^3), the permeability coefficient (Kp) was calculated to be 1.31 x 10^{-4} cm/h.

Following a 10-minute exposure to a finite application of p-dichlorobenzene in isopropyl myristate, a total of 1.66 µg equivalents of p-dichlorobenzene was detected in the receptor fluid, with 2.36 µg equivalents in the skin. Based on the amount of p-dichlorobenzene 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 penetration rate was calculated to be 36.9 µg equiv/cm^2/h

Following a 60-minute exposure to a finite application of p-dichlorobenzene in isopropyl myristate, a total of 7.06 µg equivalents of p-dichlorobenzene was detected in the receptor fluid and 2.74 µg equivalents in the skin. Based on the amount of p-dichlorobenzene in the receptor fluid and skin, an exposure area of 0.64 cm^2 and an exposure time of one hour, the short-term penetration rate was calculated to be 15.3 µg equiv/cm^2/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, for a period of at least 10 years.

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
NA	not applicable, sample result was below the limit of detection
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**
41.5	5.11	49.8	16.7	1.20	0.42

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

Time (hours)	Mean	SD
0.5	NA	NA
1	5.31	0.75
2	15.2	3.38
3	23.1	5.62
4	42.0	8.27
5	54.2	8.76
6	66.2	13.8
8	100.6	20.9
10	123.5	27.7
12	154.6	33.0
18	227.1	57.4
24	298.6	68.8
30	429.8	87.0

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

	Mean	SD
Steady-state penetration rate (µg equiv/cm^2/h)	12.8	3.49
Percent absorbed at 30 hours (%)	4.67	0.95
Permeability coefficient (Kp;cm/h)	1.31×10^{-4}	3.55×10^{-5}

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

	Mean	SD
Receptor Fluid	4.67	0.95
Skin Wash	71.4	4.27
Skin	0.12	0.05
Donor Chamber	0.037	0.018
Total Recovery	76.2	3.58

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	40.4	5.28	40.9	5.19	1.02	0.14
60	39.8	4.15	33.1	21.5	0.82	0.47

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

Exposure Time	p-Dichlorobenzene in RF (µg equiv)		p-Dichlorobenzene in Skin (µg equiv)		Total Absorbed RF + Skin (µg equiv)		Penetration Rate (µg equiv/cm^2/h)	
(minutes)	Mean	SD	Mean	SD	Mean	SD	Mean	SD
10	1.66	0.65	2.36	0.84	4.02	1.29	36.9	11.8
60	7.06	1.65	2.74	1.21	9.80	1.93	15.3	3.02

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

	10 Minutes		60 Minutes	
	Mean	**SD**	**Mean**	**SD**
Receptor Fluid	0.09	0.04	0.38	0.09
Skin Wash	81.0	10.5	73.1	8.4
Skin	0.13	0.05	0.1	0.1
Donor Chamber	0.05	0.05	0.05	0.02
Total Recovery	81.2	10.5	73.7	8.27

FIGURES

FIGURES

EXPLANATORY NOTES

ABBREVIATIONS:

dpm	disintegrations per minute
h	hour(s)

Figure 1: Static (glass) diffusion cell

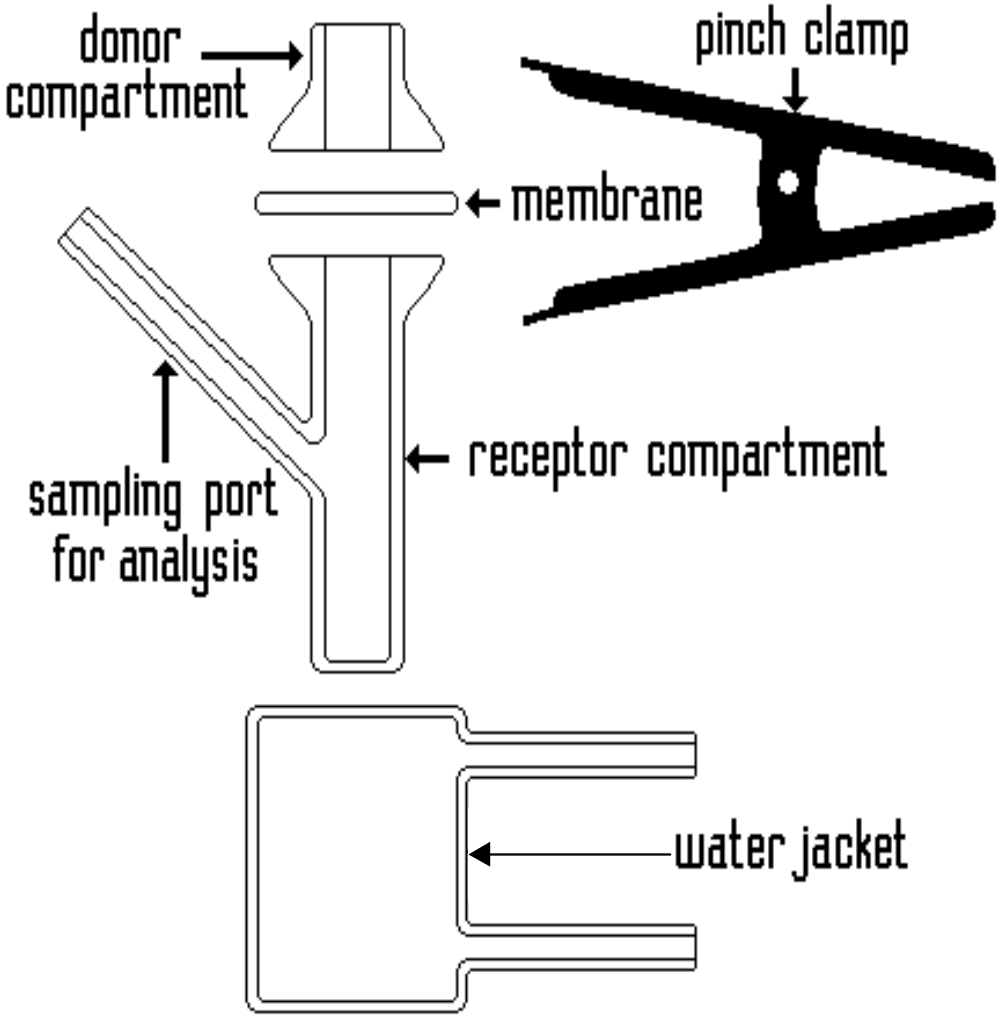


Figure 2: Radiochromatogram of [^{14}C]p-dichlorobenzene in isopropyl myristate

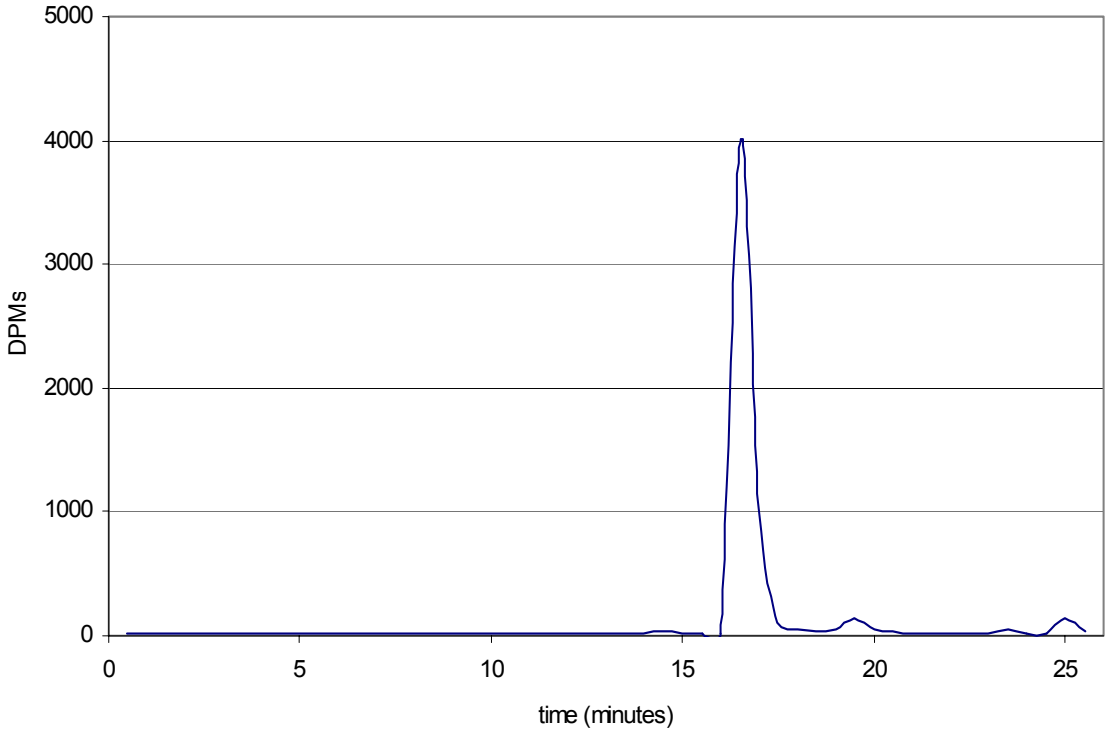
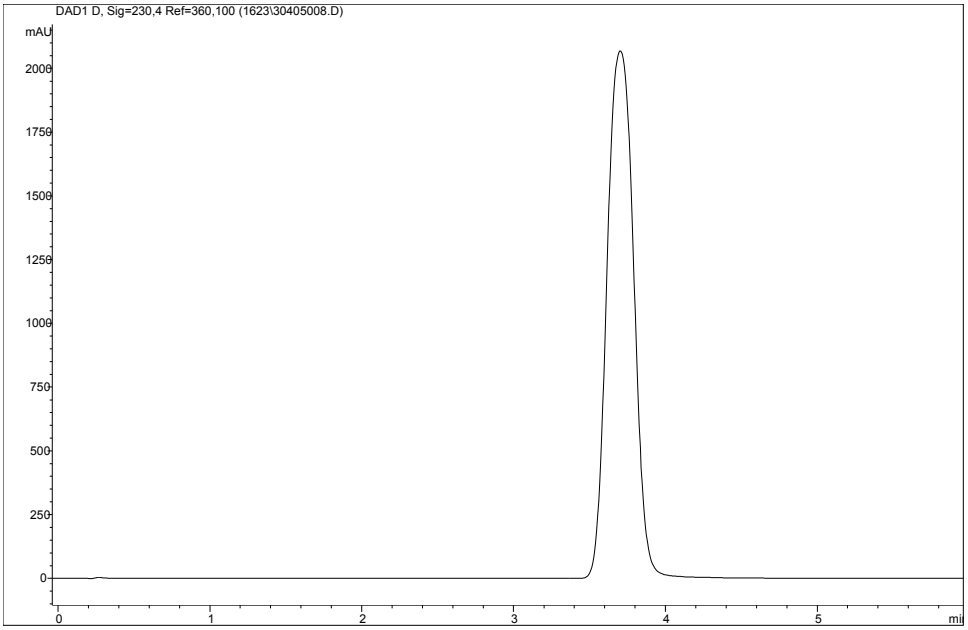


Figure 3: Representative HPLC-UV chromatogram of p-dichlorobenzene

(a) p-Dichlorobenzene analytical standard



(b) p-Dichlorobenzene in isopropyl myristate

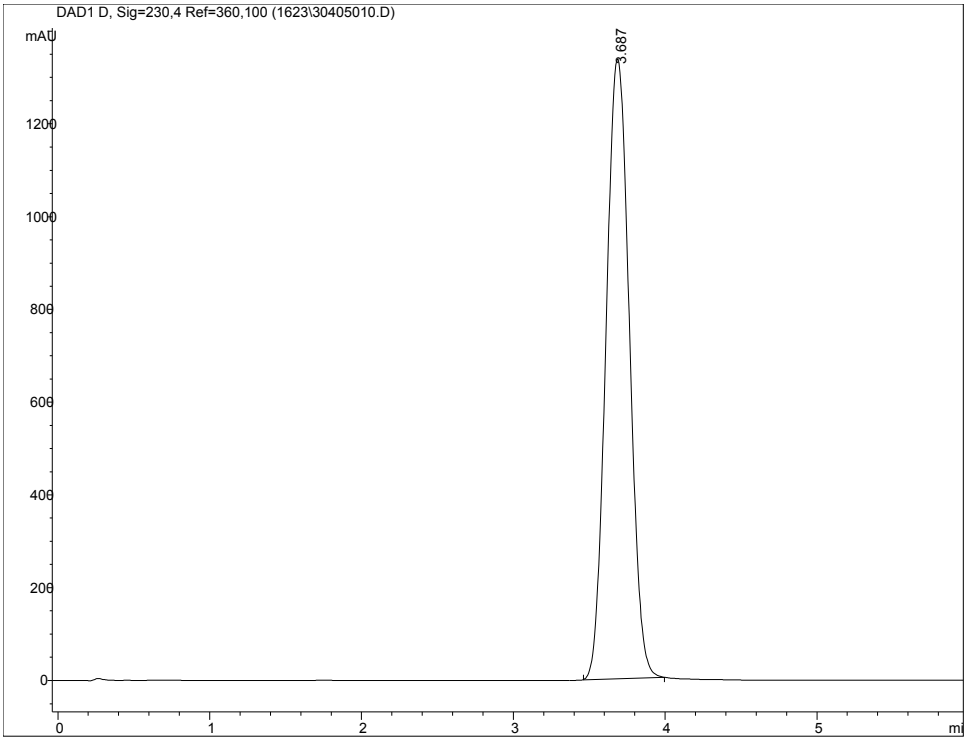
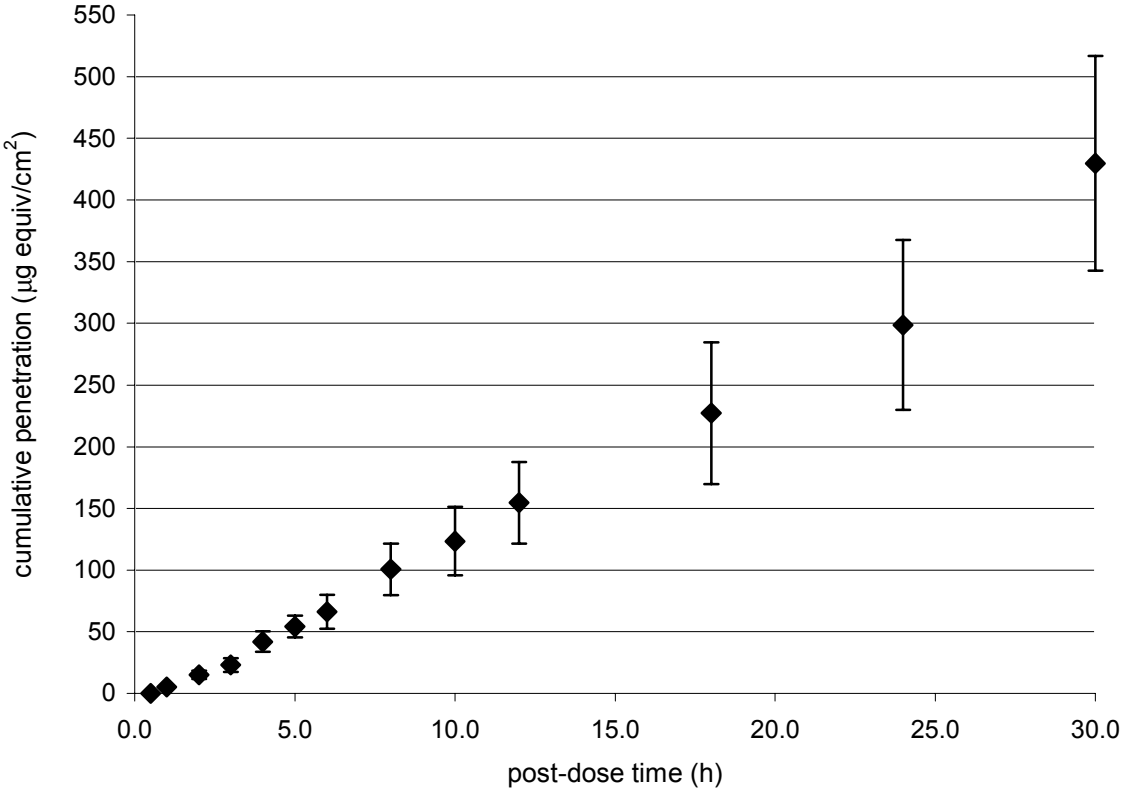


Figure 4: Permeability coefficient, cumulative amount penetrated (µg equiv/cm^2)



APPENDICES

APPENDICES

EXPLANATORY NOTES

ABBREVIATIONS:

EI	electrical impedance
h	hour(s)
HCMA-80B	Human, Caucasian, Male, Abdomen, sample 80B
Kp	permeability coefficient
NA	not applicable, sample result was below the limit of detection
RF	receptor fluid
SD	standard deviation

Appendix A:

Certificates of Analysis

NB E99681-CH
WR 15690 SC1623
LRM 11 Jan 05

SIGMA®
3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: sigma-techserv@sial.com
http://www.sigma-aldrich.com

ProductInformation

PROD NAME: 1,4-DICHLOROBENZENE-UL-14C
LOT NUMBER: 080K9418/19 ->
PROD NUMBER: D4653-14C
ANALYST: Quality Control
DATE: 1/4/05 1:28:48 PM Inj Volume: 1.00ul

=====

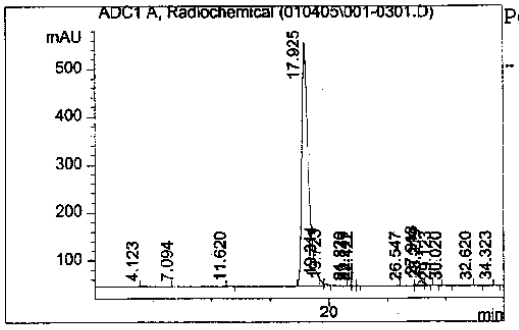
*****METHOD FOR D4653 1,4-DICHLOROBENZENE-UL-14C*****

Column: Supelco Discovery C18, 250 x 2.1 mm, 5 micron
Column Temp: 35°C
Flow Rate: 0.3 ml/min
Mobil Phase: A=0.1% Phosphoric Acid (V/V) in Water
B=Acetonitrile
50% A For 10 minutes to 0% A over 15 minutes, hold for 10 minutes
Detection: Radiochemical

*****PRODUCT INFORMATION*****

Specific Activity : 19.9 mCi/mmol
Molecular Weight : 147.0
Concentration : Solid
Storage Temp : 2-8°C
Packaging : Combi Vial

=====



Peak #	RetTime [min]	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.123	0.237	17.478	0.890	0.086
2	7.094	0.307	15.250	0.629	0.075
3	11.620	0.204	25.582	1.623	0.126
4	17.925	0.470	1.936e4	506.278	95.733
5	19.314	0.189	156.047	11.281	0.772
6	19.723	0.249	92.450	4.466	0.457
7	21.820	0.148	12.998	1.357	0.064
8	22.147	0.163	15.267	1.181	0.075
9	22.422	0.139	21.135	2.227	0.104
10	26.547	0.331	34.469	1.262	0.170
11	27.918	0.302	305.122	12.444	1.509
12	28.219	0.224	101.506	5.918	0.502
13	29.123	0.188	22.156	1.505	0.110
14	30.020	0.175	14.002	1.001	0.069
15	32.620	0.148	11.005	1.006	0.054
16	34.323	0.159	18.676	1.522	0.092

SIGMA-ALDRICH

Certificate of Analysis

Product Name	1,4-Dichlorobenzene, ≥99%
Product Number	D5,682-9
Product Brand	ALDRICH
CAS Number	106-46-7
Molecular Formula	$C_6H_4Cl_2$
Molecular Weight	147.00

TEST	SPECIFICATION	LOT 06205KA RESULTS
APPEARANCE	COLORLESS TO WHITE CRYSTALLINE SOLID	COLORLESS CRYSTALS
INFRARED SPECTRUM	CONFORMS TO STRUCTURE AND STANDARD AS	CONFORMS TO STRUCTURE AND STANDARD
TITRATION	99.0%-101.0%(WITH AGNO3 AFTER O2 COMBUSTION)	100.5 % (WITH AGNO3 AFTER OXYGEN COMBUSTION)
GAS LIQUID CHROMATOGRAPHY	99.0% (MINIMUM)	99.7 %
QUALITY CONTROL ACCEPTANCE DATE		AUGUST 2002

Ronnie J. Martin, Supervisor
Quality Control
Milwaukee, Wisconsin USA

Appendix B:

Permeability Coefficient (Kp) Data

Mock Dose

Replicate aliquots for liquid formulations

NB	E99681-CH
WR	15690
SC	1623
Formulation	p-Dichlorobenzene
Group	A

input cell

Replicate	Aliquot (µL)	Dilution (mL)	Aliquot (mL)	Radioactivity in aliquot (dpm)	Radioactivity in aliquot (dpm) - background
1	64	10	0.05	21959	21940
				21937	21918
				21981	21962
2	64	10	0.05	19396	19377
				19296	19277
				19555	19536
3	64	10	0.05	19368	19349
				20204	20185
				19862	19843

Background sample	19
Sum (dpm)	183387
Average (dpm)	20376
Amount in mock (dpm)	4075267

A - Radioactivity applied (dpm)	4075267	
B - Radioactivity applied (µCi)	1.84	(=A/2.22 x 10^6 dpm/µCi)
C - Verified specific activity (µCi/mg)	0.3118	
D - Specific activity (dpm/µg)	692	(=[C*2.22 x 10^6 dpm/µCi]/1000 µg/mg)
E - Total compound applied (µg)	5888	(=A/D)
F - Application rate (µg/cm^2)	9200	(=E/0.64 cm^2)

Pre-and post-EI values

Cell ID	Skin ID	Pre EI (k-ohms)	Post EI (k-ohms)	Ratio: Post/Pre
A2	HCMA-121	62.0	4.30	0.07
D2	HCMA-121	47.9	43.6	0.91
F2	HCMA-122	37.4	64.5	1.72
H2	HCMA-122	43.7	51.0	1.17
K2	HCFA-130	35.3	24.8	0.70
M2	HCFA-130	43.4	64.9	1.50
	Mean	41.5	49.8	1.20
	SD	5.11	16.7	0.42

Cell A2 was excluded from calculation of Mean/SD due to damage during the wash process.

Cumulative amount penetrated (µg equiv/cm^2)

	Time after dosing (hr)						
Cell ID	0.5	1	2	3	4	5	6
A2	NA	NA	9.82	15.4	30.0	40.8	45.9
D2	NA	NA	12.4	17.5	37.2	47.3	53.7
F2	NA	4.29	15.9	23.3	38.3	54.0	65.4
H2	NA	5.31	18.4	25.8	52.1	62.4	77.0
K2	NA	6.10	16.6	26.4	46.1	58.1	75.5
M2	NA	5.53	18.0	30.1	48.4	62.9	79.6
MEAN	NA	5.31	15.2	23.1	42.0	54.2	66.2
SD	NA	0.75	3.38	5.62	8.27	8.76	13.8

	Time after dosing (hr)					
Cell ID	8	10	12	18	24	30
A2	70.2	83.0	107.7	154.0	200.7	299.4
D2	82.8	98.5	124.0	168.1	235.3	349.5
F2	99.0	121.7	152.2	222.5	295.1	433.3
H2	119.8	145.6	183.7	259.3	358.7	505.3
K2	109.7	140.0	170.6	256.0	328.8	491.0
M2	122.4	152.2	189.4	302.8	373.2	500.2
MEAN	100.6	123.5	154.6	227.1	298.6	429.8
SD	20.9	27.7	33.0	57.4	68.8	87.0

Percent absorbed, steady-state penetration, Kp

Cell ID	Percent absorbed at 30 hours	Steady State Penetration rate (µg equiv/cm^2/h)	Kp (cm/h)
A2	3.25	8.23	8.36×10^{-5}
D2	3.80	9.43	9.58×10^{-5}
F2	4.71	12.3	1.25×10^{-4}
H2	5.49	15.5	1.58×10^{-4}
K2	5.34	14.6	1.48×10^{-4}
M2	5.44	17.0	1.73×10^{-4}
Mean	4.67	12.8	1.31×10^{-4}
SD	0.95	3.49	3.55×10^{-5}

Recovery data, percent of applied dose

Cell ID	Receptor Fluid	Skin Wash	Skin	Donor Chamber	Total Recovery
A2	3.25	76.7	0.213	0.066	80.3
D2	3.80	75.9	0.089	0.020	79.8
F2	4.71	68.0	0.095	0.050	72.9
H2	5.49	69.3	0.112	0.020	74.9
K2	5.34	66.3	0.122	0.035	71.8
M2	5.44	72.1	0.113	0.035	77.7
MEAN	4.67	71.4	0.124	0.037	76.2
SD	0.95	4.27	0.045	0.018	3.58

Appendix C:

Short-Term Absorption Rate Data – 10 and 60 Minutes

Mock Dose

Replicate aliquots for liquid formulations

NB	E99681-CH	input cell
WR	15690	
SC	1623	
Formulation	p-Dichlorobenzene	
Group	B,C,D	

Replicate	Aliquot (µL)	Dilution (mL)	Aliquot (mL)	Radioactivity in aliquot (dpm)	Radioactivity in aliquot (dpm) - background
1	19.2	10	0.05	6272	6253
				6355	6336
				6315	6296
2	19.2	10	0.05	6798	6779
				6891	6872
				6887	6868
3	19.2	10	0.05	6154	6135
				6017	5998
				6128	6109

Background sample	19
Sum (dpm)	57646
Average (dpm)	6405
Amount applied (dpm)	1281022

A - Radioactivity applied (dpm)	1281022	
B - Radioactivity applied (µCi)	0.58	(=A/2.22 x 10^6 dpm/µCi)
C - Verified specific activity (µCi/mg)	0.3118	
D - Specific activity (dpm/µg)	692	(=[C*2.22 x 10^6 dpm/µCi]/1000 µg/mg)
E - Total compound applied (µg)	1850	(=A/D)
F - Application rate (µg/cm^2)	2892	(=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	B2	HCMA-122	37.3	35.6	0.95
	D2	HCMA-122	39.9	37.2	0.93
	P2	HCMA-121	42.1	7.1	0.17
	E2	HCMA-121	48.0	45.7	0.95
	L2	HCFA-130	36.4	44.9	1.23
	M2	HCFA-130	48.7	6.7	0.14
		Mean	40.4	40.9	1.02
		SD	5.28	5.19	0.14

Cells P2 and M2 were excluded from calculation of Mean/SD due to damage during the wash process.

60 Minutes

Exposure Time	Cell ID	Skin ID	Pre EI (k-ohms)	Post EI (k-ohms)	Ratio: Post/Pre
60 minutes	F2	HCMA-122	40.0	42.6	1.07
	G2	HCMA-122	39.9	31.1	0.78
	H2	HCMA-121	44.2	10.0	0.23
	I2	HCMA-121	33.6	15.6	0.46
	O2	HCFA-130	44.3	70.1	1.58
	Q2	HCFA-130	37.0	29.4	0.79
		Mean	39.8	33.1	0.82
		SD	4.15	21.5	0.47

Penetration rate data

10 Minutes

Cell ID	Skin ID	p-Dichlorobenzene in RF (µg equiv)	p-Dichlorobenzene in Skin (µg equiv)	Total absorbed RF+Skin (µg equiv)	Penetration rate (µg equiv/cm^2/h)
B2	HCMA-122	0.85	2.11	2.96	27.2
D2	HCMA-122	1.30	2.07	3.37	31.0
P2	HCMA-121	1.67	1.75	3.42	31.5
E2	HCMA-121	1.70	1.45	3.15	29.0
L2	HCFA-130	1.59	3.46	5.05	46.4
M2	HCFA-130	2.82	3.33	6.16	56.6
	Mean	1.66	2.36	4.02	36.9
	SD	0.65	0.84	1.29	11.8

60 Minutes

Cell ID	Skin ID	p-Dichlorobenzene in RF (µg equiv)	p-Dichlorobenzene in Skin (µg equiv)	Total absorbed RF+Skin (µg equiv)	Penetration rate (µg equiv/cm^2/h)
F2	HCMA-122	5.35	1.74	7.08	11.1
G2	HCMA-122	7.13	2.19	9.33	14.6
H2	HCMA-121	5.02	3.22	8.24	12.9
I2	HCMA-121	7.27	4.97	12.2	19.1
O2	HCFA-130	8.30	2.43	10.7	16.8
Q2	HCFA-130	9.26	1.90	11.2	17.4
	Mean	7.06	2.74	9.80	15.3
	SD	1.65	1.21	1.93	3.02

Recovery data, percent of applied dose

10 Minutes

Cell ID	Receptor fluid	Skin Wash	Skin	Donor Chamber	Total Recovery
B2	0.05	73.8	0.11	0.01	73.9
D2	0.07	95.4	0.11	0.06	95.6
P2	0.09	87.8	0.09	0.01	87.9
E2	0.09	71.2	0.08	0.03	71.4
L2	0.09	70.5	0.19	0.03	70.8
M2	0.15	87.1	0.18	0.14	87.6
MEAN	0.09	81.0	0.13	0.05	81.2
SD	0.04	10.5	0.05	0.05	10.5

60 Minutes

Cell ID	Receptor fluid	Skin Wash	Skin	Donor Chamber	Total Recovery
F2	0.29	82.9	0.09	0.042	83.3
G2	0.39	80.6	0.12	0.055	81.2
H2	0.27	71.8	0.17	0.041	72.3
I2	0.39	59.7	0.27	0.087	60.4
O2	0.45	74.4	0.13	0.035	75.0
Q2	0.50	69.5	0.10	0.034	70.1
MEAN	0.38	73.1	0.15	0.049	73.7
SD	0.09	8.36	0.07	0.02	8.27