



May 23, 2023

Office of Chemical Safety and Pollution Prevention
Environmental Protection Agency
1200 Pennsylvania Ave., NW.
Washington, DC 20460-0001

RE: **OECD 428 Report Submission to EPA Test Order for 1,1,2-Trichloroethane (CASRN 79-00-5)**

The Vinyl Institute¹ (VI) represents manufacturers and processors of 1,1,2-Trichloroethane as part of a consortium created to respond to EPA's TSCA Test Orders. The VI Test Order Consortia (SEC4-CON-21-410800) Members include:

- | | |
|-----------------------------------|--------------------------|
| • Formosa Plastics Corporation | TO-2018-0421-643881-01-A |
| • Occidental Chemical Corporation | TO-2018-0421-461112-01-A |
| • Olin Corporation | TO-2018-0421-547336-01-A |
| • Westlake Chemical Corporation | TO-2018-0421-530411-01-A |

The VI is submitting the attached report containing data requested by the Test Order for OECD 428 titled 1,1,2-Trichloroethane: Rates of penetration through human skin using a flow through *in vitro* system, on behalf of the Members of the VI Test Order Consortia.

Please do not hesitate to contact me with any questions.

Sincerely, |

A handwritten signature in black ink, appearing to read "Domenic DeCaria".

Domenic DeCaria
Vice President, Regulatory and Technical Affairs
e-mail: ddecaria@vinylinfo.org

¹ The Vinyl Institute (VI), established in 1982, represents the leading producers of vinyl resins and monomers, and ingredient and additive producers for vinyl compounds. The VI serves as the collective voice for the vinyl industry. More information can be found at www.vinylinfo.org.

Amended Final Report 1

Study Title	1,1,2-Trichloroethane : Rates of penetration through human skin using a flow through <i>in vitro</i> system
Study Director	Adele Jones
Guidelines:	OECD 428 (2004) OECD Environmental Health and Safety Publications Series on Testing and Assessment No. 28 (2004)
Test Facility	Labcorp Early Development Laboratories Ltd Woolley Road Alconbury Huntingdon Cambridgeshire PE28 4HS UK
Study Number	8479196
Sponsor	Stantec ChemRisk (formerly Cardno ChemRisk) on behalf of Vinyl Institute 1,1,2-Trichloroethane Test Order Consortium
Amended Report 1 Issue Date:	19 May 2023
Final Report Issue Date	16 May 2023
Page Number	1 of 126

AMENDED FINAL REPORT 1

This Amended Final Report includes changes to the following portions of the original final report. The report pagination and table of contents were updated to reflect the changes in the report.

This amended final report replaces the final report issued on 16 May 2023 in its entirety.

1. **Cover Page**

Reason for Change: The cover page was modified to change *Report* to *Amended Final Report*, to include the date the amended report was issued.

2. **Compliance Statement and Signature Page**

Reason for Change: The compliance statement and signature page was re-signed because the report is being reissued as an amended final report.

3. **Quality Assurance Statement**

Reason for Change: The quality assurance statement was reissued to include the review of the amended report.

4. **Section 1, Table**

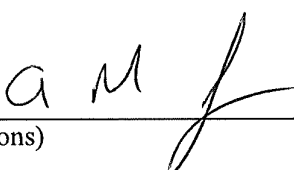
Reason for Change: An incorrect footnote has been removed from the report.

COMPLIANCE WITH GOOD LABORATORY PRACTICE

The study described in this Amended Final Report was conducted in compliance with the following Good Laboratory Practice standards and I consider the data generated to be valid.

- The United Kingdom Good Laboratory Practice Monitoring Authority, Medicines and Healthcare Products Regulatory Agency (MHRA): Good Laboratory Practice Regulations 1999, Statutory Instrument 1999 No. 3106 (as amended)
- The Organisation for Economic Co-Operation and Development (OECD): Principles on Good Laboratory Practice, ENV/MC/CHEM (98) 17 (revised in 1997, issued January 1998)

These principles are compatible with Good Laboratory Practice regulations specified by regulatory authorities throughout the European Community, the United States (EPA and FDA), Japan (MHLW, MAFF and METI), and other countries that are signatories to the OECD Mutual Acceptance of Data Agreement.



Adele Jones, BSc (Hons)
Study Director
Labcorp Early Development Laboratories Ltd

19 MAY 2023
Date

QUALITY ASSURANCE STATEMENT

1,1,2-Trichloroethane : Rates of penetration through human skin using a flow through *in vitro* system

This study has been reviewed by the Quality Assurance Unit of Labcorp, and the report accurately reflects the raw data. The following study-specific inspections were conducted and findings reported to the Study Director (SD) and associated management.

Critical procedures performed routinely in an operational area may be audited as part of a risk based process inspection programme. This can be in addition to phases scheduled on an individual study basis. Selected process inspections conducted and considered applicable to this study are included in the following.

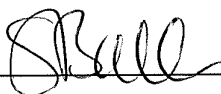
In addition to the inspection programme detailed in the following, a facility inspection programme is also operated. Details of this programme, which covers all areas of the facility annually (at a minimum), are set out in standard operating procedures.

Audit Number	Audit Dates		Phase	Date Reported to SD and SD Management
	Start	End		
AUD-120461	12 Aug 2022	12 Aug 2022	Protocol/Study Plan Review	12 Aug 2022
AUD-142841	19 Jan 2023	26 Jan 2023	Protocol/Study Plan Amendment Review	26 Jan 2023
AUD-149030	07 Mar 2023	07 Mar 2023	Protocol/Study Plan Amendment Review	07 Mar 2023
AUD-121089	20 Mar 2023	30 Mar 2023	Draft Report and Data Review	30 Mar 2023
AUD-156657	03 May 2023	03 May 2023	Protocol/Study Plan Amendment Review	03 May 2023
AUD-121090	12 May 2023	12 May 2023	Final Report Review	12 May 2023
AUD-084394	19 May 2023	19 May 2023	Amended Final Report and Data Review	19 May 2023
AUD-159161	19 May 2023	19 May 2023	Final Amended Report Review	19 May 2023

Process					
Audit Number	Department	Phase	Audit Dates		Date Reported to Management
			Start	End	
AUD-089388	Archives	Archiving Procedures	07 Dec 2022	19 Dec 2022	19 Dec 2022
AUD-084053	Metabolism	Study Direction	07 Oct 2022	14 Dec 2022	15 Dec 2022
AUD-084056	Metabolism	Study Type	24 Oct 2022	06 Nov 2022	06 Nov 2022
AUD-084059	Metabolism	Analytical Procedures	24 Oct 2022	11 Nov 2022	11 Nov 2022
AUD-084062	Metabolism	Test Systems	15 Aug 2022	16 Aug 2022	19 Dec 2022
AUD-084063	Metabolism	Metabolism Procedures	26 Oct 2022	13 Dec 2022	14 Dec 2022
AUD-084065	Metabolism	Dosing Procedures	08 Nov 2022	15 Nov 2022	15 Nov 2022
AUD-084071	Metabolism	Test Article/Item Receipt	02 Nov 2022	06 Nov 2022	06 Nov 2022
AUD-138049	Metabolism	Study Direction	31 Jan 2023	07 Feb 2023	07 Feb 2023

S. Baumber

Sandra Baumber
Quality Assurance Unit



19 May 2023.
Date

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STUDY CONTACTS

Sponsor	Stantec ChemRisk (formerly Cardno ChemRisk) on behalf of Vinyl Institute 1,1,2- Trichloroethane Test Order Consortium 20 Stanwix Street, Suite 505 Pittsburgh PA 15222 UNITED STATES
Study Monitor	Marisa Kreider (PhD, DABT)
Test Facility	Labcorp Early Development Laboratories Ltd Woolley Road Alconbury Huntingdon Cambridgeshire PE28 4HS UK

1. SUMMARY

1,1,2-Trichloroethane is used primarily in the manufacture of other chemicals. Information was required on rates of penetration of [^{14}C]-1,1,2-Trichloroethane in human membranes following dermal application as the neat test item and up to three lower dose levels (50%, 10% and 1% solutions in IPM (isopropyl myristate) or 1,2-Dichloroethane) under both infinite and finite dose conditions.

At least six flow-through diffusion cells were prepared at each dose level. Skin membranes, (approximately 200 – 400 μm thickness) were maintained in the cells at approximately 32°C. The integrity of the skin membranes was first assessed using trans epidermal water loss (TEWL).

For the infinite dose groups, receptor fluid samples were collected at 16 intervals throughout the duration of the experiment (24 hours). The solubility of 1,1,2-Trichloroethane in the receptor fluid was demonstrated to be sufficient for the study and not to be rate limiting to the absorption process. Radioactivity in the receptor fluid was analyzed to determine the rate of penetration and the permeability coefficient (K_p) of the test item.

Following infinite application of 1,1,2-Trichloroethane, as the neat test item and 50%, and 10% solutions in IPM and 1,2-Dichloroethane, there were no observed solvent dependent differences in absorption. There was also no observed difference in maximum absorption rate or permeability coefficients when applied as the neat material or 50% solution, however, there was a proportionate decrease in absorption between the 50% and 10% solutions for both solvents.

The data indicates that true sink conditions were not maintained for some replicates following application of the neat material and the 50% solution in 1,2-Dichloroethane due to the volatility of both the test item and the diluent. It is considered that sufficient data was obtained to calculate the permeability coefficient over the steady state portion of the absorption profile.

The group mean permeability coefficient (K_p) are summarized in the following table.

Dose level	Permeability coefficient (K_p) ($\times 10^{-3} \text{ cm/hr}$)
Neat	3.33
50% IPM solution	3.40
10% IPM solution	0.94
50% 1,2-Dichloroethane solution	4.49
10% 1,2-Dichloroethane solution	0.86

For the finite dose groups, the skin samples were exposed to the test material for 8 hours, after which time the dose remaining on the skin was washed off the skin with a mild detergent solution. Receptor fluid samples were collected at 16 intervals throughout the duration of the experiment (24 hours). Volatile radioactivity was trapped using a charcoal filter.

At the end of the experiment, the skin samples were tape stripped to remove residual surface dose and stratum corneum. The distribution of radioactivity in the skin was determined by radioassay of the various samples collected.

The group mean distributions of radioactivity are summarised below.

Dose level	Neat	IPM solutions			1,2-Dichloroethane solutions		
		50%	10%	1%	50%	10%	1%
Total dislodgeable dose ^a	0.35 ± 0.06	0.57 ± 0.31	0.56 ± 0.05	0.71 ± 0.16	0.70 ± 0.10	0.97 ± 0.22	0.24 ± 0.60
Tape strip (1-2)	<0.01 ± 0.01	nd	nd	nd	nd	nd	nd
Tape strip (3 onwards)	<0.01 ± 0.01	nd	nd	nd	nd	nd	nd
Total unabsorbed ^b	96.27 ± 5.92	99.79 ± 1.29	95.31 ± 3.91	105.43 ± 3.85	92.96 ± 1.60	95.29 ± 1.36	91.91 ± 1.25
Total absorbed ^c	0.39 ± 0.04	0.19 ± 0.15	0.02 ± 0.02	nd	0.59 ± 0.20	0.40 ± 0.07	0.34 ± 0.24
Dermal delivery ^d	0.45 ± 0.07	0.24 ± 0.13	0.02 ± 0.02	nd	0.59 ± 0.20	0.40 ± 0.07	0.34 ± 0.24
Mass balance	96.72 ± 5.89	100.03 ± 1.38	95.33 ± 3.90	105.43 ± 3.85	93.54 ± 1.76	95.70 ± 1.33	92.25 ± 1.33

Results expressed as % applied radioactivity ± standard deviation

nd Not detected

^a Sum of radioactivity in the skin wash (8 and 24 hours) and donor chamber

^b Sum of total dislodgeable dose and radioactivity in the volatile charcoal trap and surface tape strips

^c Sum of radioactivity detected in the receptor fluids and receptor chamber

^d Sum of absorbed dose and radioactivity in exposed skin

Following finite application of 1,1,2-Trichloroethane as the neat test item and 50%, 10% and 1% solutions in IPM and 1,2-Dichloroethane, under semi-occlusive conditions, dermal delivery was very low accounting for 0.02 – 0.59% of applied dose, where detectable. At each concentration, the majority of radioactivity was detected in the volatile traps indicating that under finite conditions, 1,1,2-Trichloroethane was most likely to quickly volatilize from the skin surface.

2. GENERAL STUDY INFORMATION

2.1 Purpose

The objectives of the study were:

- (a) To determine and compare the rates of absorption of [¹⁴C]-1,1,2-trichloroethane following *in vitro* dermal applications to excised human skin at multiple dose levels (1%, 10%, 50% and neat), using multiple potential solvents under finite and infinite dosing scenarios and to determine the retention of radioactivity at the site of application (finite dose groups only).
- (b) Satisfy USEPA TSCA Test Order for OECD testing of 1,1,2-trichloroethane.

2.2 Study Schedule

2.2.1 Time Schedule

Experimental start date 30 August 2022

Experimental completion date 15 February 2023

2.3 Regulatory Testing Guidelines

This study was conducted in compliance with the following guidelines:

- OECD Test Guideline 428 (April 2004) and the corresponding OECD Guidance Document for conducting *in vitro* skin absorption studies (March 2004).

2.4 Quality Assurance

Details of the Quality Assurance inspections and audits undertaken at Labcorp Early Development are presented on the Quality Assurance Statement.

3. METHODS

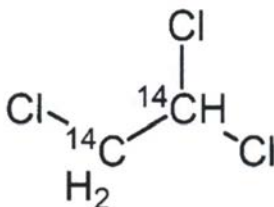
3.1 Test item and Supporting Information

3.1.1 General Information

Name:	1,1,2-Trichloroethane
CAS number:	79-00-05
Molecular formula:	C ₂ H ₃ Cl ₃
Molecular weight:	133.4 g/mol
Density:	1.44 g/mL
Physical state:	Liquid

3.1.2 Radiolabeled 1,1,2-Trichloroethane

Structural formula:



Position of Radiolabel:	Shown in above structural formula
Specific activity:	0.05 mCi/mmol
Source:	Moravek Biochemical Inc.
Batch number:	369-166-050-A-20210917-DRE
Radiochemical purity:	> 95.1 % (Appendix 11.1)
Storage:	- 20 ± 10°C

3.1.3 Non-radiolabeled Test Item

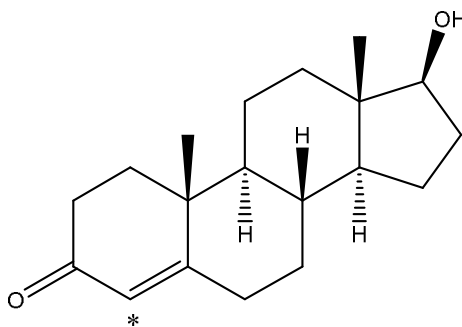
Name: 1,1,2-Trichloroethane
Source: Sigma Aldrich
Batch number: BCCB7564
Purity: 98.2 %
Storage: $20 \pm 5^{\circ}\text{C}$
Expiry date: 31 May 2024

3.2 Reference Item

3.2.1 Radiolabeled testosterone

Name: [^{14}C]-Testosterone

Structural formula:



Position of Radiolabel: * Denotes position of C-14 labeling

Source: ARC

Batch number: 210415

Radiochemical purity: 97.7 % ([Appendix 11.1](#))

Storage: $5 \pm 3^{\circ}\text{C}$

3.2.2 Non-radiolabeled Testosterone

Source:	Sigma Aldrich
Batch number:	SLCF3521
Purity:	100%
Storage:	5 ± 3°C
Retest date:	10 February 2023

The certificates of analysis are shown in [Appendix 11.1](#). A record of use of the test items was maintained using Samuel (in-house electronic tracking system).

3.3 Test Systems

3.3.1 Rationale for Selection

Assessing the hazard of skin contact with potentially toxic materials requires a combination of estimates of uptake *via* the skin and quantitative toxicology. Diffusion across the non-living outer layer of skin, the stratum corneum, is normally the rate-limiting step for percutaneous absorption. The permeability properties of the stratum corneum are unchanged by removal from the body and, therefore, *in vitro* experiments using this membrane are appropriate and offer a number of advantages over whole animal or human volunteer experiments.

Since the dermal route may be potentially significant for human exposure, the rate and extent of absorption were studied using human skin. The dose levels selected in this study were selected to be representative of potential in-life exposure levels.

The performance and reliability of the *in vitro* test system have been previously demonstrated in the performing laboratory. Data for a number of reference substances of different lipophilicity were generated during a multi-centre comparison study ([Van de Sandt et al, 2004](#)). In addition, data for a reference substance, testosterone, was generated as part of this study. This data showed the expected distribution of the reference item and results were comparable with those obtained in the multi-centre comparison study.

3.3.2 Solubility of 1,1,2-Trichloroethane in the Receptor Fluid

Adequate solubility of 1,1,2-Trichloroethane in the receptor fluid was considered to have been demonstrated if the equivalent amount potentially applied in the highest dose was completely soluble in the total volume of receptor fluid passed for the duration of the experiment (i.e. 36 mL). This ensured that the dissolution of 1,1,2-Trichloroethane was not rate-limiting for its potential dermal penetration. The method used for the conduct of the solubility test is described below.

A radiodiluted stock was prepared by mixing radiolabeled 1,1,2-Trichloroethane (2.43mg) and non-radiolabeled 1,1,2-Trichloroethane (497 mg). Triplicate aliquots of the stock, equivalent to

125 mg was added to three separate vessels containing sufficient 6% Polyoxyethylene 20 oleyl ether in water to prepare a 25 mg/mL solution if fully soluble. The vessels were magnetically stirred for approximately 24 hours at approximately 32°C. A sub-sample from each vessel was centrifuged at 1992 g for 10 minutes at 4°C. Duplicate aliquots (0.25 mL) of the supernatant were taken for radioassay.

3.3.3 Human Skin

Full thickness skin samples were obtained from human donors following elective surgery and were supplied by Tissue Solutions or Bio IVT. Samples were stored at -20°C ± 10°C.

The specification associated with each skin sample was as follows:

Skin number	Sex	Anatomical region	Age
H1	Female	Abdomen	35
H2	Female	Abdomen	31
H3	Female	Abdomen	51
H4	Female	Abdomen	31
H5	Female	Abdomen	48
H6	Female	Abdomen	31
H7	Female	Abdomen	31
H8	Female	Abdomen	70
H9	Female	Abdomen	38
H10	Female	Abdomen	32
H11	Female	Abdomen	57
H12	Female	Abdomen	34
H13	Female	Abdomen	41
H14	Female	Abdomen	65
H15	Female	Abdomen	31

3.3.4 Human Skin preparation

Skin was pre-dermatomed at Labcorp Early Development Laboratories Ltd as follows:

Skin samples were thawed to room temperature. Each full thickness skin membrane was then wiped to remove residual fat and blood, wiped dry and re-hydrated with purified water prior to dermatoming. The full thickness skin sample was pinned out on a dermatome board (cork board with raised rubber cutting surface) and a mini-dermatome was used to cut slices of skin which contained epidermis and some dermis (dermatomed skin was 200 – 400 µm thick, measured using a micrometer).

3.3.5 Membrane Integrity and Membrane Selection

The integrity of the selected skin samples was checked by measuring trans epidermal water loss (TEWL) from the skin surface under closed chamber conditions using a VaporMeter (Delfin Technologies) prior to dose application and at termination (prior to skin washing and tape stripping). A TEWL measurement of $\leq 13 \text{ gm}^{-2}\text{h}^{-1}$ was considered acceptable (Guth *et al*, 2015).

Pre exposure, cells failing to meet the acceptance criteria following the initial measurement were allowed to dry for an additional period and were then remeasured to ensure the skin was completely dry prior to an additional measurement. Any skins failing to meet the acceptance criteria prior to dose application were discarded and replaced.

Cell 38 failed to meet the acceptance criteria prior to administration and was dosed in error. Data from this cell was excluded from the mean calculation.

Post exposure, if considered necessary, any cells failing to meet the acceptance criteria following the initial measurement were allowed to dry for an additional period and were then remeasured to ensure the skin was completely dry prior to an additional measurement. Cells failing to meet the acceptance criteria, were considered to have been potentially damaged during the course of the experiment and data from these cells was excluded from the mean calculations.

3.4 Study Conduct

3.4.1 [^{14}C]-Testosterone Dose Preparation

Initial Dose Preparation

[^{14}C]-Testosterone in ethanol (nominally 0.2 mg, 0.38 mL) was reduced to dryness under nitrogen gas and resuspended in 40% aqueous ethanol solution to prepare a 1 mg/mL solution.

Repeat Dose Preparation

[^{14}C]-Testosterone was radiodiluted with non-radiolabeled testosterone. An aliquot of [^{14}C]-Testosterone (0.025 mg, 0.048 mL) was dispensed into a vial and an aliquot of a non-radiolabeled 1,1,2-Trichloroethane stock solution (0.1125 mL, 0.225 mg) was added. A 40% aqueous ethanol solution (0.25 mL) was added and the solution magnetically stirred.

3.4.2 1,1,2-Trichloroethane Dose Preparation

[^{14}C]-1,1,2-Trichloroethane was radiodiluted with non-radiolabeled 1,1,2-Trichloroethane (if required). The test item was applied neat or was further diluted with either IPM (isopropyl myristate) or 1,2-Dichloroethane to prepare 1%, 10% and 50% application solutions.

Neat Material (Groups A2 and A7)

An aliquot of [^{14}C]-1,1,2-Trichloroethane (nominally 0.238 mL) was dispensed into a vial and non-radiolabeled 1,1,2-Trichloroethane (0.762 mL) was added.

50% dose solution in IPM (Groups A3 and A8)

An aliquot of [^{14}C]-1,1,2-Trichloroethane (0.06 or 0.357 mL) was dispensed into a vial and non-radiolabeled 1,1,2-Trichloroethane (0.191 or 1.143 mL) was added. Sufficient IPM was added to prepare a 1 mg/mL solution.

10% dose solution in IPM (Groups A4 and A9)

An aliquot of the 50% dose solution (0.32 mL or 1.5 mL) was diluted to 2.9 or 7.5 mL using IPM.

1% dose solution in IPM (Group A10)

An aliquot of [^{14}C]-1,1,2-Trichloroethane (0.01 mL) was diluted to 1 mL using IPM.

50% dose solution in 1,2-Dichloroethane (Groups A5 and A11)

An aliquot of [^{14}C]-1,1,2-Trichloroethane (0.25 mL) was dispensed into a vial and non-radiolabeled 1,1,2-Trichloroethane (0.8 mL) was added. 1,2-Dichloroethane (1.05 mL) was added.

10% dose solution in 1,2-Dichloroethane (Groups A6 and A12)

An aliquot of the 50% dose solution (1 mL) was diluted to 5 mL using 1,2-Dichloroethane.

1% dose solution in 1,2-Dichloroethane (Group A13)

An aliquot of [^{14}C]-1,1,2-Trichloroethane (2.43 μL) was dispensed into a vial and non-radiolabeled 1,1,2-Trichloroethane (7.62 μL) was diluted to 1 mL using 1,2-Dichloroethane.

The above solutions were magnetically stirred if considered necessary.

3.4.3 Homogeneity Assessment

The activity and homogeneity of each dose preparation was assessed by taking aliquots (6.4 – 10 μL) from the top, middle and bottom of the formulation whilst being constantly stirred. The aliquots were either diluted to a volume (10 mL) with ethanol and duplicate aliquots (0.25 mL) taken for radioassay or analysed directly. All dose solutions were considered to be homogenous.

3.4.4 Diffusion Cell Design and Set-up

The flow-through diffusion cell is constructed from PCTFE ([Figure 9.1](#)). The skin membranes were maintained at approximately 32 °C using a water-heated manifold. The humidity was generally in the range 30 – 70 %. The humidity fell below 30% on three occasions however the humidity remained above 25%.

The flow-rate of approximately 1.5 mL/h allowed approximately six receptor chamber content changes per hour. The receptor fluid used was 6% polyoxyethylene 20 oleyl ether in water.

Skin samples were placed onto the receptor chamber of the flow-through diffusion cell. The donor chamber was then fixed in place providing an exposure area of 0.64 cm² of skin and the assembled diffusion cell inserted in-line in the flow-through set-up.

3.4.5 Cell Assignment

The assignment of cells was as follows:

Phase	Group	Dose level	Cell numbers	Donor numbers
Reference (finite)	A1	1 mg/mL Testosterone	81 – 84 (1 – 4, 1R – 4R) ^a	H4, H8, H14, H15
Infinite	A2	Neat	5 – 8 9R, 10R (9-10) ^b	H1, H2, H5, H6
	A3	50% in IPM	11R – 16R (11 – 16) ^b	H3 – H7
	A4	10% in IPM	17R – 22R + 77 – 80 (17 – 22) ^b	H3 – H7, H10 – H13
	A5	50% in 1,2-Dichloroethane	65 – 70	H5, H8 – H11
	A6	10% in 1,2-Dichloroethane	71 – 76	H5, H8, H10 – H12
Finite	A7	Neat	23 – 28	H3 – H7
	A8	50% in IPM	29R – 34R (29 – 34) ^c	H6, H8 – H10, H12
	A9	10% in IPM	35 – 40	H4, H6, H8, H9, H11
	A10	1% in IPM	41R – 46R (41 – 46) ^c	H6, H8 – H11
	A11	50% in 1,2-Dichloroethane	47 – 52	H6, H8, H10 – H12
	A12	10% in 1,2-Dichloroethane	53 – 58	H6, H8, H10 – H12
	A13	1% in 1,2-Dichloroethane	59 – 64	H6, H8, H10 – H12

^a Original cell numbers. Cells 1 – 4 were not used as the skin was semi-occluded with charcoal filters in error and cells 1R – 4R were not used as the QC's taken from the dose solution during dose application were variable, indicating that the test item was not fully in solution. Data from these cells was not considered valid.

^b Original cell numbers. Experiment repeated as TEWL measurements were not recorded in error at the terminal timepoint therefore it could not be confirmed if membrane integrity was maintained. Data from these cells was not considered valid.

^c Original cell numbers. Experiment repeated as the pump was not restarted in error following completion of the cell wash at 8 hours which resulted in no receptor fluid collections between 8 and 24 hours.

3.5 Infinite Dose Experiments

3.5.1 Application

Prior to dose application, samples of receptor fluid were collected and analysed for background radioactivity. All cells used showed acceptably low radioactivity levels in the receptor fluid prior to application of the test formulations.

Each dose formulation was applied to the skin membrane with a calibrated pipette at the rate of approximately 100 µL 1,1,2-Trichloroethane /cm² exposed skin area (or 64 µL per 0.64 cm² application area). The applications rates corresponded to 64 µL dose for the neat 1,1,2-Trichloroethane, 128 µL dose solution for the 50 % solution and 640 µL of dose solution for the

10% solution. Following application, the cells were occluded using a chemically resistant (PTFE) cap.

To accurately determine the quantity of 1,1,2-Trichloroethane administered to each cell, quality control (QC) aliquots (6.4 - 640 μ L) of each dose formulation were taken throughout the dosing procedure. These were diluted to volume (10 mL) with solvent and duplicate aliquots (0.05 - 0.25 mL) were taken radioassay.

3.5.2 Assessment of the Skin Absorption and Distribution of Radioactivity

The receptor fluid passing through the receptor chamber was collected into vials held in a fraction collector. Samples were then collected and retained for analysis at 10 minutes, 30 minutes, 1 hour, 2 hours and then every two hours for the duration of the experiment (24 hours).

After 24 hours exposure, any residual 1,1,2-Trichloroethane remaining on the skin was removed and membrane integrity was reassessed. No further samples were collected for analysis.

3.6 Reference Item Experiment (Group A1)

This experiment was conducted using the methods described for the finite dose groups in [Section 3.7](#), however, the cells were not semi occluded with charcoal filters.

3.7 Finite dose experiments (Groups A7 – A13)

3.7.1 Administration

Prior to dosing samples of receptor fluid were taken and analysed for background radioactivity. All cells used showed acceptably low radioactivity levels in the receptor fluid prior to application of the test formulations.

Each dose formulation was applied to the skin membrane with a calibrated pipette at the rate of approximately 10 μ L/cm² exposed skin area (6.4 μ L dose). Following application, the cell was semi occluded by placing a charcoal filter in the neck of the donor chamber as shown in [Figure 9.1](#). For groups A8, A9 and A10, it was not recorded that the cells were semi-occluded immediately following dose application, however, no losses of radioactivity were observed indicating that this procedure had been performed.

To accurately determine the quantity of 1,1,2-Trichloroethane (or testosterone) administered to each cell, quality control (QC) aliquots (6.4 μ L) of each dose formulation were taken throughout the dosing procedure. These were either diluted to volume (10 mL) with ethanol and duplicate aliquots (0.25 - 1 mL) were taken radioassay or were analysed directly.

Portions (10 mL) of solvent were added to the pipette tips used to apply the dose to the skin membranes and aliquot the QC samples. Samples were sonicated for 10 - 30 minutes and duplicate aliquots (0.25 - 1 mL) were taken for radioassay.

3.7.2 Assessment of the Skin Absorption and Distribution of Radioactivity

The receptor fluid passing through the receptor chamber was collected into vials held in a fraction collector. Samples were then collected for one-hour pre-dose and at 10 minutes, 30 minutes, 1 hour, 2 hours and then two-hourly intervals for the duration of the experiment (24 hours).

At 8 hours, the flow of receptor fluid was halted. The charcoal filter was removed and collected into a vial containing ethanol. The surface of the membrane was rinsed three times (3 x 0.5 mL) with a mild solution of Dove™ soap in water (ca. 1%). The skin surface was swabbed twice with cotton buds soaked with the liquid soap solution. Finally, the membrane was rinsed with a small volume (0.5 mL) of water and a further cotton bud used to swab the surface of the membrane until dry. The flow of receptor fluid was then restarted to continue the collection of samples from 8 - *ca* 24 hours. The rinses and swabs were retained as a single sample for analysis.

At termination (*ca* 24 hours), the charcoal filter was collected into the same pot as the 8-hour filter. Following TEWL measurement, the skin was washed again using the above procedure.

The skin membranes were then tape-stripped using D-squame adhesive tape. Each tape was pressed onto the skin surface. For groups 8 and 10, a pressure device (D-Squame Pressure Device) used to achieve a standardized pressure of 225 g/m². Removal of the stratum corneum was indicated by the shiny appearance of the skin. A total of up to five tape strips were collected for each sample and were retained individually for analysis. The remaining exposed skin was retained for analysis.

The receptor fluid remaining in the cell and outlet tubing at the end of the experiment was retained and analysed for mass balance purposes. The diffusion cell components were also retained, washed and the washings analysed for mass balance purposes.

All samples that were not analysed immediately after collection were stored at -20°C ± 10°C.

3.8 Analytical Methods

Sample weights and disintegration rates were generally automatically captured from balances and liquid scintillation counters via interfaces into a Laboratory Information Management System which calculated proportions of administered radioactivity present in samples.

3.8.1 Processing of Samples for Radioactivity Measurement

The receptor fluid was retained and radioassayed directly. At the end of the exposure period, the receptor chamber and outlet tubing contents were retained and radioassayed.

The carbon filter traps were extracted up to four times using ethanol by sonication for 10 minutes. The extract was then weighed and duplicate weighed aliquots (0.25 - 2 mL) taken for radioassay.

The swabs and aqueous rinses of the application site taken at 8 and 24 hours were mixed with ethanol and shaken for 10 minutes followed by sonication for 10 minutes. The ethanol:water extract was then weighed and duplicate weighed aliquots (0.1 - 2 mL) taken for radioassay. The extraction procedure was then repeated with ethanol:water 1:1 v/v and the extract analysed as before. If considered necessary to improve the recovery of radioactivity, the remaining swabs were further analysed by combustion and radioassay.

The tape strips were solubilised in 2M methanolic sodium hydroxide solution in an incubator at *ca* 55°C. The solubilised tape strips were weighed and duplicate weighed aliquots (0.25 - 1 g) taken for radioassay.

The tape stripped skin membranes were solubilised in a mixture of purified water, methanol, and Triton X-405 (6 : 3 : 1 v/v/v) containing sodium hydroxide (80 g/L) ('SHT') in an incubator at 55°C. The solubilised tape stripped skin was weighed and duplicate weighed aliquots (0.25 - 1 g) taken for radioassay.

The diffusion cell components were extracted with ethanol:water 1:1 v/v by sonication for 10 minutes. The cell components were removed, the extract weighed and duplicate weighed aliquots (1 - 5 mL) taken for radioassay.

3.8.2 Measurement of Radioactivity

Radioactivity was measured by liquid scintillation counting (LSC) using either LKB-Wallace model 1219 Rackbeta Spectral or Wallac models 1409 or 1410 liquid scintillation counters with automatic quench correction. Radioactivity in gross amounts of less than twice background was considered to be below the limit of accurate detection. Example calculations are shown in [Appendix 11.8](#).

Aliquots of liquid samples were mixed with Ultima Gold scintillator for measurement of radioactivity.

Solid samples were combusted in oxygen using a Packard 307 sample oxidiser. The efficiency of the oxidiser was determined using aliquots of Spec-Chec-¹⁴C check source for sample oxidisers (PerkinElmer) and was greater than 95%. Measurements of radioactivity were corrected for oxidiser efficiency.

3.8.3 High Performance Liquid Chromatography

Radiochemical purity checks of [¹⁴C]-1,1,2-Trichloroethane prepared as a solution in ethanol were performed using HPLC with radiodetection as follows:

Column	: Luna Omega PS C18 (250 x 4.6 mm internal diameter)
Mobile phase	: 0.1% Formic acid in water Acetonitrile

Gradient	:	Time (minutes)	%A	%B
		0	70	30
		5	70	30
		25	0	100
		29	0	100
		30	70	30
		35	70	30

Flow rate : 0.7 mL/minute

Column temperature : 30°C

Radioactivity detector : β-RAM, 500 μL liquid cell, 1 : 3 split with Pro-flow G+

Software : Laura, version 4.1 (LabLogic Systems Ltd)

UV wavelength : 210 nm

Radiochemical purity checks of [¹⁴C]-testosterone were performed using HPLC with radiodetection as follows:

Column : μBondapak C18
(300 x 3.9 mm internal diameter)

Mobile phase : Methanol: Water: Acetonitrile 39: 60: 1 v/v/v
Methanol: Water: Acetonitrile 80: 18: 2 v/v/v

Gradient	:	Time (minutes)	%A	%B
		0	90	10
		25	0	100
		30	0	100
		35	90	10

Flow rate : 1.5 mL/minute

Column temperature : 30°C

Radioactivity detector : β-RAM, 500 μL liquid cell, 1 : 3 split with Pro-flow G+

Software : Laura, version 4.1 (LabLogic Systems Ltd)

UV wavelength : 254 nm

3.9 Calculations

Definitions

Surface area of skin (0.64 cm ²)	= A [cm ²]
Volume of sample	= V [mL]
Radioactivity (sample dpm – background dpm) in aliquot of receptor fluid or sample analysed	= R [dpm]
Concentration of radioactivity in receptor fluid or sample	$C = R/V$ [dpm/mL]
Specific radioactivity of test substance	= S [kBq/mg]
Weight of test substance applied to each preparation	= D [mg/cm ²]
Radioactive dose administered to each preparation	= S x D x A [kBq]
Time period over which the rate of penetration is calculated	= Δt [hours]
Rate of penetration, taken from the initial linear portion of the graph [μg equivalents absorbed/cm ² /time]	= ΔP

Calculation of Rates of Penetration

Concentration of test substance in receptor fluid, [μg equivalents/aliquot of receptor fluid]	$\frac{R}{60 \times S} = K$
Cumulative amount of test substance penetrated at time t [μg equivalents]	$\sum_t K \times \frac{1}{A} = P_t$
Rate of penetration of test substance [μg equivalents/cm ² /hour]	$J = \frac{\Delta P}{\Delta t}$

Calculation of Percentage Recovery of Radioactivity

Per cent of radioactive dose/sample	$= \frac{K}{10 \times D}$
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Calculation of Permeability Coefficients

Permeability coefficient [cm/hour]	$K_p = \frac{J}{\Delta C}$
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where ΔC = test substance concentration difference across skin, assumed to be the formulation concentration on the surface of the skin in true sink conditions. The K_p was calculated over the steady state (linear) portion of the absorption profile.

3.10 Major Computerized Systems

Samuel	Test item records
Debra	Metabolism LIMS system
Laura	HPLC data capture and evaluation
REES	Temperature monitoring system for test item and sample storage
Veeva Unified Quality Vault	Quality management and document control

Computer system version numbers are maintained by Labcorp Early Development Laboratories Ltd.

4. DEVIATIONS FROM STUDY PLAN

The following deviations from the study plan occurred:

TEWL measurements were generally conducted on the day prior to dose application. As further TEWL measurements were taken at the end of the experiment to confirm if membrane integrity was maintained throughout the experiment, this was considered acceptable.

The TEWL measurements were not conducted at termination for the initial group A3 and A4 experiments in error. As the integrity of the skin could not be confirmed, data from these cells was not considered valid and these groups were repeated.

The initial replicates prepared for group A1 were semi-occluded with charcoal filters in error. The experiment was therefore repeated.

Four additional replicates were conducted for Group A4 as the terminal TEWL measurements from the initial six replicates were considered anomalous. The absorption profiles obtained from the additional four replicates were considered comparable with the original replicates indicating that it was likely that membrane integrity was maintained during the original experiment. Therefore all ten replicates for this group were used to evaluate absorption.

With the exception of groups A8 and A10, it cannot be confirmed from the study data that the D-squame pressure device was used as this was not recorded in error. The raw data confirms that the stratum corneum was removed therefore this was considered to have no impact on the outcomes of the study.

Following completion of the skin rinses at 8 hours, the pump was not restarted for the initial group A8 and A10 experiment in error. As no receptor fluid was collected following swabbing, the experiment was repeated.

The pump stopped flowing for a period of approximately 5 – 10 minutes during the 20 – 22-hour collection period due to a power outage for the repeat group A8 and A10 experiments. No receptor fluid was lost during this period.

A batch of non-radiolabeled 1,1,2-Trichloroethane which was not included in the Study Plan was used for HPLC co-chromatography.

Cell 38 failed to meet the membrane integrity criteria; however, this cell was dosed in error. Data from this cell was excluded from the mean calculation.

An 18-hour receptor fluid sample was collected; however, this was not stated in the study plan in error. A study plan amendment was issued on 17 January 2023 to correct this error.

The charcoal trap was removed at 8 hours and replaced with a new charcoal trap for all groups, however, this was not stated in the study plan in error. A study plan amendment was issued on 17 January 2023 to correct this error.

The above deviations are considered to have no impact on the outcomes or integrity of the study.

5. ARCHIVING

Records and documentation relating to this study (including electronic records) will be maintained in the archives of Labcorp– Huntingdon, UK for a period of one year from the issue of the final report. This will include raw data and further material that supports the reconstruction of the study. Testing Facility-generated electronic raw data will be stored on the computer system on which the data application resides or archived off-line.

Due to the inherently unstable nature of radiolabeled compounds, a subsample of the test item will not be archived. Metabolism studies are short term studies and as such there is no requirement to archive a sample of the non-radiolabeled test item.

At termination of the aforementioned period, the Sponsor will be contacted in order to determine the final disposition of these records. After the specified period, the Sponsor is responsible for all costs associated with the retention, retrieval, onward transfer or destruction/disposal of these materials. If the Sponsor is unresponsive the records will be destroyed in accordance with the Labcorp– Huntingdon, UK Standard Operating Procedure.

In case records are transferred, the Sponsor should ensure that the materials and records in support of regulatory studies are retained and maintained under conditions that guarantee their integrity and continued access according to archiving requirements of the principles of GLP. The Sponsor should also ensure that such materials and records are retained for as long as required by relevant authorities.

Labcorp will retain the study plan, final report and any amendments indefinitely.

6. RESULTS

6.1 Solubility of [^{14}C]-1,1,2-Trichloroethane in Receptor Fluid

The solubility of [^{14}C]-1,1,2-Trichloroethane at a mean concentration of 24.9 mg/mL (target concentration of 25.0 mg/mL) in the selected receptor fluid, 6% polyoxyethylene 20 oleyl ether in water, was demonstrated after incubation for approximately 24 hours at ca 32°C ([Appendix 11.2](#)). The solubility of the 1,1,2-Trichloroethane in the receptor fluid was therefore demonstrated to be adequate and not to be rate limiting to the absorption process.

6.2 Limit of Detection

Radioactivity in gross amounts of less than twice the background level was considered to be below the limit of accurate determination. Example calculations are shown in [Appendix 11.8](#).

6.3 Achieved Doses of 1,1,2-Trichloroethane

The achieved dose levels of 1,1,2-Trichloroethane applied to the skin were determined by analysis of QC samples taken during the dosing of the cells. The amount of dose applied to each skin sample is shown [Appendix 11.3](#).

The mean achieved doses levels are shown below:

Phase	Group	Dose level	Applied dose	
			mg per cell	mg/cm ²
Reference (finite)	A1	Testosterone 1 mg/mL	0.007	0.011
Infinite	A2	Neat 1,1,2-Trichloroethane	88.9	139
	A3	50% in IPM	88.7	139
	A4	10% in IPM	78.7	123
	A5	50% in 1,2-Dichloroethane	75.2	117
	A6	10% in 1,2-Dichloroethane	76.9	120
Finite	A7	Neat 1,1,2-Trichloroethane	8.96	14.0
	A8	50% in IPM	4.11	6.42
	A9	10% in IPM	0.83	1.29
	A10	1% in IPM	0.086	0.13
	A11	50% in 1,2-Dichloroethane	4.57	7.13
	A12	10% in 1,2-Dichloroethane	0.901	1.41
	A13	1% in 1,2-Dichloroethane	0.091	0.142

The achieved dose levels were considered acceptable to fulfil the objectives of the study.

6.4 Reference Test

Data is shown in [Appendix 11.5](#). Following a single application of [^{14}C]-testosterone at a nominal dose level of 1 mg/mL to dermatomed human skin, the majority of radioactivity was associated with the dislodgeable dose (79.37%). The receptor fluid contained a mean of 9.98%, with a further 1.97% detected in the exposed skin. The mean maximum absorption rate was 0.337 $\mu\text{g}/\text{cm}^2/\text{hr}$.

The absorption profiles and distribution of radioactivity obtained from this experiment showed the expected trends and the data was comparable with results obtained in the multi-centre comparison study conducted by [Van de Sandt *et al*, 2004](#).

6.5 Infinite Dose Experiments

6.5.1 Neat 1,1,2- Trichloroethane (Group A2)

Data are reported for five cells (dermatomed skin from three donors: H1, H2 and H6).

The mean permeability coefficient (Kp) and maximum absorption rates are shown in [Table 10.1](#). Individual cell data are shown [Appendix 11.6](#).

The maximum absorption rate was 527 $\mu\text{g}/\text{cm}^2/\text{hr}$ which generally occurred between 2 – 6 hours. The data indicated that true sink conditions may not have been maintained for all replicates over the entire 24-hour duration of the experiment, however, it was considered that there were sufficient timepoints to calculate a Kp value over the linear portion of the absorption profile. Kp calculated following application of the neat test item was $3.33 \times 10^{-3} \text{ cm/hr}$.

6.5.2 50% 1,1,2- Trichloroethane in IPM (Group A3)

Data are reported for six cells (dermatomed skin from five donors: H3 – H7).

Mean permeability coefficients (Kp) and maximum absorption rates are shown in [Table 10.1](#). Individual cell data are shown [Appendix 11.6](#).

The maximum absorption rate was 652 $\mu\text{g}/\text{cm}^2/\text{hr}$. The data indicated that true sink conditions were maintained throughout the duration of the experiment. Kp calculated following application of a 50% solution in IPM was $3.37 \times 10^{-3} \text{ cm/hr}$.

6.5.3 10% 1,1,2- Trichloroethane in IPM (Group A4)

Data are reported for ten cells (dermatomed skin from nine donors: H3 – H7, H10 – H13). The TEWL measurements taken at termination for the initial six cells were considered to be anomalously low when compared to the measurements taken from other groups indicating a possible malfunction with the equipment. Therefore, a further four cells were tested. Data from all ten cells were comparable.

Mean permeability coefficients (Kp) and maximum absorption rates are shown in [Table 10.1](#). Individual cell data are shown [Appendix 11.6](#).

The maximum absorption rate was 113 $\mu\text{g}/\text{cm}^2/\text{hr}$. The data indicated that true sink conditions were maintained throughout the duration of the experiment. Kp calculated following application of a 10% solution in IPM was $0.76 \times 10^{-3} \text{ cm/hr}$.

6.5.4 50% 1,1,2- Trichloroethane in 1,2-Dichloroethane (Group A5)

Data are reported for six cells (dermatomed skin from five donors: H5, H8 – H11).

Mean permeability coefficients (Kp) and maximum absorption rates are shown in [Table 10.1](#). Individual cell data are shown [Appendix 11.6](#).

The maximum absorption rate was 562 $\mu\text{g}/\text{cm}^2/\text{hr}$. The data indicated that true sink conditions may not have been maintained for all replicates over the entire 24-hour duration of the experiment, however, it was considered that there were sufficient timepoints to calculate a Kp value over the linear portion of the absorption profile. Kp calculated following application of 50% solution in 1,2-dichloroethane was $4.49 \times 10^{-3} \text{ cm/hr}$.

6.5.5 10% 1,1,2- Trichloroethane in 1,2-Dichloroethane (Group A6)

Data are reported for six cells (dermatomed skin from five donors: H5, H8, H10 – H12).

Mean permeability coefficients (Kp) and maximum absorption rates are shown in [Table 10.1](#). Individual cell data are shown [Appendix 11.6](#).

The maximum absorption rate was 155 $\mu\text{g}/\text{cm}^2/\text{hr}$. The data indicated that true sink conditions were maintained throughout the duration of the experiment. Kp calculated following application of a 10% solution in 1,2-dichloroethane was $0.91 \times 10^{-3} \text{ cm/hr}$.

6.6 Finite Dose Experiments

6.6.1 Neat 1,1,2- Trichloroethane (Group A7)

Data are reported for four cells (dermatomed skin from four donors: H3 – H6).

Mean recoveries and distributions of radioactivity expressed as % applied dose and μg equivalents of 1,1,2-Trichloroethane are shown in [Table 10.2](#). Absorption profiles are graphically presented in [Figure 9.7](#) and [Figure 9.8](#), respectively. Individual cell data are shown [Appendix 11.7](#). The mean distribution of radioactivity is discussed below.

Following a single application of [^{14}C]-1,1,2-Trichloroethane to dermatomed human skin, the majority of radioactivity was detected in the charcoal trap extract (95.92%). The skin wash taken at 8 hours contained 0.31%, with a further 0.04% removed in the skin wash taken after 24 hours.

A total 0.39% was recovered in the receptor fluid over the 0 - 24hour period. The dose remaining on the exposed skin accounted for 0.06%.

Radioactivity remaining on the tape strips taken to remove the residual surface dose and stratum corneum contained were below the limit of detection. Radioactivity remaining on the donor and receptor chambers of the diffusion cell was also below the limit of detection.

Dermal delivery (sum of radioactivity in the receptor fluid, on the receptor chamber and in the exposed skin) accounted for 0.45%. Total unabsorbed dose (sum of radioactivity in the skin washes, on the donor chamber, in the surface tape strips and in the charcoal trap) accounted for 96.27% applied dose. The overall recovery of radioactivity was 96.72%.

6.6.2 50% 1,1,2- Trichloroethane in IPM (Group A8)

Data are reported for five cells (dermatomed skin from four donors: H8 – H10 and H12).

Mean recoveries and distributions of radioactivity expressed as % applied dose and µg equivalents of 1,1,2-Trichloroethane are shown in [Table 10.3](#). Absorption profiles are graphically presented in [Figure 9.9](#) and [Figure 9.10](#), respectively. Individual cell data are shown [Appendix 11.7](#). The mean distribution of radioactivity is discussed below.

Following a single application of [^{14}C]-1,1,2-Trichloroethane to dermatomed human skin, the majority of radioactivity was detected in the charcoal trap extract (99.23%). The skin wash taken at 8 hours contained 0.44%, with a further 0.11% removed in the skin wash taken after 24 hours.

A total of 0.17% was recovered in the receptor fluid over the 0 - 24 hour period. The dose remaining on the exposed skin accounted for 0.05%.

Radioactivity remaining on the tape strips taken to remove the residual surface dose and stratum corneum contained were below the limit of detection. Radioactivity remaining on the donor and receptor chambers of the diffusion cell accounted for 0.01%.

Dermal delivery accounted for 0.24%. Total unabsorbed dose accounted for 99.79%. The overall recovery of radioactivity was 100.03%.

6.6.3 10% 1,1,2- Trichloroethane in IPM (Group A9)

Data are reported for four cells (dermatomed skin from three donors: H4 and H8 – H9).

Mean recoveries and distributions of radioactivity expressed as % applied dose and µg equivalents of 1,1,2-Trichloroethane are shown in [Table 10.4](#). Absorption profiles are graphically presented in [Figure 9.11](#) and [Figure 9.12](#), respectively. Individual cell data are shown [Appendix 11.7](#). The mean distribution of radioactivity is discussed below.

Following a single application of [^{14}C]-1,1,2-Trichloroethane to dermatomed human skin, the majority of radioactivity was detected in the charcoal trap extract (94.75%). The skin wash taken at 8 hours contained 0.54%, with a further 0.02% removed in the skin wash taken after 24 hours.

A total of 0.02% was recovered in the receptor fluid over the 0 - 24hour period. The dose remaining on the exposed skin was below the limit of detection.

Radioactivity remaining on the tape strips taken to remove the residual surface dose and stratum corneum was below the limit of detection. Radioactivity remaining on the donor and receptor chambers of the diffusion cell was also below the limit of detection.

Dermal delivery accounted for 0.02%. The total unabsorbed dose accounted for 95.31%. The overall recovery of radioactivity was 95.33%.

6.6.4 1% 1,1,2- Trichloroethane in IPM (Group A10)

Data are reported for four cells (dermatomed skin from three donors: H8 – H10).

Mean recoveries and distributions of radioactivity expressed as % applied dose and µg equivalents of 1,1,2-Trichloroethane are shown in [Table 10.5](#). Absorption profiles are not shown as radioactivity was below the limit of quantification in the receptor fluid. Individual cell data are shown [Appendix 11.7](#). The mean distribution of radioactivity is discussed below.

Following a single application of [^{14}C]-1,1,2-Trichloroethane to dermatomed human skin, the majority of radioactivity was detected in the charcoal trap extract (104.72%). The skin wash taken at 8 hours contained 0.70%, with a further 0.01% removed in the skin wash taken after 24 hours.

Radioactivity remaining in the receptor fluid and on the tape strips taken to remove the residual surface dose and stratum corneum, exposed skin and diffusion cell components was below the limit of quantification.

The total unabsorbed dose accounted for 105.43%. The overall recovery of radioactivity was 105.43%.

6.6.5 50% 1,1,2- Trichloroethane in 1,2-Dichloroethane (Group A11)

Data are reported for six cells (dermatomed skin from five donors: H6, H8, H10 – H12).

Mean recoveries and distributions of radioactivity expressed as % applied dose and µg equivalents of 1,1,2-Trichloroethane are shown in [Table 10.6](#). Absorption profiles are graphically presented in [Figure 9.13](#) and [Figure 9.14](#), respectively. Individual cell data are shown [Appendix 11.7](#). The mean distribution of radioactivity is discussed below.

Following a single application of [^{14}C]-1,1,2-Trichloroethane to dermatomed human skin, the majority of radioactivity was detected in the charcoal trap extract (92.26%). The skin wash taken at 8 hours contained 0.69%, with a further 0.01% removed in the skin wash taken after 24 hours.

A total of 0.59% was recovered in the receptor fluid over the 0 - 24 hour period.

Radioactivity remaining on the tape strips taken to remove the residual surface dose and stratum corneum, exposed skin and diffusion cell components was below the limit of quantification.

Dermal delivery accounted for 0.59%. Total unabsorbed dose accounted for 92.96%. The overall recovery of radioactivity was 93.54%.

6.6.6 10% 1,1,2- Trichloroethane in 1,2-Dichloroethane (Group A12)

Data are reported for five cells (dermatomed skin from five donors: H6, H8, H10 – H12).

Mean recoveries and distributions of radioactivity expressed as % applied dose and µg equivalents of 1,1,2-Trichloroethane are shown in [Table 10.7](#). Absorption profiles are graphically presented in [Figure 9.15](#) and [Figure 9.16](#), respectively. Individual cell data are shown [Appendix 11.7](#). The mean distribution of radioactivity is discussed below.

Following a single application of [^{14}C]-1,1,2-Trichloroethane to dermatomed human skin, the majority of radioactivity was detected in the charcoal trap extract (94.32%). The skin wash taken at 8 hours contained 0.97%. The skin wash at 24 hours was below the limit of detection.

A total of 0.40% was recovered in the receptor fluid over the 0 - 24 hour period.

Radioactivity remaining on the tape strips taken to remove the residual surface dose and stratum corneum, exposed skin and diffusion cell components was below the limit of quantification.

Dermal delivery accounted for 0.40%. Total unabsorbed dose accounted for 95.29%. The overall recovery of radioactivity was 95.70%.

6.6.7 1% 1,1,2- Trichloroethane in 1,2-Dichloroethane (Group A13)

Data are reported for six cells (dermatomed skin from five donors: H6, H8, H10 – H12).

Mean recoveries and distributions of radioactivity expressed as % applied dose and µg equivalents of 1,1,2-Trichloroethane are shown in [Table 10.8](#). Absorption profiles are graphically presented in [Figure 9.17](#) and [Figure 9.18](#), respectively. Individual cell data are shown [Appendix 11.7](#). The mean distribution of radioactivity is discussed below.

Following a single application of [^{14}C]-1,1,2-Trichloroethane to dermatomed human skin, the majority of radioactivity was detected in the charcoal trap extract (91.66%). The skin wash taken at 8 hours contained 0.24%. The skin wash at 24 hours was below the limit of detection.

A total of 0.34% was recovered in the receptor fluid over the 0 - 24 hour period.

Radioactivity remaining on the tape strips taken to remove the residual surface dose and stratum corneum, exposed skin and diffusion cell components was below the limit of quantification.

Dermal delivery accounted for 0.34%. Total unabsorbed dose accounted for 91.91%. The overall recovery of radioactivity was 92.25%.

7. CONCLUSION

Following infinite application of 1,1,2-Trichloroethane, as the neat test item and 50%, and 10% solutions in IPM and 1,2-Dichloroethane, there were no significant observed solvent dependent differences in absorption. There was also no significant difference in maximum absorption rate or permeability coefficients when applied as the neat material or 50% solution, however, there was a proportionate decrease in absorption between the 50% and 10% solutions for both solvents.

The data indicates that true sink conditions were not maintained for some replicates following application of the neat material and the 50% solution in 1,2-Dichloroethane due to the volatility of both the test item and the diluent. It is considered that sufficient data was obtained to calculate the permeability coefficient over the steady state portion of the absorption profile.

Following finite application of 1,1,2-Trichloroethane as the neat test item and 50%, 10% and 1% solutions in IPM and 1,2-Dichloroethane, under semi-occlusive conditions, dermal delivery was very low accounting for 0.02 – 0.59% of applied dose, where detectable. At each concentration, the majority of radioactivity was detected in the volatile traps indicating that under finite conditions, 1,1,2-Trichloroethane was most likely to quickly volatilize from the skin surface.

8. REFERENCES

van de Sandt JJM, van Burgsteden JA, Cage S, Carmichael PL, Dick I, Kenyon S, Korinth G, Larese F, Limasset JC, Maas WJM, Montomoli L, Nielson JB, Payan J-P, Robinson E, Sartorelli P, Schaller KH, Wilkinson SC and Williams FM (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.

Guth K, Schafer-Korting M, Fabian E, Landsiedel R, and Van Ravenzwaay B (2015). Suitability of skin integrity tests for dermal absorption studies *in vitro*. *Toxicology In vitro*, **29**, 113-123.

9. FIGURES

Figure 9.1: Diagrammatic Representation of the Flow Through Diffusion Cell

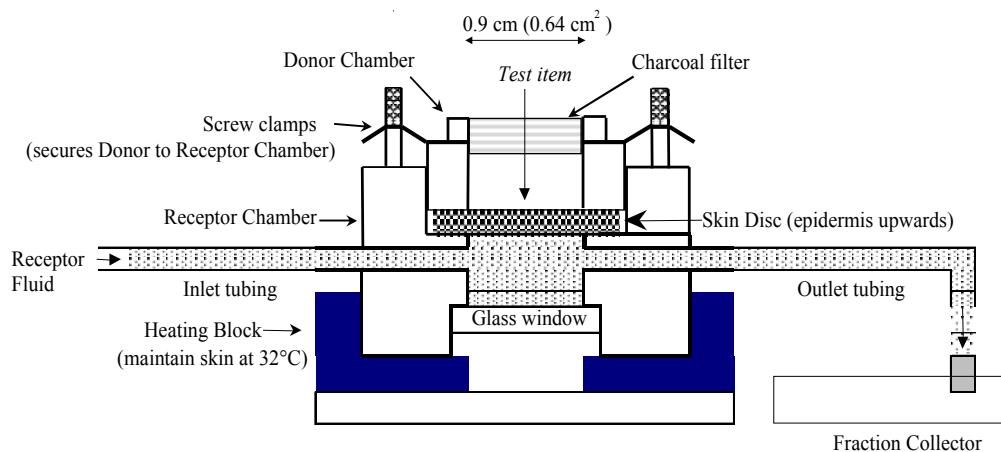
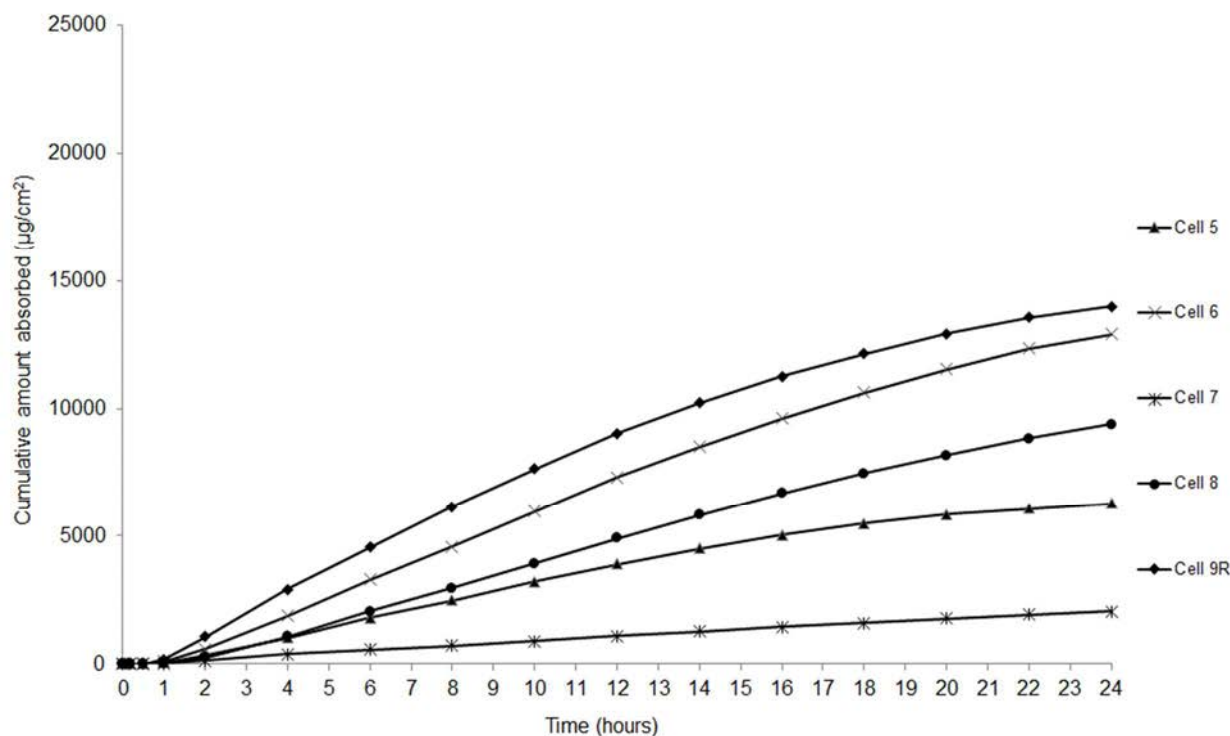


Figure 9.2: Amount of Radiolabeled Material Absorbed Following a Single Infinite Application of Neat [^{14}C]-1,1,2-Trichloroethane to Human Skin (Group A2)

Results are expressed as cumulative μg equivalents 1,1,2-Trichloroethane/ cm^2



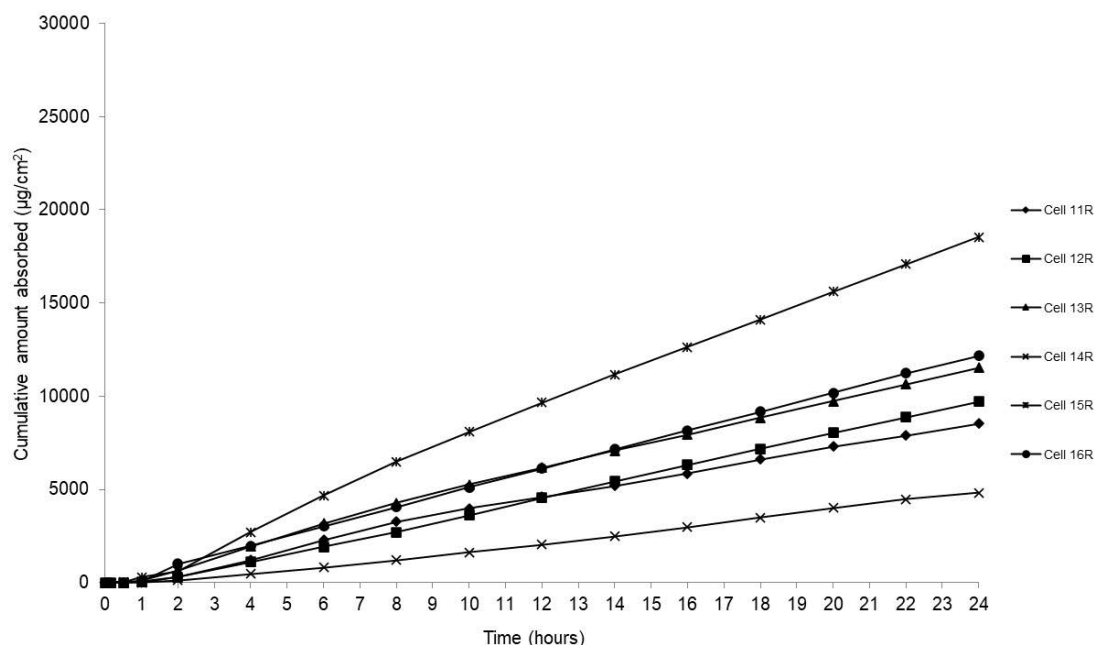
Cumulative amount absorbed (μg equivalents 1,1,2-Trichloroethane/ cm^2)

Time (hours)	Cell 5	Cell 6	Cell 7	Cell 8	Cell 9R
0	nd	nd	nd	nd	nd
0.16	nd	nd	nd	nd	nd
0.5	4.29	4.67	1.26	0.580	21.0
1	61.2	100	22.8	21.9	189
2	329	588	123	256	1100
4	1020	1880	385	1060	2970
6	1790	3290	563	2060	4670
8	2480	4590	709	2970	6140
10	3210	5980	893	3930	7810
12	3910	7310	1090	4900	9230
14	4520	8500	1270	5830	10500
16	5030	9610	1440	6670	11500
18	5490	10600	1600	7460	12400
20	5830	11500	1770	8180	13200
22	6080	12300	1920	8840	13900
24	6280	12900	2050	9370	14000

nd Not detected

Figure 9.3: Amount of Radiolabeled Material Absorbed Following a Single Infinite Application of 50% [^{14}C]-1,1,2-Trichloroethane in IPM to Human Skin (Group A3)

Results are expressed as cumulative μg equivalents 1,1,2-Trichloroethane/ cm^2



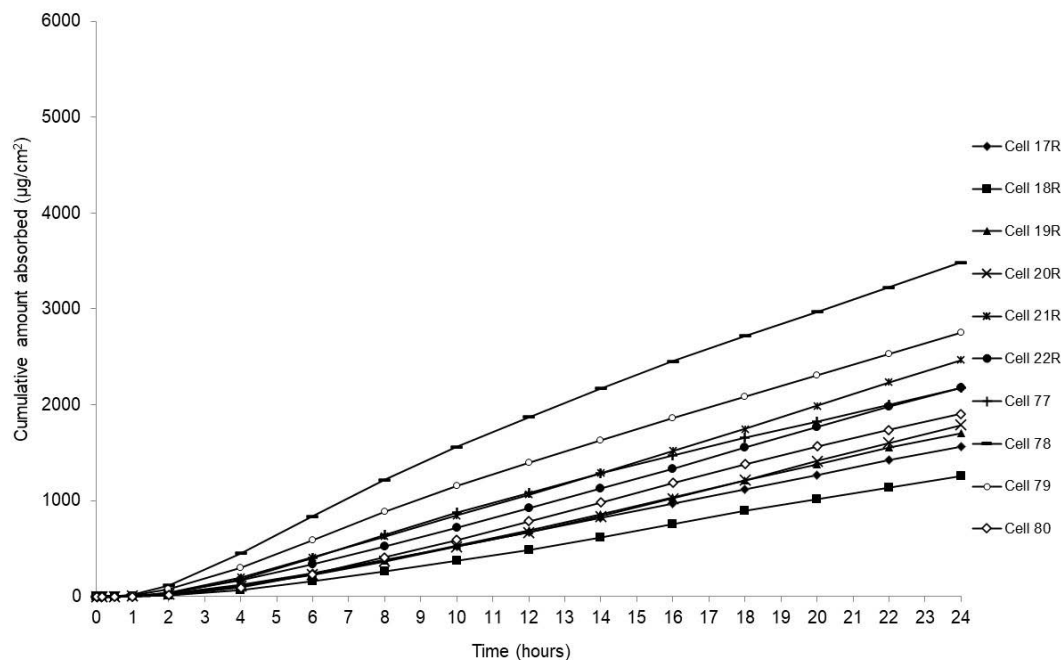
Cumulative amount absorbed (μg equivalents 1,1,2-Trichloroethane/ cm^2)

Time (hours)	Cell 11R	Cell 12R	Cell 13R	Cell 14R	Cell 15R	Cell 16R
0	nd	nd	nd	nd	nd	nd
0.16	0.255	0.237	0.237	0.404	1.20	0.272
0.5	2.34	1.69	7.43	1.93	27.4	3.92
1	53.6	37.1	116	19.4	310	59.1
2	329	305	679	127	626	1020
4	1210	1110	1960	469	2720	1980
6	2290	1930	3190	829	4680	3040
8	3270	2710	4290	1200	6500	4070
10	3990	3620	5270	1620	8100	5110
12	4600	4550	6180	2050	9660	6130
14	5200	5450	7080	2500	11200	7150
16	5850	6320	7950	2990	12600	8160
18	6630	7190	8850	3500	14100	9170
20	7300	8040	9740	4010	15600	10200
22	7910	8880	10600	4480	17100	11200
24	8530	9720	11500	4840	18500	12200

nd Not detected

Figure 9.4: Amount of Radiolabeled Material Absorbed Following a Single Infinite Application of 10% [^{14}C]-1,1,2-Trichloroethane in IPM to Human Skin (Group A4)

Results are expressed as cumulative μg equivalents 1,1,2-Trichloroethane/ cm^2



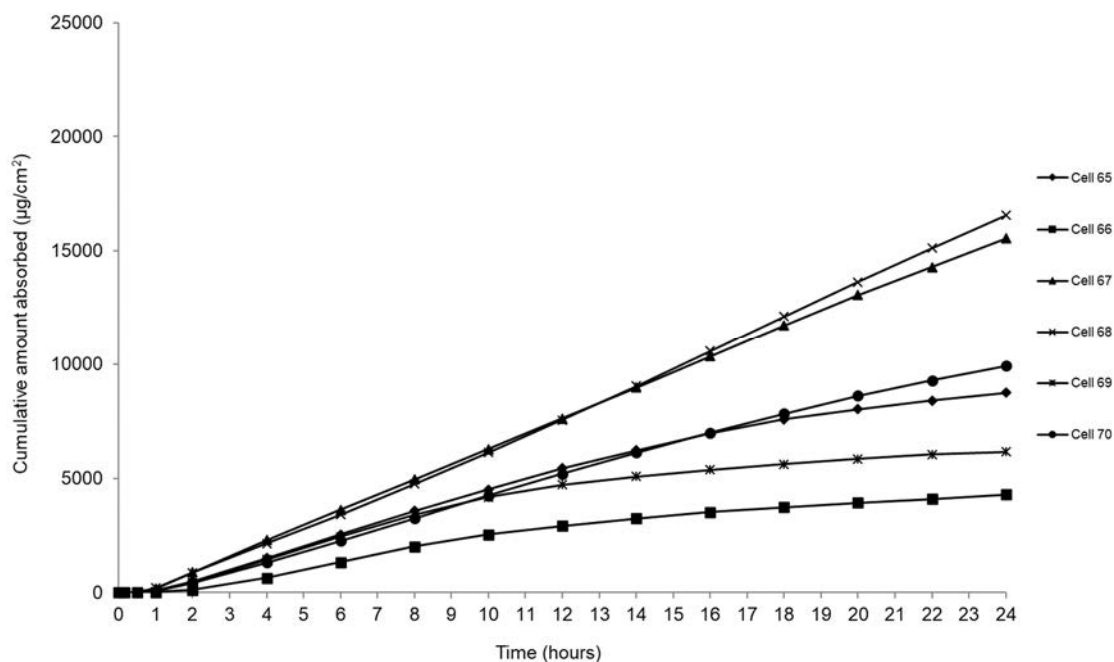
Cumulative amount absorbed (μg equivalents 1,1,2-Trichloroethane/ cm^2)

Time (hours)	Cell 17R	Cell 18R	Cell 19R	Cell 20R	Cell 21R	Cell 22R	Cell 77	Cell 78	Cell 79	Cell 80
0	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
0.16	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
0.5	nd	nd	nd	0.299	nd	0.334	0.541	1.66	1.25	nd
1	2.31	1.42	1.22	2.72	3.87	6.09	5.31	19.0	11.2	2.08
2	26.0	10.1	15.0	21.5	40.4	40.8	35.1	119	73.1	15.9
4	128	70.2	105	110	198	168	180	453	302	95
6	249	165	233	228	407	335	401	837	588	231
8	380	264	378	367	629	521	647	1220	884	408
10	519	371	528	522	845	717	873	1560	1150	591
12	668	488	690	668	1060	918	1080	1870	1400	784
14	819	615	857	837	1290	1130	1280	2170	1630	982
16	970	754	1030	1020	1520	1340	1480	2450	1860	1180
18	1120	895	1210	1220	1750	1550	1660	2720	2090	1380
20	1270	1020	1380	1410	1990	1770	1830	2970	2310	1570
22	1420	1130	1550	1610	2240	1980	2000	3220	2530	1740
24	1560	1250	1710	1790	2470	2180	2180	3490	2750	1910

nd Not detected

Figure 9.5: Amount of Radiolabeled Material Absorbed Following a Single Infinite Application of 50% [^{14}C]-1,1,2-Trichloroethane in 1,2 Dichloroethane to Human Skin (Group A5)

Results are expressed as cumulative μg equivalents 1,1,2-Trichloroethane/ cm^2



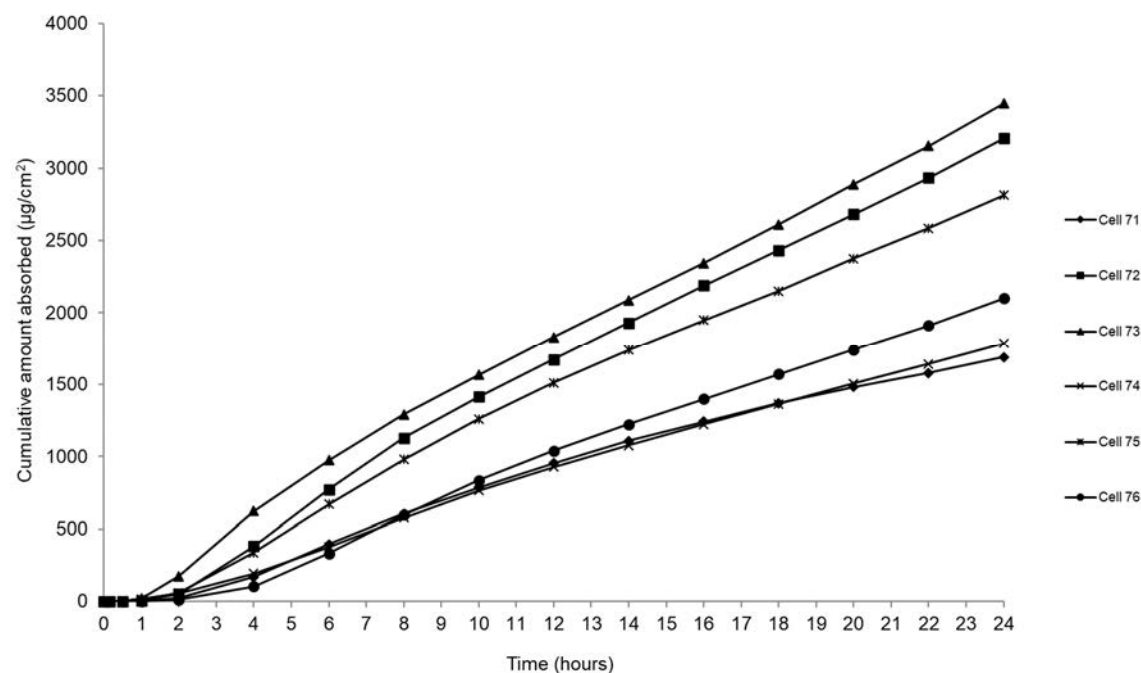
Cumulative amount absorbed (μg equivalents 1,1,2-Trichloroethane/ cm^2)

Time (hours)	Cell 65	Cell 66	Cell 67	Cell 68	Cell 69	Cell 70
0	nd	nd	nd	nd	nd	nd
0.16	nd	nd	nd	nd	nd	nd
0.5	3.27	0.922	8.52	19.3	1.41	2.30
1	75.8	11.8	157	189	43.1	44.2
2	484	120	891	862	435	421
4	1510	639	2290	2150	1440	1310
6	2540	1320	3610	3410	2440	2250
8	3570	2020	4950	4750	3410	3240
10	4500	2530	6280	6130	4180	4240
12	5430	2920	7610	7550	4710	5190
14	6230	3240	8970	9030	5070	6120
16	6970	3520	10400	10600	5370	6980
18	7580	3740	11700	12100	5620	7820
20	8020	3920	13100	13600	5850	8610
22	8400	4090	14300	15100	6040	9280
24	8750	4290	15500	16600	6160	9920

nd Not detected

Figure 9.6: Amount of Radiolabeled Material Absorbed Following a Single Infinite Application of 10% [^{14}C]-1,1,2-Trichloroethane in 1,2 Dichloroethane to Human Skin (Group A6)

Results are expressed as cumulative μg equivalents 1,1,2-Trichloroethane/ cm^2



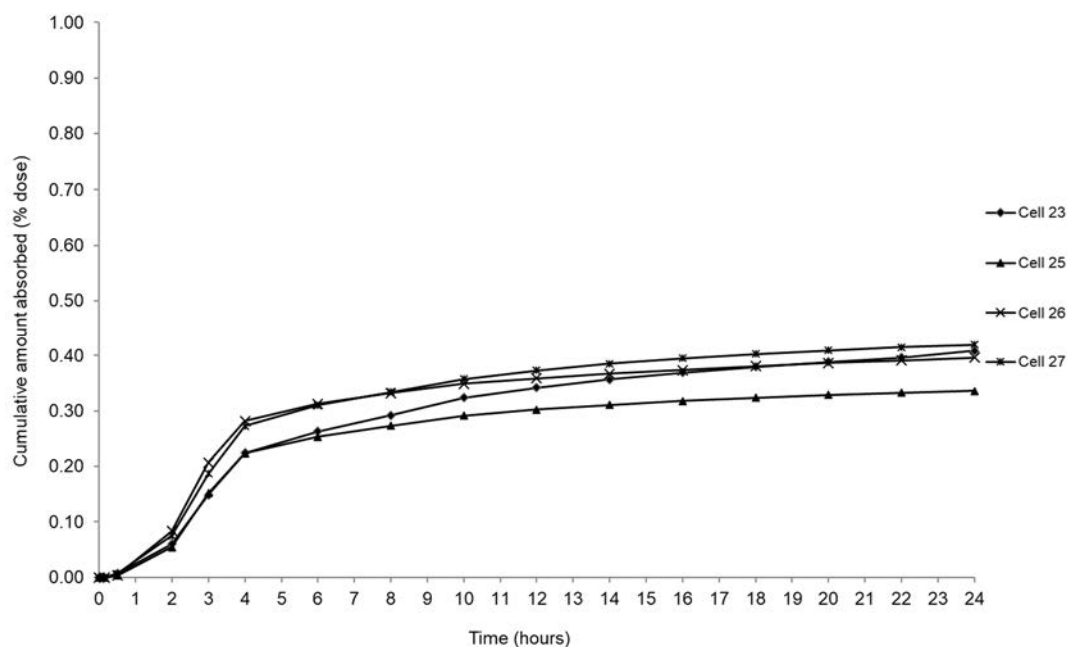
Cumulative amount absorbed (μg equivalents 1,1,2-Trichloroethane/ cm^2)

Time (hours)	Cell 71	Cell 72	Cell 73	Cell 74	Cell 75	Cell 76
0	nd	nd	nd	nd	nd	nd
0.16	nd	nd	nd	nd	nd	nd
0.5	0.421	0.869	1.94	3.70	0.992	nd
1	3.64	7.65	20.2	18.8	10.2	2.14
2	23.3	50.7	175	57.7	59.1	12.3
4	169	381	623	194	337	105
6	396	772	975	378	671	332
8	605	1130	1290	575	980	597
10	784	1410	1570	765	1260	834
12	952	1670	1830	928	1510	1040
14	1110	1930	2090	1080	1740	1220
16	1240	2190	2340	1220	1950	1400
18	1370	2430	2610	1370	2150	1570
20	1480	2680	2890	1510	2380	1740
22	1580	2930	3150	1640	2590	1910
24	1690	3210	3450	1790	2810	2100

nd Not detected

Figure 9.7: Amount of Radiolabeled Material Absorbed Following a Single Finite Application of Neat [^{14}C]-1,1,2-Trichloroethane to Human Skin (Group A7)

Results are expressed as cumulative percent applied dose



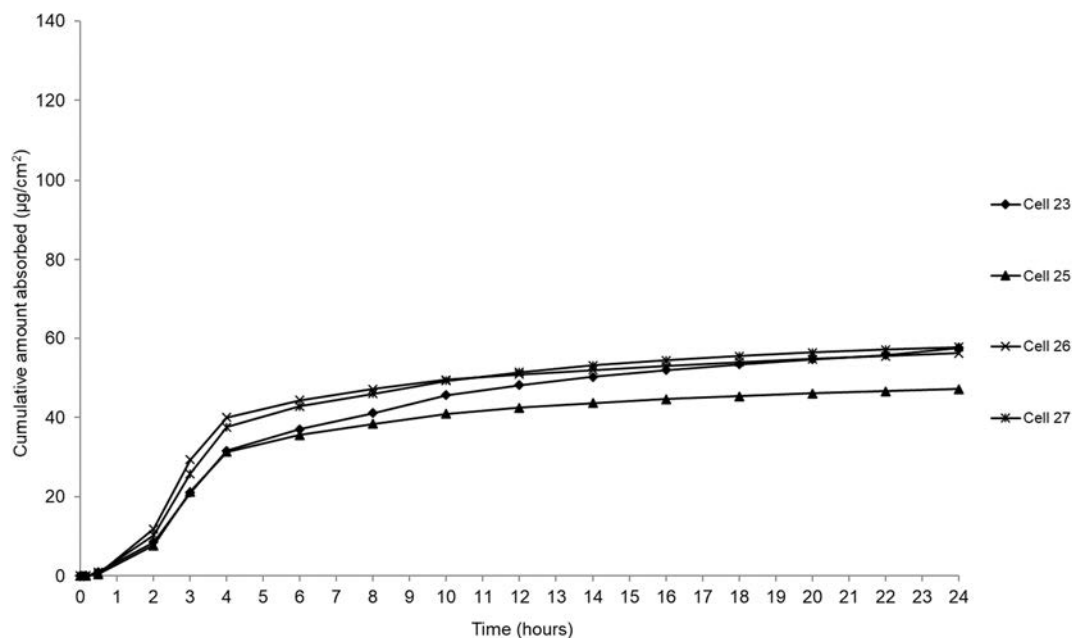
Cumulative amount absorbed (% dose)

Time (hours)	Cell 23	Cell 25	Cell 26	Cell 27
0	nd	nd	nd	nd
0.16	nd	nd	nd	nd
0.5	0.007	0.003	0.004	0.007
1	0.060	0.054	0.083	0.075
2	0.149	0.152	0.207	0.187
4	0.224	0.224	0.282	0.273
6	0.263	0.253	0.313	0.311
8	0.292	0.274	0.332	0.334
10	0.324	0.292	0.349	0.358
12	0.342	0.303	0.359	0.373
14	0.357	0.311	0.367	0.386
16	0.369	0.318	0.374	0.395
18	0.380	0.324	0.381	0.403
20	0.389	0.329	0.386	0.410
22	0.396	0.333	0.391	0.415
24	0.409	0.336	0.397	0.419

nd Not detected

Figure 9.8: Amount of Radiolabeled Material Absorbed Following a Single Finite Application of Neat [^{14}C]-1,1,2-Trichloroethane to Human Skin (Group A7)

Results are expressed as cumulative μg equivalents 1,1,2-Trichloroethane/ cm^2



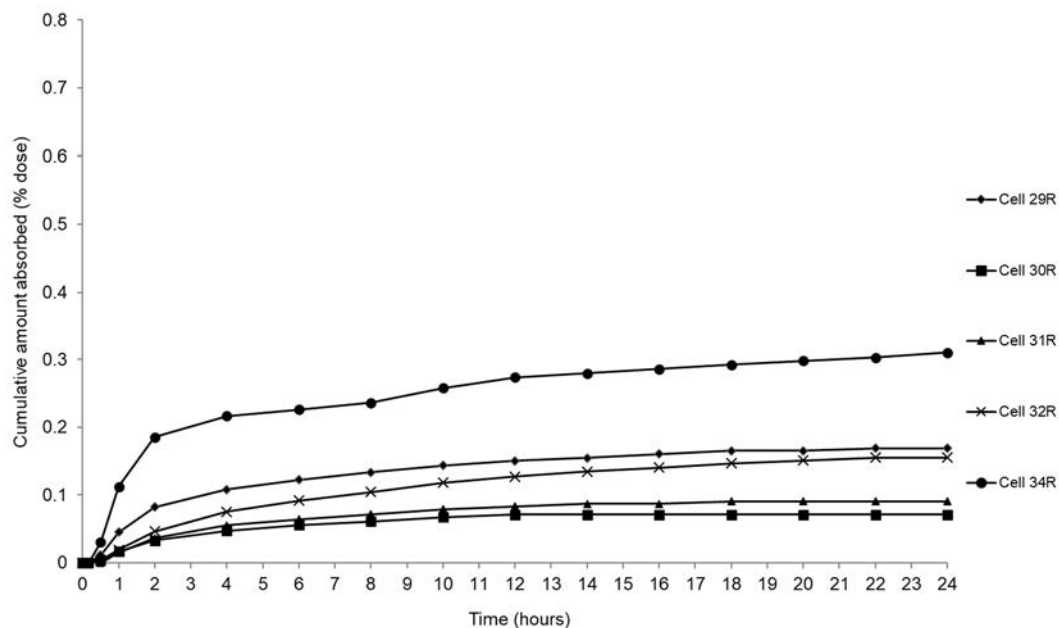
Cumulative amount absorbed (μg equivalents 1,1,2-Trichloroethane/ cm^2)

Time (hours)	Cell 23	Cell 25	Cell 26	Cell 27
0	nd	nd	nd	nd
0.16	nd	nd	nd	nd
0.5	0.969	0.419	0.559	0.908
1	8.48	7.64	11.8	10.3
2	21.0	21.3	29.3	25.8
4	31.6	31.3	40.0	37.6
6	37.0	35.5	44.3	42.8
8	41.1	38.4	47.1	46.0
10	45.6	40.9	49.5	49.3
12	48.1	42.4	50.8	51.4
14	50.2	43.6	52.0	53.1
16	51.9	44.6	53.0	54.4
18	53.4	45.4	53.9	55.5
20	54.7	46.1	54.7	56.4
22	55.7	46.6	55.4	57.1
24	57.5	47.2	56.2	57.7

nd Not detected

Figure 9.9: Amount of Radiolabeled Material Absorbed Following a Single Finite Application of 50% [^{14}C]-1,1,2-Trichloroethane in IPM to Human Skin (Group A8)

Results are expressed as cumulative percent applied dose



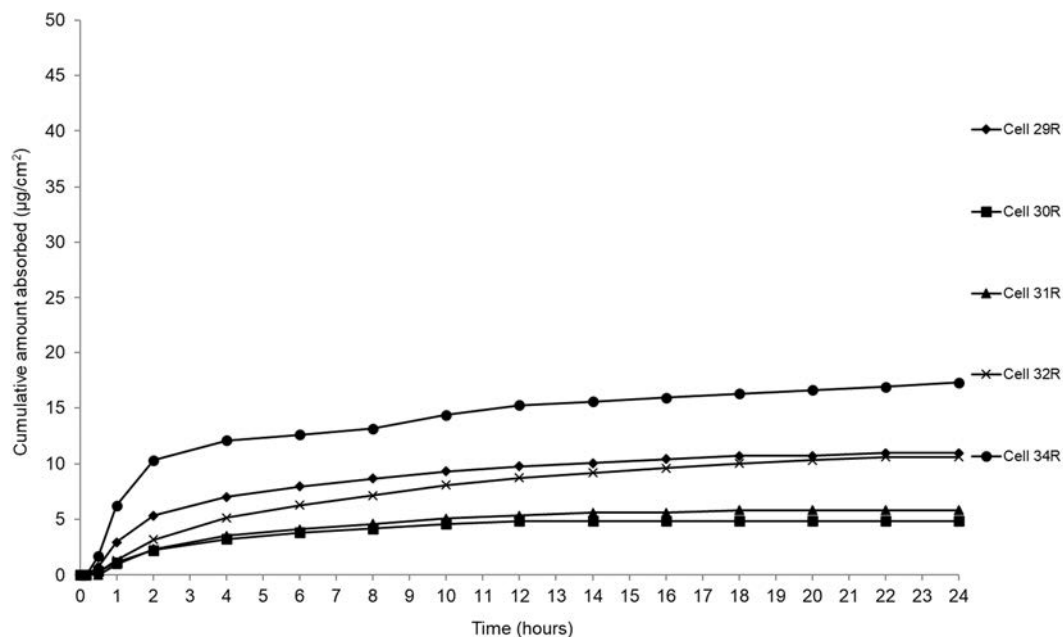
Cumulative amount absorbed (% dose)

Time (hours)	Cell 29R	Cell 30R	Cell 31R	Cell 32R	Cell 34R
0	nd	nd	nd	nd	nd
0.16	nd	nd	nd	nd	nd
0.5	0.012	0.005	nd	0.004	0.030
1	0.045	0.017	0.015	0.019	0.112
2	0.082	0.033	0.036	0.046	0.185
4	0.108	0.047	0.055	0.075	0.216
6	0.122	0.056	0.064	0.092	0.226
8	0.134	0.061	0.072	0.105	0.236
10	0.144	0.068	0.079	0.118	0.258
12	0.150	0.071	0.083	0.127	0.273
14	0.155	0.071	0.087	0.134	0.279
16	0.160	0.071	0.087	0.141	0.286
18	0.165	0.071	0.091	0.147	0.292
20	0.165	0.071	0.091	0.151	0.298
22	0.169	0.071	0.091	0.155	0.303
24	0.169	0.071	0.091	0.155	0.310

nd Not detected

Figure 9.10: Amount of Radiolabeled Material Absorbed Following a Single Finite Application of 50% [^{14}C]-1,1,2-Trichloroethane in IPM to Human Skin (Group A8)

Results are expressed as cumulative μg equivalents 1,1,2-Trichloroethane/ cm^2



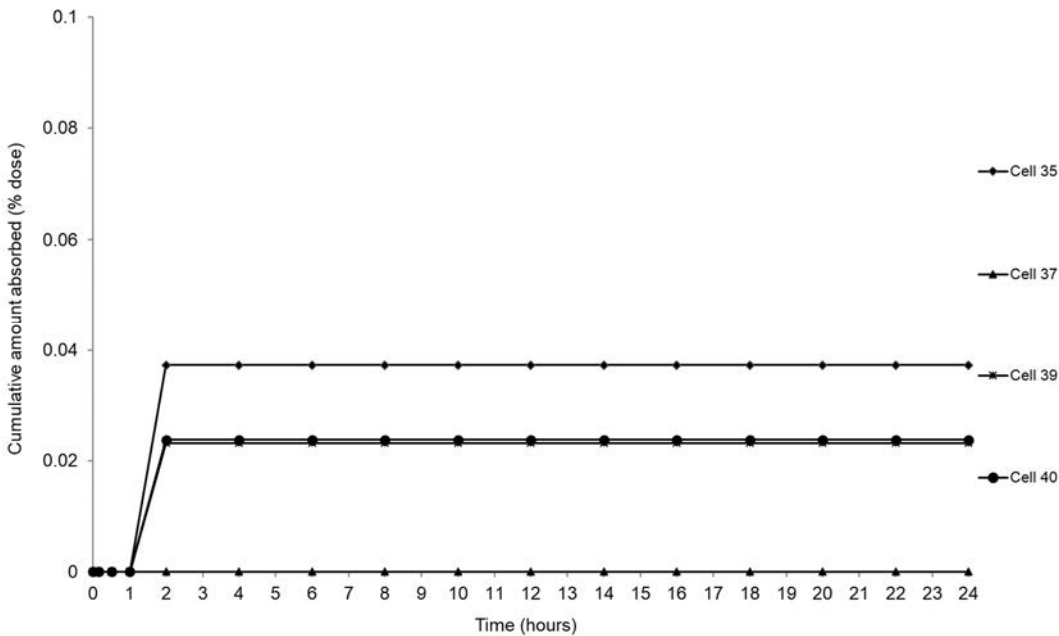
Cumulative amount absorbed (μg equivalents 1,1,2-Trichloroethane/ cm^2)

Time (hours)	Cell 29R	Cell 30R	Cell 31R	Cell 32R	Cell 34R
0	nd	nd	nd	nd	nd
0.16	nd	nd	nd	nd	nd
0.5	0.746	0.307	nd	0.246	1.69
1	2.94	1.14	0.966	1.31	6.25
2	5.32	2.24	2.33	3.17	10.3
4	7.01	3.21	3.53	5.14	12.1
6	7.94	3.80	4.12	6.27	12.6
8	8.67	4.15	4.59	7.15	13.2
10	9.31	4.60	5.07	8.07	14.4
12	9.74	4.84	5.34	8.71	15.2
14	10.0	4.84	5.59	9.19	15.6
16	10.4	4.84	5.59	9.60	15.9
18	10.7	4.84	5.82	10.0	16.3
20	10.7	4.84	5.82	10.3	16.6
22	11.0	4.84	5.82	10.6	16.9
24	11.0	4.84	5.82	10.6	17.3

nd Not detected

Figure 9.11: Amount of Radiolabeled Material Absorbed Following a Single Finite Application of 10% [¹⁴C]-1,1,2-Trichloroethane in IPM to Human Skin (Group A9)

Results are expressed as cumulative percent applied dose



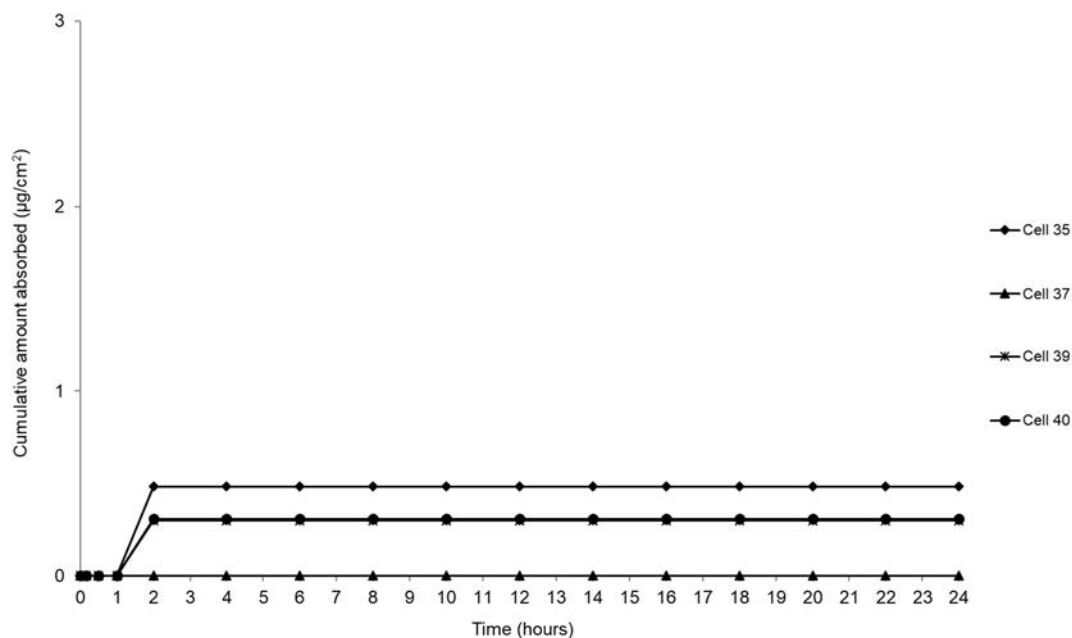
Cumulative amount absorbed (% dose)

Time (hours)	Cell 35	Cell 37	Cell 39	Cell 40
0	nd	nd	nd	nd
0.16	nd	nd	nd	nd
0.5	nd	nd	nd	nd
1	nd	nd	nd	nd
2	0.037	nd	0.023	0.024
4	0.037	nd	0.023	0.024
6	0.037	nd	0.023	0.024
8	0.037	nd	0.023	0.024
10	0.037	nd	0.023	0.024
12	0.037	nd	0.023	0.024
14	0.037	nd	0.023	0.024
16	0.037	nd	0.023	0.024
18	0.037	nd	0.023	0.024
20	0.037	nd	0.023	0.024
22	0.037	nd	0.023	0.024
24	0.037	nd	0.023	0.024

nd Not detected

Figure 9.12: Amount of Radiolabeled Material Absorbed Following a Single Finite Application of 10% [^{14}C]-1,1,2-Trichloroethane in IPM to Human Skin (Group A9)

Results are expressed as cumulative μg equivalents 1,1,2-Trichloroethane/ cm^2



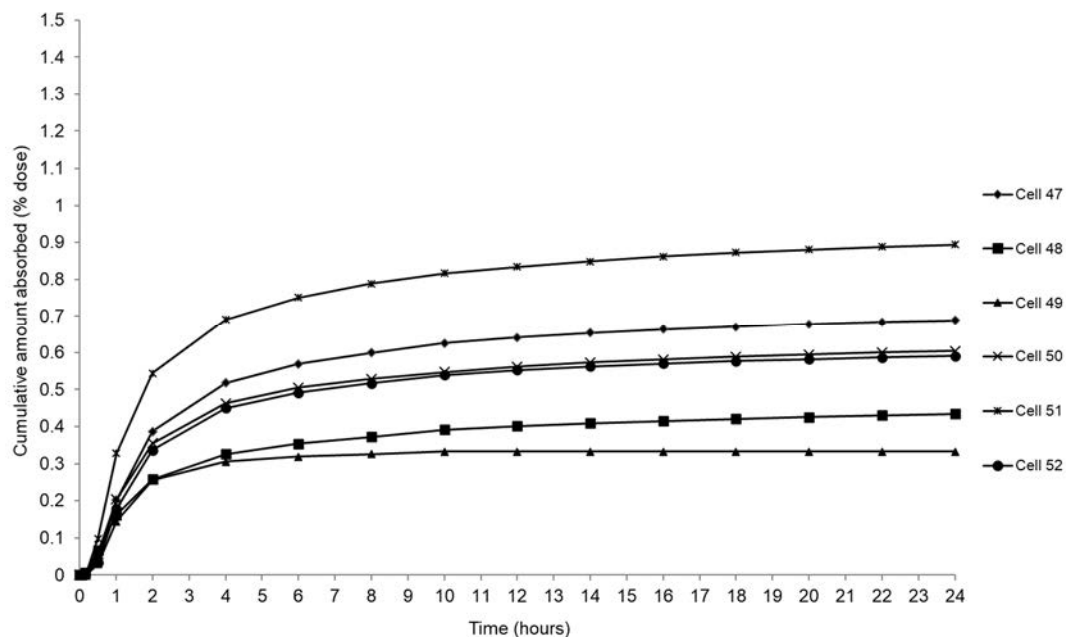
Cumulative amount absorbed (μg equivalents 1,1,2-Trichloroethane/ cm^2)

Time (hours)	Cell 35	Cell 37	Cell 39	Cell 40
0	nd	nd	nd	nd
0.16	nd	nd	nd	nd
0.5	nd	nd	nd	nd
1	nd	nd	nd	nd
2	0.483	nd	0.298	0.307
4	0.483	nd	0.298	0.307
6	0.483	nd	0.298	0.307
8	0.483	nd	0.298	0.307
10	0.483	nd	0.298	0.307
12	0.483	nd	0.298	0.307
14	0.483	nd	0.298	0.307
16	0.483	nd	0.298	0.307
18	0.483	nd	0.298	0.307
20	0.483	nd	0.298	0.307
22	0.483	nd	0.298	0.307
24	0.483	nd	0.298	0.307

nd Not detected

Figure 9.13: Amount of Radiolabeled Material Absorbed Following a Single Finite Application of 50% [^{14}C]-1,1,2-Trichloroethane in 1,2 Dichloroethane to Human Skin (Group A11)

Results are expressed as cumulative percent applied dose



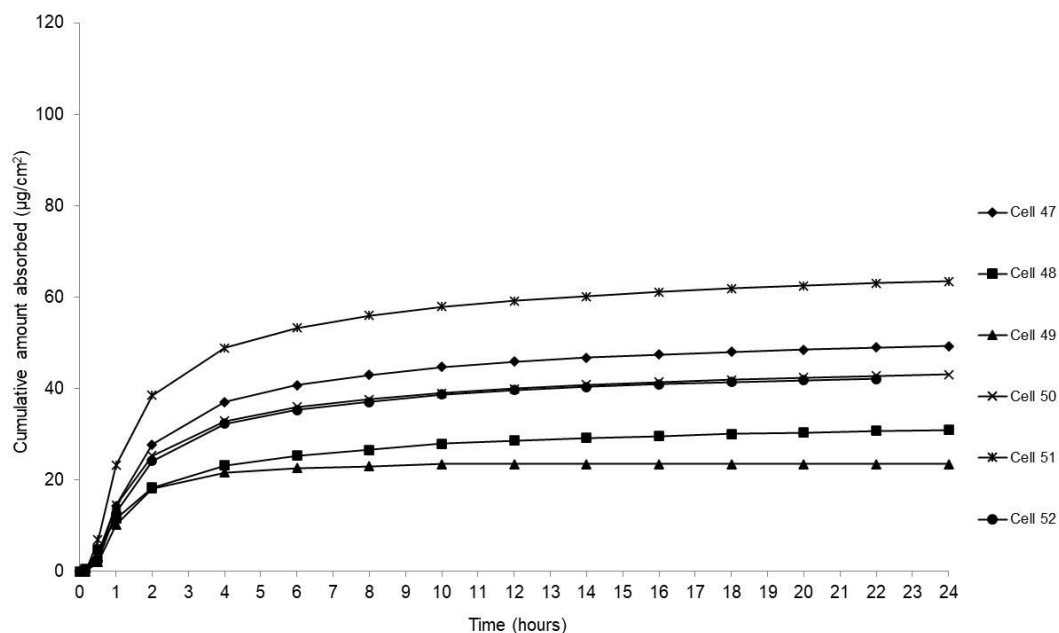
Cumulative amount absorbed (% dose)

Time (hours)	Cell 47	Cell 48	Cell 49	Cell 50	Cell 51	Cell 52
0	nd	nd	nd	nd	nd	nd
0.16	nd	0.007	nd	nd	nd	nd
0.5	0.044	0.067	0.029	0.057	0.098	0.033
1	0.201	0.162	0.145	0.203	0.328	0.179
2	0.387	0.258	0.256	0.355	0.544	0.336
4	0.518	0.326	0.306	0.462	0.690	0.450
6	0.569	0.354	0.319	0.505	0.750	0.491
8	0.600	0.372	0.325	0.528	0.789	0.517
10	0.625	0.392	0.332	0.547	0.816	0.540
12	0.641	0.401	0.332	0.561	0.834	0.553
14	0.653	0.409	0.332	0.573	0.848	0.562
16	0.663	0.415	0.332	0.581	0.862	0.570
18	0.670	0.421	0.332	0.588	0.872	0.577
20	0.678	0.426	0.332	0.594	0.881	0.582
22	0.684	0.430	0.332	0.600	0.889	0.586
24	0.688	0.434	0.332	0.604	0.894	0.590

nd Not detected

Figure 9.14: Amount of Radiolabeled Material Absorbed Following a Single Finite Application of 50% [^{14}C]-1,1,2-Trichloroethane in 1,2 Dichloroethane to Human Skin (Group A11)

Results are expressed as cumulative μg equivalents 1,1,2-Trichloroethane/ cm^2



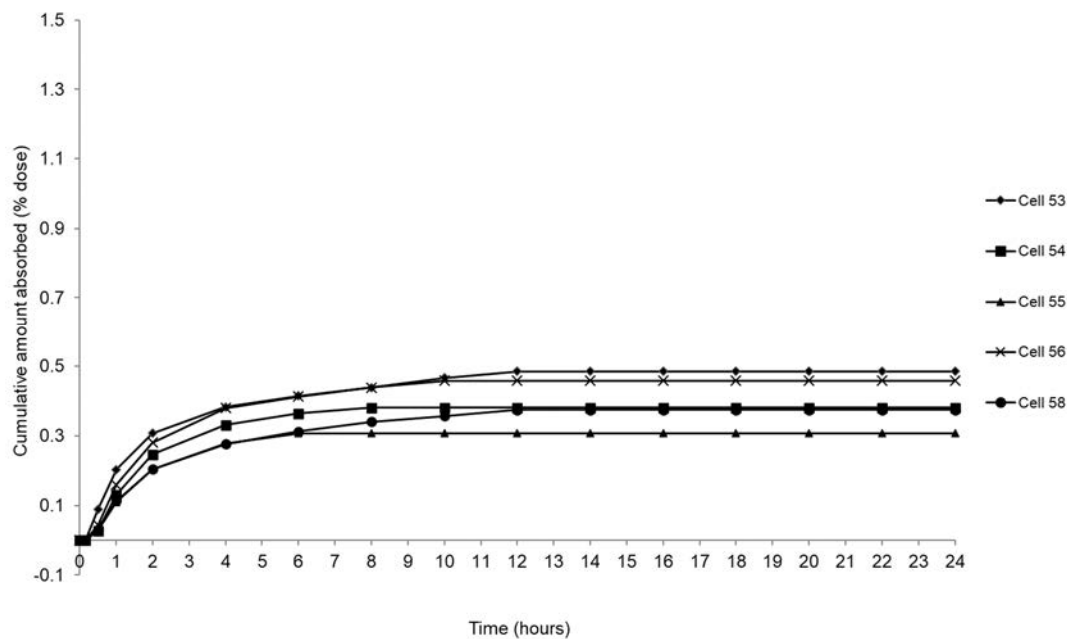
Cumulative amount absorbed (μg equivalents 1,1,2-Trichloroethane/ cm^2)

Time (hours)	Cell 47	Cell 48	Cell 49	Cell 50	Cell 51	Cell 52
0	nd	nd	nd	nd	nd	nd
0.16	nd	0.496	nd	nd	nd	nd
0.5	3.14	4.79	2.07	4.05	6.96	2.38
1	14.4	11.6	10.3	14.5	23.3	12.8
2	27.7	18.4	18.2	25.3	38.6	24.1
4	37.1	23.3	21.7	33.0	48.9	32.3
6	40.8	25.3	22.6	36.0	53.2	35.3
8	43.0	26.6	23.1	37.7	56.0	37.1
10	44.8	28.0	23.5	39.0	57.9	38.8
12	45.9	28.7	23.5	40.0	59.2	39.7
14	46.8	29.2	23.5	40.8	60.2	40.4
16	47.5	29.6	23.5	41.4	61.1	41.0
18	48.0	30.1	23.5	42.0	61.9	41.4
20	48.6	30.4	23.5	42.4	62.5	41.8
22	49.0	30.7	23.5	42.8	63.0	42.1
24	49.3	31.0	23.5	43.1	63.4	42.4

nd Not detected

Figure 9.15: Amount of Radiolabeled Material Absorbed Following a Single Finite Application of 10% [^{14}C]-1,1,2-Trichloroethane in 1,2 Dichloroethane to Human Skin (Group A12)

Results are expressed as cumulative percent applied dose



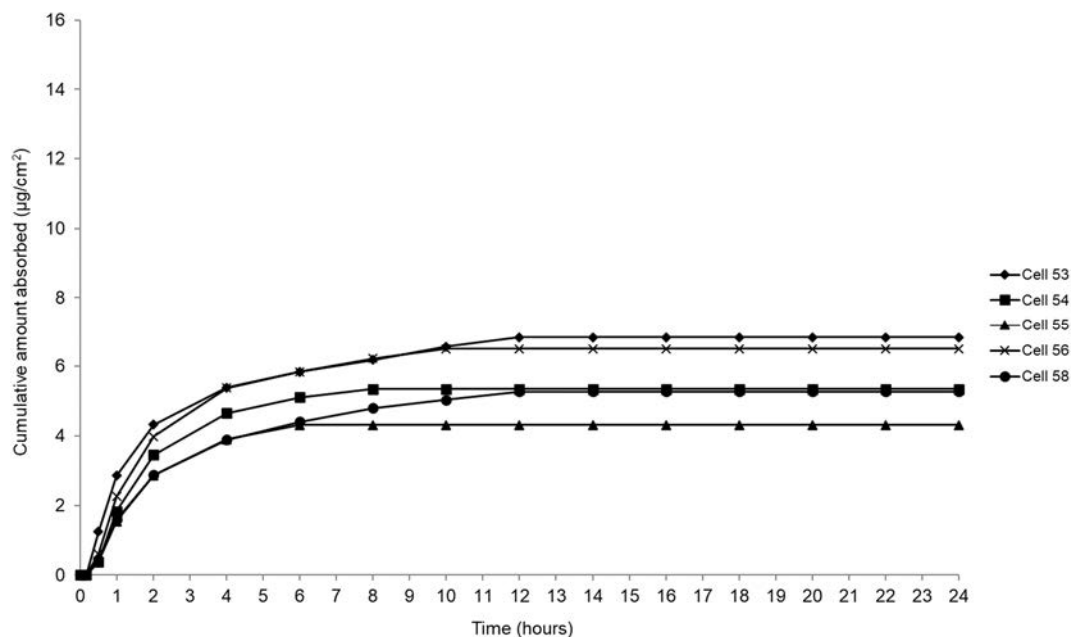
Cumulative amount absorbed (% dose)

Time (hours)	Cell 53	Cell 54	Cell 55	Cell 56	Cell 58
0	nd	nd	nd	nd	nd
0.16	nd	nd	nd	nd	nd
0.5	0.088	0.027	0.027	0.043	0.031
1	0.203	0.131	0.110	0.159	0.113
2	0.307	0.246	0.204	0.281	0.204
4	0.383	0.331	0.278	0.380	0.276
6	0.415	0.364	0.307	0.413	0.313
8	0.439	0.381	0.307	0.439	0.341
10	0.466	0.381	0.307	0.459	0.357
12	0.486	0.381	0.307	0.459	0.375
14	0.486	0.381	0.307	0.459	0.375
16	0.486	0.381	0.307	0.459	0.375
18	0.486	0.381	0.307	0.459	0.375
20	0.486	0.381	0.307	0.459	0.375
22	0.486	0.381	0.307	0.459	0.375
24	0.486	0.381	0.307	0.459	0.375

nd Not detected

Figure 9.16: Amount of Radiolabeled Material Absorbed Following a Single Finite Application of 10% [^{14}C]-1,1,2-Trichloroethane in 1,2 Dichloroethane to Human Skin (Group A12)

Results are expressed as cumulative μg equivalents 1,1,2-Trichloroethane/ cm^2



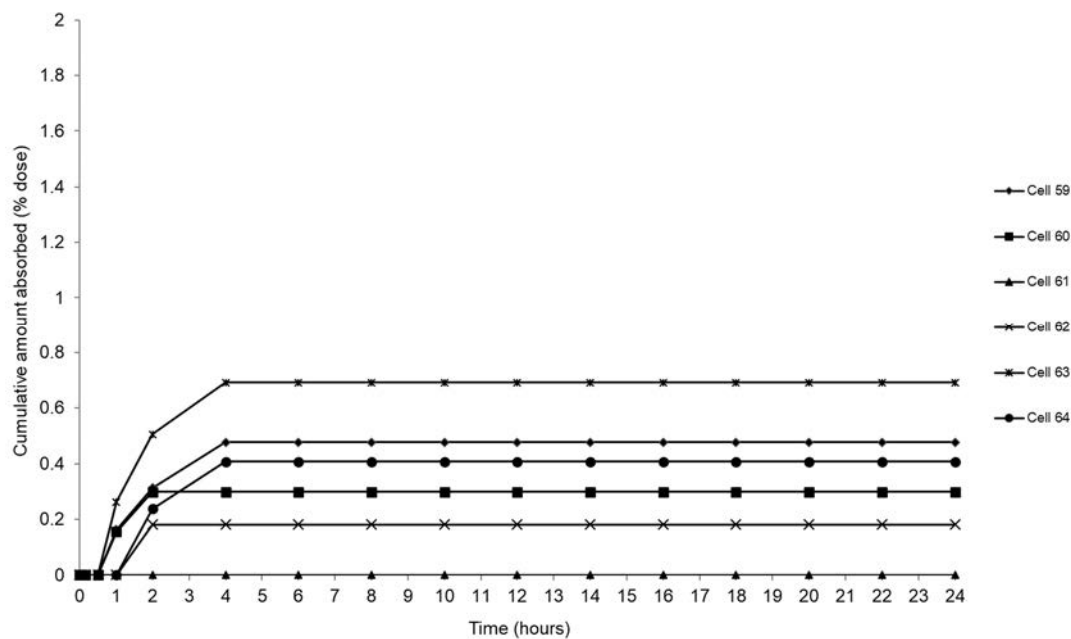
Cumulative amount absorbed (μg equivalents 1,1,2-Trichloroethane/ cm^2)

Time (hours)	Cell 53	Cell 54	Cell 55	Cell 56	Cell 58
0	nd	nd	nd	nd	nd
0.16	nd	nd	nd	nd	nd
0.5	1.24	0.384	0.384	0.608	0.440
1	2.85	1.83	1.54	2.25	1.59
2	4.32	3.45	2.86	3.98	2.87
4	5.39	4.65	3.90	5.38	3.89
6	5.84	5.11	4.31	5.85	4.41
8	6.18	5.34	4.31	6.22	4.80
10	6.57	5.34	4.31	6.50	5.03
12	6.84	5.34	4.31	6.50	5.28
14	6.84	5.34	4.31	6.50	5.28
16	6.84	5.34	4.31	6.50	5.28
18	6.84	5.34	4.31	6.50	5.28
20	6.84	5.34	4.31	6.50	5.28
22	6.84	5.34	4.31	6.50	5.28
24	6.84	5.34	4.31	6.50	5.28

nd Not detected

Figure 9.17: Amount of Radiolabeled Material Absorbed Following a Single Finite Application of 1% [^{14}C]-1,1,2-Trichloroethane in 1,2 Dichloroethane to Human Skin (Group A13)

Results are expressed as cumulative percent applied dose



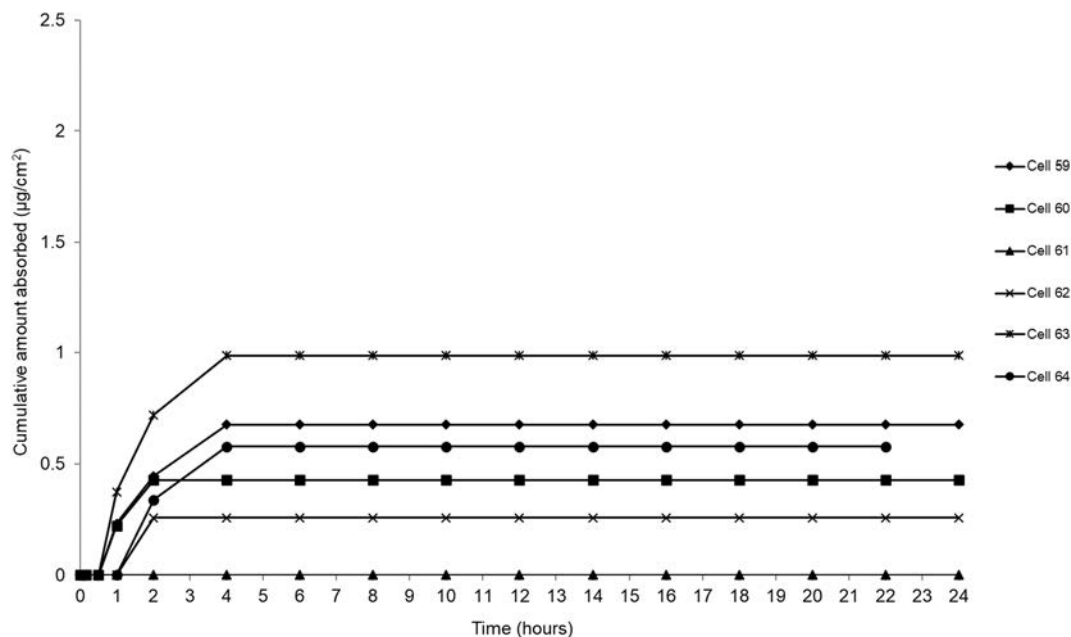
Cumulative amount absorbed (% dose)

Time (hours)	Cell 59	Cell 60	Cell 61	Cell 62	Cell 63	Cell 64
0	nd	nd	nd	nd	nd	nd
0.16	nd	nd	nd	nd	nd	nd
0.5	nd	nd	nd	nd	nd	nd
1	0.163	0.155	nd	nd	0.262	nd
2	0.313	0.298	nd	0.181	0.505	0.238
4	0.476	0.298	nd	0.181	0.692	0.408
6	0.476	0.298	nd	0.181	0.692	0.408
8	0.476	0.298	nd	0.181	0.692	0.408
10	0.476	0.298	nd	0.181	0.692	0.408
12	0.476	0.298	nd	0.181	0.692	0.408
14	0.476	0.298	nd	0.181	0.692	0.408
16	0.476	0.298	nd	0.181	0.692	0.408
18	0.476	0.298	nd	0.181	0.692	0.408
20	0.476	0.298	nd	0.181	0.692	0.408
22	0.476	0.298	nd	0.181	0.692	0.408
24	0.476	0.298	nd	0.181	0.692	0.408

nd Not detected

Figure 9.18: Amount of Radiolabeled Material Absorbed Following a Single Finite Application of 1% [^{14}C]-1,1,2-Trichloroethane in 1,2 Dichloroethane to Human Skin (Group A13)

Results are expressed as cumulative μg equivalents 1,1,2-Trichloroethane/ cm^2



Cumulative amount absorbed (μg equivalents 1,1,2-Trichloroethane/ cm^2)

Time (hours)	Cell 59	Cell 60	Cell 61	Cell 62	Cell 63	Cell 64
0	nd	nd	nd	nd	nd	nd
0.16	nd	nd	nd	nd	nd	nd
0.5	nd	nd	nd	nd	nd	nd
1	0.231	0.222	nd	nd	0.373	nd
2	0.444	0.426	nd	0.258	0.719	0.337
4	0.675	0.426	nd	0.258	0.985	0.577
6	0.675	0.426	nd	0.258	0.985	0.577
8	0.675	0.426	nd	0.258	0.985	0.577
10	0.675	0.426	nd	0.258	0.985	0.577
12	0.675	0.426	nd	0.258	0.985	0.577
14	0.675	0.426	nd	0.258	0.985	0.577
16	0.675	0.426	nd	0.258	0.985	0.577
18	0.675	0.426	nd	0.258	0.985	0.577
20	0.675	0.426	nd	0.258	0.985	0.577
22	0.675	0.426	nd	0.258	0.985	0.577
24	0.675	0.426	nd	0.258	0.985	0.577

nd Not detected

10. TABLES

Table 10.1: Permeability Co-efficient (Kp) and Maximal Absorption Rates Following an Infinite Application of [¹⁴C]-1,1,2-Trichloroethane to Human Skin (Group A2 – A6)

Group	Dose level	Kp Steady State (× 10 ⁻³ cm/hr)		Maximum absorption rate (µg/cm ² /hr)	
		Mean	sd	Mean	sd
A2	Neat	3.33	1.88	527	299
A3	50% in IPM	3.40	1.39	652	303
A4	10% in IPM	0.94	0.23	113	36.2
A5	50% in 1,2-Dichloroethane	4.49	1.19	562	159
A6	10% in 1,2-Dichloroethane	0.86	0.21	155	49.0

sd Standard deviation

Table 10.2: Mean Distribution of Radioactivity Following a Single Finite Application of Neat [^{14}C]-1,1,2-Trichloroethane to Human Skin (Group A7)

	Dose in receptor fluid (0-24 h)	Dose on receptor chamber	Total absorbed dose ^a (%)	Dose in exposed skin	Dermal Delivery ^b (%)	Dose in stratum corneum	Dislodgeable dose (Skin swab, 8 h)	Dislodgeable dose (Skin swab, 24 h)	Dose on donor chamber	Total dislodgeable dose ^c (%)	Dose in trap extract	Dose on surface tape strips (1 - 2)	Total unabsorbed dose ^d (%)	Mass balance (%)
%	0.39	nd	0.39	0.06	0.45	nd	0.31	0.04	nd	0.35	95.92	nd	96.27	96.72
µg	34.96	nd		5.38		nd	27.79	3.59	nd		8598.27	nd		

Mean dose recovery was calculated from the mean of the individual recoveries and not the sum of the mean components

nd Not detected

^a Sum of radioactivity detected in the receptor fluids and receptor chamber

^b Sum of absorbed dose and radioactivity in exposed skin

^c Sum of radioactivity detected in the skin wash (8h and 24h) and donor chamber

^d Sum of total dislodgeable dose and radioactivity in the charcoal trap and on the surface tape strips.

Table 10.3: Mean Distribution of Radioactivity Following a Single Finite Application 50% [¹⁴C]-1,1,2-Trichloroethane in IPM to Human Skin (Group A8)

	Dose in receptor fluid (0-24 h)	Dose on receptor chamber	Total absorbed dose ^a (%)	Dose in exposed skin	Dermal Delivery ^b (%)	Dose in stratum corneum	Dislodgeable dose (Skin swab, 8 h)	Dislodgeable dose (Skin swab, 24 h)	Dose on donor chamber	Total dislodgeable dose ^c (%)	Dose in trap extract	Dose on surface tape strips (1 - 2)	Total unabsorbed dose ^d (%)	Mass balance (%)
%	0.17	0.01	0.19	0.05	0.24	nd	0.44	0.11	0.01	0.57	99.23	nd	99.79	100.03
µg	6.99	0.41		2.06		nd	18.09	4.52	0.41		4079.35	nd		

Mean dose recovery was calculated from the mean of the individual recoveries and not the sum of the mean components

nd Not detected

^a Sum of radioactivity detected in the receptor fluids and receptor chamber

^b Sum of absorbed dose and radioactivity in exposed skin

^c Sum of radioactivity detected in the skin wash (8h and 24h) and donor chamber

^d Sum of total dislodgeable dose and radioactivity in the charcoal trap and on the surface tape strips.

Table 10.4: Mean Distribution of Radioactivity Following a Single Finite Application of 10% [¹⁴C]-1,1,2-Trichloroethane in IPM Trichloroethane to Human Skin (Group A9)

	Dose in receptor fluid (0-24 h)	Dose on receptor chamber	Total absorbed dose ^a (%)	Dose in exposed skin	Dermal Delivery ^b (%)	Dose in stratum corneum	Dislodgeable dose (Skin swab, 8 h)	Dislodgeable dose (Skin swab, 24 h)	Dose on donor chamber	Total dislodgeable dose ^c (%)	Dose in trap extract	Dose on surface tape strips (1 - 2)	Total unabsorbed dose ^d (%)	Mass balance (%)
%	0.02	nd	0.02	nd	0.02	nd	0.54	0.02	nd	0.56	94.75	nd	95.31	95.33
µg	0.17	nd		nd		nd	4.46	0.17	nd		782.64	nd		

Mean dose recovery was calculated from the mean of the individual recoveries and not the sum of the mean components

nd Not detected

^a Sum of radioactivity detected in the receptor fluids and receptor chamber

^b Sum of absorbed dose and radioactivity in exposed skin

^c Sum of radioactivity detected in the skin wash (8h and 24h) and donor chamber

^d Sum of total dislodgeable dose and radioactivity in the charcoal trap and on the surface tape strips.

Table 10.5: Mean Distribution of Radioactivity Following a Single Finite Application of 1% [^{14}C]-1,1,2-Trichloroethane in IPM to Human Skin (Group A10)

	Dose in receptor fluid (0-24 h)	Dose on receptor chamber	Total absorbed dose ^a (%)	Dose in exposed skin	Dermal Delivery ^b (%)	Dose in stratum corneum	Dislodgeable dose (Skin swab, 8 h)	Dislodgeable dose (Skin swab, 24 h)	Dose on donor chamber	Total dislodgeable dose ^c (%)	Dose in trap extract	Dose on surface tape strips (1 - 2)	Total unabsorbed dose ^d (%)	Mass balance (%)
%	nd	nd	nd	nd	nd	nd	0.70	0.01	nd	0.71	104.72	nd	105.43	105.43
µg	nd	nd		nd		nd	0.60	0.01	nd		90.06	nd		

Mean dose recovery was calculated from the mean of the individual recoveries and not the sum of the mean components

nd Not detected

^a Sum of radioactivity detected in the receptor fluids and receptor chamber

^b Sum of absorbed dose and radioactivity in exposed skin

^c Sum of radioactivity detected in the skin wash (8h and 24h) and donor chamber

^d Sum of total dislodgeable dose and radioactivity in the charcoal trap and on the surface tape strips.

Table 10.6: Mean Distribution of Radioactivity Following a Single Finite Application of 50% [¹⁴C]-1,1,2-Trichloroethane in 1,2-Dichloroethane to Human Skin (Group A11)

	Dose in receptor fluid (0-24 h)	Dose on receptor chamber	Total absorbed dose ^a (%)	Dose in exposed skin	Dermal Delivery ^b (%)	Dose in stratum corneum	Dislodgeable dose (Skin swab, 8 h)	Dislodgeable dose (Skin swab, 24 h)	Dose on donor chamber	Total dislodgeable dose ^c (%)	Dose in trap extract	Dose on surface tape strips (1 - 2)	Total unabsorbed dose ^d (%)	Mass balance (%)
%	0.59	nd	0.59	nd	0.59	nd	0.69	0.01	nd	0.70	92.26	nd	92.96	93.54
µg	26.94	nd		nd		nd	31.51	0.46	nd		4212.59	nd		

Mean dose recovery was calculated from the mean of the individual recoveries and not the sum of the mean components.

nd Not detected

^a Sum of radioactivity detected in the receptor fluids and receptor chamber

^b Sum of absorbed dose and radioactivity in exposed skin

^c Sum of radioactivity detected in the skin wash (8h and 24h) and donor chamber

^d Sum of total dislodgeable dose and radioactivity in the charcoal trap and on the surface tape strips.

Table 10.7: Mean Distribution of Radioactivity Following a Single Finite Application of 10% [¹⁴C]-1,1,2-Trichloroethane in 1,2-Dichloroethane to Human Skin (Group A12)

	Dose in receptor fluid (0-24 h)	Dose on receptor chamber	Total absorbed dose ^a (%)	Dose in exposed skin	Dermal Delivery ^b (%)	Dose in stratum corneum	Dislodgeable dose (Skin swab, 8 h)	Dislodgeable dose (Skin swab, 24 h)	Dose on donor chamber	Total dislodgeable dose ^c (%)	Dose in trap extract	Dose on surface tape strips (1 - 2)	Total unabsorbed dose ^d (%)	Mass balance (%)
%	0.40	nd	0.40	nd	0.40	nd	0.97	nd	nd	0.97	94.32	nd	95.29	95.70
µg	3.60	nd		nd		nd	8.74	nd	nd		849.82	nd		

Mean dose recovery was calculated from the mean of the individual recoveries and not the sum of the mean components

nd Not detected

^a Sum of radioactivity detected in the receptor fluids and receptor chamber

^b Sum of absorbed dose and radioactivity in exposed skin

^c Sum of radioactivity detected in the skin wash (8h and 24h) and donor chamber

^d Sum of total dislodgeable dose and radioactivity in the charcoal trap and on the surface tape strips.

Table 10.8: Mean Distribution of Radioactivity Following a Single Finite Application of 1% [¹⁴C]-1,1,2-Trichloroethane in 1,2-Dichloroethane to Human Skin (Group A13)

	Dose in receptor fluid (0-24 h)	Dose on receptor chamber	Total absorbed dose ^a (%)	Dose in exposed skin	Dermal Delivery ^b (%)	Dose in stratum corneum	Dislodgeable dose (Skin swab, 8 h)	Dislodgeable dose (Skin swab, 24 h)	Dose on donor chamber	Total dislodgeable dose ^c (%)	Dose in trap extract	Dose on surface tape strips (1 - 2)	Total unabsorbed dose ^d (%)	Mass balance (%)
%	0.34	nd	0.34	nd	0.34	nd	0.24	nd	nd	0.24	91.66	nd	91.91	92.25
µg	0.31	nd		nd		nd	0.22	nd	nd		83.41	nd		

Mean dose recovery was calculated from the mean of the individual recoveries and not the sum of the mean components

nd Not detected

^a Sum of radioactivity detected in the receptor fluids and receptor chamber

^b Sum of absorbed dose and radioactivity in exposed skin

^c Sum of radioactivity detected in the skin wash (8h and 24h) and donor chamber

^d Sum of total dislodgeable dose and radioactivity in the charcoal trap and on the surface tape strips.

11. APPENDICES

Appendix 11.1: Certificates of Analysis and Radiochemical Purity Data

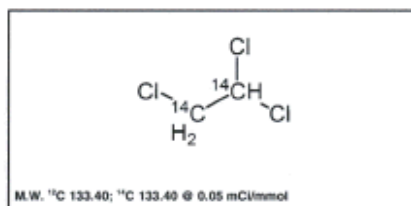
Radiolabeled 1,1,2-Trichloroethane



Certificate of Analysis

MC-1002709

1,1,2-Trichloroethane, [¹⁴C]-



Lot #: 369-166-050-A-20210917-DRE

Specific Activity by GC-MS: 0.05 mCi/mmol

Packaged as: Neat liquid

Date of Analysis: September 22, 2021

Chemical Purity by GC-MS: 100%

ID by GC-MS: Conforms to reference standard

ID by ¹H NMR: Results conform to expected structure

Storage Recommendation: Store at -20°C.

Quality Level Disclaimer: Results were obtained utilizing non-qualified instrumentation, methods that have not been validated or transferred, and analyses not performed according to a formal, documented, analytical protocol.

Note: SA is based on a dilution of 15mg ¹⁴C material at 9.2 mCi/mmol : 2.645g cold material.

When handling this radiochemical, please refer to the safe handling of isotopes document provided with your shipment. If the document is misplaced, a link to a copy is available at www.moravek.com/customer-service.

In addition to the hazard connected with radiation, this compound also has chemical safety characteristics that need to be taken into account before it can be safely worked with. For assistance with designing a safe working environment and procedures for this and all chemicals at your workplace, please contact OSHA at 800-321-6742 (OSHA) or https://www.osha.gov/html/Feed_Back.html.

Caution: This material was manufactured under non-GLP conditions and is intended for investigational or manufacturing use only. Formulations using this material are not suitable for use in humans or clinical diagnosis. Responsibility for use of this material and compliance with federal law rests solely with the purchaser. An expiry date has not been determined. In the absence of stability data, a purity check is recommended before use.

Moravek, Inc. 577 Mercury Lane, Brea, California 92821 www.moravek.com info@moravek.com Phone (714) 990-2018 Fax (714) 990-1824

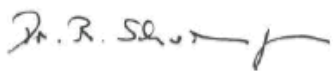
Appendix 11.1: Certificates of Analysis and Radiochemical Purity Data (continued)

Non-radiolabeled 1,1,2-Trichloroethane

SIGMA-ALDRICH®3050 Spruce Street, Saint Louis, MO 63103 USA
Email USA: techserv@sigmaaldrich.com Outside USA: eurtechserv@sigmaaldrich.com**Certificate of Analysis**

Product Name: 1,1,2-Trichloroethane
analytical standard
Product Number: 46262
Batch Number: BCCB7564
Brand: Sigma-Aldrich
CAS Number: 79-00-5
Formula: $\text{ClCH}_2\text{CHCl}_2$
Formula Weight: 133.40
Expiration Date: MAY 2024
Quality Release Date: 08 JUL 2019

TEST	SPECIFICATION	RESULT
APPEARANCE (COLOR)	COLORLESS TO FAINT YELLOW	COLORLESS
APPEARANCE (FORM)	LIQUID	LIQUID
PURITY (GC AREA %)	≥ 98.0 %	98.2 %
REFRACTIVE INDEX N20/D	1.4696 - 1.4736	1.4716
PROTON NMR SPECTRUM	CONFORMS TO STRUCTURE	CONFORMS



Dr. Reinhold Schwenninger
Quality Assurance
Buchs, Switzerland

Sigma-Aldrich warrants that at the time of the quality release or subsequent retest date this product conformed to the information contained in this publication. The current specification sheet may be available at Sigma-Aldrich.com. For further inquiries, please contact Technical Service. Purchaser must determine the suitability of the product for its particular use. See reverse side of invoice or packing slip for additional terms and conditions of sale.

SIGMA-ALDRICH3050 Spruce Street, Saint Louis, MO 63103 USA
Email USA: techserv@sigma.com Outside USA: eurtechserv@sigma.com**Certificate of Analysis****46262 1,1,2-Trichloroethane**

Lot Number: BCCB7564		Sample Name: 0000000671853_001_GC	
Shimadzu GC-2010 Plus			
Injector :	250 °C	GC Serial Number:	C11804802868
Detector (TCD):	280 °C	Injection Time:	19.07.19 20:57
Carrier Gas:	Helium	Sample Type:	unknown
Velocity:	35.0 cm/s	Channel:	GC_1
Temp. Control:	see Figure 1	Sequence Creation Time:	18.07.19 14:32
Split Ratio:	100:1	Sequence Created By:	Nicolas Dosch
Range:	-	Time Processed:	15.10.19 18:58
Injection Volume:	1.0 µl	Processed By:	Mikael Berthet
Vial Number:	2		
Run Time:	40.00 min		
Column:	Supelco SPB-624, 30 m x 0.32 mm, 1.8 µm		
Sample Prep.:	neat		

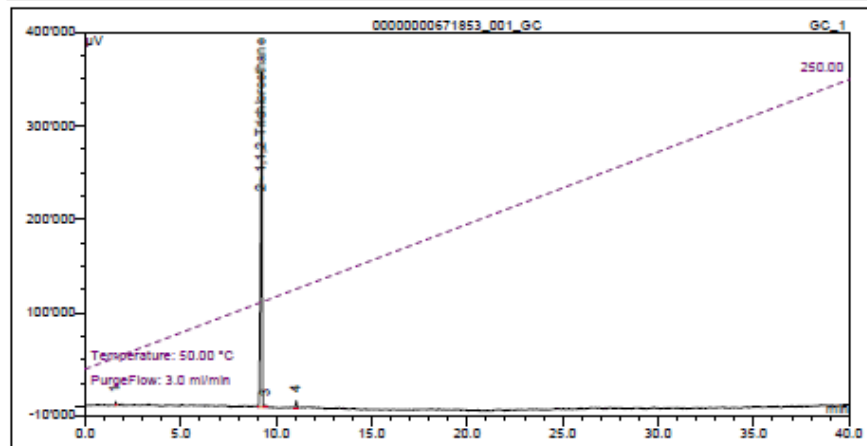


Figure 1: Zoomed Chromatogram and Temperature Program

No.	Ret.Time min	Peak Name	Type	Height µV	Area µV*min	Amount	Rel.Area %
1	1.600	n.a.	BMB*	3585.0000	99.6642	n.a.	0.271
2	9.245	1,1,2-Trichloroethane	BMB	358791.0000	36154.1400	n.a.	98.174
3	9.475	n.a.	BMB	1870.0000	99.5867	n.a.	0.270
4	11.052	n.a.	BMB	6749.0000	473.2525	n.a.	1.285
Total:				370995	36826.6433		100.000

Appendix 11.1: Certificates of Analysis and Radiochemical Purity Data (continued)

Radiolabeled testosterone



American
Radiolabeled
Chemicals, Inc.

101 ARC Dr.
St. Louis, MO 63146 U.S.A.
Ph. (314) 991-4545 or (800) 331-6661
Fax (314) 991-4692 or (800) 999-9925
Web: <http://www.arc-inc.com>
E-mail: arcinc@arc-inc.com

TECHNICAL DATA SHEET ARC 0858 Testosterone [$4\text{-}^{14}\text{C}$]

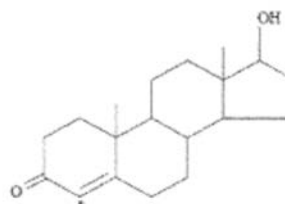
SPECIFIC LOT DATA:

Lot Number: 210415

Specific activity: 55 mCi/mmol

Solvent: EtOH

Concentration: 0.1 mCi/ml



M.W. 288.41

PACKAGING INFORMATION:

ARC 0858 S.A. 40-60 mCi/mmol. Packaged in solution in ethanol at 0.1mCi/ml in screw cap vials.

STABILITY AND STORAGE RECOMMENDATIONS:

The recommended storage temperature for ARC 0858 is 0-5°C. When testosterone [$4\text{-}^{14}\text{C}$] is stored at 0-5°C in its original solvent and at its original concentration, the rate of decomposition is approximately 1% for the first 12 months from the date of purification. Lot to lot variation may occur and it is advisable to check purity prior to use.

RADIOCHEMICAL AND CHEMICAL PURITY:

It was determined on 4/15/21 and found to be greater than 99 % by using the following method:

TLC (silica gel):

toluene:ethyl acetate (2:1)

HPLC:

Column: Zorbax SB-C18 (250 x 4.6mm) 5 μ

Solvent A: Water

Solvent B: Acetonitrile

Gradient: 10-100% B over 30 min

Flow Rate: 1ml/min

Detector A: β -Ram [^{14}C]

Detector B: UV at 260 nm

At the time of shipment all products are guaranteed to be free from defects in material and workmanship and to conform to the accompanying technical specifications and purity data. ARC will offer a 30 day money back guarantee of free replacement of products that are found to be unsatisfactory in respect to product specifications and purity. ARC makes no other warranty, expressed or implied, pertaining to the suitability of the product for any specific application. In case of breach of this warranty the entire liability of ARC will be limited to the invoice price of the goods. In no case will ARC be liable for any special, incidental or consequential damages resulting from the use of its products. ARC hereby expressly disclaims any warranty regarding results obtained through use of the products, including without limitation any claim of inaccurate, invalid, or incomplete results. Products are not suitable for human use.

Appendix 11.1: Certificates of Analysis and Radiochemical Purity Data (continued)

Non-radiolabeled testosterone

SIGMA-ALDRICH®

sigma-aldrich.com

3050 Spruce Street, Saint Louis, MO 63103, USA

Website: www.sigmaaldrich.comEmail USA: techserv@sial.comOutside USA: eurtechserv@sial.com**Certificate of Analysis**

Product Name:

Testosterone - $\geq 98\%$

Product Number:

T1500

Batch Number:

SLCF3521

Brand:

SIGMA

CAS Number:

58-22-0

MDL Number:

MFCD00003654

Formula:

C₁₉H₂₈O₂

Formula Weight:

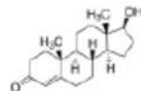
288.42 g/mol

Quality Release Date:

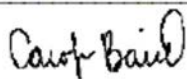
10 FEB 2020

Recommended Retest Date:

FEB 2023



Test	Specification	Result
Appearance (Color)	White to Off White	White
Appearance (Form)	Powder	Powder
Solubility (Color)	Colorless to Very Faint Yellow	Colorless
Solubility (Turbidity)	Clear	Clear
100 mg/mL, CHCl ₃		
¹ H NMR Spectrum	Conforms to Structure	Conforms
¹³ C NMR Spectrum	Conforms to Structure	Conforms
Purity (HPLC)	$\geq 98\%$	100 %



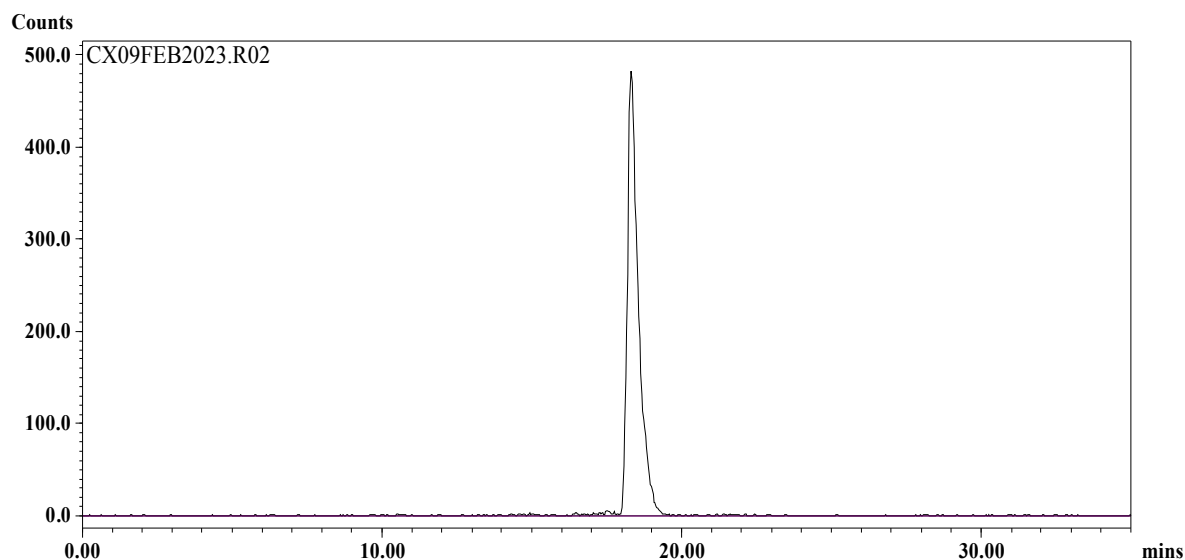
Carolyn Baird, Supervisor
Quality Assurance
St. Louis, Missouri US

Sigma-Aldrich warrants, that at the time of the quality release or subsequent retest date this product conformed to the information contained in this publication. The current Specification sheet may be available at Sigma-Aldrich.com. For further inquiries, please contact Technical Service. Purchaser must determine the suitability of the product for its particular use. See reverse side of invoice or packing slip for additional terms and conditions of sale.

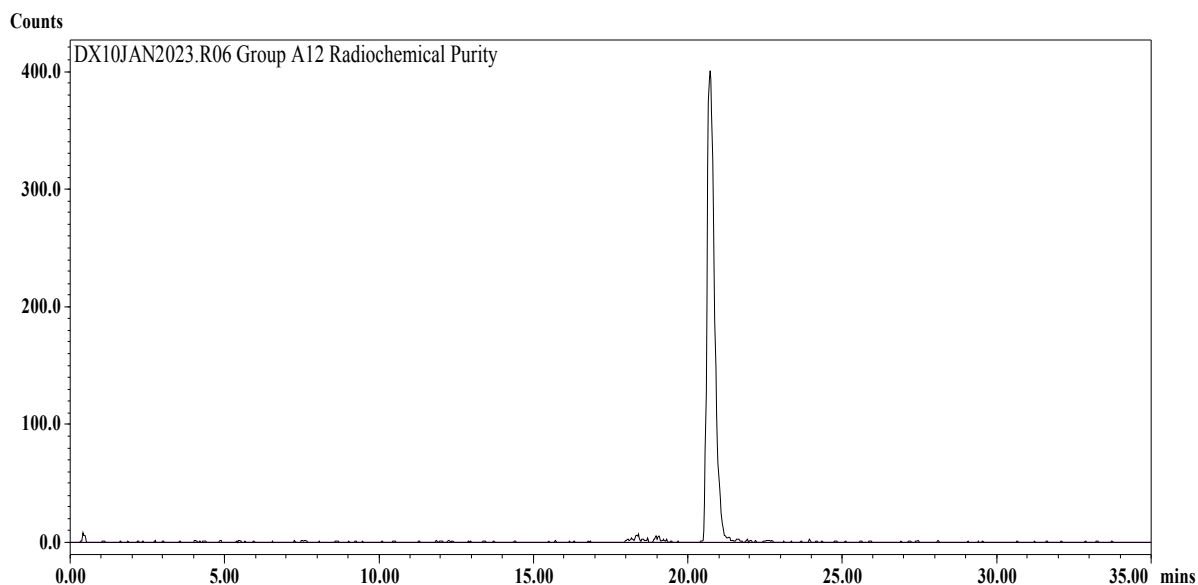
Appendix 11.1: Certificates of Analysis and Radiochemical Purity Data (continued)

Example radiochromatograms

1% Testosterone solution



10% 1,1,2-Trichloroethane in 1,2-Dichloroethane (Group A12)



Appendix 11.1: Certificates of Analysis and Radiochemical Purity Data (continued)

Phase	Group	Dose level (g/L)	Radiochemical purity (%) ^a
Testosterone reference (finite)	A1	1 mg/mL	97.7
Infinite	A2	Neat	95.6 (initial dosing) 95.6 (Cells 9R - 10R)
	A3	50% in IPM	95.1
	A4	10% in IPM	95.2 (initial) 96.2 (additional dosing for Cells 77 - 80)
	A5	50% in 1,2-Dichloroethane	96.0
	A6	10% in 1,2-Dichloroethane	96.4
	A7	Neat	95.6
Finite	A8	50% in IPM	96.3 (initial) 96.3 (repeat)
	A9	10% in IPM	97.3
	A10	1% in IPM	96.7
	A11	50% in 1,2-Dichloroethane	97.1
	A12	10% in 1,2-Dichloroethane	96.7
	A13	1% in 1,2-Dichloroethane	98.3

^a The radiochemical purity value is calculated from a mean of two analyses.

Appendix 11.2: Amount of [¹⁴C]-1,1,2-Trichloroethane dissolved in 6% polyoxyethylene 20 oleyl ether in water

	Aliquot activity (dpm)	Aliquot volume (mL)	Mean dpm/mL*	Actual concentration in receptor fluid (µg/mL)	Concentration target (µg/mL)	% Calculated solubility
Vessel 1	25066	0.25	100020	24775.8	25000	99.1
	24992	0.25				
Vessel 2	24813	0.25	100260	24835.3	25000	99.3
	25365	0.25				
Vessel 3	25021	0.25	100776	24963.1	25000	99.9
	25415	0.25				
			Mean	24858.1		99.4
			sd	95.7		0.4

* Calculated using a background value of 24 dpm

Specific activity of [¹⁴C]-1,1,2-Trichloroethane = 4.037 dpm/µg

sd Standard deviation

Appendix 11.3: Applied Dose

Group number	Dose level	Cell number	Achieved dose		
			(dpm)	(mg)	($\mu\text{g}/\text{cm}^2$)
A1	1 mg/mL	81	293847	0.007	0.011
		82	293147	0.007	0.011
		83	294607	0.007	0.011
		84	294267	0.007	0.011
		Mean (all cells)	293967	0.007	0.011

Group number	Dose level	Cell number	Achieved dose		
			(dpm)	(mg)	(mg/cm^2)
A2	Neat	5	15368832	87.8	137
		6	15368832	87.8	137
		7	15368832	87.8	137
		8	15368832	87.8	137
		9R	16676670	93.2	146
		<i>10R</i>	<i>16676670</i>	<i>93.2</i>	<i>146</i>
		Mean (all cells)	15804778	89.6	140
		Mean (reported cells)	15630400	88.9	139

Group number	Dose level	Cell number	Achieved dose		
			(dpm)	(mg)	(mg/cm^2)
A3	50% in IPM	11R	15789360	88.7	139
		12R	15789360	88.7	139
		13R	15789360	88.7	139
		14R	15789360	88.7	139
		15R	15789360	88.7	139
		16R	15789360	88.7	139
		Mean (all cells)	15789360	88.7	139

Data shown in italics was excluded from the mean calculation.

Appendix 11.3: Applied Dose (continued)

Group number	Dose level	Cell number	Achieved dose		
			(dpm)	(mg)	(mg/cm ²)
A4	10% in IPM	17R	15181247	85.3	133
		18R	15181247	85.3	133
		19R	15181247	85.3	133
		20R	15181247	85.3	133
		21R	15181247	85.3	133
		22R	15181247	85.3	133
		77	12333300	68.9	108
		78	12333300	68.9	108
		79	12333300	68.9	108
		80	12333300	68.9	108
		Mean (all cells)	14042068	78.7	123

Group number	Dose level	Cell number	Achieved dose		
			(dpm)	(mg)	(mg/cm ²)
A5	50% in 1,2-Dichloroethane	65	13378260	75.2	117
		66	13378260	75.2	117
		67	13378260	75.2	117
		68	13378260	75.2	117
		69	13378260	75.2	117
		70	13378260	75.2	117
		Mean (all cells)	13378260	75.2	117

Group number	Dose level	Cell number	Achieved dose		
			(dpm)	(mg)	(mg/cm ²)
A6	10% in 1,2-Dichloroethane	71	13692700	76.9	120
		72	13692700	76.9	120
		73	13692700	76.9	120
		74	13692700	76.9	120
		75	13692700	76.9	120
		76	13692700	76.9	120
		Mean (all cells)	13692700	76.9	120

Appendix 11.3: Applied Dose (continued)

Group number	Dose level	Cell number	Achieved dose		
			(dpm)	(mg)	(mg/cm ²)
A7	Neat	23	1611607	9.00	14.1
		24	1609907	8.99	14.1
		25	1605687	8.97	14.0
		26	1623007	9.07	14.2
		27	1577707	8.81	13.8
		28	1615207	9.02	14.1
		Mean (all cells)	1607187	8.98	14.0
		Mean (reported cells)	1604502	8.96	14.0

Group number	Dose level	Cell number	Achieved dose		
			(dpm)	(mg)	(mg/cm ²)
A8	50% in IPM	29R	739073	4.15	6.49
		30R	774833	4.35	6.80
		31R	731253	4.11	6.42
		32R	778473	4.37	6.83
		33R	786053	4.42	6.90
		34R	635313	3.57	5.58
		Mean (all cells)	740833	4.16	6.50
		Mean (reported cells)	731789	4.11	6.42

Group number	Dose level	Cell number	Achieved dose		
			(dpm)	(mg)	(mg/cm ²)
A9	10% in IPM	35	147433	0.828	1.29
		36	147513	0.829	1.30
		37	147373	0.828	1.29
		38	147553	0.829	1.30
		39	146453	0.823	1.29
		40	146893	0.825	1.29
		Mean (all cells)	147203	0.827	1.29
		Mean (reported cells)	147038	0.826	1.29

Data shown in italics is excluded from the mean calculation.

Appendix 11.3: Applied Dose (continued)

Group number	Dose level	Cell number	Achieved dose		
			(dpm)	(mg)	(mg/cm ²)
A10	1% in IPM	<i>41R</i>	<i>71636</i>	<i>0.085</i>	<i>0.133</i>
		42R	72036	0.086	0.134
		<i>43R</i>	<i>71616</i>	<i>0.085</i>	<i>0.133</i>
		44R	72016	0.086	0.134
		45R	71846	0.086	0.134
		46R	72246	0.086	0.134
		Mean (all cells)	71899	0.086	0.134
		Mean (reported cells)	72036	0.086	0.134

Group number	Dose level	Cell number	Achieved dose		
			(dpm)	(mg)	(mg/cm ²)
A11	50% in 1,2-Dichloroethane	47	765787	4.59	7.17
		48	763407	4.57	7.14
		49	757347	4.54	7.09
		50	762347	4.57	7.13
		51	758227	4.54	7.09
		52	767707	4.60	7.18
		Mean (all cells)	762470	4.57	7.13

Group number	Dose level	Cell number	Achieved dose		
			(dpm)	(mg)	(mg/cm ²)
A12	10% in 1,2-Dichloroethane	53	150513	0.901	1.41
		54	149953	0.898	1.40
		55	150013	0.898	1.40
		56	151473	0.907	1.42
		<i>57</i>	<i>150293</i>	<i>0.900</i>	<i>1.41</i>
		58	150493	0.901	1.41
		Mean (all cells)	150456	0.901	1.41
		Mean (reported cells)	150489	0.901	1.41

Data shown in italics is excluded from the mean calculation.

Appendix 11.3: Applied Dose (continued)

Group number	Dose level	Cell number	Achieved dose		
			(mg)	(mg/cm ²)	(mg)
A13	1% in 1,2-Dichloroethane	59	15957	0.091	0.142
		60	16107	0.092	0.144
		61	15977	0.091	0.142
		62	16027	0.091	0.142
		63	16037	0.091	0.142
		64	15947	0.091	0.142
		Mean (all cells)	16009	0.091	0.142

Appendix 11.4: TEWL Measurements

Group number	Dose level	Cell number	Skin source	TEWL measurement (g/m ² /h)	
				Pre dose ^a	24 hour ^c
A1	10 mg/cm ²	81	H4	12.0	12.9
		82	H14	8.5	4.2
		83	H8	8.3	4.5
		84	H15	11.7	9.9

Group number	Dose level	Cell number	Skin source	TEWL measurement (g/m ² /h)	
				Pre dose ^a	24 hour
A2	Neat	5	H1	12.3	8.5
		6	H1	9.3	7.8
		7	H2	11.6	8.8
		8	H2	9.5	5.5
		9R	H6	11.5	10.0
		10R	H5	12.0	27.9

Group number	Dose level	Cell number	Skin source	TEWL measurement (g/m ² /h)	
				Pre dose ^a	24 hour
A3	50% in IPM	11R	H3	10.5	5.6
		12R	H3	10.3	3.3
		13R	H4	6.7	7.2
		14R	H5	6.0	11.1
		15R	H6	10.0	3.3
		16R	H7	7.3	4.6

Group number	Dose level	Cell number	Skin source	TEWL measurement (g/m ² /h)	
				Pre dose ^a	24 hour
A4	10% in IPM	17R	H3	11.7	1.4 ^b
		18R	H5	9.0	1.0 ^b
		19R	H4	8.2	0.4 ^b
		20R	H5	8.3	0.1 ^b
		21R	H6	9.0	4.9
		22R	H7	9.2	1.4 ^b
		77	H13	8.9	10.4
		78	H11	12.4	6.3
		79	H10	10.9	8.9
		80	H12	8.4	12.1

^a Cells failing to meet the acceptance criteria following the initial measurement were allowed to air dry for a further period of time prior to additional measurements being taken. Cells failing to meet the membrane acceptance criteria following the second measurement were discarded and replaced.

^b TEWL readings were considered to be anomalously low in comparison to the other measurements obtained in the study indicating a possible equipment malfunction.

^c Mean of two readings.

Appendix 11.4: TEWL Measurements (continued)

Group number	Dose level	Cell number	Skin source	TEWL measurement (g/m ² /h)	
				Pre dose ^a	24 hour
A5	50% in 1,2-Dichloroethane	65	H5	9.0	9.0
		66	H9	7.6	7.0
		67	H11	9.6	9.8
		68	H11	12.7	8.6
		69	H8	8.4	7.0
		70	H10	8.7	11.9

Group number	Dose level	Cell number	Skin source	TEWL measurement (g/m ² /h)	
				Pre dose ^a	24 hour
A6	10% in 1,2-Dichloroethane	71	H5	5.3	12.4
		72	H11	8.0	10.8
		73	H11	11.1	11.6
		74	H8	12.4	5.1
		75	H10	3.3	10.3
		76	H12	7.1	6.7

Group number	Dose level	Cell number	Skin source	TEWL measurement (g/m ² /h)	
				Pre dose ^a	24 hour
A7	Neat	23	H3	11.5	9.5
		24	H4	9.0	61.0 ^b
		25	H4	7.6	9.1
		26	H5	8.3	7.7
		27	H6	11.2	9.2
		28	H7	11.6	11.3

Group number	Dose level	Cell number	Skin source	TEWL measurement (g/m ² /h)	
				Pre dose ^a	24 hour
A8	50% in IPM	29R	H10	6.8	8.3
		30R	H8	8.0	9.4
		31R	H9	9.9	9.3
		32R	H10	10.9	12.8
		33R	H6	10.6	15.8
		34R	H12	8.0	12.6

^a Cells failing to meet the acceptance criteria following the initial measurement were allowed to air dry for a further period of time prior to additional measurements being taken. Cells failing to meet the membrane acceptance criteria following the second measurement were discarded and replaced.

Appendix 11.4: TEWL Measurements (continued)

Group number	Dose level	Cell number	Skin source	TEWL measurement (g/m ² /h)	
				Pre dose ^a	24 hour
A9	10% in IPM	35	H8	11.8	9.5
		36	H6	12.3	15.1
		37	H9	12.4	12.4
		38 ^d	H11	13.5	18.1
		39	H8	6.9	9.6
		40	H4	9.3	12.2

Group number	Dose level	Cell number	Skin source	TEWL measurement (g/m ² /h)	
				Pre dose ^a	24 hour
A10	1% in IPM	41R	H6	7.8	16.0
		42R	H9	8.7	12.2
		43R	H11	12.7	22.6
		44R	H8	10.9	12.9
		45R	H10	11.2	11.2
		46R	H9	8.2	10.0

Group number	Dose level	Cell number	Skin source	TEWL measurement (g/m ² /h)	
				Pre dose ^a	24 hour
A11	50% in 1,2-Dichloroethane	47	H6	10.1	11.2
		48	H11	9.7	10.2
		49	H8	5.5	5.4
		50	H12	7.5	6.5
		51	H12	9.9	8.2
		52	H10	7.2	8.1

Group number	Dose level	Cell number	Skin source	TEWL measurement (g/m ² /h)	
				Pre dose ^a	24 hour
A12	10% in 1,2-Dichloroethane	53	H6	8.1	11.0
		54	H11	8.5	7.8
		55	H8	4.2	6.2
		56	H10	9.4	9.6
		57	H12	9.8	7.9
		58	H12	5.2	8.2

^a Cells failing to meet the acceptance criteria following the initial measurement were allowed to air dry for a further period of time prior to additional measurements being taken. Cells failing to meet the membrane acceptance criteria following the second measurement were discarded and replaced.

^d Replicate dosed in error as initial TEWL measurement did not meet the acceptance criteria. Data from this cell has been excluded.

Appendix 11.4: TEWL Measurements (continued)

Group number	Dose level	Cell number	Skin source	TEWL measurement (g/m ² /h)	
				Pre dose ^a	24 hour
A13	1% in 1,2-Dichloroethane	59	H6	7.8	9.9
		60	H11	7.6	9.1
		61	H8	3.7	5.5
		62	H10	7.9	9.0
		63	H12	5.2	7.1
		64	H12	5.9	6.2

^a Cells failing to meet the acceptance criteria following the initial measurement were allowed to air dry for a further period of time prior to additional measurements being taken. Cells failing to meet the membrane acceptance criteria following the second measurement were discarded and replaced.

Appendix 11.5: Reference Test Data

Table 1: Distribution of Radioactivity Following a Single Finite Application of [¹⁴C]-Testosterone at a Nominal 1 mg/mL to Dermatomed Human Skin (Group A1)

Cell number	Skin source	Dose in receptor fluid (0-24 h)	Dose on receptor chamber	Total absorbed dose ^a	Dose in exposed skin	Dermal Delivery ^b	Dose in stratum corneum	Dislodgeable dose (Skin wash, 8 h)	Dislodgeable dose (Skin wash, 24 h)	Dose on donor chamber	Total dislodgeable dose ^c	Dose on surface tape strips (1 - 2)	Total unabsorbed dose ^d	Mass balance	Maximum absorption rate (µg/cm ² /hr)
81	H4	15.68	nd	15.68	0.10	15.78	0.77	73.56	1.82	nd	75.38	0.22	75.60	92.15	0.718
82	H14	4.53	nd	4.53	3.27	7.80	1.73	71.34	9.36	1.59	82.29	2.26	84.55	94.08	0.077
83	H8	7.08	nd	7.08	0.90	7.98	0.41	82.35	1.96	nd	84.31	0.23	84.54	92.93	0.212
84	H15	12.61	nd	12.61	3.62	16.23	1.47	69.54	5.95	nd	75.49	1.26	76.75	94.45	0.339
	Mean	9.98	nd	9.98	1.97	11.95	1.10	74.20	4.77	0.40	79.37	0.99	80.36	93.40	0.337
	sd	5.08	-	5.08	1.74	4.69	0.61	5.68	3.61	0.80	4.62	0.98	4.86	1.06	0.276
	cv	50.90	-	50.90	88.32	39.25	55.45	7.65	75.68	200.00	5.82	98.99	6.05	1.13	81.99

Results expressed as percent applied radiochemical dose

nd Not detected

sd Standard deviation

cv Coefficient of variance

- Not applicable

^a Sum of radioactivity detected in the receptor fluids and receptor chamber

^b Sum of absorbed dose and radioactivity in exposed skin

^c Sum of radioactivity detected in the skin wash (8h and 24h) and donor chamber

^d Sum of total dislodgeable dose and radioactivity in the charcoal trap and on the surface tape strips.

Values below the limit of detection (nd) are regarded as being equal to zero for the calculation of mean and standard deviation

Appendix 11.5: Reference Test Data (continued)**Table 2: Distribution of Radioactivity in the Tape Strips Following a Single Application of [¹⁴C]-Testosterone at a Nominal 1 mg/mL to Dermatomed Human Skin (Group A1)**

Results are expressed as percent applied radiochemical dose

Cell Number		81	82	83	84	Mean	sd
Tape strip	1	0.11	1.42	0.10	0.69	0.58	0.62
	2	0.11	0.84	0.13	0.57	0.41	0.36
Total in tape surface		0.22	2.26	0.23	1.26	0.99	0.98
Tape strip	3	0.60	0.70	0.15	0.42	0.47	0.24
	4	0.17	0.40	0.17	0.44	0.30	0.15
	5	ns	0.63	0.09	0.61	0.44	0.31
Total in stratum corneum		0.77	1.73	0.41	1.47	1.10	0.61

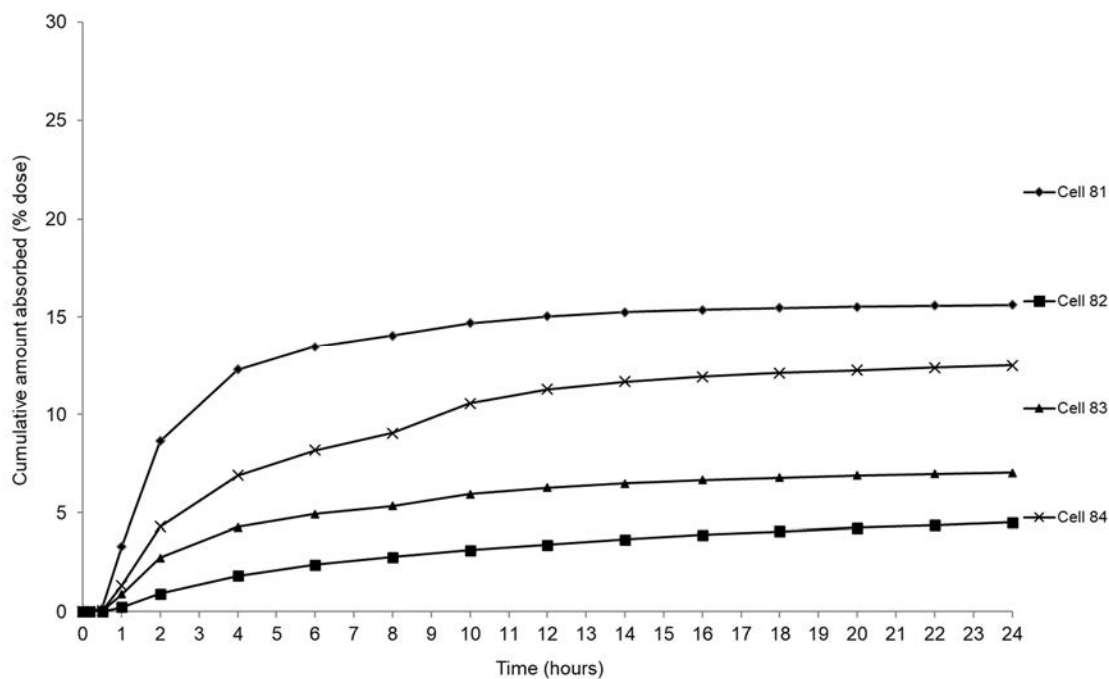
Results expressed as percent applied radiochemical dose

sd Standard deviation

ns No sample (stratum corneum removed)

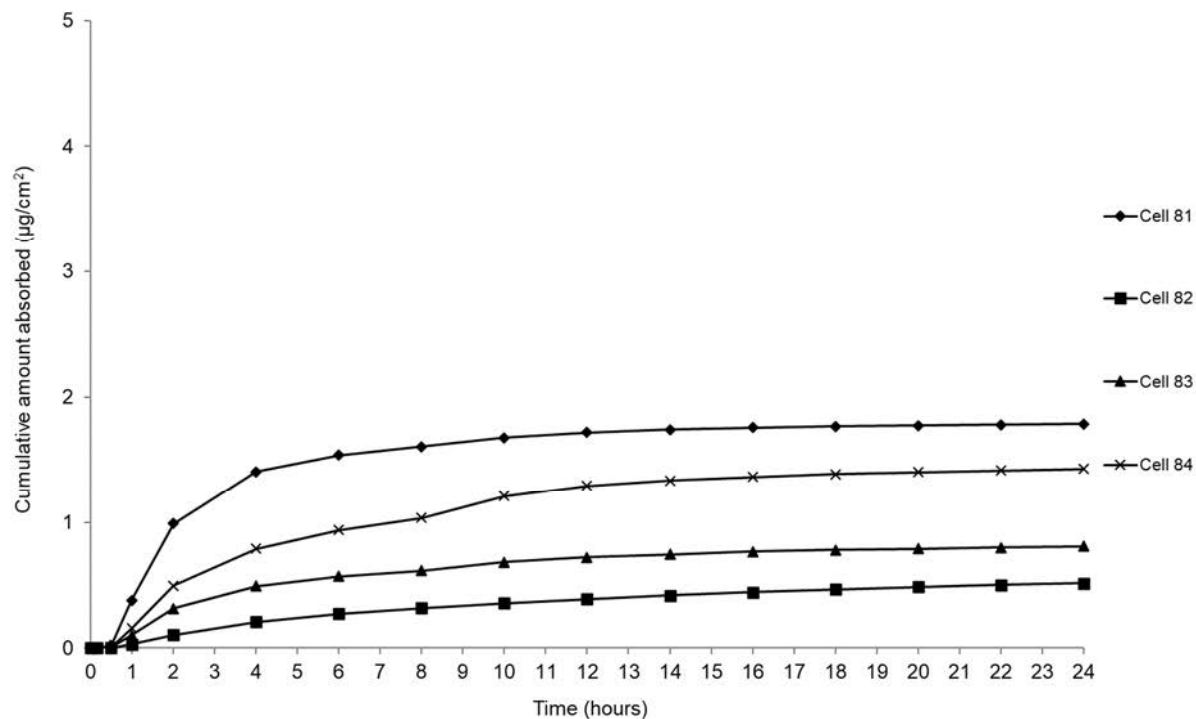
Appendix 11.5: Reference Test Data (continued)**Figure 1: Amount of Radiolabeled Material Absorbed Following a Single Application of [^{14}C]-Testosterone at a Nominal 1 mg/mL to Dermatomed Human Skin (Group A1)**

Results are expressed as cumulative percent applied dose



Appendix 11.5: Reference Test Data (continued)**Figure 2: Amount of Radiolabeled Material Absorbed Following a Single Application of [¹⁴C]-Testosterone at a Nominal 1 mg/mL to Human Skin (Group A1)**

Results are expressed as cumulative μg equivalents testosterone/cm²



Appendix 11.6: Infinite Experiment Individual Data

Neat 1,1,2-Trichloroethane (Group A2)

Cell Number	Kp ($\times 10^{-3}$ cm/hr)	Steady state time range (hours)	Maximum absorption rate ($\mu\text{g}/\text{cm}^2/\text{hr}$)	Maximum absorption time range (hours)
5	2.46	4 – 16	388	4 – 6
6	4.85	4 – 14	706	4 – 6
7	0.62	4 – 24	131	2 – 4
8	3.47	4 – 14	496	4 – 6
9R	5.26	4 – 12	912	2 – 4
mean	3.33	-	527	-
sd	1.88	-	299	-

50% 1,1,2-Trichloroethane in IPM (Group A3)

Cell Number	Kp ($\times 10^{-3}$ cm/hr)	Steady state time range (hours)	Maximum absorption rate ($\mu\text{g}/\text{cm}^2/\text{hr}$)	Maximum absorption time range (hours)
11R	2.67	1 – 24	542	4 – 6
12R	3.09	1 – 24	463	10 – 12
13R	3.53	1 – 24	641	2 – 4
14R	1.59	2 – 24	256	16 – 18
15R	5.78	1 – 24	1050	2 – 4
16R	3.73	1 – 24	962	1 – 2
mean	3.40	-	652	-
sd	1.39	-	303	-

10% 1,1,2-Trichloroethane in IPM (Group A4)

Cell Number	Kp ($\times 10^{-3}$ cm/hr)	Steady state time range (hours)	Maximum absorption rate ($\mu\text{g}/\text{cm}^2/\text{hr}$)	Maximum absorption time range (hours)
17R	0.74	2 – 24	76.5	20 – 22
18R	1.15	2 – 24	70.5	16 – 18
19R	0.91	2 – 24	87.8	18 – 20
20R	0.70	2 – 24	98.8	18 – 20
21R	0.84	2 – 24	123	20 – 22
22R	0.75	2 – 24	109	16 – 18
77	0.92	1 – 24	123	6 – 8
78	1.43	1 – 24	192	4 – 6
79	1.13	1 – 24	148	6 – 8
80	0.87	4 – 24	101	14 – 16
mean	0.94	-	113	-
sd	0.23	-	36.2	-

sd Standard deviation
 - Not applicable

Appendix 11.6: Infinite Experiment Individual Data (continued)

50% 1,1,2-Trichloroethane in 1,2-Dichloroethane (Group A5)

Cell Number	Kp ($\times 10^{-3}$ cm/hr)	Steady state time range (hours)	Maximum absorption rate ($\mu\text{g}/\text{cm}^2/\text{hr}$)	Maximum absorption time range (hours)
65	4.11	1 – 14	515	6 – 8
66	2.93	4 – 8	350	6 – 8
67	5.71	1 – 24	734	1 – 2
68	6.10	1 – 24	769	14 – 16
69	4.03	2 – 10	503	2 – 4
70	4.08	2 – 14	500	8 – 10
mean	4.49	-	562	-
sd	1.19	-	159	-

10% 1,1,2-Trichloroethane in 1,2-Dichloroethane (Group A6)

Cell Number	Kp ($\times 10^{-3}$ cm/hr)	Steady state time range (hours)	Maximum absorption rate ($\mu\text{g}/\text{cm}^2/\text{hr}$)	Maximum absorption time range (hours)
71	0.64	8 – 18	114	4 – 6
72	1.07	8 – 24	196	4 – 6
73	1.12	6 – 24	224	2 – 4
74	0.64	6 – 24	98.5	6 – 8
75	0.93	8 – 24	167	4 – 6
76	0.74	10 – 24	133	6 – 8
mean	0.86	-	155	-
sd	0.21	-	49.0	-

sd Standard deviation
 - Not applicable

Appendix 11.7: Finite Experiment Individual Skin Distribution Data

Table 1: Distribution of Radioactivity Following a Single Finite Application of Neat [^{14}C]-1,1,2-Trichloroethane to Dermatomed Human Skin (Group A7, all cells)

Cell number	Skin source	Dose in receptor fluid	Dose on receptor chamber	Total absorbed dose ^a	Dose in exposed skin	Dermal Delivery ^b	Dose in stratum corneum	Dislodgeable dose (Skin wash, 8 h)	Dislodgeable dose (Skin wash, 24 h)	Dose on donor chamber	Total dislodgeable dose ^c	Dose in trap extract	Dose on surface tape strips (1 - 2)	Total unabsorbed dose ^d	Mass balance
23	H3	0.41	nd	0.41	0.05	0.46	nd	0.29	0.05	nd	0.34	91.60	nd	91.94	92.40
24	H4	9.71	nd	9.71	0.01	9.72	0.02	0.29	0.00	nd	0.29	86.33	nd	86.62	96.36
25	H4	0.34	nd	0.34	0.02	0.36	0.01	0.41	0.02	nd	0.43	103.27	0.01	103.71	104.08
26	H5	0.40	nd	0.40	0.13	0.53	nd	0.25	0.03	nd	0.28	98.05	nd	98.33	98.86
27	H6	0.42	nd	0.42	0.04	0.46	nd	0.30	0.04	nd	0.34	90.75	nd	91.09	91.55
28	H7	0.89	nd	0.89	0.13	1.02	nd	0.34	0.03	nd	0.37	73.02	nd	73.39	74.41
	Mean	2.03	nd	2.03	0.06	2.09	0.01	0.31	0.03	nd	0.34	90.50	<0.01	90.85	92.94
	sd	3.77	-	3.77	0.05	3.74	0.01	0.06	0.02	-	0.05	10.43	-	10.44	10.16
	cv	185.71	-	185.71	83.33	178.95	100.00	19.35	66.67	-	14.71	11.52	-	11.49	10.93

Results expressed as percent applied radiochemical dose

nd Not detected

sd Standard deviation

cv Coefficient of variance

- Not applicable

^a Sum of radioactivity detected in the receptor fluids and receptor chamber

^b Sum of absorbed dose and radioactivity in exposed skin

^c Sum of radioactivity detected in the skin wash (8h and 24h) and donor chamber

^d Sum of total dislodgeable dose, radioactivity in the charcoal trap and on the surface tape strips.

Values below the limit of detection (nd) are regarded as being equal to zero for the calculation of mean and standard deviation

Appendix 11.7: Finite Experiment Individual Data (continued)

Table 2: Distribution of Radioactivity Following a Single Finite Application of Neat [^{14}C]-1,1,2-Trichloroethane to Dermatomed Human Skin (Group A7, reported cells)

Cell number	Skin source	Dose in receptor fluid	Dose on receptor chamber	Total absorbed dose ^a	Dose in exposed skin	Dermal Delivery ^b	Dose in stratum corneum	Dislodgeable dose (Skin wash, 8 h)	Dislodgeable dose (Skin wash, 24 h)	Dose on donor chamber	Total dislodgeable dose ^c	Dose in trap extract	Dose on surface tape strips (1 - 2)	Total unabsorbed dose ^d	Mass balance
23	H3	0.41	nd	0.41	0.05	0.46	nd	0.29	0.05	nd	0.34	91.60	nd	91.94	92.40
25	H4	0.34	nd	0.34	0.02	0.36	0.01	0.41	0.02	nd	0.43	103.27	0.01	103.71	104.08
26	H5	0.40	nd	0.40	0.13	0.53	nd	0.25	0.03	nd	0.28	98.05	nd	98.33	98.86
27	H6	0.42	nd	0.42	0.04	0.46	nd	0.30	0.04	nd	0.34	90.75	nd	91.09	91.55
	Mean	0.39	nd	0.39	0.06	0.45	<0.01	0.31	0.04	nd	0.35	95.92	<0.01	96.27	96.72
	sd	0.04	-	0.04	0.05	0.07	0.01	0.07	0.01	-	0.06	5.89	0.01	5.92	5.89
	cv	10.26	-	10.26	83.33	15.56	-	22.58	25.00	-	17.14	6.14	-	6.15	6.09

Results expressed as percent applied radiochemical dose

nd Not detected

sd Standard deviation

cv Coefficient of variance

- Not applicable

^a Sum of radioactivity detected in the receptor fluids and receptor chamber

^b Sum of absorbed dose and radioactivity in exposed skin

^c Sum of radioactivity detected in the skin wash (8h and 24h) and donor chamber

^d Sum of total dislodgeable dose, radioactivity in the charcoal trap and on the surface tape strips.

Values below the limit of detection (nd) are regarded as being equal to zero for the calculation of mean and standard deviation

Appendix 11.7: Finite Experiment Individual Data (continued)

Table 3: Distribution of Radioactivity Following a Single Finite Application of 50% [¹⁴C]-1,1,2-Trichloroethane in IPM to Dermatomed Human Skin (Group A8, all cells)

Cell number	Skin source	Dose in receptor fluid	Dose on receptor chamber	Total absorbed dose ^a	Dose in exposed skin	Dermal Delivery ^b	Dose in stratum corneum	Dislodgeable dose (Skin wash, 8 h)	Dislodgeable dose (Skin wash, 24 h)	Dose on donor chamber	Total dislodgeable dose ^c	Dose in trap extract	Dose on surface tape strips (1 - 2)	Total unabsorbed dose ^d	Mass balance
29R	H10	0.17	nd	0.17	0.06	0.23	nd	0.31	0.10	nd	0.41	98.98	nd	99.39	99.62
30R	H8	0.07	nd	0.07	0.04	0.11	nd	0.40	0.09	nd	0.49	99.52	nd	100.01	100.12
31R	H9	0.09	nd	0.09	0.07	0.16	nd	0.36	0.07	nd	0.43	97.42	nd	97.85	98.01
32R	H10	0.16	nd	0.16	0.10	0.26	nd	0.32	0.07	nd	0.39	100.01	nd	100.40	100.66
33R	H6	0.34	nd	0.34	0.17	0.51	nd	0.30	nd	0.07	0.37	77.49	0.03	77.89	78.40
34R	H12	0.37	0.07	0.44	nd	0.44	nd	0.82	0.23	0.06	1.11	100.21	nd	101.32	101.76
	Mean	0.20	0.01	0.21	0.07	0.29	nd	0.42	0.09	0.02	0.53	95.61	0.01	96.14	96.43
	sd	0.13	0.03	0.15	0.06	0.16	-	0.2	0.08	0.03	0.29	8.93	0.01	9.02	8.92
	cv	65.00	300.00	71.43	85.71	55.17	-	47.62	88.89	150.00	54.72	9.34	100.00	9.38	9.25

Results expressed as percent applied radiochemical dose

nd Not detected

sd Standard deviation

cv Coefficient of variance

- Not applicable

^a Sum of radioactivity detected in the receptor fluids and receptor chamber

^b Sum of absorbed dose and radioactivity in exposed skin

^c Sum of radioactivity detected in the skin wash (8h and 24h) and donor chamber

^d Sum of total dislodgeable dose, radioactivity in the charcoal trap and on the surface tape strips.

Values below the limit of detection (nd) are regarded as being equal to zero for the calculation of mean and standard deviation

Appendix 11.7: Finite Experiment Individual Data (continued)

Table 4: Distribution of Radioactivity Following a Single Finite Application of 50% [¹⁴C]-1,1,2-Trichloroethane in IPM to Dermatomed Human Skin (Group A8, reported cells)

Cell number	Skin source	Dose in receptor fluid	Dose on receptor chamber	Total absorbed dose ^a	Dose in exposed skin	Dermal Delivery ^b	Dose in stratum corneum	Dislodgeable dose (Skin wash, 8 h)	Dislodgeable dose (Skin wash, 24 h)	Dose on donor chamber	Total dislodgeable dose ^c	Dose in trap extract	Dose on surface tape strips (1 - 2)	Total unabsorbed dose ^d	Mass balance
29R	H10	0.17	nd	0.17	0.06	0.23	nd	0.31	0.10	nd	0.41	98.98	nd	99.39	99.62
30R	H8	0.07	nd	0.07	0.04	0.11	nd	0.40	0.09	nd	0.49	99.52	nd	100.01	100.12
31R	H9	0.09	nd	0.09	0.07	0.16	nd	0.36	0.07	nd	0.43	97.42	nd	97.85	98.01
32R	H10	0.16	nd	0.16	0.10	0.26	nd	0.32	0.07	nd	0.39	100.01	nd	100.40	100.66
34R	H12	0.37	0.07	0.44	nd	0.44	nd	0.82	0.23	0.06	1.11	100.21	nd	101.32	101.76
	Mean	0.17	0.01	0.19	0.05	0.24	nd	0.44	0.11	0.01	0.57	99.23	nd	99.79	100.03
	sd	0.12	0.03	0.15	0.04	0.13	-	0.21	0.07	0.03	0.31	1.12	-	1.29	1.38
	cv	70.59	300.00	78.95	80.00	54.17	-	47.73	63.64	300.00	54.39	1.13	-	1.29	1.38

Results expressed as percent applied radiochemical dose

nd Not detected

sd Standard deviation

cv Coefficient of variance

- Not applicable

^a Sum of radioactivity detected in the receptor fluids and receptor chamber

^b Sum of absorbed dose and radioactivity in exposed skin

^c Sum of radioactivity detected in the skin wash (8h and 24h) and donor chamber

^d Sum of total dislodgeable dose, radioactivity in the charcoal trap and on the surface tape strips.

Values below the limit of detection (nd) are regarded as being equal to zero for the calculation of mean and standard deviation

Appendix 11.7: Test Formulation Skin Distribution Data (continued)

Table 5: Distribution of Radioactivity Following a Single Finite Application of 10% [¹⁴C]-1,1,2-Trichloroethane in IPM to Dermatomed Human Skin (Group A9, all cells)

Cell number	Skin source	Dose in receptor fluid	Dose on receptor chamber	Total absorbed dose ^a	Dose in exposed skin	Dermal Delivery ^b	Dose in stratum corneum	Dislodgeable dose (Skin wash, 8 h)	Dislodgeable dose (Skin wash, 24 h)	Dose on donor chamber	Total dislodgeable dose ^c	Dose in trap extract	Dose on surface tape strips (1 - 2)	Total unabsorbed dose ^d	Mass balance
35	H8	0.04	nd	0.04	nd	0.04	nd	0.53	nd	nd	0.53	95.20	nd	95.73	95.77
36	H6	0.09	nd	0.09	nd	0.09	nd	0.72	nd	nd	0.72	91.14	nd	91.86	91.95
37	H9	nd	nd	nd	nd	nd	nd	0.59	nd	nd	0.59	99.50	nd	100.09	100.09
38	H11	0.26	nd	0.26	nd	0.26	nd	0.67	nd	nd	0.67	93.96	nd	94.63	94.89
39	H8	0.02	nd	0.02	nd	0.02	nd	0.51	nd	nd	0.51	90.04	nd	90.55	90.57
40	H4	0.02	nd	0.02	nd	0.02	nd	0.54	0.07	nd	0.61	94.27	nd	94.88	94.90
	Mean	0.07	nd	0.07	nd	0.07	nd	0.59	0.01	nd	0.61	94.02	nd	94.62	94.70
	sd	0.10	-	0.10	-	0.10	-	0.08	0.03	-	0.08	3.34	-	3.33	3.31
	cv	142.86	-	142.86	-	142.86	-	13.56	300.00	-	13.11	3.55	-	3.52	3.50

Results expressed as percent applied radiochemical dose

nd Not detected

sd Standard deviation

cv Coefficient of variance

- Not applicable

^a Sum of radioactivity detected in the receptor fluids and receptor chamber

^b Sum of absorbed dose and radioactivity in exposed skin

^c Sum of radioactivity detected in the skin wash (8h and 24h) and donor chamber

^d Sum of total dislodgeable dose, radioactivity in the charcoal trap and on the surface tape strips.

Values below the limit of detection (nd) are regarded as being equal to zero for the calculation of mean and standard deviation

Appendix 11.7: Test Formulation Skin Distribution Data (continued)**Table 6: Distribution of Radioactivity Following a Single Finite Application of 10% [¹⁴C]-1,1,2-Trichloroethane in IPM to Dermatomed Human Skin (Group A9, reported cells)**

Cell number	Skin source	Dose in receptor fluid	Dose on receptor chamber	Total absorbed dose ^a	Dose in exposed skin	Dermal Delivery ^b	Dose in stratum corneum	Dislodgeable dose (Skin wash, 8 h)	Dislodgeable dose (Skin wash, 24 h)	Dose on donor chamber	Total dislodgeable dose ^c	Dose in trap extract	Dose on surface tape strips (1 - 2)	Total unabsorbed dose ^d	Mass balance
35	H8	0.04	nd	0.04	nd	0.04	nd	0.53	nd	nd	0.53	95.20	nd	95.73	95.77
37	H9	nd	nd	nd	nd	nd	nd	0.59	nd	nd	0.59	99.50	nd	100.09	100.09
39	H8	0.02	nd	0.02	nd	0.02	nd	0.51	nd	nd	0.61	90.04	nd	90.55	90.57
40	H4	0.02	nd	0.02	nd	0.02	nd	0.54	0.07	nd	0.54	94.27	nd	94.81	94.90
	Mean	0.02	nd	0.02	nd	0.02	nd	0.54	0.02	nd	0.56	94.75	nd	95.31	95.33
	sd	0.02	-	0.02	-	0.02	-	0.03	0.04	-	0.05	3.88	-	3.91	3.90
	cv	100.00	-	100.00	-	100.00	-	5.56	200.00	-	8.93	4.09	-	4.10	4.09

Results expressed as percent applied radiochemical dose

nd Not detected

sd Standard deviation

cv Coefficient of variance

- Not applicable

^a Sum of radioactivity detected in the receptor fluids and receptor chamber^b Sum of absorbed dose and radioactivity in exposed skin^c Sum of radioactivity detected in the skin wash (8h and 24h) and donor chamber^d Sum of total dislodgeable dose, radioactivity in the charcoal trap and on the surface tape strips.

Values below the limit of detection (nd) are regarded as being equal to zero for the calculation of mean and standard deviation

Appendix 11.7: Test Formulation Skin Distribution Data (continued)**Table 7: Distribution of Radioactivity Following a Single Finite Application of 1% [¹⁴C]-1,1,2-Trichloroethane in IPM to Dermatomed Human Skin (Group 10, all cells)**

Cell number	Skin source	Dose in receptor fluid	Dose on receptor chamber	Total absorbed dose ^a	Dose in exposed skin	Dermal Delivery ^b	Dose in stratum corneum	Dislodgeable dose (Skin wash, 8 h)	Dislodgeable dose (Skin wash, 24 h)	Dose on donor chamber	Total dislodgeable dose ^c	Dose in trap extract	Dose on surface tape strips (1 - 2)	Total unabsorbed dose ^d	Mass balance
41R	H6	nd	nd	nd	nd	nd	nd	0.72	nd	nd	0.72	107.59	nd	108.31	108.31
42R	H9	nd	nd	nd	nd	nd	nd	0.79	nd	nd	0.79	109.56	nd	110.35	110.35
43R	H11	0.20	nd	0.20	nd	0.20	nd	0.59	nd	nd	0.59	107.24	nd	107.83	108.03
44R	H8	nd	nd	nd	nd	nd	nd	0.43	0.04	nd	0.47	106.17	nd	106.64	106.64
45R	H10	nd	nd	nd	nd	nd	nd	0.77	nd	nd	0.77	101.61	nd	102.38	102.38
46R	H9	nd	nd	nd	nd	nd	nd	0.81	nd	nd	0.81	101.53	nd	102.34	102.34
	Mean	0.03	nd	0.03	nd	0.03	nd	0.69	0.01	nd	0.69	105.62	nd	106.31	106.34
	sd	0.08	-	0.08	-	0.08	-	0.15	0.02	-	0.13	3.32	-	3.28	3.30
	cv	266.67	-	266.67	-	266.67	-	21.74	200.00	-	18.84	3.14	-	3.09	3.10

Results expressed as percent applied radiochemical dose

nd Not detected

sd Standard deviation

cv Coefficient of variance

- Not applicable

^a Sum of radioactivity detected in the receptor fluids and receptor chamber^b Sum of absorbed dose and radioactivity in exposed skin^c Sum of radioactivity detected in the skin wash (8h and 24h) and donor chamber^d Sum of total dislodgeable dose, radioactivity in the charcoal trap and on the surface tape strips.

Values below the limit of detection (nd) are regarded as being equal to zero for the calculation of mean and standard deviation

Appendix 11.7: Test Formulation Skin Distribution Data (continued)**Table 8: Distribution of Radioactivity Following a Single Finite Application of 1% [¹⁴C]-1,1,2-Trichloroethane in IPM to Dermatomed Human Skin (Group A10, reported cells)**

Cell number	Skin source	Dose in receptor fluid	Dose on receptor chamber	Total absorbed dose ^a	Dose in exposed skin	Dermal Delivery ^b	Dose in stratum corneum	Dislodgeable dose (Skin wash, 8 h)	Dislodgeable dose (Skin wash, 24 h)	Dose on donor chamber	Total dislodgeable dose ^c	Dose in trap extract	Dose on surface tape strips (1 - 2)	Total unabsorbed dose ^d	Mass balance
42R	H9	nd	nd	nd	nd	nd	nd	0.79	nd	nd	0.79	109.56	nd	110.35	110.35
44R	H8	nd	nd	nd	nd	nd	nd	0.43	0.04	nd	0.47	106.17	nd	106.64	106.64
45R	H10	nd	nd	nd	nd	nd	nd	0.77	nd	nd	0.77	101.61	nd	102.38	102.38
46R	H9	nd	nd	nd	nd	nd	nd	0.81	nd	nd	0.81	101.53	nd	102.34	102.34
	Mean	nd	nd	nd	nd	nd	nd	0.70	0.01	nd	0.71	104.72	nd	105.43	105.43
	sd	-	-	-	-	-	-	0.18	0.02	-	0.16	3.89	-	3.85	3.85
	cv	-	-	-	-	-	-	25.71	200.00	-	22.54	3.71	-	3.65	3.65

Results expressed as percent applied radiochemical dose

nd Not detected

sd Standard deviation

cv Coefficient of variance

- Not applicable

^a Sum of radioactivity detected in the receptor fluids and receptor chamber^b Sum of absorbed dose and radioactivity in exposed skin^c Sum of radioactivity detected in the skin wash (8h and 24h) and donor chamber^d Sum of total dislodgeable dose, radioactivity in the charcoal trap and on the surface tape strips.

Values below the limit of detection (nd) are regarded as being equal to zero for the calculation of mean and standard deviation

Appendix 11.7: Test Formulation Skin Distribution Data (continued)

Table 9: Distribution of Radioactivity Following a Single Finite Application of 50% [¹⁴C]-1,1,2-Trichloroethane in 1,2 Dichloroethane to Dermatomed Human Skin (Group A11, all cells)

Cell number	Skin source	Dose in receptor fluid	Dose on receptor chamber	Total absorbed dose ^a	Dose in exposed skin	Dermal Delivery ^b	Dose in stratum corneum	Dislodgeable dose (Skin wash, 8 h)	Dislodgeable dose (Skin wash, 24 h)	Dose on donor chamber	Total dislodgeable dose ^c	Dose in trap extract	Dose on surface tape strips (1 - 2)	Total unabsorbed dose ^d	Mass balance
47	H6	0.69	nd	0.69	nd	0.69	nd	0.72	0.05	nd	0.77	92.53	nd	93.30	93.99
48	H11	0.43	nd	0.43	nd	0.43	nd	0.81	nd	nd	0.81	89.61	nd	90.42	90.85
49	H8	0.33	nd	0.33	nd	0.33	nd	0.57	0.03	nd	0.60	91.11	nd	91.71	92.04
50	H12	0.60	nd	0.60	nd	0.60	nd	0.74	nd	nd	0.74	93.84	nd	94.58	95.18
51	H12	0.89	nd	0.89	nd	0.89	nd	0.72	nd	nd	0.72	93.64	nd	94.36	95.25
52	H10	0.59	nd	0.59	nd	0.59	nd	0.56	nd	nd	0.56	92.80	nd	93.36	93.95
	Mean	0.59	nd	0.59	nd	0.59	nd	0.69	0.01	nd	0.70	92.26	nd	92.96	93.54
	sd	0.20	-	0.20	-	0.20	-	0.10	0.02	-	0.10	1.62	-	1.60	1.76
	cv	33.90	-	33.90	-	33.90	-	14.49	200.00	-	14.29	1.76	-	1.72	1.88

Results expressed as percent applied radiochemical dose

nd Not detected

sd Standard deviation

cv Coefficient of variance

- Not applicable

^a Sum of radioactivity detected in the receptor fluids and receptor chamber

^b Sum of absorbed dose and radioactivity in exposed skin

^c Sum of radioactivity detected in the skin wash (8h and 24h) and donor chamber

^d Sum of total dislodgeable dose, radioactivity in the charcoal trap and on the surface tape strips.

Values below the limit of detection (nd) are regarded as being equal to zero for the calculation of mean and standard deviation

Appendix 11.7: Test Formulation Skin Distribution Data (continued)

Table 10: Distribution of Radioactivity Following a Single Finite Application of 10% [¹⁴C]-1,1,2-Trichloroethane in 1,2 Dichloroethane to Dermatomed Human Skin (Group A12, all cells)

Cell number	Skin source	Dose in receptor fluid	Dose on receptor chamber	Total absorbed dose ^a	Dose in exposed skin	Dermal Delivery ^b	Dose in stratum corneum	Dislodgeable dose (Skin wash, 8 h)	Dislodgeable dose (Skin wash, 24 h)	Dose on donor chamber	Total dislodgeable dose ^c	Dose in trap extract	Dose on surface tape strips (1 - 2)	Total unabsorbed dose ^d	Mass balance
53	H6	0.49	nd	0.49	nd	0.49	nd	0.95	nd	nd	0.95	93.93	nd	94.88	95.37
54	H11	0.38	nd	0.38	nd	0.38	nd	0.90	nd	nd	0.90	92.58	nd	93.48	93.86
55	H8	0.31	nd	0.31	nd	0.31	nd	0.66	nd	nd	0.66	96.49	nd	97.15	97.46
56	H10	0.46	nd	0.46	nd	0.46	nd	1.12	nd	nd	1.12	93.89	nd	95.01	95.47
57	H12	0.59	nd	0.59	nd	0.59	nd	1.32	nd	nd	1.32	69.47	nd	70.79	71.38
58	H12	0.37	nd	0.37	nd	0.37	nd	1.22	nd	nd	1.22	94.73	nd	95.95	96.32
	Mean	0.43	nd	0.43	nd	0.43	nd	1.03	nd	nd	1.03	90.18	nd	91.21	91.64
	sd	0.10	-	0.10	-	0.10	-	0.24	-	-	0.24	10.23	-	10.08	10.00
	cv	23.26	-	23.26	-	23.26	-	23.30	-	-	23.30	11.34	-	11.05	10.91

Results expressed as percent applied radiochemical dose

nd Not detected

sd Standard deviation

cv Coefficient of variance

- Not applicable

^a Sum of radioactivity detected in the receptor fluids and receptor chamber

^b Sum of absorbed dose and radioactivity in exposed skin

^c Sum of radioactivity detected in the skin wash (8h and 24h) and donor chamber

^d Sum of total dislodgeable dose, radioactivity in the charcoal trap and on the surface tape strips.

Values below the limit of detection (nd) are regarded as being equal to zero for the calculation of mean and standard deviation

Appendix 11.7: Test Formulation Skin Distribution Data (continued)

Table 11: Distribution of Radioactivity Following a Single Finite Application of 10% [¹⁴C]-1,1,2-Trichloroethane in 1,2 Dichloroethane to Dermatomed Human Skin (Group A12, reported cells)

Cell number	Skin source	Dose in receptor fluid	Dose on receptor chamber	Total absorbed dose ^a	Dose in exposed skin	Dermal Delivery ^b	Dose in stratum corneum	Dislodgeable dose (Skin wash, 8 h)	Dislodgeable dose (Skin wash, 24 h)	Dose on donor chamber	Total dislodgeable dose ^c	Dose in trap extract	Dose on surface tape strips (1 - 2)	Total unabsorbed dose ^d	Mass balance
53	H6	0.49	nd	0.49	nd	0.49	nd	0.95	nd	nd	0.95	93.93	nd	94.88	95.37
54	H11	0.38	nd	0.38	nd	0.38	nd	0.90	nd	nd	0.90	92.58	nd	93.48	93.86
55	H8	0.31	nd	0.31	nd	0.31	nd	0.66	nd	nd	0.66	96.49	nd	97.15	97.46
56	H10	0.46	nd	0.46	nd	0.46	nd	1.12	nd	nd	1.12	93.89	nd	95.01	95.47
58	H12	0.37	nd	0.37	nd	0.37	nd	1.22	nd	nd	1.22	94.73	nd	95.95	96.32
	Mean	0.40	nd	0.40	nd	0.40	nd	0.97	nd	nd	0.97	94.32	nd	95.29	95.70
	sd	0.07	-	0.07	-	0.07	-	0.22	-	-	0.22	1.44	-	1.36	1.33
	cv	17.50	-	17.50	-	17.50	-	22.68	-	-	22.68	1.53	-	1.43	1.39

Results expressed as percent applied radiochemical dose

nd Not detected

sd Standard deviation

cv Coefficient of variance

- Not applicable

^a Sum of radioactivity detected in the receptor fluids and receptor chamber

^b Sum of absorbed dose and radioactivity in exposed skin

^c Sum of radioactivity detected in the skin wash (8h and 24h) and donor chamber

^d Sum of total dislodgeable dose, radioactivity in the charcoal trap and on the surface tape strips.

Values below the limit of detection (nd) are regarded as being equal to zero for the calculation of mean and standard deviation

Appendix 11.7: Test Formulation Skin Distribution Data (continued)

Table 12: Distribution of Radioactivity Following a Single Finite Application of 1% [¹⁴C]-1,1,2-Trichloroethane in 1,2-Dichloroethane to Dermatomed Human Skin (Group A13)

Cell number	Skin source	Dose in receptor fluid	Dose on receptor chamber	Total absorbed dose ^a	Dose in exposed skin	Dermal Delivery ^b	Dose in stratum corneum	Dislodgeable dose (Skin wash, 8 h)	Dislodgeable dose (Skin wash, 24 h)	Dose on donor chamber	Total dislodgeable dose ^c	Dose in trap extract	Dose on surface tape strips (1 - 2)	Total unabsorbed dose ^d	Mass balance
59	H6	0.48	nd	0.48	nd	0.48	nd	nd	nd	nd	nd	92.86	nd	92.86	93.34
60	H11	0.30	nd	0.30	nd	0.30	nd	nd	nd	nd	nd	92.00	nd	92.00	92.30
61	H8	nd	nd	nd	nd	nd	nd	1.46	nd	nd	1.46	91.32	nd	92.78	92.78
62	H10	0.18	nd	0.18	nd	0.18	nd	nd	nd	nd	nd	89.47	nd	89.47	89.65
63	H12	0.69	nd	0.69	nd	0.69	nd	nd	nd	nd	nd	92.32	nd	92.32	93.01
64	H12	0.41	nd	0.41	nd	0.41	nd	nd	nd	nd	nd	92.01	nd	92.01	92.42
	Mean	0.34	nd	0.34	nd	0.34	nd	0.24	nd	nd	0.24	91.66	nd	91.91	92.25
	sd	0.24	-	0.24	-	0.24	-	0.60	-	-	0.60	1.19	-	1.25	1.33
	cv	70.59	-	70.59	-	70.59	-	250.00	-	-	250.00	1.30	-	1.36	1.44

Results expressed as percent applied radiochemical dose

nd Not detected

sd Standard deviation

cv Coefficient of variance

- Not applicable

^a Sum of radioactivity detected in the receptor fluids and receptor chamber

^b Sum of absorbed dose and radioactivity in exposed skin

^c Sum of radioactivity detected in the skin wash (8h and 24h) and donor chamber

^d Sum of total dislodgeable dose, radioactivity in the charcoal trap and on the surface tape strips.

Values below the limit of detection (nd) are regarded as being equal to zero for the calculation of mean and standard deviation.

Appendix 11.8: Example Raw Data and Calculations

Measurement of Radioactivity Applied to Skin

Example : Group A7

The specific activity of the radiolabelled formulation was calculated to be 179 dpm/μg. Aliquots (6.4 μL, QC checks) were taken from the dose formulation before, during and after dosing, diluted to volume (10 mL) with ethanol. Duplicate aliquots (250 μL) were taken for radioassay.

The dose pipette tips used to apply the dose to the skin membranes were also retained and were extracted with a portion (10 mL) ethanol. Duplicate aliquots (0.25 mL) were taken for radioassay.

Amounts of radioactivity in QC checks (6.4 μl of formulation):

QC number	QC dilution (mL)	Aliquot Volume (mL)	Mean dpm/mL	Total dpm dispensed
13	10	0.25	165594	1655940
14	10	0.25	162756	1627560
15	10	0.25	164188	1641880

Amounts of radioactivity associated with QC tip extracts:

QC number	QC tip wash (mL)	Aliquot Volume (mL)	Mean dpm/mL	Total dpm in QC tip residue
13	10	0.25	3000	30000
14	10	0.25	2280	22800
15	10	0.25	2482	24820

Total radioactivity in 6.4 μl formulation = (QC activity) + (QC tip extract activity):

QC number	QC dispensed (dpm)	QC pipette tip residue activity (dpm)	Total QC activity (dpm)	MeanQC activity (dpm)
13	1655940	30000	1685940	1667667
14	1627560	22800	1650360	
15	1641880	24820	1666700	

Appendix 11.8: Example Raw Data and Calculations (continued)

Amount of radioactivity applied to each cell = (mean QC activity) - (dose pipette tip):

$$\begin{aligned}\therefore \text{For cell 23 radioactivity applied} &= 1667667 \text{ dpm} - 56060 \text{ dpm} \\ &= 1611607 \text{ dpm}\end{aligned}$$

$$\text{Specific activity of 1,1,2-Trichloroethane} = 179 \text{ dpm}/\mu\text{g}$$

$$\text{Therefore cell 23 received} = \frac{1611607 \text{ dpm}}{179000 \text{ dpm/mg}}$$

$$\mu\text{g equivalents of 1,1,2-Trichloroethane} = 9.00 \mu\text{g (or } 14.1 \mu\text{g/cm}^2\text{)}$$

Percentage of Dose in Receptor Fluid (0 - 24 hours)

Example: Group A7, Cell 23

$$\begin{aligned}\text{Cumulative dose (\%)} &= \left[\frac{\text{Cumulative activity at 24 hours (dpm)}}{\text{dpm applied to cell (Appendix 11.3)}} \right] \times 100 \\ &= \left[\frac{6589 \text{ dpm}}{1611607 \text{ dpm}} \right] \times 100 \\ &= 0.41 \%\end{aligned}$$

Cumulative amount of 1,1,2-Trichloroethane absorbed in $\mu\text{g/cm}^2$

$$= \frac{\text{Cumulative amount in receptor fluid } (\mu\text{g})}{\text{Skin exposure area (cm}^2\text{)}}$$

$$\text{Where skin area of exposure} = 0.64 \text{ cm}^2$$

Cumulative amount of 1,1,2-Trichloroethane (μg equivalents) after 24 hours

$$= 36.81 \mu\text{g}$$

$\therefore$ cumulative amount of 1,1,2-Trichloroethane after 24 hours per cm^2

$$= \frac{36.8101 \mu\text{g}}{0.64 \text{ cm}^2}$$

$$= 57.52 \mu\text{g/cm}^2$$

Appendix 11.8: Example Raw Data and Calculations (continued)**Total Radioactivity in Remaining Skin Following Tape-stripping (calculated using the Debra automated data capture system)**

Calculation demonstrated for tape stripped skin, but a similar method used to determine the percentage of applied dose associated with skin wash extracts, donor and receptor chambers extracts and tape-strips of the stratum corneum.

Example: Group A7, Cell 26

Tape stripped skin were solubilised in *toto*. Aliquots of tape stripped skin were analysed by LSC.

Tape stripped skin aliquot number	Tape stripped skin aliquot weight (g)	Gross dpm per aliquot	Net dpm per aliquot	Mean net dpm per g
1	1.0384	399.9	372.5	354.8
2	1.0404	392.6	365.2	

Calculated using a background of 27.4 dpm

Total weight of tape stripped skin = 6.0630 g

∴ total radioactivity associated with tape stripped skin

$$= 354.8 \times 6.0630 \text{ g}$$

$$= 2151 \text{ dpm}$$

$$\% \text{ dose remaining in tape stripped skin} = \frac{2151 \text{ dpm}}{1623007 \text{ dpm}} \times 100$$

$$= 0.13\% \text{ (Appendix 11.7, Table 1)}$$

Appendix 11.8: Example Raw Data and Calculations (continued)**Limit of Detection**

Radioactivity in gross amounts of less than twice background was considered to be below the limit of accurate determination and is reported as not detected.

The following section provides some information on detection limits in terms of µg equiv/g for Cell 34R, receptor chamber extract.

Cell	Sample	Background dpm	Aliquot weight (g)	Dpm/g	Mean Dpm/g	Detection limit (µg equiv/g)
34R	Receptor chamber	20.5	2.3654	<8.7		
	extract	20.5	2.3582	<8.7	<8.7	0.05

The specific activity was 178 dpm/µg

$$\begin{aligned}\text{Limit of detection} &= \frac{< 8.7 \text{ dpm/g}}{178 \text{ dpm/}\mu\text{g}} \\ &= <0.05 \mu\text{g equiv/g}\end{aligned}$$

as % applied dose

Total weight of extract is 44.6205 g

$$\begin{aligned}\text{Therefore total dpm/sample} &= <8.7 \text{ dpm/g} \times 44.6205 \text{ g} \\ &= <388 \text{ dpm}\end{aligned}$$

$$\text{Radioactivity dosed to Cell 34R} = 635313 \text{ dpm}$$

$$\begin{aligned}\text{Therefore} &= \frac{<388}{635313} \times 100 \\ &= <0.06\% \text{ AR}\end{aligned}$$

Appendix 11.9: Sample Repeat Analysis

The following samples were reanalysed during the course of the study:

Cell Number	Samples	Reason /Justification
28	Trap extract 1	High variability between aliquots. Repeat data used as this showed improved variability.
49	Trap extract 1	High variability between aliquots. Repeat data used as this showed improved variability.
34R	Trap extract 2	High variability between aliquots. Repeat data used as this showed improved variability.

12. ANNEXES

Annex 12.1: Study Plan and Amendments



Study Plan Amendment 3

1,1,2-Trichloroethane: Rates of penetration through human skin using a flow through *in vitro* system

Study Number:	8479196
Sponsor Name:	<u>Stantec ChemRisk (formerly</u> Cardno ChemRisk) on behalf of Vinyl Institute 1,1,2-Trichloroethane Test Order Consortium
Version ID:	Final
Issue Date:	27 April 2023
Study Director:	Adele Jones
Testing Facility:	Labcorp Early Development Laboratories Ltd. Woolley Road Alconbury Huntingdon Cambridgeshire PE28 4HS UK

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Study Plan – Log of Revisions

Final Study Plan	Issue date: 11 August 2022
Amendment No. 1	Issue date: 17 January 2023
Section(s)	Revision detail and reason
5.2	Lot number for the second batch of radiolabel added following receipt from the supplier.
7.7	Dose levels corrected due to incorrect rounding.
7.8, 7.9.1	To include an 18 hours collection as this timepoint was not included in error in the original study plan
7.9.5	To state that the charcoal filter will be removed and replaced with a fresh filter at washing. This was previously omitted in error.
10	Veeva QMS updated to Veeva Unified Quality Vault
14	Sentence removed as this was a duplication of a previous paragraph.
Amendment No. 2	Issue date: 03 March 2023
5.2	The lot number for the second batch of test item was not amended in error in Study Plan Amendment 1. As the second batch of test item was not used, reference to this batch has been removed in this amendment.
7.7	To correct the target dose level for Group A1 as the values was expressed in µg and not mg.
Amendment No. 3	Issue date: 27 April 2023
Page 1, 18 and 19	Sponsor company name and email amended
1.1	To amend the objectives to state this study was related to OECD 428 testing only
5.4.2	Section added to include details of non-radiolabeled testosterone

Any changes to the study design after the date of this authorizing signature will be documented in further versions of the study plan as Study Plan Amendments. Modifications will be described in the above summary of changes, and within the body of the Study Plan Amendment any deleted statements are struck through and new statements are underlined and in bold. Any previous amendment changes are shown as clean text.

1. Introduction and Purpose

[¹⁴C]-1,1,2-Trichloroethane is used primarily in the manufacture of other chemicals. Information is required on rates of penetration of [¹⁴C]-1,1,2-Trichloroethane in human membranes following dermal application as the neat test item and up to three lower dose levels.

Assessing the hazard of skin contact with potentially toxic materials requires a combination of estimates of uptake *via* the skin and quantitative toxicology. Diffusion across the non-living outer layer of skin, the stratum corneum, is normally the rate-limiting step for percutaneous absorption. The permeability properties of the stratum corneum are unchanged by removal from the body and, therefore, *in vitro* experiments using this membrane are appropriate and offer a number of advantages over whole animal or human volunteer experiments.

1.1 Objectives

- (a) To determine and compare the rates of absorption of [¹⁴C]-1,1,2-Trichloroethane following *in vitro* dermal applications to excised human skin at multiple dose levels (1%, 10%, 50% and neat), using multiple potential solvents under finite and infinite dosing scenarios and to determine the retention of radioactivity at the site of application (finite dose groups only).
- (b) Satisfy USEPA TSCA Test Order for **OECD 428 testing** ~~risk evaluations~~ of 1,1,2-trichloroethane.

2. Justification

Dermal contamination is a potential route of human exposure.

The concentration and application rate have been chosen to represent possible worker exposure to the neat and diluted test item. An 8-hour exposure period has been selected to represent the average length of the working day.

Data generated in this study will be used in the risk evaluation of the test item.

3. Proposed Schedule

Experimental start date	22 August 2022
Experimental termination date	20 January 2023
Unaudited Draft report date	03 March 2023

This schedule is based on the experimental work described in the study plan. Any additional work, subject to a separate contract with the Sponsor, will be included in an amendment to the study plan and a new schedule will be agreed once the scope of the additional work is determined.

4. Regulatory Information

4.1 Good Laboratory Practice

The study will be conducted in compliance with principles of Good Laboratory Practice Standards as set forth in:

- The United Kingdom Good Laboratory Practice Monitoring Authority, Medicines and Healthcare Products Regulatory Agency (MHRA): Good Laboratory Practice Regulations 1999, Statutory Instrument 1999 No. 3106 (as amended)
- The Organisation for Economic Co-Operation and Development (OECD): Principles on Good Laboratory Practice, ENV/MC/CHEM (98) 17 (revised in 1997, issued January 1998)

These principles are compatible with Good Laboratory Practice regulations specified by regulatory authorities throughout the European Community, the United States (EPA and FDA), and Japan (MHLW, MAFF and METI), and other countries that are signatories to the OECD Mutual Acceptance of Data Agreement.

4.2 Regulatory Testing Guidelines

The study will be performed in compliance with the following regulations or guidelines:

- OECD Test Guideline 428 (April 2004) and the corresponding OECD Guidance Document for conducting in vitro skin absorption studies (March 2004).

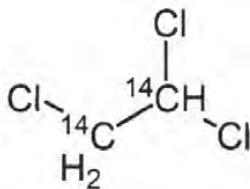
5. Test Item and Supporting Information

5.1 General information

Name:	1,1,2-Trichloroethane
CAS number:	79-00-05
Molecular formula:	C ₂ H ₃ Cl ₃
Molecular weight:	133.4 g/mol
Physical state:	Liquid

5.2 Radiolabeled test item

Structural Formula:



Position of Radiolabel:	Shown in above structural formula
Specific activity:	0.05 mCi/mmol
Source:	Moravek Biochemical Inc.
Lot number (batch 1):	369-166-050-A-20210917-DRE
Radiochemical purity:	The radiochemical purity of each batch will be measured prior to application and should be greater than 95%.
Storage:	- 20 ± 10°C

5.3 Non-radiolabeled test item

Name:	1,1,2-Trichloroethane
Source:	Sigma Aldrich
Batch number:	BCCB7564
Purity:	98.2 %
Storage:	20 ± 5°C
Expiry date:	31 May 2024

5.4 Control Reference

5.4.1 Radiolabelled testosterone

Name:	[¹⁴ C]-Testosterone
Source:	ARC
Batch number:	210415
Radiochemical purity:	The radiochemical purity of each batch will be measured prior to application and should be greater than 95%.

Storage: $5 \pm 3^{\circ}\text{C}$

5.4.2 Non-radiolabeled Testosterone

Source: Sigma Aldrich
Batch number: SLCF3521
Purity: 100%
Storage: $5 \pm 3^{\circ}\text{C}$
Retest date: 10 February 2023

6. Test System

6.1 Human

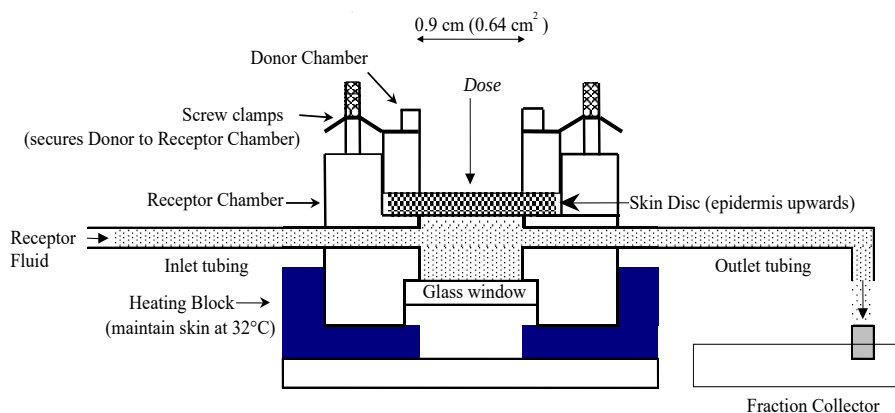
Human skin (from at least 4 donors) will be obtained from a recognized Tissue Bank/supplier and stored at -10 to -30°C prior to use. The anatomical site, age and sex of donor and date of arrival at the laboratory will be recorded.

Dermatomed skin will be used (approximately 200-400 μm). Details of preparation will be recorded in the study data.

7. Study Conduct

7.1 Cell design

The flow-through diffusion cell will be used to study the absorption of the test item, providing an exposure area of 0.64 cm^2 skin. The cells will be maintained at $32^{\circ}\text{C} \pm 1^{\circ}\text{C}$ using a water-heated manifold; the relative humidity (target range 30 – 70%) will be monitored during the study. The receptor chamber contents will be pumped at a flow rate of 1.5 ml/hr. ($\pm 25\%$) (equivalent to approximately 6 receptor chamber content changes per hour). A diagrammatic representation of the diffusion cell is shown below. In this experiment the aperture of the donor chamber will be either occluded with a PTFE screw lid for the infinite dose groups or semi occluded by a charcoal filter placed into the donor chamber aperture.



7.2 Membrane integrity

On the day of application, the integrity of the epidermal surface will be checked by TEWL assessment (Transepidermal Water Loss) using a VapoMeter.

Intact skin preparations will be those which meet the following criteria:

- Visual inspection shows no signs of physical damage to the membranes during the experiment.
- Transepidermal Water Loss is $< 13 \text{ gm}^{-2}\text{h}^{-1}$ (Guth *et al*, 2015).

Skin replicates failing to meet the above criteria will be discarded and replaced prior to dose application. Four replicates will be performed for the reference phase. Six replicates will be performed for the remaining phases.

The integrity of the epidermal surface will be checked again at 24 hours after application, following removal of the remaining dose formulation for the infinite dose group and immediately prior to the 24-hour surface wash for the finite group.

Data from all intact samples will be reported. If fewer than 4 intact samples are achieved, additional replicates will be performed until 4 intact replicates are achieved.

Excised skin will be assigned an experimental number as it is placed in the diffusion cell.

7.3 Solubility of [^{14}C]-test item in the receptor fluid

Appropriate amounts of radiolabelled and unlabeled test item will be transferred to each of three glass vessels. A calculated volume of receptor fluid will then be added to give a prospective concentration equivalent to ten times the highest dose level applied to the skin dissolved in the total volume of receptor fluid passed over the 24 hour duration of the experiment i.e. approximately 915 mg per 36 ml, equivalent to a concentration of 25 mg/ml. The vessels will be tightly stoppered and agitated at approximately 32°C. After a period of time (24 hours), the vessels (per receptor fluid) will be removed and the mixture, or samples thereof, will be centrifuged and aliquots of the resulting solution taken for scintillation counting.

If adequate solubility is not considered to be achieved, at the discretion of the Study Director, an alternative receptor fluid will be selected and the solubility test repeated for that receptor fluid.

7.4 Reference Phase

A reference experiment (group A1) will be performed alongside the main study. Radiolabelled Testosterone will be applied to the reference skin cells and this phase will be processed in the same way as the rest of the study, using the same equipment and techniques.

The performance and reliability of the in vitro test system have been previously demonstrated in the performing laboratory. Data for a number of reference substances of different lipophilicity were generated during a multi-centre comparison study (Van de Sandt et al, 2004). Results consistent with those obtained for testosterone during this study will be considered to validate the performance of the test in the performing laboratory.

7.5 Preparation, Stability and Homogeneity of Radiolabelled Dose

Reference phase (group A1): [^{14}C] Testosterone will be diluted in 40% ethanol in water to achieve the required concentration (1 mg/mL).

Groups A2 and A7: [^{14}C]-1,1,2-Trichloroethane will be applied neat.

Groups A3, A4, A8, A9, and A10: [^{14}C]-1,1,2-Trichloroethane will be diluted in isopropyl myristate (IPM) at a radioactivity concentration of approximately 0.25 – 4 MBq/mL.

Groups A5, A6, A11, A12, and A13: [^{14}C]-1,1,2-Trichloroethane will be diluted in 1,2-dichloroethane (ethylene dichloride) at a radioactivity concentration of approximately 0.25 – 4 MBq/mL.

The [^{14}C]-1,1,2-Trichloroethane will be adjusted to a suitable specific activity if necessary. The method of preparation will be documented in the study records.

The radiochemical purity of each [^{14}C]-1,1,2-Trichloroethane dose will be measured at appropriate times, using suitable chromatographic methods. The radiochemical content and homogeneity of each formulation will be checked prior to dosing.

7.6 Administration

The test item will be administered as a single dermal application.

Infinite doses: The target dose will be ca 100 μL of neat test item/ cm^2 , with the surface area of each skin preparation being 0.64 cm^2 .

Group A2 (100%) will correspond to 64 μL test item.

Groups A3 and A5 (50%) will correspond to 128 μL (64 μL test item diluted with 64 μL of vehicle).

Groups A4 and A6 (10%) will correspond to will correspond to 640 μL (64 μL test item diluted with 576 μL of vehicle).

Finite doses : The target dose will be ca. 10 μL formulation/ cm^2 , with the surface area of each skin preparation being 0.64 cm^2 .

The test formulation be applied to the skin membranes with an appropriate pipette at the desired application rate. The actual amount of radiolabelled test item will be determined from the quality control (QC) checks taken before, during and after dosing.

For the infinite phase, the donor chamber will be occluded with a PTFE screw lid. Following dosing of the finite groups, activated charcoal trap will be added to the donor chamber of each cell and the screw the lid will remain unattached (off). The cells for the finite phase will therefore not be sealed, but the activated charcoal trap will allow for semi-occlusion of each cell.

7.7 Group assignment

Phase	Group	Species	Number of skin cells	Condition	Nominal dose level (mg/ cm^2)
Reference (finite)	A1	Human	4	Non-occluded	0.01, [^{14}C]-Testosterone
Infinite	A2	Human	6	Occluded	144 (high, neat, 100%)
Infinite	A3	Human	6	Occluded	144 (50% in isopropyl myristate)
Infinite	A4	Human	6	Occluded	144 (10% in isopropyl myristate)
Infinite	A5	Human	6	Occluded	144 (50% in 1,2-dichlorethane)
Infinite	A6	Human	6	Occluded	144 (10% in 1,2-dichlorethane)
Finite	A7	Human	6	Semi-occluded, with traps	14.4 (neat, 100%)
Finite	A8	Human	6	Semi-occluded, with traps	7.2 (50% in isopropyl myristate)
Finite	A9	Human	6	Semi-occluded, with traps	1.4 (10% in isopropyl myristate)
Finite	A10	Human	6	Semi-occluded, with traps	0.14 (1% in isopropyl myristate)
Finite	A11	Human	6	Semi-occluded, with traps	7.2 (50% in 1,2-dichlorethane)
Finite	A12	Human	6	Semi-occluded, with traps	1.4 (10% in 1,2-dichlorethane)
Finite	A13	Human	6	Semi-occluded, with traps	0.14 (1% in 1,2-dichlorethane)

7.8 Infinite Dose Groups (groups A2-A6)

The equipment used for skin penetration studies is the ILC07 Automated Diffusion System, obtained from PermeGear.

The flow rate of the receptor fluid will be set to ca. 1.5 mL/h and the flow through cells will be set to collect the following fractions:

One-hour pre-dose; 10 minutes, 30 minutes, 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 hours post dose (16 samples per cell).

Fractions of receptor-fluid will be collected directly into scintillation fluid and will be retained for radioassay.

No other samples will be retained for analysis for these groups.

7.9 Finite Dose Groups (groups A7-A13)

7.9.1 Sampling of Receptor-fluid

The equipment used for skin penetration studies is the ILC07 Automated Diffusion System, obtained from PermeGear.

The flow rate of the receptor fluid will be set to ca. 1.5 mL/h and the flow through cells will be set to collect the following fractions:

One-hour pre-dose; 10 minutes, 30 minutes, 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 hours post dose (16 samples per cell).

Fractions of receptor-fluid will be collected directly into scintillation fluid and will be retained for radioassay.

7.9.2 Surface Washings

Following completion of the 7-8 hour sample of receptor-fluid for the finite dose groups, the flow of receptor fluid will be halted and the surface of the membrane will be rinsed three times (3 x 0.5 mL) with a mild solution of Dove™ liquid soap in water (ca. 1%). The skin surface will then be gently swabbed twice with cotton buds soaked with the liquid soap solution. Finally, the membrane will be rinsed with a small volume (0.5 mL) of deionised water and a further cotton bud will be used to swab the surface of the membrane until dry. The flow of receptor fluid will then be restarted to continue the collection of samples from 8-24 hours.

Following completion of the 24-hour sample of receptor fluid, the flow of receptor fluid will be halted and the membrane surface will be washed and rinsed as described above.

All cotton buds, washings and the water rinse will be placed into suitable containers at each sampling time and a volume of ethanol added to give an approximate 1/1 (v/v) ethanol/water solution. The container will be placed on an orbital shaker and shaken followed by sonication in a sonic water bath. The cotton buds will be removed from the container, rinsed in a small

volume of deionised water and the rinsing combined with the washings and water rinse. The cotton buds will be retained for further extraction (approximately 1:1 (v/v) ethanol/water). All resulting solutions will be analysed for radioactivity. If considered necessary, residual radioactivity in the cotton buds analysed by combustion and radioassay.

7.9.3 Skin Samples

Following sampling at the terminal time point (24 hours), the skin sample will be removed from the diffusion cell and be tape-stripped until the stratum corneum is removed (indicated by the glistening appearance of the viable epidermis). Tape stripping will be performed with D-squame adhesive tape. Each tape will be pressed onto the skin surface with a pressure device (D-Squame Pressure Device) to achieve a standardized pressure of 225 g/m² for approximately 5 seconds. Tape strips containing viable epidermis will be analyzed separately. Tape strips of the stratum corneum will be retained and analyzed individually. The remaining skin will be retained for analysis.

7.9.4 Diffusion Chamber Washings

Following removal of the skin sample from the diffusion cell, the cell will be split into donor and receptor chambers and each chamber will be placed in a suitable container and covered in a suitable volume of water:ethanol (ca. 1:1, v/v). The apparatus will be removed from the chamber and the washings retained.

7.9.5 Charcoal filter extracts

Following washing at 8 hours and at termination at 24 hours, the charcoal filter will be removed and placed into a suitable container and covered in a suitable volume of ethanol. The resultant extract will be retained and analysed for radioactivity.

Following completion of the washing, the charcoal filter will be replaced with a fresh filter.

7.10 Sample Storage

Any samples not analyzed immediately after collection will be placed in a freezer at -20 ± 10°C as soon as possible after collection.

7.11 Determination of radioactivity

Samples will be counted by liquid scintillation counting (LSC) with automatic quench correction using an external standard. Disintegration rates from appropriate blank sample vials will be subtracted from sample disintegration rates to give net dpm for each sample. In general, gross radioactivity levels below twice background level will be considered to be below the limit of reliable measurement. The limit of detection may vary with the counting time and will be documented. If considered necessary, solid samples may be combusted in an automatic sample oxidiser and radioactivity trapped in a suitable absorber before radioactivity measurement.

8. Disposal of Samples and Test Formulations

Test samples generated during the study will be stored deep-frozen until the final report has been issued at which time they will be disposed of. The Sponsor will be informed before disposal occurs.

9. Data Presentation and Analysis

Individual and mean data will be tabulated and presented in graphical form where appropriate. The results will be expressed up to either 2 or 3 significant figures. The following information will be reported:

- recovery of radioactivity from receptor-fluid, surface wash samples, tape strips, skin, charcoal filter extracts and chamber washings, providing an overall mass balance of applied radioactivity (% recovery);
- amount of [^{14}C]-1,1,2-Trichloroethane recovered from receptor-fluid, tape strips, skin and chamber washings;
- percent absorption of radioactivity in receptor-fluid for all timepoints (finite dose) for all tested materials;
- permeability coefficient (K_p) for all timepoints for each infinite dose for all tested materials

Analysis may be repeated where sufficient sample remains to allow reanalysis and it can be demonstrated that the data is anomalous or non-representative. LSC measurements may need to be repeated because of either poor reproducibility or high variance (typically greater than 15% CV, where sufficient radioactivity concentrations are present). Radio-LC may be repeated due to chromatographic reasons (e.g. poor peak response, atypical retention times). The justification for repeat analysis will be fully documented in the study data and approved by the Study Director/Responsible Scientist. All data (even if excluded) will be retained with the original data set in accordance with GLP requirements. The reasons for repeat analysis will be considered during data review and reporting.

10. Major Computerized Systems

Computer systems used for the generation, measurement and assessment of data may include Laura (Radio HPLC data capture and evaluation), Tina (Radio TLC image data capture and evaluation), Debra (Metabolism LIMS system) and validated Excel spreadsheets. Test item storage and usage may be recorded using Samuel (in-house electronic tracking system) and Xybion Pristima used for Pharmacy test item management. Veeva Unified Quality Vault (Quality Management and Document Control System) may be used for recording deviations and other quality issues. Any additional computer systems used during the course of the study will be documented in the study records.

11. Quality Assurance

The Labcorp GLP Quality Assurance Unit (QAU) will perform authorised Study Plan verifications, inspections of procedures, data and final/draft report audits for activities performed at the Test Facility according to their established standard operating procedures.

QA findings will be reported to the Study Director and Test Facility Management promptly on completion of each action.

12. Study Plan Amendment and Deviation

Any intended change to the study plan will result in an amendment to study plan approved by the Study Director and the Sponsor. Modifications will be described in the summary of changes, and within the body of the Study Plan Amendment any deleted statements are struck through and new statements are underlined and in bold. Amendments will be distributed to all recipients of the study plan.

Deviations (unplanned changes) from the study plan and study plan amendment will be documented and acknowledged by the Study Director.

13. Reporting

Status Report or Periodic Updates

Periodic verbal and written updates on study progress will be provided by the Study Director.

Draft Report

A GLP compliant report will be prepared. Following experimental completion an unaudited draft report, in Labcorp report format will be provided to the Sponsor for their review and submitted to Labcorp Quality Assurance Unit for audit. The Draft report will be provided electronically in PDF (non-hyperlinked) format. In addition, an MS Word copy of the text will also be provided electronically at Sponsor request.

After receipt and review of the Sponsor's comments, appropriate changes will be made and revisions provided to the Sponsor.

Final Report

Once authorized by the Sponsor, the audited, signed final report will be issued. The Final report will be provided electronically in eCTD compliant fully hyperlinked PDF format.

Any additions or corrections to an authorized final report will be documented as a formal addendum/amendment to the final report.

A paper copy of the authorized final report, including original signatures, will be held in the Labcorp archives.

In the absence of ongoing communications and after notification in writing to the Sponsor, Labcorp reserves the right to finalize, sign and issue the Final report from this study six months after the issue of the draft. Any subsequent requests for modifications, corrections or additions to the Final Report will be the subject of a formal report amendment (or new study, as appropriate) and will be subject to additional cost.

14. Archiving

Records and documentation relating to this study (including electronic records) will be maintained in the archives of Labcorp– Huntingdon, UK for a period of one year from the issue of the final report. This will include raw data and further material that supports the reconstruction of the study. Testing Facility-generated electronic raw data will be stored on the computer system on which the data application resides or archived off-line.

Due to the inherently unstable nature of radiolabeled compounds, a subsample of the test item will not be archived. Metabolism studies are short term studies and as such there is no requirement to archive a sample of the non-radiolabeled test item.

At termination of the aforementioned period, the Sponsor will be contacted in order to determine the final disposition of these records. After the specified period, the Sponsor is responsible for all costs associated with the retention, retrieval, onward transfer or destruction/disposal of these materials. If the Sponsor is unresponsive the records will be destroyed in accordance with the Labcorp– Huntingdon, UK Standard Operating Procedure.

In case records are transferred, the Sponsor should ensure that the materials and records in support of regulatory studies are retained and maintained under conditions that guarantee their integrity and continued access according to archiving requirements of the principles of GLP. The Sponsor should also ensure that such materials and records are retained for as long as required by relevant authorities.

Labcorp will retain the study plan, final report and any amendments indefinitely.

15. References

Guth K, Schafer-Korting M, Fabian E, Landsiedel R, and Van Ravenzwaay B (2004). Suitability of skin integrity tests for dermal absorption studies *in vitro*. *Toxicology In vitro*, **29**, 113-123.

Van de Sandt, J.J.M. et al 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.

16. Contact Details/Shipping Addresses

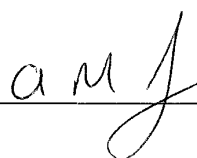
In temporary absence of the Study Director, routine duties and technical questions will be attended to by the alternative Labcorp study contact.

Sponsor	Stantec ChemRisk (formerly Cardno ChemRisk) on behalf of Vinyl Institute 1,1,2-Trichloroethane Test Order Consortium 20 Stanwix Street, Suite 505 Pittsburgh PA 15222 UNITED STATES
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17. Signatures

1,1,2-Trichloroethane: Rates of penetration through human skin using a flow through *in vitro* system

This is the Final Study Plan Amendment 3; it has been reviewed and approved by:



Study Director
Adele Jones
Labcorp Early Development Laboratories Ltd.

27 APRIL 2023
Date



Study Monitor
Marisa Kreider
Stantec ChemRisk (formerly Cardno ChemRisk)

05/03/2023

Date

Annex 1 Calculations**Definitions**

Surface area of skin (0.64 cm ²)	= A [cm ²]
Volume of sample	= V [mL]
Radioactivity (sample dpm – background dpm) in aliquot of receptor fluid or sample analysed	= R [dpm]
Concentration of radioactivity in receptor fluid or sample	C = R/V [dpm/mL]
Specific radioactivity of test substance	= S [kBq/mg]
Weight of test substance applied to each preparation	= D [mg/cm ²]
Radioactive dose administered to each preparation	= S x D x A [kBq]
Time period over which the rate of penetration is calculated	= Δt [hours]
Rate of penetration, taken from the initial linear portion of the graph [μg equivalents absorbed/cm ² /time]	= ΔP

Calculation of Rates of Penetration

Concentration of test substance in receptor fluid, [μg equivalents/aliquot of receptor fluid]	$\frac{R}{60 \times S} = K$
Cumulative amount of test substance penetrated at time t [μg equivalents]	$\sum_t K \times \frac{1}{A} = P_t$
Rate of penetration of test substance [μg equivalents/cm ² /hour]	$J = \frac{\Delta P}{\Delta t}$

Calculation of Percentage Recovery of Radioactivity

Per cent of radioactive dose/sample	$= \frac{K}{10 \times D}$
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Calculation of Permeability Coefficients

Permeability coefficient [cm/hour]	$K_p = \frac{J}{\Delta C}$
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where ΔC = test substance concentration difference across skin, assumed to be the formulation concentration on the surface of the skin in true sink conditions.

Mean relative permeation into the receptor fluid
occurring within half of the sampling time [%]

$$100\% \sum_{i=1}^n \frac{RF_{12i}}{RF_{24i}} \times \frac{1}{n} = t_{0.5}$$

where n is the number of replicates.

Calculation of Lag Times

Lag time [hours]

$$= \frac{-1 \times \text{intercept on P axis}}{J}$$

where the intercept on the P axis is obtained directly by linear regression analysis.