Data Supplement

Caprylhydroxamic Acid

CIR EXPERT PANEL MEETING
JUNE 6-7, 2019

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Memorandum

To: CIR Expert Panel Members and Liaisons
From: Monice M. Fiume MMF
Senior Director
Date: May 28, 2019
Subject: Safety Assessment of Caprylhydroxamic Acid as Used in Cosmetics – Wave 2

A Final Report entitled "The *In Vitro* Percutaneous Absorption of Radiolabeled Caprylhydroxamic Acid in Three Formulations Through Human Skin" was submitted directly to CIR on May 10, 2019. This submission was received after the original Panel materials on Caprylhydroxamic Acid were prepared and distributed to the Panel; therefore this study is attached for distribution as a Wave 2 document (*caphyd062019wave2_data*).

The rate and extent of dermal absorption of Caprylhydroxamic Acid following topical application of three suspensions (oil in water, silicone in water, and clear lotion) was examined in vitro using split-thickness human abdominal skin. The concentration of Caprylhydroxamic Acid in each of the three suspensions was *ca* 0.15% (w/w). Split-thickness human skin membranes were mounted into static diffusion cells. 1-[^{14}C]-Caprylhydroxamic Acid (specific activity, 360 µCi/mg; 99.6% pure) was incorporated into the blank formulation to produce the three suspensions, and absorption was assessed by collecting samples of the receptor fluid prior to dosing and at 2, 4, 6, 8, and 12-h post-dose. At 24-h post dose, the skin was washed, rinsed with a dilute 2% (v/v) soap solution, and then dried. The process was repeated, the skin samples removed from the diffusion cells, and the stratum corneum was removed by tape stripping. Exposed and unexposed skin was separated, and exposed skin was further separated into the dermis and epidermis.

Dermal absorption of Caprylhydroxamic Acid was greatest with the oil in water suspension, followed by the silicone in water suspension, and then the clear lotion. With these preparations, the total absorbed dose (cumulative receptor fluid + receptor chamber was) was 41.89% (2971 ng equiv/cm^2), 31.75% (2747 ng equiv/cm^2), and 22.93% (1824 ng equiv/cm^2) of the applied dose, respectively. Dermal delivery (absorbed dose + epidermis + dermis + clingfilm) using these preparations was 51.45% (3649 ng equiv/cm^2), 43.84% (3793 ng equiv/cm^2), and 36.87% (2933 ng equiv/cm^2) of the applied dose, respectively. The total unabsorbed dose (total dislodgeable dose + stratum corneum + unexposed skin) was 43.99% (3120 ng equiv/cm^2), 52.67% (4558 ng equiv/cm^2), and 60.23% (4792 ng equiv/cm^2) of the applied dose for the oil in water, silicone in water, and clear lotion suspensions of Caprylhydroxamic Acid, respectively.

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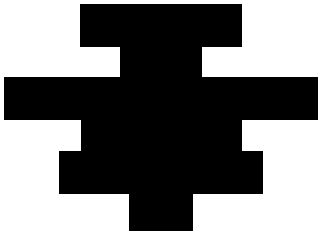
FINAL REPORT

Test Facility Study No. 798232, Report No. 37551

The *In Vitro* Percutaneous Absorption of Radiolabelled Caprylhydroxamic Acid in Three Formulations Through Human Skin

TEST FACILITY:
Charles River Laboratories Edinburgh Ltd
Elphinstone Research Centre
Tranent, East Lothian
EH33 2NE, UK



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1 COMPLIANCE STATEMENT

Study Title: The *In Vitro* Percutaneous Absorption of Radiolabelled Caprylhydroxamic Acid in Three Formulations Through Human Skin

I, the undersigned, hereby declare that this study was performed in accordance with the OECD Principles of Good Laboratory Practice as incorporated into the United Kingdom Statutory Instrument for GLP and as accepted by Regulatory Authorities throughout the European Union, United States of America (FDA and EPA) and Japan (MHLW, MAFF and METI), and other countries that are signatories to the OECD Mutual Acceptance of Data Agreement.

The study was conducted according to the procedures herein described and this report represents a true and accurate record of the results obtained.

Joanne Vinall, BSc, MSc, PhD
Study Director

28 Nov 2016
Date

2 QUALITY ASSURANCE STATEMENT

Study Title: The *In Vitro* Percutaneous Absorption of Radiolabelled Caprylhydroxamic Acid in Three Formulations Through Human Skin

The Charles River Quality Assurance Unit conducted a protocol review, protocol amendment review, study-based inspections and report audits on this study, as detailed below.

		Dates Findings Submitted to:	
Date(s) of Audit	**Phase(s) Audited**	**Study Director**	**Study Director Management**
01-Feb-2016	Final Protocol	01-Feb-2016	01-Feb-2016
10-Mar-2016	Protocol Amendment 1	10-Mar-2016	10-Mar-2016
16-Mar-2016	Dose Administration	05-Apr-2016	05-Apr-2016
17-Mar-2016 - 05-Apr-2016	Dose Preparation Review	05-Apr-2016	05-Apr-2016
17-Jun-2016 20-Jun-2016 - 24-Jun-2016 27-Jun-2016	Draft Report	27-Jun-2016	27-Jun-2016
19-Sep-2016	Protocol Amendment 2	19-Sep-2016	19-Sep-2016
18-Nov-2016	Final Report	21-Nov-2016	21-Nov-2016

Process-based inspections relevant to this study are scheduled once every quarter. The outcome of each inspection is reported to Management and, where relevant, the Study Director.

Facilities relevant to this study are included in Charles River's annual facility inspection programme. The outcome of each inspection is reported to Management.

This report is considered to describe accurately and completely the procedures used in the study and the results obtained.

Alison McNaughton

28 Nov 2016.

Alison McNaughton, HNC
Quality Assurance

Date

3 RESPONSIBLE PERSONNEL

Study Director:	Joanne Vinall, BSc, MSc, PhD
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Quality Assurance:	Jean-François Selfslagh, BSc Alison McNaughton, HNC Leanne Frace, BSc

4 SUMMARY

The test item, Caprylhydroxamic Acid, is used in three cosmetic suspensions (Oil in Water, Silicone in Water and Clear Lotion). The concentration of Caprylhydroxamic Acid in each of the three suspensions is 0.15% (w/w).

As part of the safety evaluation of Caprylhydroxamic Acid, a study was required to assess the rate and extent of absorption of Caprylhydroxamic Acid following topical application of the three suspensions to human skin.

The study was conducted in accordance with the OECD Principles of Good Laboratory Practice as incorporated into the United Kingdom Statutory Instrument for Good Laboratory Practice and was performed in accordance with the following documents: OECD Guideline for Testing of Chemicals, Guideline 428: Skin Absorption: *In Vitro* Method (2004); OECD Environmental Health and Safety Publications Series on Testing and Assessment No. 28. Guidance Document for the Conduct of Skin Absorption Studies (2004); SCCS (Scientific Committee on Consumer Safety). Basic Criteria for the *In Vitro* Assessment of Dermal Absorption of Cosmetic Ingredients. SCCS/1358/10 adopted 22 June 2010; Scientific Committee on Consumer Products. Notes of Guidance for Testing of Cosmetic Ingredients and their Safety Evaluation, 9th Revision, 29 September 2015; COLIPA (1997). Cosmetic Ingredients: Guidelines for Percutaneous Absorption/Penetration. The European Cosmetic, Toiletry and Perfumery Association.

Split-thickness human skin membranes were mounted into static diffusion cells. The absorption process was followed by taking samples of the receptor fluid (phosphate buffered saline containing polyoxyethylene 20-oleyl ether (PEG, *ca* 6%, w/v), sodium azide (*ca* 0.01%, w/v), streptomycin (*ca* 0.1 mg/mL) and penicillin (*ca* 100 units/mL) at recorded intervals throughout the experimental period. The skin surface temperature was maintained at 32°C ± 1°C throughout the experiment. An electrical resistance barrier integrity test was performed and any skin sample exhibiting a resistance lower than 4 kΩ was excluded from subsequent absorption measurements.

[^{14}C]-Caprylhydroxamic Acid was incorporated into the blank formulation, prepared from the appropriate supplied excipients, to produce Test Preparation 1, the Oil in Water Suspension; Test Preparation 2, the Silicone in Water Suspension; and Test Preparation 3, the Clear Lotion Suspension each containing *ca* 0.15% (w/w) of Caprylhydroxamic Acid.

Absorption of Caprylhydroxamic Acid was assessed by collecting samples of the receptor fluid prior to dosing and at 2, 4, 6, 8 and 12 h post dose. At 24 h post dose, the skin was washed with a concentrated commercial hand wash soap and rubbed in with a tissue swab. This was followed by rinsing the skin with a dilute 2% (v/v) soap solution and drying the skin surface with tissue paper (tissue swabs); this process was repeated. The skin was removed

from the cells and dried with a tissue swab. The donor chamber was transferred to a pre-weighed pot containing methanol. The stratum corneum was removed by tape stripping and the skin divided into exposed and unexposed skin. The exposed skin was then further separated into dermis and epidermis by heat separation. The skin samples were solubilised with Solvable® tissue solubiliser. The bulk receptor fluid was collected from the receptor chamber. The receptor chambers were rinsed with methanol and the samples retained for analysis. All samples were analysed by liquid scintillation counting.

A summary of the mean % applied dose and ng equiv./cm^2 results is provided in the following table.

Test Preparation	1	2	3
Target Concentration (%, w/w)	0.15%		
Concentration by Radioactivity (%, w/w)	0.14	0.17	0.16
	(% Applied Dose)		
Total Dislodgeable Dose	41.03	47.38	54.17
Stratum Corneum	2.79	5.15	5.93
Total Unabsorbed Dose	43.99	52.67	60.23
Total Absorbed Dose	41.89	31.75	22.93
Dermal Delivery	51.45	43.84	36.87
Mass Balance	95.44	96.52	97.10
	ng equiv./cm^2		
Total Dislodgeable Dose	2910	4099	4310
Stratum Corneum	198	446	472
Total Unabsorbed Dose	3120	4558	4792
Total Absorbed Dose	2971	2747	1824
Dermal Delivery	3649	3793	2933
Mass Balance	6770	8351	7725

Total Dislodgeable Dose = Skin Wash 24 h + Pipette Tip 24 h + Donor Chamber Wash + Tissue Swab 24 h
Total Unabsorbed Dose = Total Dislodgeable Dose + Stratum Corneum + Unexposed Skin
Absorbed Dose = Cumulative Receptor Fluid + Receptor Chamber Wash
Dermal Delivery = Absorbed Dose + Epidermis + Dermis + Clingfilm
Mass Balance = Total Unabsorbed Dose + Dermal Delivery

[^{14}C]-Caprylhydroxamic Acid in Test Preparation 1 (*ca* 0.15%, w/w) was applied to human split-thickness skin *in vitro*. At 24 h post dose, the total dislodgeable dose was 41.03% (2910 ng equiv./cm^2) of the applied dose. The stratum corneum retained 2.79% (198 ng equiv./cm^2), of the applied dose with 0.44% (31.3 ng equiv./cm^2) being removed with the first two tape strips. The total unabsorbed dose was 43.99% (3120 ng equiv./cm^2) of the applied dose. The total absorbed dose and dermal delivery were 41.89% (2971 ng equiv./cm^2) and 51.45% (3649 ng equiv./cm^2) of the applied dose, respectively.

[^{14}C]-Caprylhydroxamic Acid in Test Preparation 2 (*ca* 0.15% w/w) was applied to human split-thickness skin *in vitro*. At 24 h post dose, the total dislodgeable dose was 47.38% (4099 ng equiv./cm^2) of the applied dose. The stratum corneum retained 5.15% (446 ng equiv./cm^2), of the applied dose with 0.73% (63.1 ng equiv./cm^2) being removed with the first two tape strips. The total unabsorbed dose was 52.67% (4558 ng equiv./cm^2) of the applied dose. The total absorbed dose and dermal delivery were 31.75% (2747 ng equiv./cm^2) and 43.84% (3793 ng equiv./cm^2) of the applied dose, respectively.

[^{14}C]-Caprylhydroxamic Acid in Test Preparation 3 (*ca* 0.15% w/w) was applied to human split-thickness skin *in vitro*. At 24 h post dose, the total dislodgeable dose was 54.17% (4310 ng equiv./cm^2) of the applied dose. The stratum corneum retained 5.93% (472 ng equiv./cm^2), of the applied dose with 1.08% (85.8 ng equiv./cm^2) being removed with the first two tape strips. The total unabsorbed dose was 60.23% (4792 ng equiv./cm^2) of the applied dose. The total absorbed dose and dermal delivery were 22.93% (1824 ng equiv./cm^2) and 36.87% (2933 ng equiv./cm^2) of the applied dose, respectively.

In conclusion, following topical application of [^{14}C]-Caprylhydroxamic Acid in Test Preparation 1, Test Preparation 2 or Test Preparation 3 to human skin *in vitro,* the absorbed dose was 41.89% (2971 ng equiv./cm^2), 31.75% (2747 ng equiv./cm^2) and 22.93% (1824 ng equiv./cm^2) of the applied dose, respectively. The dermal delivery was 51.45% (3649 ng equiv./cm^2), 43.84% (3793 ng equiv./cm^2) and 36.87% (2933 ng equiv./cm^2) of the applied dose, respectively. The mass balance for [^{14}C]-Caprylhydroxamic Acid from Test Preparation 1, Test Preparation 2 and Test Preparation 3 was 95.44% (6770 ng equiv./cm^2), 96.52% (8351 ng equiv./cm^2) and 97.10% (7725 ng equiv./cm^2) of the applied dose, respectively.

5 INTRODUCTION

The test item, Caprylhydroxamic Acid, is used in three cosmetic suspensions (Oil in Water, Silicone in Water and Clear Lotion). The concentration of Caprylhydroxamic Acid in the three suspensions is 0.15% (w/w).

As part of the safety evaluation of Caprylhydroxamic Acid, a study was required to assess the rate and extent of absorption of Caprylhydroxamic Acid following topical application of the three suspensions to human skin.

5.1 Study Location

The study was conducted at Charles River Laboratories Edinburgh Ltd., Elphinstone Research Centre, Tranent, East Lothian, EH33 2NE.

5.2 Study Dates

Key dates in the conduct of the study were as follows:

Study Initiation Date:	27 January 2016
Experimental Start Date:	27 January 2016
Experimental Completion Date:	24 March 2016
Study Completion Date:	See Compliance Statement page for date of Study Director's Signature.

5.3 Archive Location

All raw data generated and recorded during this study will be stored in the Scientific Archive of Charles River Laboratories Edinburgh Ltd., Elphinstone Research Centre for 2 years after the issue of the final report. After the 2 year period the Sponsor will be consulted regarding the disposal, transfer or continued storage of the raw data.

A reserve sample of the test item and formulation excipients, with the exception of the radiolabelled test item, will be retained in the Scientific Archive of Charles River Laboratories Edinburgh Ltd., Elphinstone Research Centre for a period of at least 2 years or until the quality of the material no longer affords evaluation.

The original signed copy of the final report will be stored indefinitely in the Scientific Archive of Charles River Laboratories Edinburgh Ltd., Elphinstone Research Centre.

5.4 Regulatory Citations

This study was performed in accordance with Good Laboratory Practice regulations. A copy of the GLP certificate for Charles River is provided in Appendix 1. This study was also performed in accordance with the following documents:

OECD Guideline for Testing of Chemicals, Guideline 428: Skin Absorption: *In Vitro* Method (2004).

OECD Environmental Health and Safety Publications Series on Testing and Assessment No. 28. Guidance Document for the Conduct of Skin Absorption Studies (2004).

SCCS (Scientific Committee on Consumer Safety). Basic Criteria for the *In Vitro* Assessment of Dermal Absorption of Cosmetic Ingredients. SCCS/1358/10 adopted 22 June 2010.

Scientific Committee on Consumer Products. Notes of Guidance for Testing of Cosmetic Ingredients and their Safety Evaluation, 9th Revision, 29 September 2015.

COLIPA (1997). Cosmetic Ingredients: Guidelines for Percutaneous Absorption/Penetration. The European Cosmetic, Toiletry and Perfume Association.

6 EXPERIMENTAL PROCEDURES

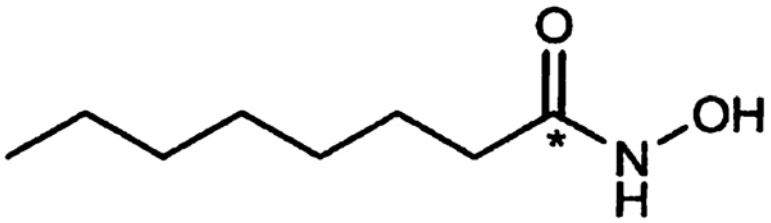
6.1 Materials

6.1.1 Radiolabelled Test Item

[^{14}C]-Caprylhydroxamic Acid (batch no. CFQ42636), was supplied by Quotient Bioresearch Ltd. on behalf of the Sponsor. This batch was tested by HPLC and found to be impure, so a replacement batch was provided and used on the study.

[^{14}C]-Caprylhydroxamic Acid (batch no. CFQ42749), supplied by Quotient Bioresearch Ltd. on behalf of the Sponsor, was stored in the dark, in a freezer set to maintain a temperature of -80°C. The specific activity and radiochemical purity were 360 µCi/mg and 99.6%, respectively. A copy of the Certificate of Analysis for the replacement batch is provided in Appendix 2.

The structure and site of labelling (*) of [^{14}C]-Caprylhydroxamic Acid are shown below.



6.1.2 Non-Radiolabelled Test Item

Non-radiolabelled Caprylhydroxamic Acid was supplied [redacted]. A Certificate of Analysis was supplied and a copy is provided in Appendix 3. The test item was stored in the dark at ambient temperature at Charles River. An archive sample has been retained at Charles River under the same storage conditions as detailed above. The name and properties of the test item are summarised below:

Test Item:	Caprylhydroxamic Acid [redacted]
Batch No:	FA8580
Expiry Date:	28 February 2017
Purity:	99.8%
Appearance:	Off-white crystals

6.2 Formulation Excipients

The formulation excipients, as summarised in the table to follow, were supplied and stored at ambient temperature.

Archive samples of the formulation excipients have been retained at Charles River, under the same storage conditions.

Name	Batch No.	Expiry Date
Butylene Glycol (1,3-Butylene glycol)	CB024851470	12 February 2019
Dimethicone 5 CS (200 Fluid, 5 cst, Xiameter PMX-200 5cs)	0007720852	29 December 2016
Laureth 4 (BRIJ L4-LQ-(AP))	0001025065	28 July 2017*
Cetearyl Glucoside (and) Cetearyl Alcohol	0011094963	06 December 2018
Cetyl Alcohol	PN 00F114217	05 August 2016
Cyclopentasiloxane (and) Dimethicone Crosspolymer	0007994859	06 October 2016
Cyclopentasiloxane (DC-245)	0007444821	27 June 2016
Dipropylene Glycol	3F2301N6HA	31 July 2016
Tocopheryl Acetate (DL-Alpha Tocopheryl Acetate (Vitamin E))	88196977L0	01 November 2016
Dimethicone 350 CS (Dow Corning 200 Fluid 350 cst)	0006722269	02 September 2016
Panthenol (D-Panthenol USP)	90844916K0	14 July 2017*
Glycerin (Glycerin USP)	HF506214322	15 November 2019
Hydroxyethylcellulose (Hydroxyethylcellulose (250 HHR Type))	24452	01 October 2016
Isopropyl Palmitate	0013991361	21 July 2020
Mineral Oil (Hydrobrite 1000)	621830	23 April 2017
PEG-100 Stearate	0000869720	09 April 2016
Polyacrylamide and C13-14 Isoparaffin and Laureth 7 (Sepigel 305)	U 50355	16 January 2017
Polyquaternium-39	EL5B2599A0	04 March 2017
Polysorbate 20	0000908387	03 August 2016
Sodium Acrylate/ Sodium Acryloyldimethyl Taurate Copolymer	150609011857	04 June 2017
Stearyl Alcohol	PN344714304	08 October 2019

* Retest date

6.3 Other Materials

Potassium dihydrogen phosphate was supplied by Alfa Aesar and Merck. Acetone, hydrochloric acid, phosphate buffered saline (PBS), penicillin-streptomycin solution, polyoxyethylene 20-oleyl ether (PEG; Brij®020), tin (II) chloride dihydrate, methanol and sodium azide were obtained from Sigma-Aldrich Ltd. Sodium hydroxide standard solution was obtained from Fluka. Solvable® was obtained from Perkin Elmer Inc. Ammonia solution and methanol were obtained from Fisher. Aquasafe 500 plus liquid scintillation fluid was obtained from Zinsser Analytic. ProFlow G+ was supplied by Meridian Biotechnologies Ltd. Simple soap was obtained from Unilever. Ethanol was obtained from Hayman Kimia. All other materials were obtained by Charles River.

6.4 Preparation of [^{14}C]-Caprylhydroxamic Acid Stock Solution

Acetone (2 mL) was added to the [^{14}C]-Caprylhydroxamic Acid (360 µCi/mg; Section 6.1.1, batch no. CFQ42749) in a vial and inverted/swirled until the test item was dissolved to produce the stock solution. Three aliquots (5 µL) of the stock solution were then transferred into scintillation vials and mixed with acetone (10 mL). The contents were mixed by inversion. Duplicate aliquots (1 mL) were removed from each vial, mixed with scintillation fluid (10 mL) and analysed by liquid scintillation counting. The concentration of test item in

the stock solution was determined to be 7.26 mg/mL with a coefficient of variation (CV) of 1.55%.

6.5 Confirmation of Radiochemical Purity of [^{14}C]-Caprylhydroxamic Acid

[^{14}C]-Caprylhydroxamic Acid (5 µL, Section 6.1.1, batch no. CFQ42749) was diluted with equal volumes of mobile phase A and mobile phase B (1100 µL, see below) and vortex mixed. A UV standard solution (1 mg/mL) was prepared by dissolving Caprylhydroxamic Acid (4 mg, Section 6.1.2) in 4 mL diluent (equal volumes (2 mL) of mobile phase A and mobile phase B, see below), vortex mixed and sonicated. An aliquot (100 µL) of the diluted [^{14}C]-Caprylhydroxamic Acid was added to a HPLC vial with UV standard (10 µL). Determination of radiochemical purity was performed using the equipment and conditions to follow:

Equipment
HPLC Model: Agilent 1100, Agilent 1260
Radiodetector Model: Beta Ram 4

Conditions

Column:	Phenomenex Luna C18 (2) (150 mm x 4.6 mm, 5 µm)
Mobile Phase A:	20 mM Potassium Dihydrogen Phosphate (pH 6.5)
Mobile Phase B:	Methanol
Run Time:	35 min
Mobile Phase Conditions:	Gradient
Flow Rate:	1.0 mL/min
Column Temperature:	40°C
Auto Sampler Temperature:	4°C
UV Wavelength:	220 nm

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
0	90	10
20	20	80
30	20	80
31	90	10
35	90	10

Data was captured by LAURA 4.0.4 (lab logic).

The radiochemical purity of the supplied [^{14}C]-Caprylhydroxamic Acid was determined to be 97.6%. A representative chromatogram is provided in Appendix 4.

6.6 Human Skin Samples

Five samples of full-thickness human skin (abdominal) were obtained from female patients aged 30 to 64 years old. Samples of full-thickness skin were obtained from NHS Lothian, UK and Tissue Solutions, UK. On arrival at Charles River, the samples of skin from NHS Lothian were cleaned of subcutaneous fat and connective tissue using a scalpel blade. The skin samples were washed in cold running water and dried using paper towels. The skin samples were then cut into smaller pieces, wrapped in aluminium foil or placed into plastic bags which were vacuum-packed and sealed, and stored in a freezer set to maintain a temperature of -20°C until they were used on the study. Skin obtained from Tissue Solutions arrived deep frozen on dry ice and was stored in a freezer set to maintain a temperature of -20°C until they were used on the study. The age and sex of the donor and site from which the skin was taken were recorded centrally and in the study records. The sample details are shown in Appendix 5.

6.7 Preparation of Split-Thickness Skin Membranes

Human skin samples were removed from -20°C storage and allowed to thaw at ambient temperature. The thickness of the full-thickness skin membranes was measured using a micrometer. Split-thickness membranes were prepared by pinning the full-thickness skin, stratum corneum uppermost, onto a raised cork board and cutting with an electric dermatome (Zimmer®) at a setting equivalent to 200-400 µm depth. The thickness of the membranes was measured using a micrometer. Membranes were then wrapped in foil and stored in a freezer, set to maintain a temperature of -20°C, for a maximum period of two months. The thickness of the full-thickness and split-thickness human skin membranes is provided in Appendix 6.

6.8 Static Diffusion Cell Apparatus

A static diffusion cell system (PermeGear Inc) was used (see photograph). The static diffusion cells were placed in a manifold on a magnetic stirrer plate heated *via* a circulating water bath to maintain the skin surface temperature at 32°C ± 1°C. The actual cell temperatures (ranging from 32.1°C to 32.6°C) were calibrated prior to mounting of the skin cells.

A Photograph of a Static Diffusion Cell and a Static Diffusion Cell in the Heated Manifold

The surface area of exposed skin within the cells was 3.14 cm^2. The receptor chamber volume was nominally 10 mL, with each receptor chamber individually marked with the actual volume by the manufacturer.

6.9 Receptor Fluid

The receptor fluid was PBS containing PEG (*ca* 6%, w/v), streptomycin (*ca* 0.1 mg/mL), penicillin (*ca* 100 units/mL) and sodium azide (*ca* 0.01%, w/v). The pH was measured and adjusted to pH 7.4 ± 0.1. The receptor fluid was degassed by sonication for *ca* 10 min after being made and was stored in a refrigerator set to maintain a temperature of 4°C prior to use on the study.

6.10 Solubility of Caprylhydroxamic Acid in Receptor Fluid

Caprylhydroxamic Acid was predicted to have a water solubility of 1.55 g/L (information from Sponsor). Caprylhydroxamic Acid (23.6 µg) would be applied for an application of 5 mg/cm^2 over a 3.14 cm^2 application area, for the formulations (0.15%, w/w). If 100% absorption in 1 h (10 mL) is assumed then, 23.6 µg/10 mL is equivalent to 2.36 mg/L, the maximum concentration of test item in receptor fluid. This receptor fluid, therefore, was not considered to be rate-limiting for solubility of the test item.

6.11 Static Diffusion Cell Preparation

Split-thickness skin was removed from a freezer set to maintain a temperature of -20°C and allowed to reach ambient temperature. The circulating water bath was set to maintain a skin surface temperature of 32°C ± 1°C (Section 6.7). Receptor fluid chambers containing

magnetic stirrer bars were filled with Receptor Fluid. Sections of split-thickness skin (*ca* 3 x 3 cm) were cut and mounted in the diffusion cells between the donor and receptor chamber. The donor chamber was tightened into place with a clamp. Each cell was filled above the calibration line on the receptor fluid arm with receptor fluid and checked visually after to confirm that no cells were leaking (leak test). No cells were found to be leaking. No air bubbles were present in the receptor fluid chamber.

6.12 Barrier Integrity Assessment

Skin samples were allowed to equilibrate at 32°C ± 1°C for *ca* 30 min. Phosphate buffered saline (3 mL) was then added to the donor chamber and the skin samples were allowed to equilibrate for a further *ca* 30 min. The electrical resistance was then measured using a Tinsley Databridge (Model 6401) set at low voltage alternating current, 1000 Hz with a maximum voltage of 300 mV root-mean-squared (rms) in the parallel equivalent circuit mode. Any skin sample exhibiting a resistance less than 4 kΩ was excluded from subsequent absorption measurements. A cross reference of skin cell number, donor number and electrical resistance (kΩ) is presented in Appendix 7. The phosphate buffered saline was removed from the skin surface; the skin was rinsed with water (2-3 mL) and dried with a tissue swab.

6.13 Predose Receptor Fluid Collection

Prior to dosing, a 300 µL (6 x 50 µL aliquots) sample of receptor fluid was removed from the receptor chamber collection arm. The receptor fluid volume was then maintained by the addition of fresh receptor fluid up to the calibration line on the receptor chamber collection arm. The receptor fluid samples were mixed with methanol: scintillation fluid (1:5, v/v; 12 mL) and analysed by liquid scintillation counting. Following sample collection, the receptor chamber collection arm was sealed with Parafilm® to prevent evaporation of receptor fluid from the receptor chamber.

6.14 Preparation of Test Preparations

Caprylhydroxamic Acid [redacted], 23.3 mg, Section 6.1.2) was added to a 2 mL volumetric flask. [^{14}C]-Caprylhydroxamic Acid stock solution (964 µL, specific activity 360 µCi/mg, Section 6.4) was added to the volumetric flask. Acetone was added up to the 2 mL calibration line and the sample vortex mixed for *ca* 30 sec. Three aliquots (5 µL) were transferred to vials and acetone (10 mL) added to each and mixed by inversion. Duplicate aliquots were taken from each (1 mL), mixed with scintillation fluid (10 mL) and analysed by liquid scintillation counting.

By radioactivity, the concentration of [^{14}C]-Caprylhydroxamic Acid in the solution was determined to be 14.9 mg/mL. [^{14}C]-Caprylhydroxamic Acid was homogeneously distributed in the solution with a CV of 3.21%. The specific activity of [^{14}C]-Caprylhydroxamic Acid was calculated to be 79.0 µCi/mg.

6.14.1 Preparation of Caprylhydroxamic Acid Premixes

Aliquots of the [^{14}C]-Caprylhydroxamic Acid solution (801 µL, 79.0 µCi/mg, Section 6.14) were transferred into 2 small glass vials (Premix 10 and Premix 11) and solvent removed under a stream of nitrogen gas.

Glycerin USP (177.53 mg) was added to CHA Premix 10, heated in a water bath to 50°C (± 2°C) and stirred gently to avoid formation of bubbles.

Glycerin USP (444.05 mg) was added to CHA Premix 11, heated in a water bath to 50°C (± 2°C) and stirred gently to avoid formation of bubbles.

6.14.2 Test Preparation 1 (Oil in Water Suspension)

For Test Preparation 1, Premix 12 and 13 were first prepared, according to the supplied method, as summarised in sections to follow. These were then mixed with Premix 10 and Polyacrylamide and C13-14 Isoparaffin and Laureth 7 to make the final formulation.

6.14.2.1 Premix 12 (Oil Phase)

Excipient	Mass (mg)
Mineral Oil	[redacted]
Isopropyl Palmitate	
Cetearyl Glucoside and Cetearyl Alcohol*	
PEG-100 Stearate*	
Stearyl Alcohol*	
Cetyl Alcohol*	
DL-Alpha Tocopheryl Acetate	
Total	

* Ground with a mortar and pestle prior to weighing

Each of the above excipients were weighed into a single 15 mL centrifuge tube before mixing the contents on a magnetic stirrer in a heated waterbath (*ca* 62°C to 69°C). To ensure Premix 12 was fully mixed, contents of tube were subjected to centrifugation (*ca* 2000 g for 6 min, *ca* 3000 g for 5 min, all at ambient temperature) and additional mixing in a heated water bath (*ca* 62°C to 69°C).

6.14.2.2 Premix 13 (Water Phase)

Excipient	Mass (mg)
D-Panthenol USP	[redacted]
Ultrapure water	
Total	

The above excipients were added to a 7 mL glass vial and mixed by magnetic stirrer in a heated water bath (*ca* 62°C to 69°C).

6.14.2.3 Preparation of Test Preparation 1 from the Premixes

CHA Premix 10 (64.92 mg, Section 6.14.1) was added to Premix 13 and mixed on a magnetic stirrer, heated in a water bath (*ca* 67°C to 69°C). The vial was further mixed by vortex. Following mixing, 1.78651 g of the mixture was added to Premix 12 and mixed on a magnetic stirrer, heated in the above water bath. Additional vortex mixing, mixing by FastPrep® (2 bursts of 20 s) and centrifugation (*ca* 3000 g for 6 min) was given to fully mix contents. Polyacrylamide and C13-14 Isoparaffin and Laureth 7 (40.40 mg) was added to the preparation and mixed by vortex and magnetic stirrer, while cooling to ambient temperature.

Six weighed aliquots (*ca* 15.7 mg) were taken, mixed by inversion with acetone (10 mL) and sonicated (*ca* 10 min). Duplicate aliquots (1 mL) were taken of each and scintillation fluid (10 mL) added, before being analysed by liquid scintillation counting.

By radioactivity, the concentration of [^{14}C]-Caprylhydroxamic Acid in Test Preparation 1 was determined to be 1.44 mg/g (0.144%, w/w). This was 95.88% of the target concentration (0.15%, w/w). [^{14}C]-Caprylhydroxamic Acid was homogenously distributed in the solution with a CV of 0.73%. Test Preparation 1 was accepted for dosing. There were some bubbles in Test Preparation 1, so this was centrifuged at 184 g for 2 min prior to dosing. The final concentrations of all the excipients are summarised in the table to follow.

Excipient	**Concentration (%, w/w)**
Mineral Oil	[redacted]
Isopropyl Palmitate	
Cetearyl Glucoside and Cetearyl Alcohol	
PEG-100 Stearate	
Stearyl Alcohol	
Cetyl Alcohol	
DL-Alpha Tocopheryl Acetate	
D-Panthenol USP	
Polyacrylamide C13-14 Isoparaffin and Laureth 7	
Ultrapure water	
Glycerin	
Caprylhydroxamic Acid	0.14
Total	100

6.14.3 Test Preparation 2 (Silicone in Water Suspension)

For Test Preparation 2, Premix 14 and 15 were first prepared, according to supplied method, as summarised in the sections to follow. These were then mixed with Premix 11 and

Sodium Acrylate/Sodium Acryloyldimethyl Taurate Copolymer to prepare the final formulation.

6.14.3.1 Premix 14 (Silicone Phase)

Excipient	Mass (mg)
Dow Corning 200 Fluid 350 cst	[redacted]
DC-245	[redacted]
200 Fluid 5cst, Xiameter PMX-200 5cs	[redacted]
Cyclopentasiloxane (and) Dimethicone	[redacted]
Brij L4-LQ-(AP)	[redacted]
Total	[redacted]

The above excipients were added to a small vial before mixing by magnetic stirrer in a heated waterbath (50°C ± 2°C).

6.14.3.2 Premix 15 (Water Phase)

Excipient	Mass (mg)
Sodium Acrylate/Sodium Acryloyldimethyl Taurate Copolymer	[redacted]
Ultrapure water	[redacted]
Total	[redacted]

The above excipients were added to a 15 mL centrifuge tube and Mixed by Fast Prep-24® (20 sec burst) and magnetic stirrer in a heated water bath (50°C ± 2°C).

6.14.3.3 Preparation of Test Preparation 2 from the Premixes

CHA Premix 11 (103.68 mg, Section 6.14.1) was added to Premix 15 and mixed both by vortex and on a magnetic stirrer, and heated in a water bath (50°C ± 2°C). Premix 14 (323.00 mg), was added to contents of centrifuge tube, mixed by vortex and a magnetic stirrer. Sodium Acrylate/Sodium Acryloyldimethyl Taurate Copolymer (19.47 mg) was added to preparation and mixed by vortex (*ca* 10 min) and centrifugation (*ca* 740 g, 1.5 min).

Six weighed aliquots (*ca* 15.7 mg) were taken, mixed by vortex with acetone (10 mL). Duplicate aliquots (1 mL) were taken of each and scintillation fluid (10 mL) added, before being analysed by liquid scintillation counting.

By radioactivity, the concentration of [^{14}C]-Caprylhydroxamic Acid in Test Preparation 2 was determined to be 1.75 mg/g (0.175%, w/w). This was 116.5% of the target concentration (0.15%, w/w). Due to the complexity of the test preparation method, it was not possible to dilute the prepared solution to achieve within 10% of the required concentration. Following discussion with the Sponsor, it was agreed that concentrations of up to 0.2% (w/w) were acceptable. [^{14}C]-Caprylhydroxamic Acid was homogenously distributed in the solution with

a CV of 1.02%, therefore, Test Preparation 2 was accepted for dosing. The final concentrations of all the excipients are summarised in the table to follow.

Excipient	Concentration (%, w/w)
Dow Corning 200 Fluid 350 cst	[redacted]
DC-245	
200 Fluid 5cst, Xiameter PMX-200 5cs	
Cyclopentasiloxane (and) Dimethicone	
Brij L4-LQ-(AP)	
Sodium Acrylate/Sodium Acryloyldimethyl Taurate Copolymer	
Ultrapure water	
Glycerin	
Caprylhydroxamic Acid	0.17
Total	100

6.14.4 Test Preparation 3 (Clear Lotion Suspension)

For Test Preparation 3, Premix 16, 17 and 18 were first prepared, according to supplied method, as summarised in the sections to follow. These were then mixed with Premix 11 to prepare the final formulation.

6.14.4.1 Premix 16 (Premix A)

Excipient	Mass (mg)
Ultrapure water	[redacted]
Polyquarternium-39	
Total	

The above excipients were added to a 4 mL vial and mixed by vortex, sonication and on a magnetic stirrer plate in a heated waterbath (*ca* 50°C).

6.14.4.2 Premix 17 (Premix B)

Excipient	Mass (mg)
Dipropylene Glycol	[redacted]
1,3-Butylene Glycol	
Hydroxyethylcellulose	
Total	

The above excipients were added to a 4 mL vial. The solution was then mixed by vortex, sonication, and by magnetic stirrer in a heated waterbath (*ca* 50°C).

6.14.4.3 Premix 18 (Premix C)

Excipient	Mass (mg)
Ultrapure water	[redacted]
Polysorbate 20	[redacted]
Total	[redacted]

The above excipients were added to a 4 mL vial. The resulting solution was then mixed by vortex and on a magnetic stirrer plate on a heated waterbath (50°C ± 2°C).

6.14.4.4 Preparation of Test Preparation 3 from the Premixes

Premix	Mass Added	Manipulation
Premix 17	[redacted]	Heated in waterbath (45°C to 50°C)
Premix 16	[redacted]	Added in 2 aliquots, vial swirled between additions, mixed by vortex and on a magnetic stirrer. Sonicated for *ca* 10 min.
Premix 18	[redacted]	Added in 2 aliquots, vial vortexed briefly between additions.
Premix 11 (Section 6.14.1)	[redacted]	Mixed on magnetic stirrer plate in a waterbath (45°C to 50°C) for *ca* 5 mins then mixed on a magnetic stirrer at ambient temperature until cooled.

The above components were added to a clean 7 mL glass vial and mixed as described. Six aliquots (*ca* 15.7 mg) of the resulting test preparation were taken, mixed by inversion with acetone (10 mL). Duplicate aliquots (1 mL) were taken of each and scintillation fluid (10 mL) added, then analysed by liquid scintillation counting.

By radioactivity, the concentration of [^{14}C]-Caprylhydroxamic Acid in Test Preparation 3 was determined to be 1.64 mg/g (0.164%, w/w). This was 109.3% of the target concentration (0.15%, w/w). [^{14}C]-Caprylhydroxamic Acid was homogenously distributed in the solution with a CV of 0.65%. Therefore, Test Preparation 3 was accepted for dosing. The final concentrations of all the excipients are summarised in the table to follow.

Excipient	Concentration (%, w/w)
Polyquaternium-39	[redacted]
Dipropylene Glycol	[redacted]
1,3-Butylene Glycol	[redacted]
Hydroxyethylcellulose	[redacted]
Polysorbate 20	[redacted]
Ultrapure water	[redacted]
Glycerin	[redacted]
Caprylhydroxamic Acid	0.16
Total	100

6.15 Test Item Stability Confirmation

Prior to dosing, an aliquot (15.73 mg, 17.24 mg, and 16.09 mg, Test Preparations 1, 2 and 3, respectively) was taken from each test preparation. The aliquots were diluted with Mobile Phase A: Mobile Phase B (1:1, v/v) (380 µL, 466 µL, and 436 µL, for Test Preparations 1, 2, and 3, respectively). The radiochemical purity of [^{14}C]-Caprylhydroxamic Acid in the test preparations was determined by HPLC following the method detailed in Section 6.5. The process described above was repeated immediately post dose. For post dose analysis aliquot weights were: 14.67 mg, 15.93 mg, and 16.01 mg, Test Preparations 1, 2 and 3, respectively. The results of this analysis are provided in the following table.

Sample Description	Radiochemical Purity (%)
Pre dose [^{14}C]-Caprylhydroxamic Acid in Test Preparation 1	98.1
Post dose [^{14}C]-Caprylhydroxamic Acid in Test Preparation 1	97.7
Pre dose [^{14}C]-Caprylhydroxamic Acid in Test Preparation 2	97.8
Post dose [^{14}C]-Caprylhydroxamic Acid in Test Preparation 2	98.3
Pre dose [^{14}C]-Caprylhydroxamic Acid in Test Preparation 3	98.0
Post dose [^{14}C]-Caprylhydroxamic Acid in Test Preparation 3	97.3

The results of the radiochemical purity assessment confirmed that the test item was stable over the dosing period in all 3 test preparations.

6.16 Application of Test Preparations to Human Skin

Test Preparation 1 was applied evenly over the entire stratum corneum surface of the exposed skin of 12 split-thickness samples using a Rainin MR25 positive displacement pipette set to deliver *ca* 15.7 mg (*ca* 5 mg/cm^2). Seven representative aliquots of Test Preparation 1 were dispensed into scintillation vials at the time of dosing, weighed, diluted with acetone (10 mL) and the contents mixed by inversion and sonicated for *ca* 10 min. Duplicate aliquots (1 mL) were taken and scintillant (10 mL) added, then analysed by liquid scintillation counting. Test Preparation 2 and Test Preparation 3 were applied as described above. Representative aliquots were also taken and processed as described above (with the exception that representative samples were not sonicated).

The results of the representative aliquots are provided in the table below.

Test Preparation	Target [^{14}C]-Caprylhydroxamic Acid Concentration (%, w/w)	Actual [^{14}C]-Caprylhydroxamic Acid Concentration	
		(%, w/w)	CV (%)
1	0.15%	0.143%	0.82
2	0.15%	0.172%	2.09
3	0.15%	0.157%	0.80

6.17 Receptor Fluid Sampling

Receptor fluid aliquots were collected at 2, 4, 6, 8 and 12 h post dose as described in Section 6.13. All receptor fluid samples were mixed with methanol (2 mL) and scintillation fluid prior to analysis by liquid scintillation counting. After each sample had been collected it was ensured that no large bubbles were present under the skin.

6.18 Terminal Procedures (24 h Post Dose)

The exposure period was terminated at 24 h post dose. Commercial hand wash soap (*ca* 50 µL) was applied to the skin and the soap gently rubbed onto the skin with a tissue swab. The skin was then rinsed with *ca* 5 mL of a *ca* 2% (v/v) commercial soap solution. The soap solution was applied in aliquots (1 mL) and each aliquot was aspirated three times with a pipette. The skin was dried with a tissue swab. The process was repeated and the skin was dried with an additional tissue swab.

The soap solution (skin wash) was pooled into a single pre-weighed vial for each cell. Methanol (10 mL) was added to all skin wash vials for Test Preparation 1. Duplicate weighed aliquots (1 mL) were taken from all skin wash samples, mixed with scintillation fluid (10 mL) and analysed by liquid scintillation counting. The tissue swabs were pooled into a single vial for each cell. The pipette tip was cut in half and retained. Methanol: scintillation fluid (1:5, v/v; 12 mL) was added to tissue swabs and pipette tips and analysed by liquid scintillation counting.

Donor chambers were transferred to pre-weighed donor wash pots containing methanol (*ca* 40 mL). Donor chambers were extracted in methanol for at least 30 min before sonication (10 min). After removal of the donor chambers, duplicate weighed aliquots (1 mL) of the solvent were mixed with scintillation fluid (10 mL) and analysed by liquid scintillation counting. The skin was removed from each cell and placed on a piece of tissue to remove any remaining receptor fluid from the underside of the skin. This tissue was placed into the pre-weighed receptor chamber wash pot for that particular cell.

The stratum corneum was removed with 20 successive tape strips (Scotch® tape). The skin sample was rotated 90° after each tape strip. Rotation was stopped if the epidermis/dermis junction became fragile or if epidermis was removed. Each tape strip was placed into an individual vial containing methanol: scintillation fluid (1:5, v/v; 12 mL) and then analysed by liquid scintillation counting. Where a piece of epidermis was removed, this was recorded and is presented in Appendix 8. Where all of the epidermis was removed tape stripping was stopped.

The skin under the cell flange (unexposed skin) was cut away from the exposed skin and placed into a vial containing Solvable® (3 mL). Exposed skin samples were placed on

clingfilm which was folded over covering the entire epidermis. A *ca* 200 g weight was heated to *ca* 65°C in a water bath and placed onto the epidermal surface for *ca* 90 s. The epidermis was peeled away from the dermis using a scalpel. Epidermis and dermis samples were collected in separate vials containing Solvable® (3 mL or 1.5 mL, for dermis and epidermis respectively). Methanol scintillation fluid (1:5, v/v; 12 mL) was added to clingfilm samples prior to analysis by liquid scintillation counting.

All skin samples were placed into a waterbath set to *ca* 60°C to aid solubilisation. When fully dissolved, unexposed and dermis samples were each split evenly across 2 vials. Stannous chloride solution (0.2 g/mL in ethanol; 150 µL) and scintillation fluid (10 mL) were added to all dermis, epidermis and unexposed skin samples and analysed by liquid scintillation counting.

The bulk receptor fluid was removed from each receptor chamber and retained in a vial. This was split into four parts, with approximately 2.5 mL being transferred to each of three new vials. Scintillation fluid (10 mL) was added to all vials before samples were analysed by liquid scintillation counting.

The receptor chambers were rinsed with methanol (40 mL). The solvent was pooled as a single sample into the pre-weighed receptor wash pot. Duplicate weighed aliquots (1 mL) of the solvent were mixed with scintillation fluid (10 mL) and analysed by liquid scintillation counting.

6.19 Re-analysis

Due to poor recovery, the epidermis sample for Cell 18 was re-analysed in the following manner. A sub sample of the epidermis for Cell 18 (6 mL) was transferred into a fresh scintillation vial and stannous chloride solution (0.2 g/mL in ethanol; 150 µL) and scintillation fluid (5 mL) were added to both the original vial and the sub sample, prior to analysis by liquid scintillation counting. Results of re-analysis, when compared to original values, indicated that there was no epidermis in the sample as values were much lower when compared to other cells. Data from Cell 18 was excluded from mean and SD calculations.

6.20 Storage of Samples

All samples were stored at ambient temperature for analysis. Following analysis, bulk samples were stored in a freezer set to maintain a temperature of -20°C.

6.21 Quantification of Total Radioactivity

All samples were counted together with representative blanks using a liquid scintillation analyser (Packard 2100-TR) with automatic quench correction by external standard. Representative blank sample values were subtracted from sample count rates to give net

d.p.m. per sample. Prior to analysis, samples were allowed to stabilise with regard to light and temperature.

6.22 Limit of Reliable Measurement

A limit of reliable measurement of 30 d.p.m. above background has been instituted in these laboratories. Counts that are below 30 d.p.m. above background represent a true value. This means that data are recorded with values that are less than the limit of reliable measurement. Results calculated from data less than 30 d.p.m above background have been highlighted with an asterisk (*) in the results tables, and the limit of reliable measurement line is included in the receptor fluid figures. The instrumental equipment used to quantify radioactivity records data to a fraction of a d.p.m, and reports these values as a mean rounded value over the counting period (5 min).

6.23 Electronic Data Acquisitions and Systems

Total radioactivity and sample weight data was acquired using DEBRA® (version 5.7.10.129) Laboratory Information System (LabLogic Systems Limited).

The data was transferred into Microsoft® Office Excel® 2007 for calculation of result tables and graphs.

Chromatography data was acquired using Laura® version 4.0.4 (LabLogic Systems Limited).

6.24 Calculations – Distribution of Radiolabelled Test Item

The following calculations were performed:

$$\text{Sample amount (ng equiv./cm}^2\text{)} = \frac{\text{sample radioactivity (d.p.m.)}}{\text{specific activity (d.p.m./ng equiv.) x exposure area (cm}^2\text{)}}$$

$$\text{Sample applied dose (\%)} = \frac{\text{sample radioactivity (d.p.m.) x 100\%}}{\text{applied dose (d.p.m.)}}$$

6.25 Data Presentation

Data presented in results, tables, figures and appendices are computer generated and rounded appropriately for inclusion in the report. As a consequence, calculation of values from data presented will, in some instances, yield minor variations.

7 DEFINITIONS

The definitions used are taken directly from the OECD Guidance Document No. 28. For the definitions see Appendix 9.

8 PROTOCOL ADHERENCE

The study was performed in accordance with the protocol for Charles River Study No. 798232 and protocol amendment 1, with the following deviations:

Protocol Section 8.9 lists the batch numbers for the formulation excipients used in the study. Due to a typographical error, the batch numbers for DL Alpha Tocopheryl Acetate (Vitamin E), D Panthenol USP and Polyquaternium 39 had the letter "O" in the protocol. These have been correctly recorded in the study files as the number "0". As the correct materials were used in the study, there is no impact on study integrity.

Protocol Section 12.2 states that the receptor fluid will be pH 7.4 + 0.1. The receptor fluid used in the study was pH 7.32, which is outside this range. However, this was an error in the protocol, the correct range should have been pH 7.4 ± 0.1, in which case the receptor fluid would have been acceptable. This very slight difference in pH would have had negligible impact on test item solubility, and the receptor fluid was appropriate for use. There is no impact on study integrity.

9 REFERENCES

Craig, Susan (2014), Charles River Study No. 992063, Report No. 35627 "The *In Vitro* Percutaneous Absorption of Radiolabelled Testosterone Through Human Skin"

10 RESULTS AND DISCUSSION

10.1 [^{14}C]-Caprylhydroxamic Acid in Test Preparation 1 (Oil in Water Suspension, ca 0.15%, w/w)

[^{14}C]-Caprylhydroxamic Acid in Test Preparation 1 (*ca* 0.15%, w/w) was applied to a total of 12 samples of human skin obtained from 5 different donors. Absorption calculated throughout the course of the experiment was consistent for all samples, and increased over the 24 h monitoring period (Figure 1). The mass balance for all samples was within 100% ± 10%. Therefore, the results presented are mean values (n = 12) obtained from 5 different donors.

The distribution of radioactivity (% applied dose) at 24 h post dose is provided in Table 1. The mean mass balance was 95.44% of the applied dose at 24 h post dose. The mean total unabsorbed dose was 43.99% of the applied dose. This consisted of the total dislodgeable dose (41.03%), unexposed skin (0.17%) and the radioactivity associated with the stratum corneum (2.79%). The first 2 tape strips contained 0.44% of the applied dose. There was a consistency in the recovery of radioactivity associated with the stratum corneum. Tape strips 3-5, 6-10, 11-15 and 16-20 contained a further 0.42%, 0.70%, 0.66% and 0.57%, respectively. The absorbed dose (41.89%) was the sum of the receptor fluid (39.08%) and receptor wash (2.81%). The exposed skin (sum of epidermis, dermis and clingfilm samples) contained 9.56% of the applied dose. Dermal delivery (51.45%) was the sum of the absorbed dose and exposed skin. The absorption profile is provided in Table 2 and Figure 2.

The distribution, by mass, of [^{14}C]-Caprylhydroxamic Acid at 24 h post dose is shown in Table 3. The mean mass balance, total dislodgeable dose, total unabsorbed dose, absorbed dose, and dermal delivery were 6770, 2910, 3120, 2971, and 3649 ng equiv./cm^2, respectively. The distribution of radioactivity through the stratum corneum is provided in Table 4 and Figure 3. The absorption profile, by mass, is provided in Table 5 and Figure 4.

10.2 [^{14}C]-Caprylhydroxamic Acid in Test Preparation 2 (Silicone in Water Suspension, *ca* 0.15%, w/w)

[^{14}C]-Caprylhydroxamic Acid in Test Preparation 2 (*ca* 0.15%, w/w) was applied to a total of 12 samples of human skin obtained from 5 different donors. The individual absorption profiles were similar for all samples, and increased over the 24 h monitoring period (Figure 5). The mass balance for all samples was within 100% ± 10%, (except for Cell 18, suspected missing epidermis sample and results excluded from calculations). Therefore, the results presented are mean values (n = 11) obtained from 5 different donors.

The distribution of radioactivity (% applied dose) at 24 h post dose is provided in Table 6. The mean mass balance was 96.52% of the applied dose at 24 h post dose. The mean total unabsorbed dose was 52.67% of the applied dose. This consisted of the total dislodgeable

dose (47.38%), unexposed skin (0.15%) and the radioactivity associated with the stratum corneum (5.15%). The first 2 tape strips contained 0.73% of the applied dose. There was a consistency in the recovery of radioactivity associated with the stratum corneum. Tape strips 3-5, 6-10, 11-15 and 16-20 contained a further 0.80%, 1.27%, 1.25% and 1.10%, respectively. The absorbed dose (31.75%) was the sum of the receptor fluid (29.35%) and receptor wash (2.41%). The exposed skin (sum of epidermis, dermis and clingfilm samples) contained 12.08% of the applied dose. Dermal delivery (43.84%) was the sum of the absorbed dose and exposed skin. The absorption profile is provided in Table 7 and Figure 6.

The distribution, by mass, of [^{14}C]-Caprylhydroxamic Acid at 24 h post dose is shown in Table 8. The mass balance, total dislodgeable dose, total unabsorbed dose, absorbed dose and dermal delivery were 8351, 4099, 4558, 2747, and 3793 ng equiv./cm^2, respectively. The distribution of radioactivity through the stratum corneum is provided in Table 9 and Figure 7. The absorption profile, by mass, is provided in Table 10 and Figure 8.

10.3 [^{14}C]-Caprylhydroxamic Acid in Test Preparation 3 (Clear Lotion Suspension, *ca* 0.15%, w/w)

[^{14}C]-Caprylhydroxamic Acid in Test Preparation 3 (*ca* 0.15%, w/w) was applied to a total of 12 samples of human skin obtained from 5 different donors. The mass balance for all samples was within 100% ± 10%. Therefore, the results presented are mean values (n = 12) obtained from 5 different donors. The individual absorption profiles were similar for all samples, and increased over the 24 h monitoring period (Figure 9).

The distribution of radioactivity (% applied dose) at 24 h post dose is provided in Table 11. The mean mass balance was 97.10% of the applied dose at 24 h post dose. The mean total unabsorbed dose was 60.23% of the applied dose. This consisted of the total dislodgeable dose (54.17%), unexposed skin (0.14%) and the radioactivity associated with the stratum corneum (5.93%). The first 2 tape strips contained 1.08% of the applied dose. There was consistency in the recovery of radioactivity associated with the stratum corneum. Tape strips 3-5, 6-10, 11-15 and 16-20 contained a further 0.96%, 1.34%, 1.35% and 1.20%, respectively. The absorbed dose (22.93%) was the sum of the receptor fluid (21.09%) and receptor wash (1.84%). The exposed skin (sum of epidermis, dermis and clingfilm samples) contained 13.94% of the applied dose. Dermal delivery (36.87%) was the sum of the absorbed dose and exposed skin. The absorption profile is provided in Table 12 and Figure 10.

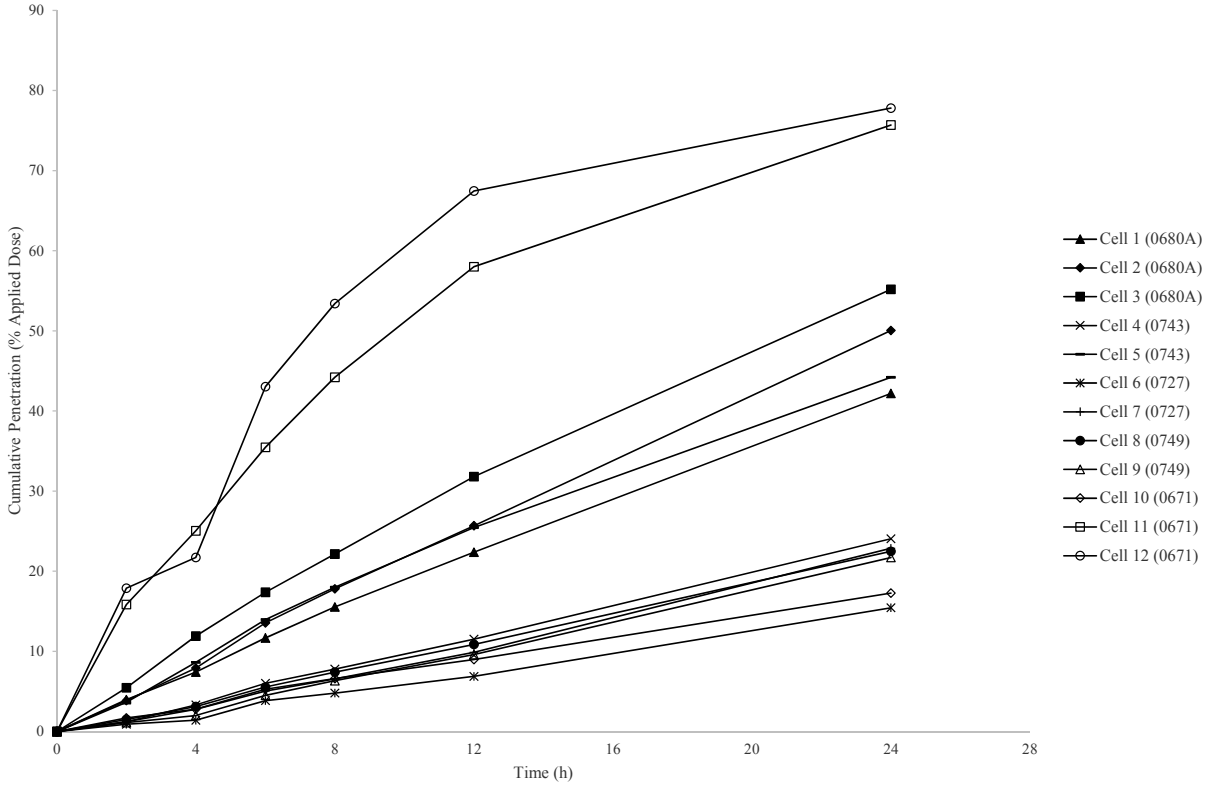
The distribution, by mass, of [^{14}C]-Caprylhydroxamic Acid at 24 h post dose is shown in Table 13. The mass balance, total dislodgeable dose, total unabsorbed dose, absorbed dose and dermal delivery were 7725, 4310, 4792, 1824, and 2933 ng equiv./cm^2, respectively. The distribution of radioactivity through the stratum corneum is provided in Table 14 and Figure 11. The absorption profile, by mass, is provided in Table 15 and Figure 12.

11 CONCLUSION

In conclusion, following topical application of [^{14}C]-Caprylhydroxamic Acid in Test Preparation 1, Test Preparation 2 and Test Preparation 3 to human skin *in vitro,* the absorbed dose was 41.89% (2971 ng equiv./cm^2), 31.75% (2747 ng equiv./cm^2) and 22.93% (1824 ng equiv./cm^2) of the applied dose, respectively. The dermal delivery was 51.45% (3649 ng equiv./cm^2), 43.84% (3793 ng equiv./cm^2) and 36.87% (2933 ng equiv./cm^2) of the applied dose, respectively. The mass balance was 95.44% (6770 ng equiv./cm^2), 96.52% (8351 ng equiv./cm^2) and 97.10% (7725 ng equiv./cm^2) of the applied dose, respectively.

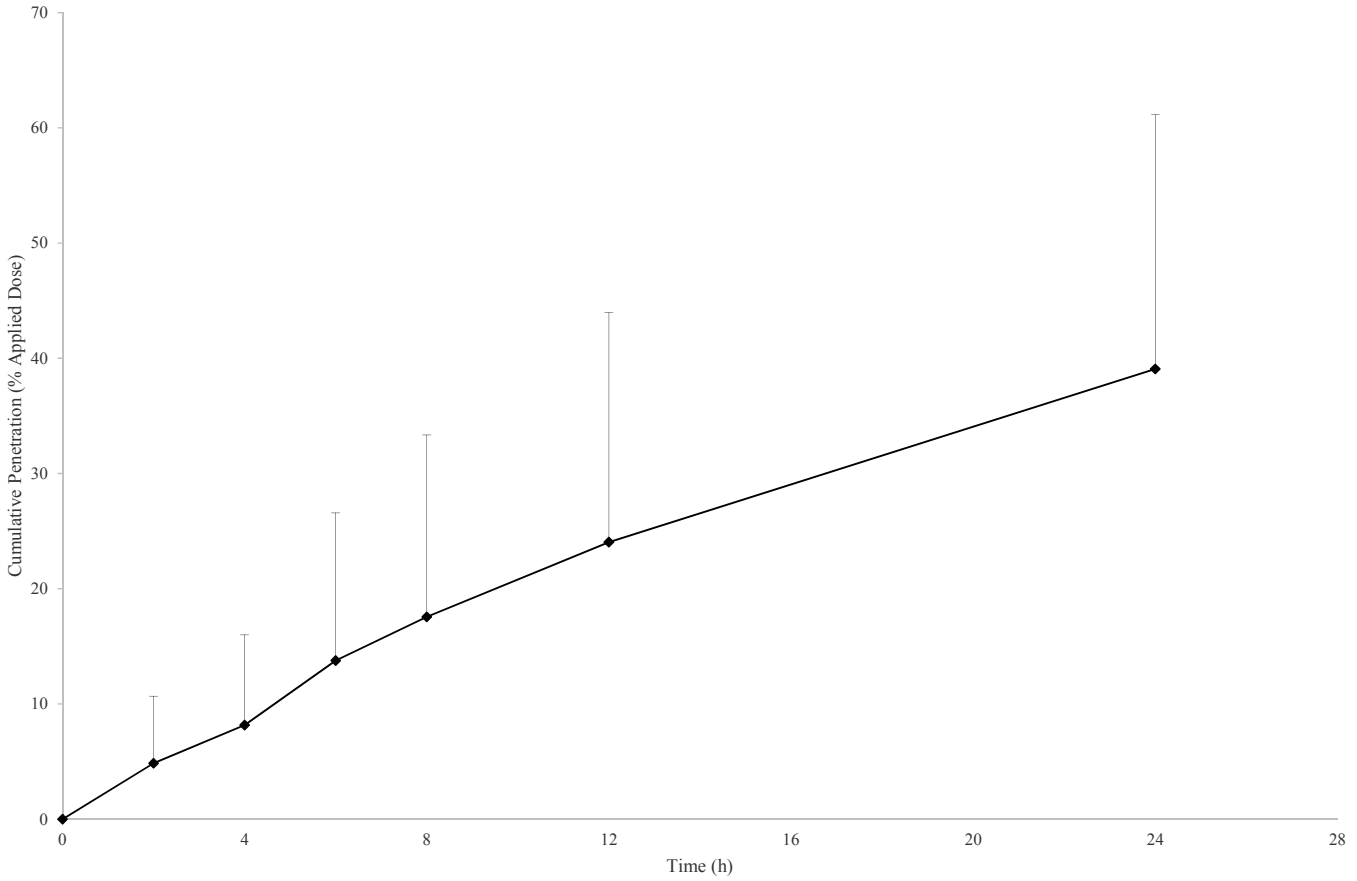
12 FIGURES

Figure 1 **Individual Absorption Profile for [14C]-Caprylhydroxamic Acid (% Applied Dose) in Receptor Fluid Following Topical Application of [14C]-Caprylhydroxamic Acid Test Preparation 1 (ca 0.15%, w/w) to Human Split-Thickness Skin**



***** Limit of reliable measurement is 0.026% (0-12 h) and 0.003% (24 h) of the applied dose

Figure 2 **Absorption Profile for [14C]-Caprylhydroxamic Acid (% Applied Dose) in Receptor Fluid Following Topical Application of [14C]-Caprylhydroxamic Acid in Test Preparation 1 (ca 0.15%, w/w) to Human Split-Thickness Skin (n =12)**



* Limit of reliable measurement is 0.026% (0-12 h) and 0.003% (24 h) of the applied dose

Figure 3 **Distribution of [14C]-Caprylhydroxamic Acid (ng equiv./cm2) in Stratum Corneum at 24 h Post Application Following Topical Application of [14C]-Caprylhydroxamic Acid in Test Preparation 1 (ca 0.15%, w/w) to Human Split-Thickness Skin (n = 12)**

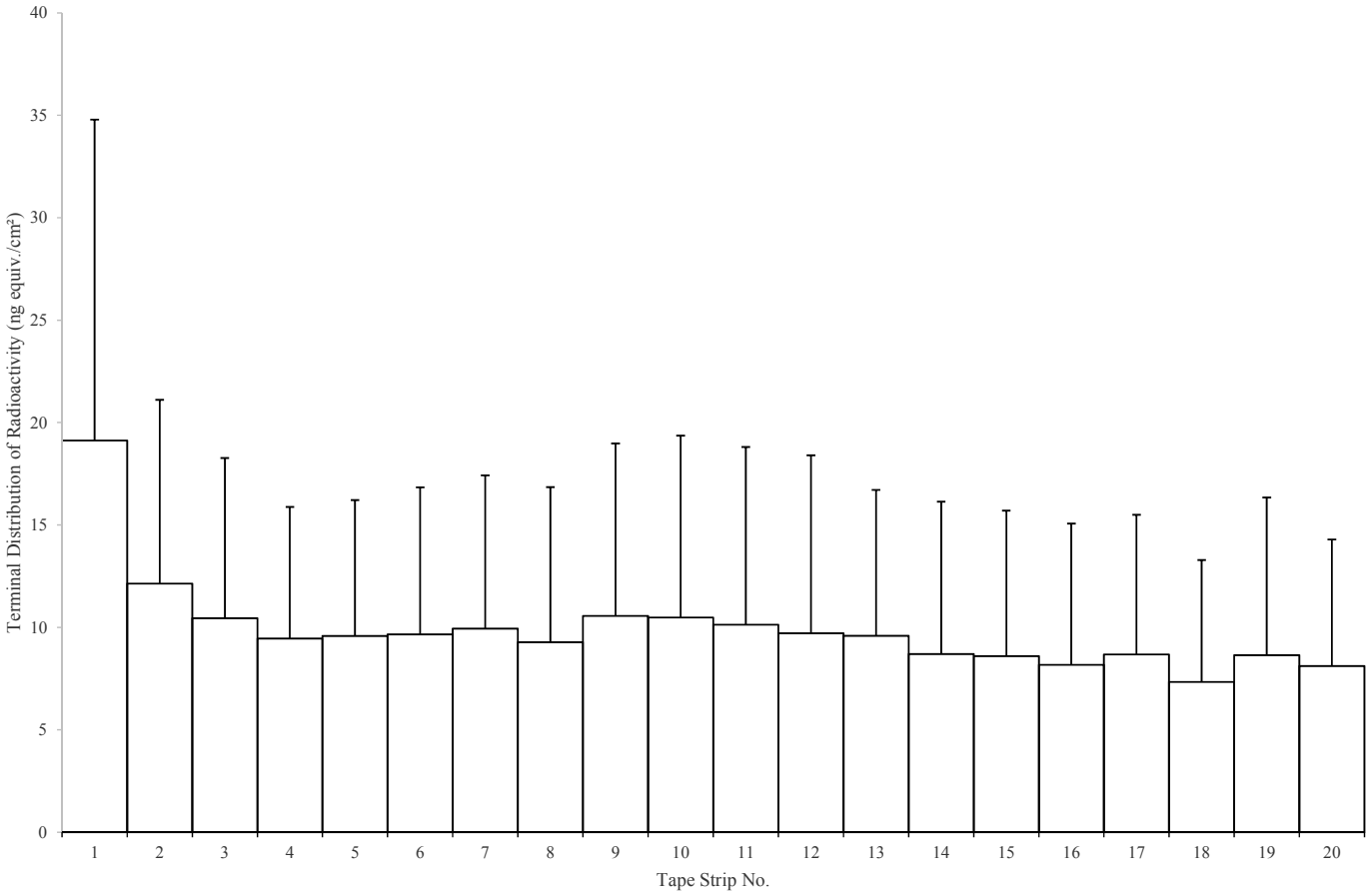
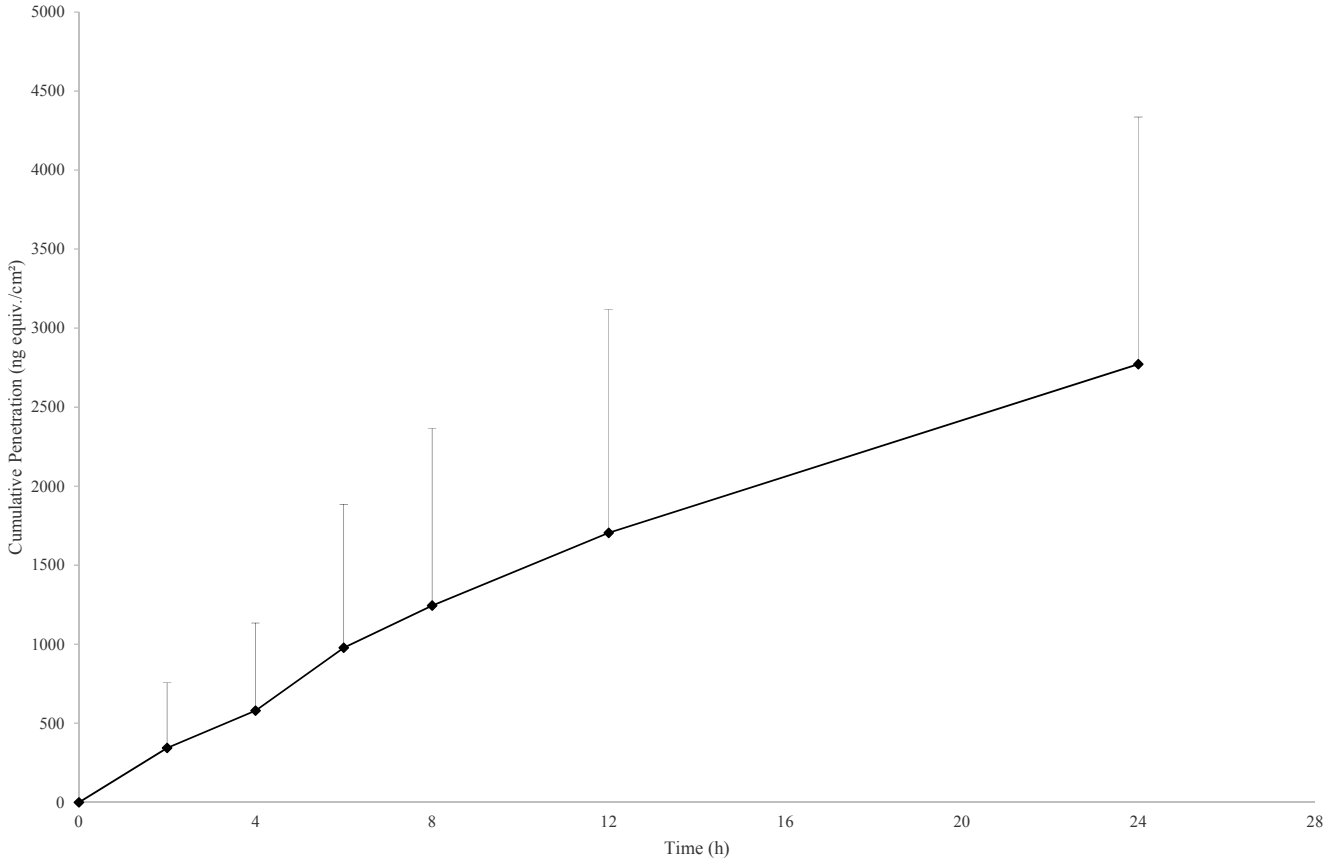
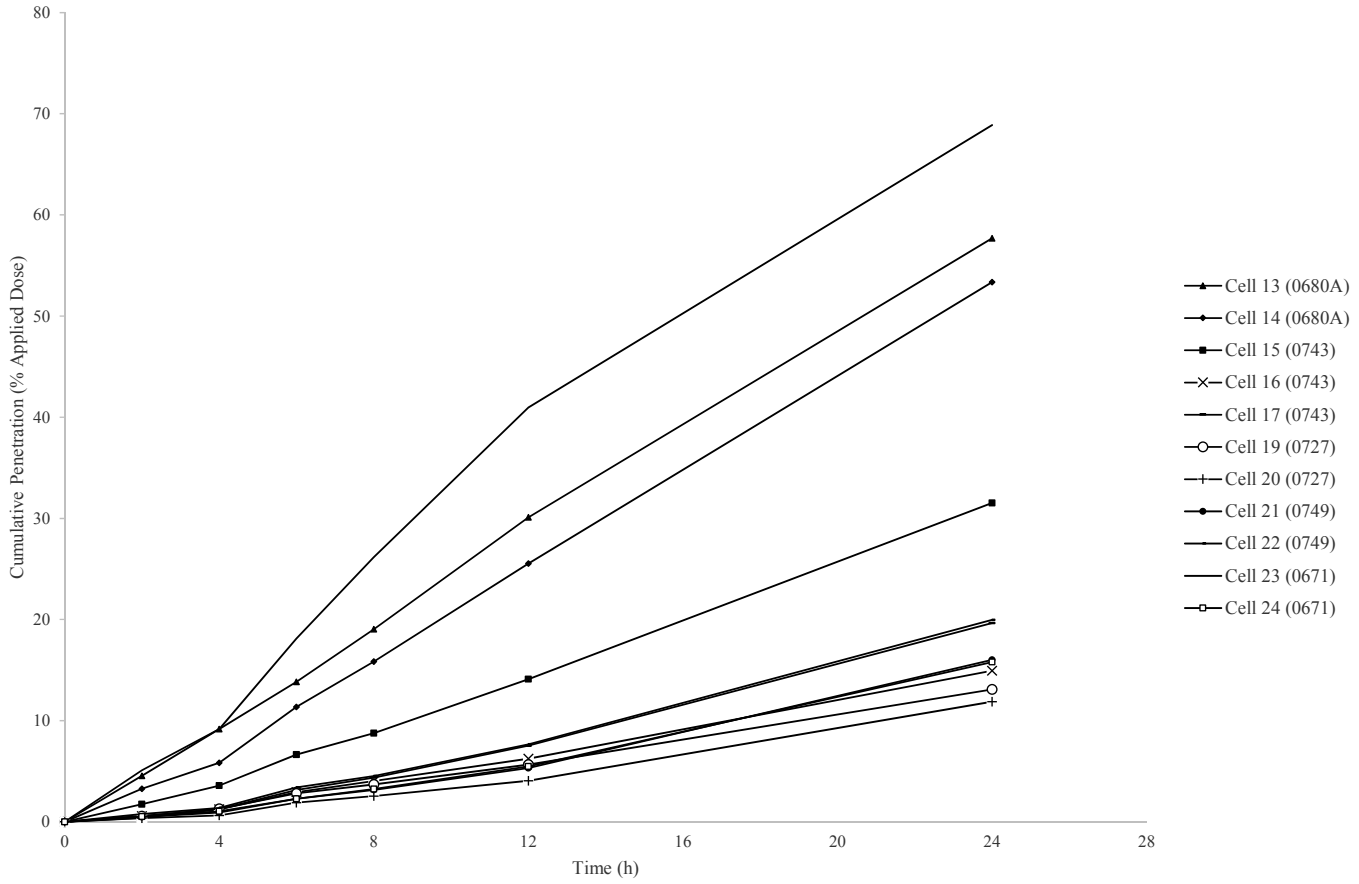


Figure 4 **Absorption Profile for [14C]-Caprylhydroxamic Acid (ng equiv./cm2) in Receptor Fluid Following Topical Application of [14C]-Caprylhydroxamic Acid in Test Preparation 1 (ca 0.15%, w/w) to Human Split-Thickness Skin (n = 12)**



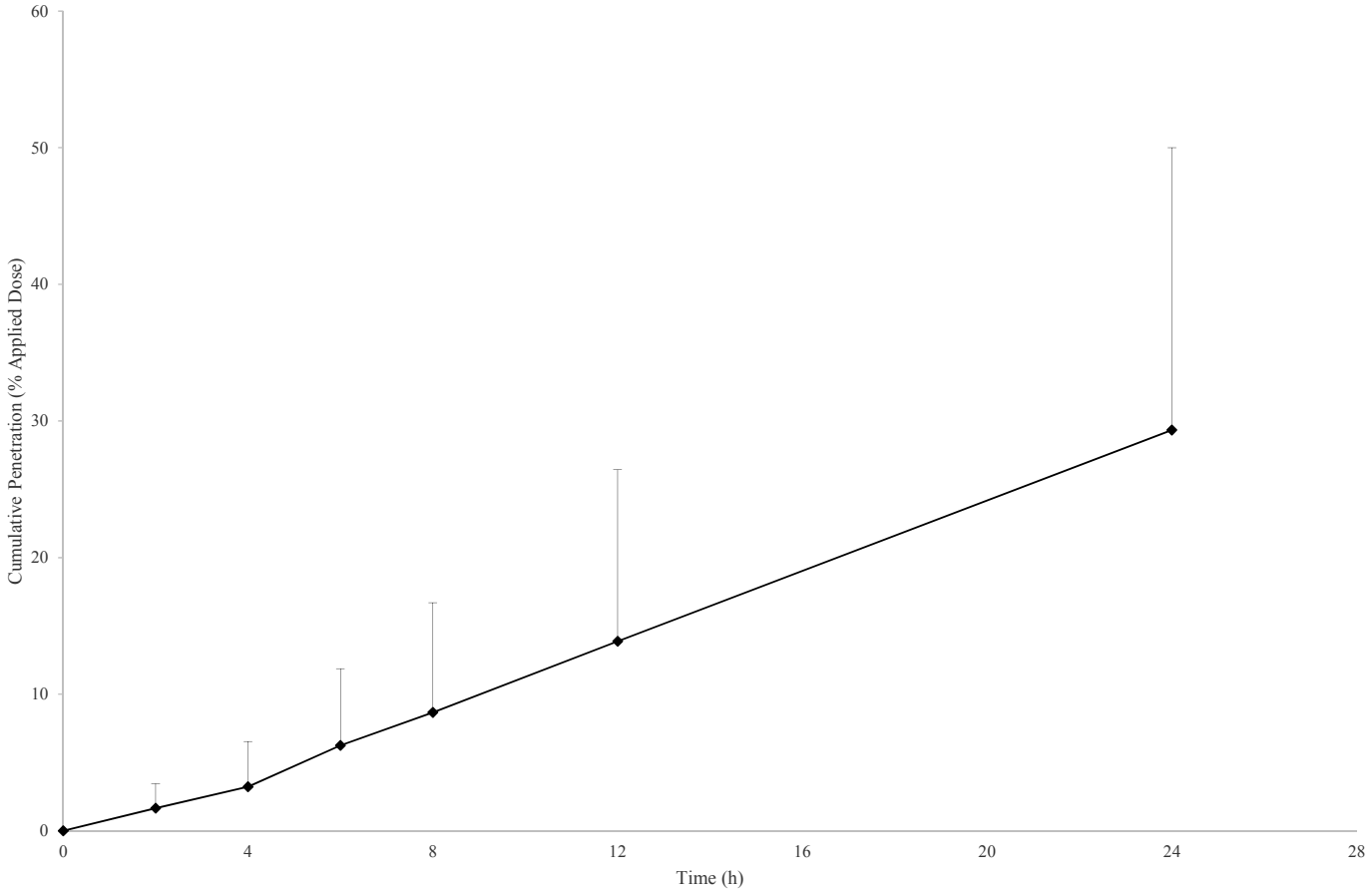
* Limit of reliable measurement is 1.815 ng equiv./cm^2 (0-12 h) and 0.218 ng equiv./cm^2 (24 h)

Figure 5 **Individual Absorption Profiles for [14C]-Caprylhydroxamic Acid (% Applied Dose) in Receptor Fluid Following Topical Application of [14C]-Caprylhydroxamic Acid in Test Preparation 2 (ca 0.15%, w/w) to Human Split-Thickness Skin**



* Limit of reliable measurement is 0.021 (0-12 h) and 0.003 (24 h) % of the applied dose

Figure 6 **Absorption Profile for [14C]-Caprylhydroxamic Acid (% Applied Dose) in Receptor Fluid Following Topical Application of [14C]-Caprylhydroxamic Acid in Test Preparation 2 (ca 0.15%, w/w) to Human Split-Thickness Skin (n =11)**



* Limit of reliable measurement is 0.021 (0-12 h) and 0.003 (24 h) % of the applied dose

Figure 7 **Distribution of [14C]-Caprylhydroxamic Acid (ng equiv./cm2) in Stratum Corneum at 24 h Post Application Following Topical Application of [14C]-Caprylhydroxamic Acid in Test Preparation 2 (ca 0.15%, w/w) to Human Split-Thickness Skin (n = 11)**

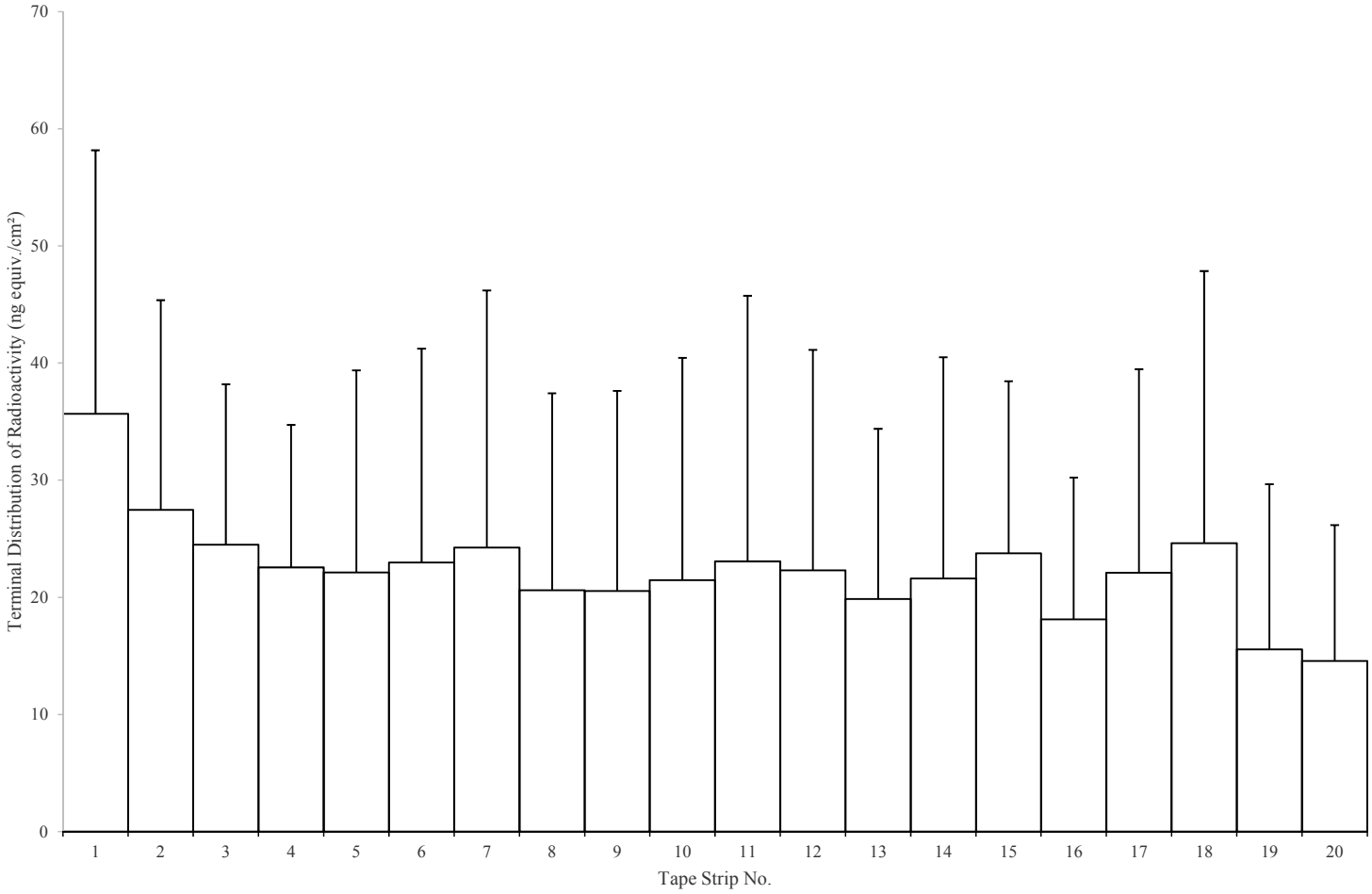
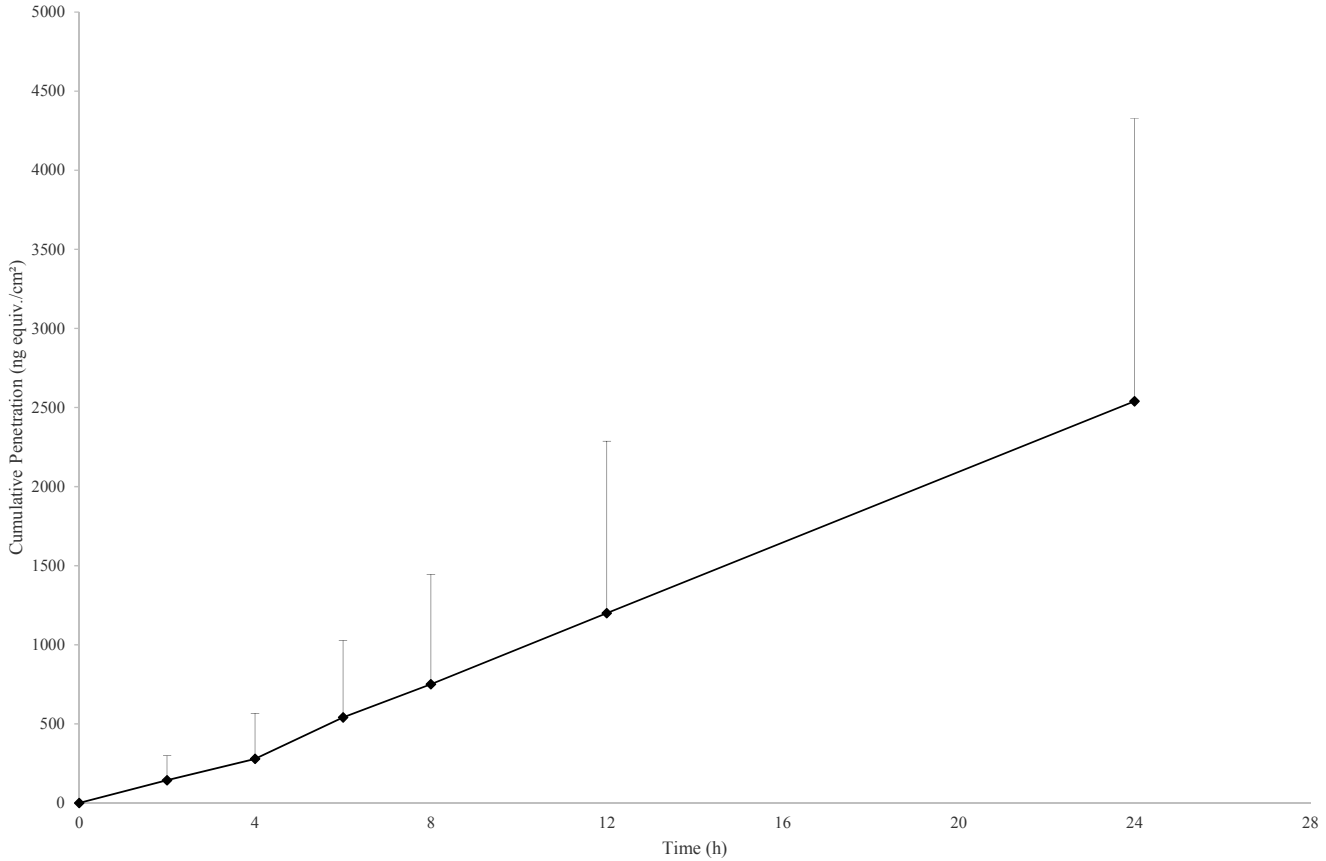
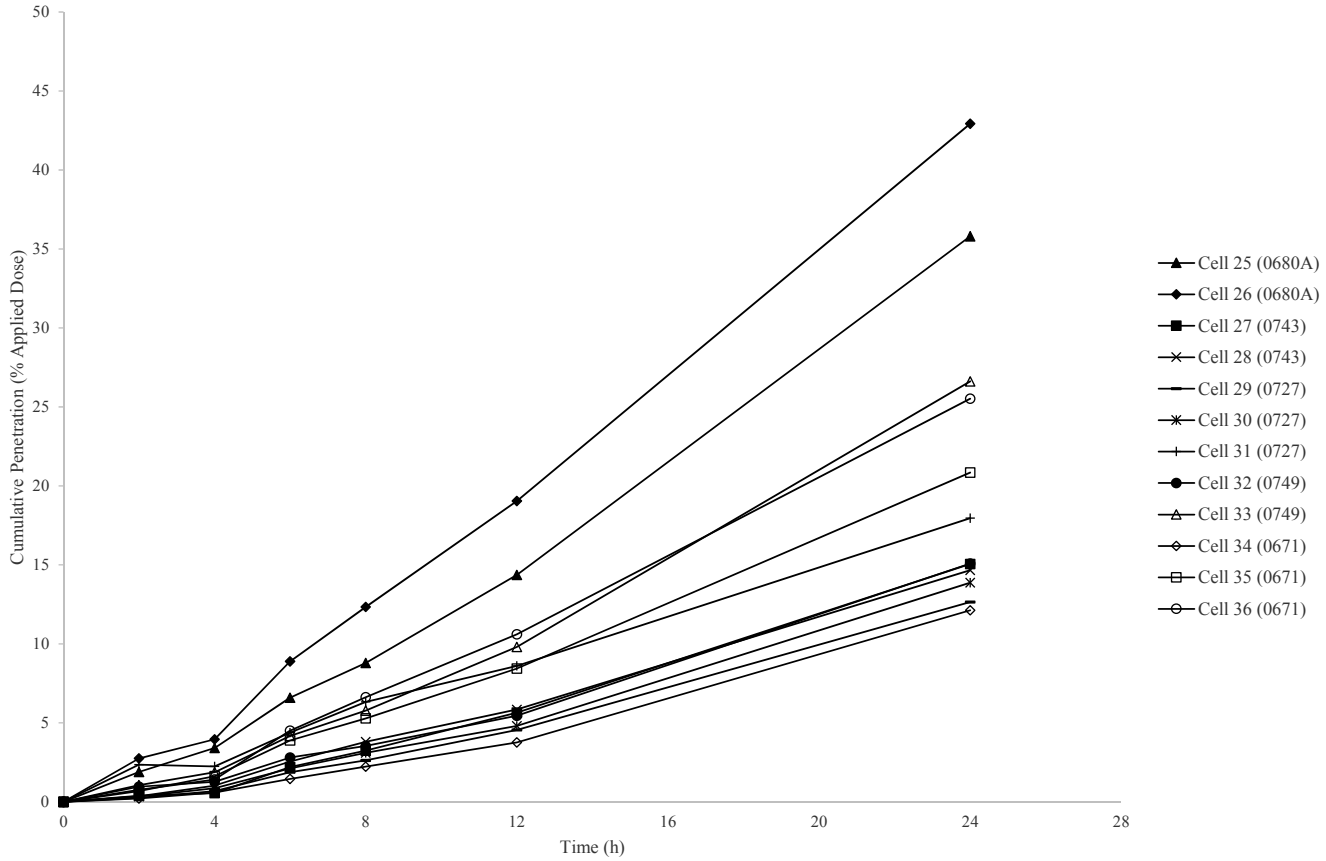


Figure 8 **Absorption Profile for [^{14}C]-Caprylhydroxamic Acid (ng equiv./cm^2) in Receptor Fluid Following Topical Application of [^{14}C]-Caprylhydroxamic Acid in Test Preparation 2 (*ca* 0.15%, w/w) to Human Split-Thickness Skin (n = 11)**



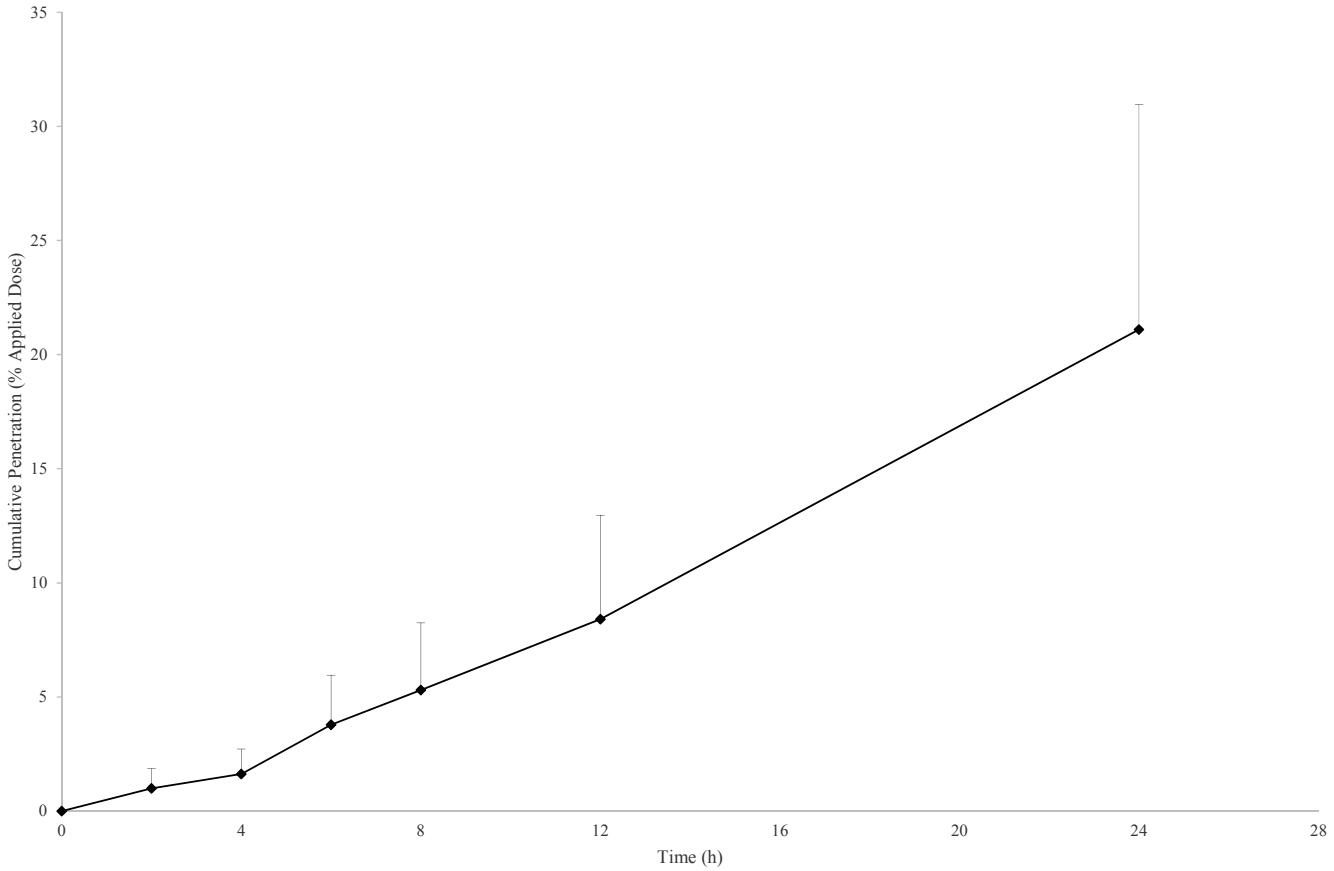
* Limit of reliable measurement is 1.815 ng equiv./cm^2 (0-12 h) and 0.218 ng equiv./cm^2 (24 h)

Figure 9 **Individual Absorption Profile for [14C]-Caprylhydroxamic Acid (% Applied Dose) in Receptor Fluid Following Topical Application of [14C]-Caprylhydroxamic Acid Test Preparation 3 (ca 0.15%, w/w) to Human Split-Thickness Skin**



* Limit of reliable measurement is 0.023 (0-12 h) and 0.003 (24 h) % of the applied dose

Figure 10 **Absorption Profile for [14C]-Caprylhydroxamic Acid (% Applied Dose) in Receptor Fluid Following Topical Application of [14C]-Caprylhydroxamic Acid in Test Preparation 3 (ca 0.15%, w/w) to Human Split-Thickness Skin (n =12)**



* Limit of reliable measurement is 0.023 (0-12 h) and 0.003 (24 h) % of the applied dose

Figure 11 **Distribution of [14C]-Caprylhydroxamic Acid (ng equiv./cm2) in Stratum Corneum at 24 h Post Application Following Topical Application of [14C]-Caprylhydroxamic Acid in Test Preparation 3 (ca 0.15%, w/w) to Human Split-Thickness Skin (n = 12)**

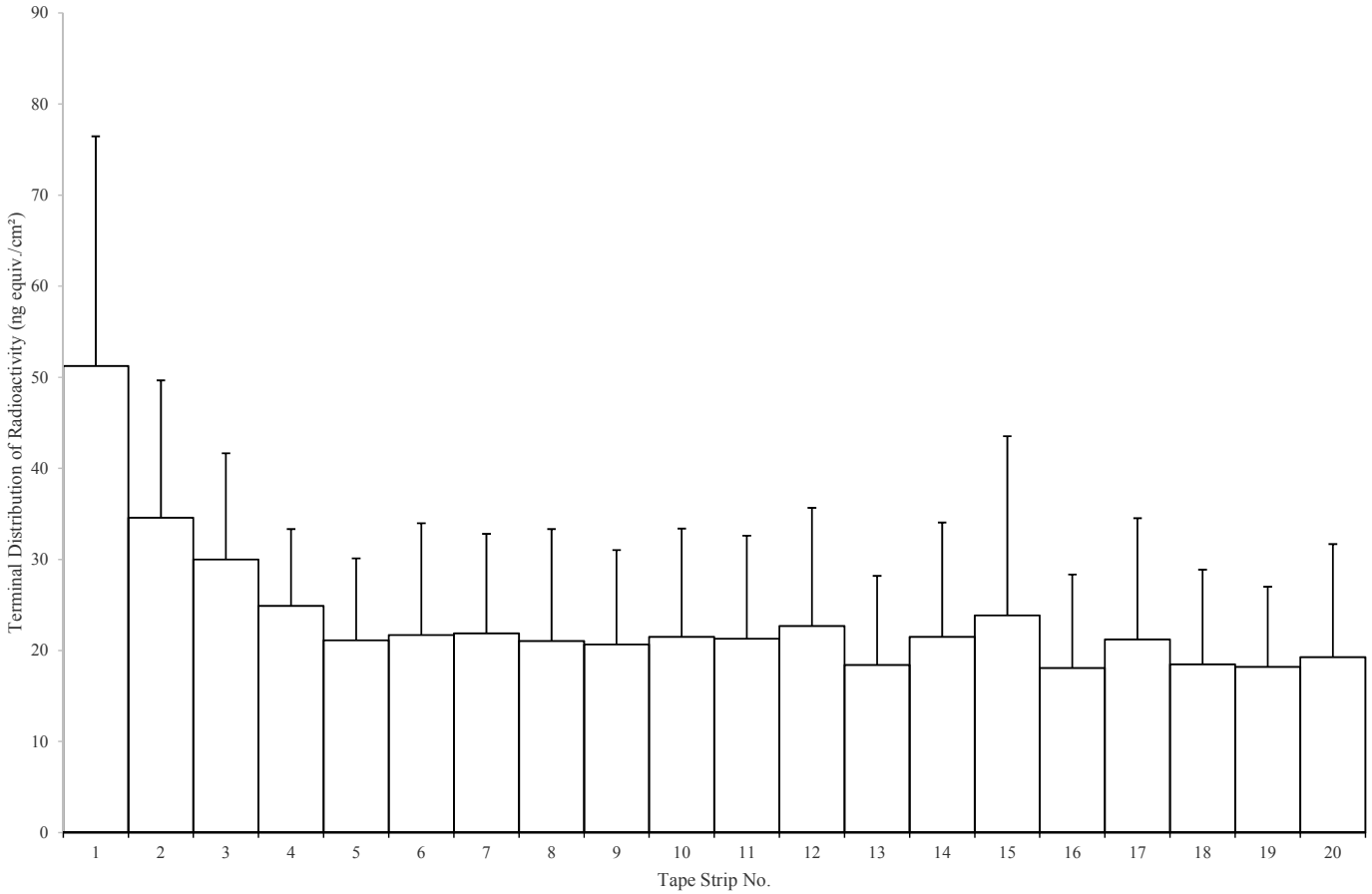
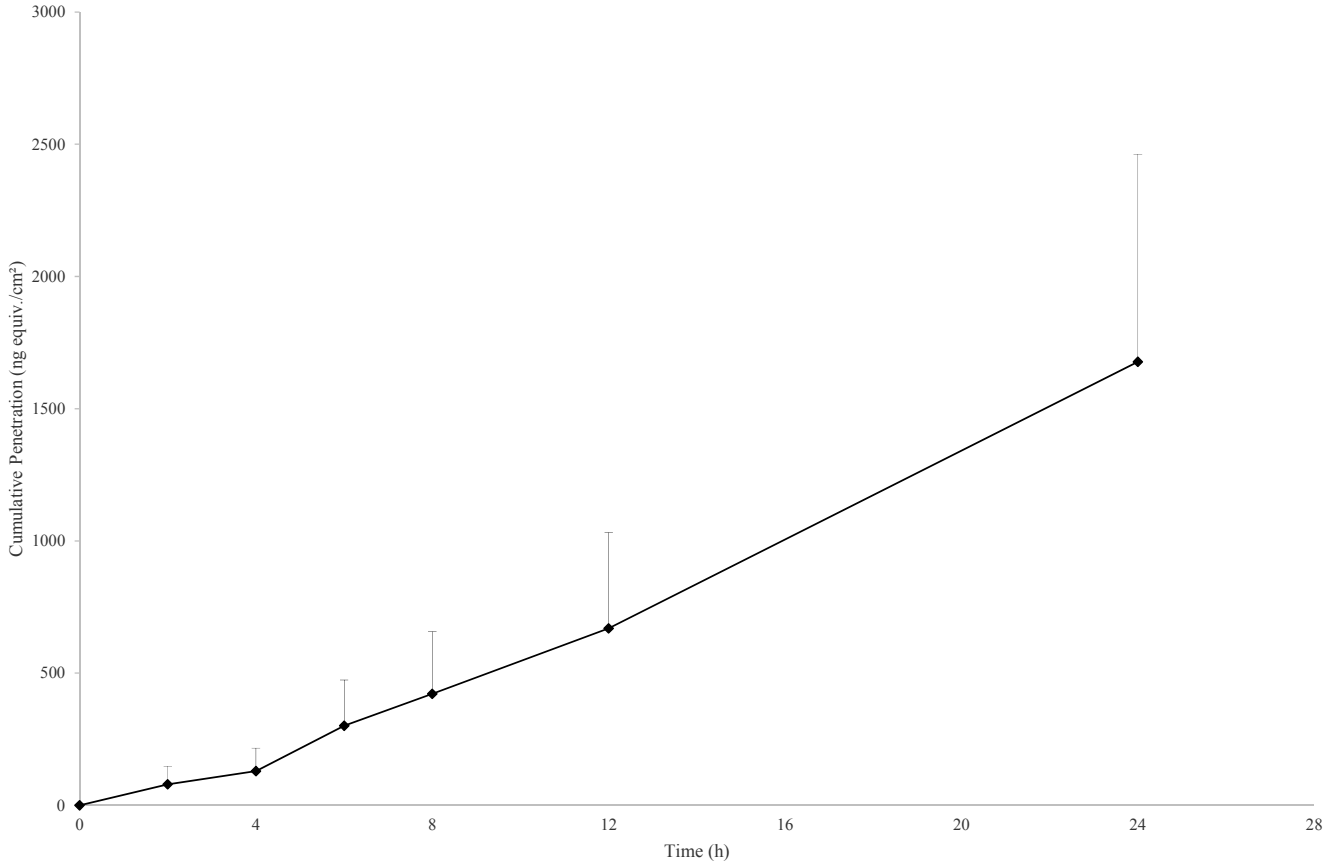


Figure 12 **Absorption Profile for [14C]-Caprylhydroxamic Acid (ng equiv./cm2) in Receptor Fluid Following Topical Application of [14C]-Caprylhydroxamic Acid in Test Preparation 3 (ca 0.15%, w/w) to Human Split-Thickness Skin (n = 12)**



* Limit of reliable measurement is 1.815 ng equiv./cm^2 (0-12 h) and 0.218 ng equiv./cm^2 (24 h)

13 TABLES

Table 1 Distribution of Radioactivity (% Applied Dose) at 24 h Post Dose Following Topical Application of [14C]-Caprylhydroxamic Acid in Test Preparation 1 (ca 0.15%, w/w) to Human Split-Thickness Skin

	Cell Number and Donor Number													
	Cell 1 0680A	Cell 2 0680A	Cell 3 0680A	Cell 4 0743	Cell 5 0743	Cell 6 0727	Cell 7 0727	Cell 8 0749	Cell 9 0749	Cell 10 0671	Cell 11 0671	Cell 12 0671	Mean	SD
Skin Wash 24 h	26.82	10.65	14.93	32.85	19.88	25.25	32.63	26.86	26.10	25.72	4.33	4.24	20.86	10.06
Tissue Swab 24 h	11.71	22.09	11.41	14.60	13.99	38.16	24.93	27.97	30.28	36.71	5.47	1.60	19.91	11.92
Pipette Tip 24 h	0.03	0.02	0.03	0.09	0.03	0.03	0.04	0.03	0.12	0.03	0.02	0.01	0.04	0.03
Donor Chamber Wash	0.30	0.15	0.21	0.22	0.18	0.39	0.24	0.28	0.26	0.17	0.23	0.07	0.23	0.08
Total Dislodgeable Dose	38.86	32.90	26.58	47.76	34.08	63.83	57.84	55.14	56.76	62.63	10.06	5.93	41.03	19.73
Stratum Corneum 1-2	0.18	0.15	0.18	0.81	0.26	0.71	0.46	1.16	0.74	0.47	0.07	0.10	0.44	0.35
Stratum Corneum 3-5	0.22	0.15	0.19	0.81	0.24	0.66	0.60	0.82	0.66	0.49	0.07	0.07	0.42	0.29
Stratum Corneum 6-10	0.43	0.17	0.27	1.24	0.45	1.29	1.70	0.72	1.12	0.88	0.09	0.07	0.70	0.54
Stratum Corneum 11-15	0.40	0.19	0.21	0.69	0.54	1.45	1.60	0.47	1.27	0.97	0.07	*0.05	°0.66	°0.54
Stratum Corneum 16-20	0.37	0.14	0.20	0.46	0.44	1.18	1.19	0.62	0.89	1.23	0.08	0.05	0.57	0.45
Stratum Corneum 3-20	1.42	0.65	0.88	3.20	1.66	4.57	5.09	2.63	3.95	3.57	0.31	0.25	2.35	1.71
Stratum Corneum	1.60	0.80	1.06	4.01	1.92	5.29	5.54	3.79	4.68	4.04	0.38	0.34	2.79	1.96
Unexposed Skin	0.30	0.18	0.22	0.15	0.34	0.09	0.07	0.10	0.14	0.07	0.21	0.15	0.17	0.09
Total Unabsorbed	40.76	33.88	27.86	51.92	36.35	69.21	63.45	59.03	61.58	66.75	10.65	6.43	43.99	21.50
Epidermis	6.83	4.76	6.19	13.46	8.10	5.06	3.42	16.86	6.52	7.03	0.67	0.47	6.61	4.71
Dermis	4.01	4.44	4.09	2.15	3.36	1.48	3.36	2.71	3.25	2.07	2.50	1.97	2.95	0.95
Receptor Fluid	42.18	50.05	55.17	24.05	44.20	15.43	22.86	22.49	21.72	17.28	75.68	77.80	39.08	22.06
Receptor Chamber Wash	2.63	2.46	4.28	1.67	4.43	1.04	1.27	1.02	1.94	1.11	4.76	7.15	2.81	1.94
Total Absorbed	44.81	52.51	59.45	25.72	48.63	16.47	24.13	23.51	23.66	18.39	80.45	84.95	41.89	23.86
Dermal Delivery	55.64	61.71	69.74	41.34	60.08	23.01	30.92	43.08	33.42	27.49	83.61	87.38	51.45	21.66
Mass Balance	96.40	95.59	97.60	93.25	96.43	92.23	94.37	102.11	95.00	94.24	94.26	93.81	95.44	2.58

*=Results calculated from data less than 30 d.p.m. above background
°=Mean includes results calculated from data less than 30 d.p.m above background
Epidermis = Epidermis + Clingfilm

Table 2 Cumulative Absorption (% Applied Dose) of [14C]-Caprylhydroxamic Acid into Receptor Fluid Following Topical Application of Test Preparation 1 (ca 0.15%, w/w) to Human Split-Thickness Skin

	Cell Number and Donor Number													
	Cell 1	Cell 2	Cell 3	Cell 4	Cell 5	Cell 6	Cell 7	Cell 8	Cell 9	Cell 10	Cell 11	Cell 12		
Time (h)	0680A	0680A	0680A	0743	0743	0727	0727	0749	0749	0671	0671	0671	Mean	SD
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	3.97	3.85	5.44	1.21	3.64	0.90	1.24	1.47	1.11	1.70	15.86	17.86	4.85	5.81
4	7.42	7.91	11.92	3.32	8.63	1.40	2.77	3.12	2.00	2.77	25.04	21.72	8.17	7.83
6	11.67	13.55	17.37	6.01	13.97	3.84	5.05	5.56	4.51	5.26	35.45	43.03	13.77	12.81
8	15.54	17.79	22.15	7.78	18.01	4.79	6.57	7.41	6.33	6.59	44.20	53.39	17.55	15.81
12	22.38	25.69	31.80	11.52	25.45	6.87	9.87	10.87	9.58	8.99	57.99	67.46	24.04	19.93
24	42.18	50.05	55.17	24.05	44.20	15.43	22.86	22.49	21.72	17.28	75.68	77.80	39.08	22.06

Table 3 Distribution of [14C]-Caprylhydroxamic Acid (ng equiv./cm2) at 24 h Post Dose Following Topical Application of Test Preparation 1 (ca 0.15%, w/w) to Human Split-Thickness Skin

	Cell Number and Donor Number													
	Cell 1 0680A	Cell 2 0680A	Cell 3 0680A	Cell 4 0743	Cell 5 0743	Cell 6 0727	Cell 7 0727	Cell 8 0749	Cell 9 0749	Cell 10 0671	Cell 11 0671	Cell 12 0671	Mean	SD
Skin Wash 24 h	1902.28	755.43	1059.19	2330.00	1410.11	1791.02	2314.33	1905.40	1851.08	1824.33	307.30	300.97	1479.29	713.71
Tissue Swab 24 h	830.70	1566.87	809.40	1035.85	992.50	2706.39	1768.31	1983.71	2148.06	2603.95	388.31	113.71	1412.31	845.73
Pipette Tip 24 h	2.38	1.10	2.43	6.21	2.08	2.28	2.73	2.05	8.63	1.92	1.35	0.62	2.81	2.29
Donor Chamber Wash	21.14	10.34	14.65	15.64	12.70	28.00	17.34	20.10	18.12	12.29	16.66	5.21	16.02	5.79
Total Dislodgeable Dose	2756.50	2333.74	1885.68	3387.71	2417.39	4527.70	4102.71	3911.26	4025.89	4442.48	713.61	420.51	2910.43	1399.13
Stratum Corneum 1-2	12.82	10.56	12.71	57.44	18.60	50.55	32.34	82.12	52.28	33.56	5.23	7.03	31.27	24.55
Stratum Corneum 3-5	15.81	10.32	13.63	57.57	16.75	46.58	42.86	58.14	47.11	34.98	4.94	5.08	29.48	20.42
Stratum Corneum 6-10	30.61	12.37	19.16	88.07	31.87	91.66	120.28	51.20	79.57	62.59	6.52	5.16	49.92	38.29
Stratum Corneum 11-15	28.02	13.77	15.13	48.90	38.32	102.61	113.38	33.18	90.32	68.61	5.20	*3.49	°46.74	°38.41
Stratum Corneum 16-20	26.18	9.88	14.21	32.29	30.88	83.59	84.27	43.85	62.92	86.95	5.35	3.65	40.33	31.67
Stratum Corneum 3-20	100.62	46.35	62.13	226.82	117.81	324.45	360.78	186.38	279.92	253.13	22.01	17.38	166.48	121.45
Stratum Corneum	113.44	56.91	74.84	284.27	136.41	375.00	393.12	268.49	332.19	286.68	27.24	24.42	197.75	139.15
Unexposed Skin	21.04	12.56	15.75	10.53	24.38	6.73	4.77	6.91	10.02	5.30	14.76	10.91	11.97	6.14
Total Unabsorbed	2890.97	2403.21	1976.27	3682.50	2578.19	4909.42	4500.60	4186.67	4368.10	4734.47	755.61	455.83	3120.15	1525.02
Epidermis	484.11	337.60	439.32	954.97	574.21	359.20	242.69	1195.88	462.23	498.41	47.42	33.24	469.11	333.82
Dermis	284.23	315.11	290.32	152.59	238.19	105.05	238.61	192.35	230.30	146.94	177.13	139.44	209.19	67.21
Receptor Fluid	2991.96	3549.97	3913.29	1705.95	3135.16	1094.51	1621.63	1595.02	1540.55	1225.50	5368.04	5518.01	2771.63	1564.82
Receptor Chamber Wash	186.22	174.31	303.39	118.56	314.05	73.66	89.95	72.62	137.41	79.03	337.93	507.42	199.54	137.36
Total Absorbed	3178.18	3724.29	4216.68	1824.51	3449.21	1168.17	1711.57	1667.65	1677.96	1304.54	5705.97	6025.43	2971.18	1692.20
Dermal Delivery	3946.52	4376.99	4946.32	2932.06	4261.61	1632.42	2192.87	3055.87	2370.49	1949.88	5930.51	6198.11	3649.47	1536.06
Mass Balance	6837.49	6780.20	6922.58	6614.56	6839.80	6541.84	6693.47	7242.54	6738.60	6684.35	6686.11	6653.94	6769.62	182.66

*=Results calculated from data less than 30 d.p.m. above background
°=Mean includes results calculated from data less than 30 d.p.m above background
Epidermis = Epidermis + Clingfilm

Table 4 Distribution of [14C]-Caprylhydroxamic Acid (ng equiv./cm2) in Stratum Corneum at 24 h Post Dose Following Topical Application of Test Preparation 1 (ca 0.15%, w/w) to Human Split-Thickness Skin

	Cell Number and Donor Number													
	Cell 1	Cell 2	Cell 3	Cell 4	Cell 5	Cell 6	Cell 7	Cell 8	Cell 9	Cell 10	Cell 11	Cell 12		
Tape Strip No.	0680A	0680A	0680A	0743	0743	0727	0727	0749	0749	0671	0671	0671	Mean	SD
1	7.10	6.14	6.68	37.44	11.94	30.96	17.74	51.90	31.34	20.88	2.98	4.45	19.13	15.66
2	5.72	4.42	6.03	20.00	6.66	19.59	14.60	30.22	20.93	12.68	2.25	2.58	12.14	8.97
3	5.54	4.36	2.98	21.85	6.00	16.08	14.09	24.14	16.05	10.18	1.87	2.27	10.45	7.81
4	4.67	3.31	5.09	15.71	5.37	18.60	13.68	16.33	15.41	12.29	1.54	1.44	9.45	6.43
5	5.60	2.65	5.57	20.00	5.38	11.90	15.09	17.67	15.65	12.51	1.52	1.37	9.58	6.64
6	6.83	2.81	3.99	15.48	4.15	13.43	22.98	16.11	14.83	12.99	1.18	1.08	9.66	7.18
7	6.95	2.61	3.18	21.18	6.00	16.97	20.69	10.30	15.89	13.25	1.22	1.12	9.94	7.48
8	3.98	2.15	4.58	19.01	5.89	13.44	24.18	8.05	16.16	11.47	1.63	0.84	9.28	7.57
9	5.81	2.48	3.89	18.26	8.84	22.41	25.18	9.09	17.44	11.27	1.05	1.00	10.56	8.42
10	7.05	2.32	3.53	14.14	7.00	25.42	27.25	7.65	15.25	13.62	1.44	1.12	10.48	8.88
11	6.20	2.76	2.74	12.28	8.35	18.92	28.66	6.77	19.51	13.34	1.16	0.91	10.13	8.68
12	4.36	2.56	2.71	11.60	7.09	22.78	25.52	6.00	19.26	12.87	1.00	0.80	9.71	8.69
13	6.06	3.17	3.95	9.56	9.66	18.65	22.40	8.10	16.73	14.75	1.01	1.05	9.59	7.12
14	5.14	2.63	2.99	8.77	6.78	20.38	21.19	5.48	16.53	13.56	0.94	*0.00	°8.70	°7.44
15	6.26	2.65	2.75	6.69	6.43	21.88	15.60	6.82	18.28	14.08	1.09	0.73	8.60	7.10
16	3.41	2.10	2.50	5.86	7.75	17.25	16.44	6.56	18.09	16.39	0.96	0.76	8.17	6.90
17	6.59	1.95	3.04	6.41	6.29	17.08	15.10	9.16	19.56	17.01	1.06	0.86	8.68	6.82
18	4.94	1.78	3.18	7.37	5.68	15.43	14.49	8.10	E.C	17.48	1.48	0.76	7.34	5.95
19	6.38	2.01	2.33	5.75	4.64	18.91	23.34	9.73	11.00	18.01	0.91	0.71	8.64	7.70
20	4.85	2.05	3.16	6.91	6.51	14.92	14.90	10.30	14.27	18.07	0.94	0.56	8.12	6.17

E.C.= Epidermal Content
*=Results calculated from data less than 30 d.p.m. above background
°=Mean includes results calculated from data less than 30 d.p.m above background

Table 5 Cumulative Absorption of [14C]-Caprylhydroxamic Acid (ng equiv./cm2) into Receptor Fluid Following Topical Application of Test Preparation 1 (ca 0.15%, w/w) to Human Split-Thickness Skin

	Cell Number and Donor Number													
Time (h)	Cell 1 0680A	Cell 2 0680A	Cell 3 0680A	Cell 4 0743	Cell 5 0743	Cell 6 0727	Cell 7 0727	Cell 8 0749	Cell 9 0749	Cell 10 0671	Cell 11 0671	Cell 12 0671	Mean	SD
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	281.32	273.16	385.78	85.66	258.18	63.63	88.24	104.59	79.00	120.30	1124.62	1267.10	344.30	412.08
4	526.06	560.72	845.31	235.66	612.33	99.43	196.19	221.28	141.55	196.49	1775.97	1540.25	579.27	555.30
6	828.00	961.12	1232.16	426.58	991.16	272.72	358.07	394.47	319.90	373.12	2514.55	3052.40	977.02	908.39
8	1101.99	1261.83	1571.00	551.68	1277.57	339.54	466.17	525.78	449.08	467.35	3135.24	3786.99	1244.52	1121.10
12	1587.13	1821.98	2255.50	817.26	1805.37	487.03	699.81	771.29	679.54	637.58	4113.39	4784.99	1705.07	1413.32
24	2991.96	3549.97	3913.29	1705.95	3135.16	1094.51	1621.63	1595.02	1540.55	1225.50	5368.04	5518.01	2771.63	1564.82

Table 6 Distribution of Radioactivity (% Applied Dose) at 24 h Post Dose Following Topical Application of [14C]-Caprylhydroxamic Acid in Test Preparation 2 (ca 0.15%, w/w) to Human Split-Thickness Skin

	Cell Number and Donor Number													
	Cell 13 0680A	Cell 14 0680A	Cell 15 0743	Cell 16 0743	Cell 17 0743	Cell 18 0727	Cell 19 0727	Cell 20 0727	Cell 21 0749	Cell 22 0749	Cell 23 0671	Cell 24 0671	Mean	SD
Skin Wash 24 h	17.54	19.81	25.25	38.75	36.62	*36.75*	36.35	42.74	33.65	31.56	8.57	37.27	29.83	10.64
Tissue Swab 24 h	9.55	11.92	17.49	13.68	17.73	*30.43*	23.40	16.38	24.79	30.16	5.10	18.21	17.13	7.15
Pipette Tip 24 h	0.05	0.04	0.03	0.04	0.03	*0.13*	0.11	0.06	0.01	0.02	0.02	0.06	0.04	0.03
Donor Chamber Wash	0.05	0.81	0.54	0.23	0.42	*0.67*	0.80	0.41	0.16	0.26	0.16	0.28	0.38	0.25
Total Dislodgeable Dose	27.20	32.58	43.32	52.69	54.80	*67.98*	60.66	59.60	58.61	62.01	13.85	55.82	47.38	16.09
Stratum Corneum 1-2	0.22	0.38	0.25	0.77	0.72	*1.23*	1.53	1.30	0.76	0.65	0.22	1.23	0.73	0.46
Stratum Corneum 3-5	0.35	0.31	0.49	0.63	0.87	*1.33*	1.78	1.31	0.91	0.67	0.20	1.28	0.80	0.49
Stratum Corneum 6-10	0.34	0.37	0.66	1.28	1.40	*1.72*	3.73	2.57	1.37	0.71	0.27	1.26	1.27	1.05
Stratum Corneum 11-15	0.21	0.28	0.73	1.57	1.41	*1.52*	3.18	2.72	1.63	0.59	0.16	1.30	1.25	1.00
Stratum Corneum 16-20	0.19	0.28	0.74	0.96	1.08	*1.29*	2.48	2.32	1.89	0.72	0.11	1.31	1.10	0.83
Stratum Corneum 3-20	1.10	1.24	2.62	4.45	4.75	5.86	11.17	8.91	5.79	2.69	0.74	5.15	4.42	3.30
Stratum Corneum	1.31	1.62	2.88	5.22	5.47	*7.09*	12.70	10.20	6.55	3.34	0.96	6.37	5.15	3.73
Unexposed Skin	0.26	0.44	0.15	0.06	0.07	*0.06*	0.03	0.04	0.10	0.10	0.31	0.09	0.15	0.13
Total Unabsorbed	28.77	34.64	46.34	57.97	60.34	*75.13*	73.39	69.84	65.27	65.45	15.12	62.29	52.67	18.90
Epidermis	5.27	5.43	14.20	15.05	17.44	**0.24*	4.80	9.85	7.16	5.18	1.08	16.07	°9.23	°5.57
Dermis	4.05	4.04	2.60	1.83	2.55	*1.16*	1.77	2.00	3.79	2.83	2.61	3.34	2.85	0.84
Receptor Fluid	57.69	53.37	31.51	14.94	19.64	*11.69*	13.09	11.87	16.02	19.96	68.89	15.80	29.35	20.66
Receptor Chamber Wash	4.98	3.19	2.75	1.75	1.94	*0.70*	1.49	1.00	1.36	1.31	4.23	2.51	2.41	1.28
Total Absorbed	62.67	56.55	34.27	16.70	21.58	*12.39*	14.58	12.87	17.38	21.27	73.12	18.30	31.75	21.84
Dermal Delivery	71.99	66.02	51.06	33.58	41.57	*13.79*	21.15	24.72	28.33	29.28	76.81	37.72	43.84	19.76
Mass Balance	100.77	100.67	97.41	91.55	101.91	*88.92*	94.54	94.56	93.59	94.73	91.93	100.01	96.52	3.77
	Cell 18 excluded due to mass balance outside 100% +- 10%, missing epidermis sample													

*=Results calculated from data less than 30 d.p.m. above background
°=Mean includes results calculated from data less than 30 d.p.m above background
Epidermis = Epidermis + Clingfilm

Table 7 Cumulative Absorption (% Applied Dose) of [14C]-Caprylhydroxamic Acid into Receptor Fluid Following Topical Application of Test Preparation 2 (ca 0.15%, w/w) to Human Split-Thickness Skin

	Cell Number and Donor Number													
Time (h)	Cell 13 0680A	Cell 14 0680A	Cell 15 0743	Cell 16 0743	Cell 17 0743	*Cell 18 0727*	Cell 19 0727	Cell 20 0727	Cell 21 0749	Cell 22 0749	Cell 23 0671	Cell 24 0671	Mean	SD
0	0.00	0.00	0.00	0.00	0.00	*0.00*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	4.56	3.26	1.74	0.57	0.40	*0.40*	0.55	0.35	0.46	0.76	5.09	0.51	1.66	1.79
4	9.18	5.83	3.57	1.24	1.24	*0.92*	1.29	0.62	0.91	1.37	9.17	1.05	3.23	3.31
6	13.83	11.36	6.65	2.93	3.14	*2.20*	2.83	1.88	2.28	3.41	18.11	2.28	6.25	5.62
8	19.04	15.84	8.77	4.03	4.37	*2.99*	3.70	2.55	3.14	4.53	26.17	3.25	8.67	8.03
12	30.11	25.52	14.10	6.24	7.52	*4.45*	5.65	4.06	5.31	7.66	40.96	5.47	13.87	12.56
24	57.69	53.37	31.51	14.94	19.64	*11.69*	13.09	11.87	16.02	19.96	68.89	15.80	29.35	20.66
	Cell 18 excluded due to mass balance outside 100% +- 10%, missing epidermis sample													

Table 8 Distribution of [14C]-Caprylhydroxamic Acid (ng equiv./cm2) at 24 h Post Dose Following Topical Application of Test Preparation 2 (ca 0.15%, w/w) to Human Split-Thickness Skin

	Cell Number and Donor Number													
	Cell 13 0680A	Cell 14 0680A	Cell 15 0743	Cell 16 0743	Cell 17 0743	*Cell 18 0727*	Cell 19 0727	Cell 20 0727	Cell 21 0749	Cell 22 0749	Cell 23 0671	Cell 24 0671	Mean	SD
Skin Wash 24 h	1517.84	1713.76	2184.85	3352.56	3168.29	*3180.15*	3144.88	3698.55	2911.42	2731.13	741.51	3225.25	2580.91	920.70
Tissue Swab 24 h	826.66	1031.11	1513.15	1184.02	1534.55	*2633.05*	2024.46	1417.63	2145.36	2610.06	441.55	1575.63	1482.20	618.67
Pipette Tip 24 h	4.06	3.83	2.92	3.15	2.45	*11.56*	9.75	4.84	1.05	1.69	1.33	4.95	3.64	2.42
Donor Chamber Wash	4.65	70.43	47.07	19.48	36.34	*57.63*	69.37	35.67	13.64	22.58	13.80	24.17	32.47	22.03
Total Dislodgeable Dose	2353.21	2819.13	3747.98	4559.21	4741.63	*5882.39*	5248.47	5156.68	5071.47	5365.46	1198.19	4830.00	4099.22	1391.85
Stratum Corneum 1-2	18.77	32.93	21.88	66.96	62.03	*106.29*	132.38	112.25	65.78	56.45	19.01	106.03	63.13	39.55
Stratum Corneum 3-5	30.16	26.92	42.05	54.92	74.91	*114.81*	154.10	113.12	78.36	57.96	17.68	110.54	69.16	42.42
Stratum Corneum 6-10	29.74	32.10	57.32	110.94	121.34	*148.57*	322.79	222.10	118.27	61.09	23.11	109.24	109.82	91.25
Stratum Corneum 11-15	18.22	24.10	63.56	136.22	122.08	*131.59*	275.24	234.94	140.90	50.82	13.88	112.73	108.43	86.82
Stratum Corneum 16-20	16.69	24.54	63.95	82.75	93.05	*111.93*	214.67	200.43	163.54	62.56	9.42	112.98	94.96	71.45
Stratum Corneum 3-20	94.81	107.66	226.89	384.83	411.37	*506.90*	966.79	770.59	501.07	232.42	64.10	445.50	382.37	285.88
Stratum Corneum	113.57	140.59	248.77	451.79	473.40	*613.19*	1099.16	882.84	566.86	288.87	83.11	551.53	445.50	322.96
Unexposed Skin	22.78	37.64	12.98	5.33	6.25	*5.41*	2.35	3.68	8.88	8.85	27.06	7.86	13.06	11.25
Total Unabsorbed	2489.56	2997.36	4009.74	5016.33	5221.28	*6500.99*	6349.98	6043.20	5647.21	5663.18	1308.36	5389.39	4557.78	1635.26
Epidermis	456.17	469.99	1228.58	1302.15	1509.20	**20.42*	415.74	852.52	619.65	448.02	93.10	1390.90	°798.73	°481.83
Dermis	350.29	349.47	224.80	158.36	220.25	*100.68*	153.23	172.70	327.85	244.86	225.86	289.32	247.00	73.03
Receptor Fluid	4991.00	4616.83	2726.24	1292.85	1699.07	*1011.08*	1132.62	1027.13	1386.13	1727.06	5959.68	1366.75	2538.67	1787.47
Receptor Chamber Wash	431.05	275.77	238.29	151.85	167.71	*60.87*	128.65	86.30	117.38	113.25	366.39	216.85	208.50	111.13
Total Absorbed	5422.05	4892.60	2964.54	1444.70	1866.78	*1071.94*	1261.26	1113.43	1503.51	1840.31	6326.07	1583.60	2747.17	1889.05
Dermal Delivery	6228.51	5712.06	4417.92	2905.21	3596.23	*1193.05*	1830.23	2138.65	2451.01	2533.18	6645.03	3263.82	3792.90	1709.49
Mass Balance	8718.07	8709.42	8427.65	7921.54	8817.51	*7694.04*	8180.21	8181.85	8098.21	8196.36	7953.39	8653.21	8350.68	326.52
	Cell 18 excluded due to mass balance outside 100% +- 10%, missing epidermis sample													

*=Results calculated from data less than 30 d.p.m. above background
°=Mean includes results calculated from data less than 30 d.p.m above background
Epidermis = Epidermis + Clingfilm

Table 9 **Distribution of [^{14}C]-Caprylhydroxamic Acid (ng equiv./cm^2) in Stratum Corneum at 24 h Post Dose Following Topical Application of Test Preparation 2 (*ca* 0.15%, w/w) to Human Split-Thickness Skin**

	Cell Number and Donor Number													
Tape Strip No.	Cell 13 0680A	Cell 14 0680A	Cell 15 0743	Cell 16 0743	Cell 17 0743	*Cell 18 0727*	Cell 19 0727	Cell 20 0727	Cell 21 0749	Cell 22 0749	Cell 23 0671	Cell 24 0671	Mean	SD
1	8.90	20.15	13.49	37.75	34.08	*67.99*	69.97	73.36	34.09	34.61	10.76	55.25	35.67	22.50
2	9.87	12.79	8.39	29.21	27.95	*38.30*	62.41	38.89	31.69	21.84	8.25	50.78	27.46	17.90
3	11.14	9.65	16.45	18.22	29.51	*39.14*	47.34	40.42	29.64	20.22	7.09	39.64	24.48	13.71
4	12.08	9.01	15.03	18.75	25.90	*36.06*	43.33	35.83	24.21	20.96	5.81	37.14	22.55	12.17
5	6.95	8.26	10.58	17.96	19.51	*39.61*	63.42	36.87	24.51	16.78	4.79	33.77	22.13	17.24
6	7.30	8.70	8.46	24.17	25.38	*27.98*	59.32	53.82	21.92	14.93	4.74	23.82	22.96	18.27
7	8.21	7.16	13.23	22.29	32.44	*37.87*	82.64	35.04	20.54	12.67	4.66	27.88	24.25	21.94
8	5.12	6.38	11.70	19.44	24.77	*21.64*	58.81	42.78	23.64	11.81	5.26	16.89	20.60	16.81
9	5.04	5.45	11.87	19.38	22.01	*33.06*	60.10	40.75	27.54	11.34	4.54	17.90	20.54	17.08
10	4.07	4.42	12.07	25.65	16.74	*28.03*	61.92	49.71	24.64	10.35	3.92	22.76	21.48	18.97
11	4.70	5.31	9.08	22.51	28.22	*28.46*	81.56	38.92	26.95	8.56	3.89	24.03	23.07	22.68
12	2.70	6.23	12.59	32.36	21.84	*30.44*	56.74	53.26	28.70	10.98	3.43	16.44	22.30	18.82
13	4.45	4.82	15.75	26.37	24.50	*25.15*	46.73	38.52	28.23	7.86	3.06	18.03	19.85	14.54
14	3.09	2.90	11.44	26.68	20.02	*28.29*	55.79	54.25	29.27	10.65	3.50	20.10	21.61	18.89
15	3.28	4.83	14.71	28.29	27.50	*19.24*	34.41	49.99	27.75	12.77	E.C	34.13	23.77	14.68
16	3.04	5.58	12.88	22.21	21.13	*24.27*	41.65	27.77	26.44	12.06	2.58	23.95	18.12	12.11
17	3.89	5.25	19.57	18.76	20.35	*23.27*	48.20	56.34	32.09	16.38	2.32	19.98	22.10	17.37
18	3.15	4.42	12.27	26.18	16.51	*21.18*	50.84	39.45	76.98	13.11	1.79	26.22	24.63	23.23
19	3.90	4.64	10.62	8.51	20.23	*18.30*	40.61	42.46	11.10	8.61	1.29	19.06	15.55	14.09
20	2.70	4.65	8.61	7.09	14.82	*24.92*	33.37	34.41	16.93	12.40	1.44	23.76	14.56	11.60
	Cell 18 excluded due to mass balance outside 100% +- 10%, missing epidermis sample													

E.C.= Epidermal Content

Table 10 Cumulative Absorption of [^{14}C]-Caprylhydroxamic Acid (ng equiv./cm^2) into Receptor Fluid FollowingTopical Application of Test Preparation 2 (*ca* 0.15%, w/w) to Human Split-Thickness Skin

	Cell Number and Donor Number													
Time (h)	Cell 13 0680A	Cell 14 0680A	Cell 15 0743	Cell 16 0743	Cell 17 0743	*Cell 18 0727*	Cell 19 0727	Cell 20 0727	Cell 21 0749	Cell 22 0749	Cell 23 0671	Cell 24 0671	Mean	SD
0	0.00	0.00	0.00	0.00	0.00	*0.00*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	394.06	282.20	150.72	49.44	34.25	*34.55*	47.95	30.63	40.05	65.47	440.75	44.29	143.62	154.83
4	794.26	504.38	309.02	107.10	107.61	*79.53*	111.51	53.90	79.10	118.79	793.35	90.52	279.05	286.45
6	1196.30	983.03	575.37	253.49	271.89	*190.55*	244.43	162.81	197.62	294.85	1566.46	197.47	540.34	485.81
8	1647.11	1370.36	758.41	348.65	378.26	*258.63*	320.42	220.22	272.05	391.92	2264.09	281.41	750.26	694.47
12	2605.21	2207.95	1219.72	539.92	650.81	*384.54*	489.09	351.57	459.60	662.66	3543.62	473.13	1200.30	1086.80
24	4991.00	4616.83	2726.24	1292.85	1699.07	*1011.08*	1132.62	1027.13	1386.13	1727.06	5959.68	1366.75	2538.67	1787.47
	Cell 18 excluded due to mass balance outside 100% +- 10%, missing epidermis sample													

Table 11 Distribution of Radioactivity (% Applied Dose) at 24 h Post Dose Following Topical Application of [^{14}C]-Caprylhydroxamic Acid in Test Preparation 3 (*ca* 0.15%, w/w) to Human Split-Thickness Skin

	Cell Number and Donor Number													
	Cell 25 0680A	Cell 26 0680A	Cell 27 0743	Cell 28 0743	Cell 29 0727	Cell 30 0727	Cell 31 0727	Cell 32 0749	Cell 33 0749	Cell 34 0671	Cell 35 0671	Cell 36 0671	Mean	SD
Skin Wash 24 h	25.60	27.07	34.26	37.27	34.36	44.54	36.01	29.49	32.29	31.89	32.91	28.99	32.89	5.08
Tissue Swab 24 h	13.81	12.14	18.04	17.10	22.85	21.84	23.66	28.70	19.03	34.17	16.72	19.88	20.66	6.18
Pipette Tip 24 h	0.03	0.04	0.03	0.05	0.07	0.07	0.04	0.04	0.05	0.06	0.02	0.03	0.04	0.02
Donor Chamber Wash	0.12	0.25	0.91	0.44	0.41	0.74	0.30	0.38	0.99	0.28	0.21	1.83	0.57	0.48
Total Dislodgeable Dose	39.56	39.49	53.24	54.86	57.70	67.19	60.01	58.62	52.36	66.41	49.86	50.72	54.17	8.79
Stratum Corneum 1-2	0.52	0.43	1.22	2.12	1.31	0.72	0.93	1.60	1.34	0.85	1.26	0.66	1.08	0.49
Stratum Corneum 3-5	0.63	0.38	1.38	1.29	1.20	0.67	1.00	1.45	0.99	0.72	1.10	0.66	0.96	0.34
Stratum Corneum 6-10	0.95	0.35	2.77	2.14	1.85	0.99	1.31	1.84	0.76	0.67	1.58	0.90	1.34	0.71
Stratum Corneum 11-15	0.76	0.36	2.22	1.86	2.93	0.98	0.99	1.97	0.54	0.60	1.78	1.26	1.35	0.79
Stratum Corneum 16-20	0.51	0.31	1.62	1.48	2.05	0.74	1.11	2.34	0.44	0.67	1.57	1.52	1.20	0.66
Stratum Corneum 3-20	2.86	1.40	7.99	6.76	8.02	3.38	4.41	7.60	2.72	2.67	6.03	4.35	4.85	2.34
Stratum Corneum	3.39	1.82	9.20	8.88	9.33	4.09	5.34	9.20	4.06	3.52	7.28	5.00	5.93	2.71
Unexposed Skin	0.12	0.25	0.26	0.21	0.04	0.03	0.13	0.05	0.28	0.03	0.12	0.14	0.14	0.09
Total Unabsorbed	43.07	41.57	62.70	63.95	67.08	71.31	65.48	67.86	56.70	69.96	57.26	55.86	60.23	9.79
Epidermis	11.80	5.70	13.10	15.34	11.64	8.99	9.83	10.18	8.81	10.19	12.76	9.09	10.62	2.50
Dermis	4.71	5.53	3.90	2.61	2.42	2.24	1.92	2.65	2.93	2.50	3.86	4.61	3.32	1.16
Receptor Fluid	35.80	42.93	15.05	14.66	12.65	13.87	17.96	15.08	26.62	12.12	20.84	25.52	21.09	9.87
Receptor Chamber Wash	2.47	3.00	1.88	1.56	1.28	1.27	1.93	0.92	1.10	1.09	2.50	3.03	1.84	0.76
Total Absorbed	38.26	45.93	16.94	16.22	13.92	15.14	19.89	16.00	27.73	13.21	23.34	28.56	22.93	10.42
Dermal Delivery	54.77	57.16	33.94	34.17	27.98	26.37	31.64	28.83	39.47	25.90	39.96	42.25	36.87	10.42
Mass Balance	97.84	98.73	96.64	98.12	95.06	97.68	97.12	96.69	96.16	95.86	97.22	98.11	97.10	1.07

Epidermis = Epidermis + Clingfilm

Table 12 Cumulative Absorption (% Applied Dose) of [^{14}C]-Caprylhydroxamic Acid into Receptor Fluid Following Topical Application of Test Preparation 3 (*ca* 0.15%, w/w) to Human Split-Thickness Skin

	Cell Number and Donor Number													
Time (h)	Cell 25 0680A	Cell 26 0680A	Cell 27 0743	Cell 28 0743	Cell 29 0727	Cell 30 0727	Cell 31 0727	Cell 32 0749	Cell 33 0749	Cell 34 0671	Cell 35 0671	Cell 36 0671	Mean	SD
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	1.88	2.75	0.38	0.35	0.26	0.34	2.35	0.96	1.06	0.21	0.68	0.77	1.00	0.86
4	3.41	3.96	0.55	1.03	0.68	0.86	2.23	1.26	1.88	0.60	1.62	1.41	1.62	1.10
6	6.60	8.89	2.20	2.55	1.87	2.11	4.39	2.80	4.17	1.46	3.89	4.50	3.78	2.17
8	8.79	12.34	3.24	3.80	2.62	3.10	6.32	3.54	5.78	2.22	5.28	6.62	5.30	2.95
12	14.37	19.05	5.65	5.84	4.55	4.81	8.61	5.45	9.81	3.77	8.44	10.60	8.41	4.55
24	35.80	42.93	15.05	14.66	12.65	13.87	17.96	15.08	26.62	12.12	20.84	25.52	21.09	9.87

Table 13 Distribution of [^{14}C]-Caprylhydroxamic Acid (ng equiv./cm^2) at 24 h Post Dose Following Topical Application of Test Preparation 3 (*ca* 0.15%, w/w) to Human Split-Thickness Skin

	Cell Number and Donor Number													
	Cell 25 0680A	Cell 26 0680A	Cell 27 0743	Cell 28 0743	Cell 29 0727	Cell 30 0727	Cell 31 0727	Cell 32 0749	Cell 33 0749	Cell 34 0671	Cell 35 0671	Cell 36 0671	Mean	SD
Skin Wash 24 h	2036.77	2153.25	2725.92	2965.40	2733.83	3543.66	2864.43	2346.46	2569.10	2537.24	2617.95	2306.27	2616.69	404.18
Tissue Swab 24 h	1098.68	965.81	1435.00	1360.48	1818.16	1737.44	1882.51	2283.13	1514.01	2718.75	1330.12	1581.44	1643.79	491.77
Pipette Tip 24 h	2.29	2.92	2.31	3.75	5.60	5.41	3.45	3.55	3.76	5.15	1.74	2.05	3.50	1.33
Donor Chamber Wash	9.61	20.03	72.05	35.06	32.95	59.11	23.91	30.62	78.68	22.20	16.76	145.44	45.54	38.42
Total Dislodgeable Dose	3147.34	3142.02	4235.28	4364.70	4590.54	5345.61	4774.30	4663.77	4165.55	5283.34	3966.58	4035.20	4309.52	699.23
Stratum Corneum 1-2	41.72	33.89	96.78	168.47	104.54	56.90	73.90	126.91	106.37	67.87	99.95	52.35	85.81	38.93
Stratum Corneum 3-5	50.48	30.62	109.79	102.28	95.08	52.91	79.28	115.60	78.67	57.33	87.50	52.33	75.99	27.08
Stratum Corneum 6-10	75.79	27.76	220.09	170.05	146.86	79.02	104.03	146.39	60.10	53.43	125.90	71.72	106.76	56.10
Stratum Corneum 11-15	60.64	28.36	176.52	147.98	233.31	77.59	79.13	156.73	42.84	47.86	141.53	100.35	107.74	63.07
Stratum Corneum 16-20	40.67	24.55	128.98	117.44	162.80	59.11	88.61	186.02	35.09	53.54	124.65	121.30	95.23	52.80
Stratum Corneum 3-20	227.59	111.29	635.38	537.74	638.06	268.64	351.05	604.74	216.71	212.17	479.58	345.71	385.72	186.21
Stratum Corneum	269.31	145.18	732.16	706.21	742.60	325.54	424.95	731.65	323.08	280.04	579.54	398.06	471.53	215.49
Unexposed Skin	9.42	19.60	20.90	16.50	3.40	2.20	10.13	3.60	22.01	2.64	9.27	11.03	10.89	7.32
Total Unabsorbed	3426.08	3306.79	4988.35	5087.41	5336.54	5673.35	5209.38	5399.02	4510.64	5566.03	4555.38	4444.29	4791.94	778.61
Epidermis	938.47	453.83	1042.24	1220.54	925.75	715.28	782.26	809.51	701.13	810.29	1015.48	723.06	844.82	199.10
Dermis	374.88	439.65	310.57	207.45	192.40	178.00	152.37	211.10	232.86	198.63	306.70	366.36	264.25	92.36
Receptor Fluid	2847.21	3414.48	1197.29	1165.85	1005.93	1103.41	1428.66	1199.48	2117.57	964.27	1657.62	2030.07	1677.65	784.85
Receptor Chamber Wash	196.22	238.95	149.92	124.37	101.45	100.93	153.81	73.21	87.88	86.76	198.66	241.43	146.13	60.14
Total Absorbed	3043.44	3653.44	1347.21	1290.22	1107.38	1204.34	1582.47	1272.68	2205.45	1051.03	1856.28	2271.50	1823.79	828.48
Dermal Delivery	4356.79	4546.92	2700.02	2718.22	2225.53	2097.61	2517.10	2293.29	3139.44	2059.96	3178.46	3360.92	2932.85	829.08
Mass Balance	7782.87	7853.71	7688.37	7805.63	7562.07	7770.97	7726.48	7692.31	7650.08	7625.99	7733.84	7805.21	7724.79	84.97

Epidermis = Epidermis + Clingfilm

Table 14 Distribution of [^{14}C]-Caprylhydroxamic Acid (ng equiv./cm^2) in Stratum Corneum at 24 h Post Dose Following Topical Application of Test Preparation 3 (*ca* 0.15%, w/w) to Human Split-Thickness Skin

	Cell Number and Donor Number													
Tape Strip No.	Cell 25 0680A	Cell 26 0680A	Cell 27 0743	Cell 28 0743	Cell 29 0727	Cell 30 0727	Cell 31 0727	Cell 32 0749	Cell 33 0749	Cell 34 0671	Cell 35 0671	Cell 36 0671	Mean	SD
1	21.82	18.67	55.47	105.43	71.03	36.34	51.57	68.15	65.34	36.64	60.05	24.30	51.23	25.22
2	19.90	15.22	41.31	63.04	33.51	20.56	22.33	58.76	41.03	31.23	39.90	28.05	34.57	15.08
3	18.51	13.76	48.92	39.20	37.17	19.89	27.69	45.03	38.37	24.61	29.80	16.91	29.99	11.65
4	18.53	9.53	31.30	31.66	23.34	19.59	26.34	41.91	24.10	18.83	31.95	21.63	24.89	8.44
5	13.45	7.32	29.57	31.41	34.57	13.44	25.25	28.66	16.20	13.89	25.75	13.79	21.11	9.01
6	15.71	5.75	50.05	28.19	31.51	12.67	21.79	31.20	11.33	13.50	25.15	13.50	21.69	12.28
7	15.90	6.20	41.84	31.18	34.99	19.74	18.64	31.31	14.04	8.90	24.15	15.59	21.87	10.94
8	13.23	4.97	48.06	36.06	23.11	13.07	21.06	30.12	13.23	12.26	25.37	11.90	21.04	12.30
9	15.40	5.58	40.25	31.66	31.24	18.09	18.88	28.50	10.35	10.33	21.87	15.88	20.67	10.36
10	15.55	5.27	39.90	42.97	26.02	15.45	23.67	25.26	11.15	8.44	29.37	14.85	21.49	11.90
11	14.53	5.75	33.64	30.03	41.98	12.87	24.53	23.87	11.68	9.58	31.66	15.50	21.30	11.29
12	12.45	5.32	34.41	32.58	48.16	17.70	17.82	33.74	8.76	10.02	29.00	22.14	22.68	12.99
13	11.54	5.55	26.27	30.13	26.63	17.66	12.35	28.34	6.48	7.54	31.62	16.86	18.41	9.78
14	11.64	6.09	34.86	29.32	44.39	16.91	11.52	34.41	7.49	10.79	24.02	26.61	21.50	12.54
15	10.49	5.64	47.34	25.92	72.15	12.46	12.91	36.37	8.44	9.92	25.23	19.24	23.84	19.68
16	8.66	4.86	23.90	21.33	36.51	10.52	15.57	33.62	6.95	11.21	20.02	23.67	18.07	10.26
17	7.34	5.28	32.28	25.32	37.89	12.04	15.79	44.50	5.74	13.37	22.77	32.32	21.22	13.30
18	8.09	5.34	22.33	22.01	23.37	9.24	25.10	37.93	5.76	11.34	29.79	21.48	18.48	10.40
19	8.48	5.17	24.31	21.63	24.63	13.60	21.76	30.68	8.09	9.81	29.38	20.88	18.20	8.79
20	8.09	3.90	26.16	27.15	40.40	13.71	10.38	39.29	8.55	7.81	22.68	22.96	19.26	12.43

Table 15 Cumulative Absorption of [^{14}C]-Caprylhydroxamic Acid (ng equiv./cm^2) into Receptor Fluid Following Topical Application of Test Preparation 3 (*ca* 0.15%, w/w) to Human Split-Thickness Skin

	Cell Number and Donor Number													
Time (h)	Cell 25 0680A	Cell 26 0680A	Cell 27 0743	Cell 28 0743	Cell 29 0727	Cell 30 0727	Cell 31 0727	Cell 32 0749	Cell 33 0749	Cell 34 0671	Cell 35 0671	Cell 36 0671	Mean	SD
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	149.63	218.56	30.06	28.14	20.36	27.26	186.63	76.50	84.52	16.68	53.91	60.87	79.43	68.77
4	271.59	314.78	43.42	81.56	54.29	68.35	177.73	100.59	149.41	47.36	128.76	112.40	129.19	87.50
6	524.59	706.87	174.92	202.98	148.44	167.65	348.89	222.79	331.48	115.79	309.66	358.19	301.02	172.75
8	699.01	981.58	257.53	302.39	208.75	246.87	502.37	281.21	459.60	176.53	420.36	526.59	421.90	234.77
12	1142.83	1515.03	449.27	464.71	362.30	382.37	684.85	433.15	780.14	299.58	671.05	843.21	669.04	362.01
24	2847.21	3414.48	1197.29	1165.85	1005.93	1103.41	1428.66	1199.48	2117.57	964.27	1657.62	2030.07	1677.65	784.85

14 APPENDICES

Appendix 1 GLP certificate for Charles River

Department of Health

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE IN ACCORDANCE WITH DIRECTIVE 2004/9/EC

TEST FACILITY	TEST TYPE(S)
Charles River Laboratories Edinburgh Limited Tranent Edinburgh EH33 2NE	Analytical/Clinical Chemistry Environmental Fate Environmental Toxicity Ecosystems Phys.Chem. Testing Residue Studies Mutagenicity Toxicology
Including satellite facilities at: Charles River Trade Terminal Dietzenbach Max-Planck-Str 6 63128, Dietzenbach Germany	Residue Studies
DATE OF INSPECTION	24 to 26 March 2015

An inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above test facility as part of the UK Good Laboratory Practice Compliance Monitoring Programme.

This statement confirms that, on the date of issue, the UK Good Laboratory Practice Monitoring Authority were satisfied that the above test facility was operating in compliance with the OECD Principles of Good Laboratory Practice.

This statement constitutes a Good Laboratory Practice Instrument (as defined in the UK Good Laboratory Practice Regulations 1999).

13/7/15

Dr. Andrew J. Gray
Head, UK GLP Monitoring Authority

MHRA

Appendix 2 Certificate of Analysis, [^{14}C]-Caprylhydroxamic Acid

CAUTION-RADIOACTIVE MATERIAL

Product Specification

Quotient Bioresearch (Radiochemicals) Ltd
The Old Glassworks
Nettlefold Road, Cardiff, CF24 5JQ
Telephone: +44(0)2920 474930

QUOTIENT BIORESEARCH

[^{14}C]Caprylhydroxamic acid
CFQ42749
5 mCi, 185 MBq

Before using this product, please read the instructions on the use, safe handling, storage and disposal of the material.

Technical Data

Specific Activity
Determined by:
Mass Spectrometry : 58 mCi/mmol 2.15 GBq/mmol

Molecular weight (at this specific activity) : 161.1

Date of analysis : 22 February 2016

Radiochemical purity by high performance liquid chromatography : 99.6%
Column: Luna C18(2) 5 µm 150 x 4.6 mm
Solvent A: 20 mN Potassium dihydrogen phosphate in water pH 6.5
Solvent B: Methanol
Gradient T(min): 0 20 30 31 35
%B: 10 80 80 10 10
Temperature: 40°C
Flow rate: 1.0mL/min
Detection: Homogeneous radiochemical detection, DAD at 220nm

Chemical Identity
The material co-chromatographs with commercially available material in the above chromatographic system.
The mass spectrum is consistent with the proposed structure and a non labelled reference.
The ^{1}H-NMR spectrum is consistent with the proposed structure and a non labelled reference.

Packaging and storage
The material is supplied as a solid in a borosilicate multidose vial with additional screw cap.
Storage at -80°C is recommended.

Purification details
Manufactured to Quotient Bioresearch (Radiochemicals) Ltd procedures.
[^{14}C]Caprylhydroxamic acid was purified by HPLC.

Appendix 2
(Continued)

Safety Warnings and precautions

USE IN HUMANS - WARNING This product is NOT suitable for direct administration in human studies. Further modification, alteration, preparation and/or testing of this product by the user will be required prior to use in clinical trials or any applications involving humans. Any such use of this product is the sole responsibility of the user, and the user must ensure compliance with all relevant international, national and local regulations. FOR RESEARCH USE ONLY.

Caution: Radioactive Material For professional users only

Instructions relating to the handling, use, storage and disposal of radioactive materials

1 Upon receipt, vials or ampoules containing radioactive material should be checked for contamination. All radioactive materials should be stored in specially designated areas. Access to these areas should be restricted to authorised personnel only.

2 Radioactive materials should be used by responsible persons only in authorised areas. Care should be taken to prevent ingestion or contact with skin or clothing. Protective clothing, such as laboratory coats, safety glasses and gloves should be worn whenever radioactive materials are handled.

3 No smoking, drinking or eating should be allowed in areas where radioactive materials are used. Avoid actions that could lead to the ingestion of radioactive materials, such as the pipetting of radioactive solutions by mouth.

4 Vials containing radioactive materials should not be touched by hand; wear suitable protective gloves as normal practice. Ampoules likely to contain volatile radioactive compounds should be opened only in a well ventilated fume cabinet.

5 Work should be carried out on a surface covered with absorbent material or in suitable trays of sufficient capacity to contain any spillage. Working areas should be monitored regularly.

6 Any spills of radioactive material should be cleaned immediately and all contaminated materials should be decontaminated or disposed of as radioactive waste via an authorised route. Contaminated surfaces should be washed with suitable detergent/solvent to remove traces of radioactivity.

7 After use, all unused radioactive materials should be stored in specifically designated areas. Any radioactive product not required or any materials that have come into contact with radioactivity should be disposed of as radioactive waste via an authorised route.

8 Hands should be washed after using radioactive materials. Hands and clothing should be monitored before leaving the designated area, using appropriate instruments to ensure that no contamination has occurred. If radioactive contamination is detected, hands should be washed again and rechecked. Any contamination persisting on hands and clothing should be reported to the responsible person so that suitable remedial actions can be taken.

9 Certain national/international organisations and agencies consider it appropriate to have additional controls during pregnancy. Users should check local regulations. Most countries have legislation governing the handling, use, storage, disposal and transportation of radioactive materials. The instructions set out above complement local regulations or codes of practice. Such regulations may require that a person be nominated to oversee radiological protection. Users of radioactive products must be aware of and observe local regulations or codes of practice which relate to such matters.

Document serial number: 2016 0224 1439

Appendix 3 Certificate of Analysis, Caprylhydroxamic Acid [redacted]

[redacted]

Certificate of Analysis

Product: [redacted] Date: Jan/20/2016

Mfg Date: Nov/16/2015

LOT #: FA8580

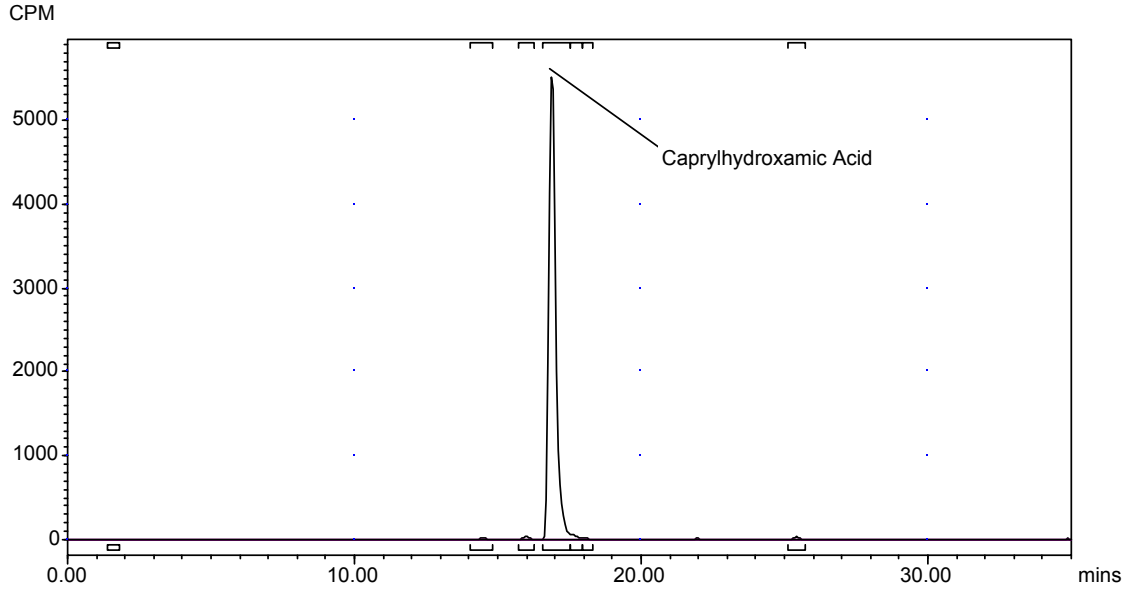
ANALYSIS	RESULT	SPECIFICATION
Melt Range °C (begin)	79	78 Min.
Melt Range °C (end)	80	81 Max.
Infrared Spectrum	Conforming	MATCHES REFERENCE
Appearance	Conforming	WHITE,OFF WHITE,AMBER CRYSTAL
CHA, %	99.8	98.0 Min.

[redacted]

Appendix 4 Confirmation of Radiochemical Purity of [^{14}C]-Caprylhydroxamic Acid

Radiochromatogram from the analysis of [^{14}C] - Caprylhydroxamic Acid

Sample Name: RCP 2
File Name: 798232_11Mar2016Run3Eval1



Peak Name	Retention Time (min)	% ROI
-	14.42	0.3
-	15.90	0.6
Caprylhydroxamic acid	16.82	97.6
-	17.58	0.9
-	18.05	0.2
-	25.37	0.5

Appendix 5 Human Skin Donor Details

Charles River Donor No.	Sex/Age	Site	Supplier
0671	Female/31Y	Abdomen	NHS Lothian, UK
0680A	Female/39Y	Abdomen	NHS Lothian, UK
0727	Female/41Y	Abdomen	NHS Lothian, UK
0743	Female/30Y	Abdomen	Tissue Solutions, UK
0749	Female/64Y	Abdomen	Tissue Solutions, UK

Appendix 6 Thickness of Full and Split-Thickness Skin Membranes

Charles River Donor No.	Membrane Thickness (μm)	
	Full Thickness Skin	Split-Thickness Skin
0671	1600-1810	380-390
0680A	1120-1380	370
0727	1310-1490	360-390
0743	1340-1850	380-390
0749	1010-1150	380

Appendix 7 Cross Reference of Cell Number with Donor Number and Electrical Resistance

Cell Number	Charles River Donor No.	Electrical Resistance (kΩ)
1	0680A	6.126
2	0680A	5.354
3	0680A	5.681
4	0743	4.890
5	0743	4.568
6	0727	8.762
7	0727	6.614
8	0749	7.509
9	0749	8.423
10	0671	6.397
11	0671	4.736
12	0671	4.724
13	0680A	5.109
14	0680A	4.974
15	0743	5.272
16	0743	5.455
17	0743	6.135
18	0727	8.920
19	0727	8.892
20	0727	8.652
21	0749	7.786
22	0749	7.312
23	0671	5.770
24	0671	5.723
25	0680A	5.291
26	0680A	5.849
27	0743	6.307
28	0743	5.922
29	0727	7.377
30	0727	8.830
31	0727	4.570
32	0749	5.191
33	0749	6.776
34	0671	5.499
35	0671	5.959
36	0671	5.832

Rejection criterion, sample rejected if electrical resistance < 4 kΩ for split-thickness skin.

Appendix 8 Epidermis Removal by Tape Stripping

The stratum corneum was removed with twenty successive tape strips. Where epidermis was removed on a tape strip this is reported in the table below, with approximate fraction removed given in parenthesis. Where epidermis was removed during tape stripping, the associated value was added to the epidermis value.

Cell Number	Tape Strip Number (Fraction of Epidermis Removed)
9	18 (Trace)
23	15(Trace)

Appendix 9 OECD Guidance Document No. 28 Glossary of Terms

ENV/JM//MONO(2004)2

GLOSSARY OF TERMS

Absorbed dose (*in vivo*): comprises that present in urine, cage wash, faeces, expired air (if measured), blood, tissues (if collected) and the remaining carcass, following removal of application site skin.

Absorbed dose (*in vitro*): mass of test substance reaching the receptor fluid or systemic circulation within a specified period of time.

Absorbable dose (*in vitro and in vivo*) represents that present on or in the skin following washing.

Absorption (Dermal, Percutaneous and Skin absorption): diffusion of chemicals from the outer surface of the skin to the receptor fluid or systemic circulation.

Absorption profile: a graphical representation of cumulative absorption as a function of time.

Absorption rate: mass of test substance passing through a unit area of skin into the receptor fluid or systemic circulation, per unit time (in $\mu g/cm^2/h$).

Adsorption: reversible binding or adherence the test substance to any component of the test system.

Applied dose: mass of test preparation containing a specified mass of test substance applied per cm^2 of skin.

Dermal delivery: sum of the applied dose found in the treated skin and the absorbed dose at the end of the experiment.

Dislodgeable dose: mass of test substance that is removable from the application site.

Exposure period: time from application of test preparation to removal at skin washing.

Finite dose: amount of test preparation applied to the skin where a maximum absorption rate of the test substance may be achieved for a certain time interval but is not maintained.

Flux: mass of test substance passing through a unit area of skin per unit of time under steady-state conditions (in $\mu g/cm^2/h$).

'in-use' preparation: the preparation of test substance which relates directly to potential human exposure (e.g. cosmetic or agrochemical formulations and dilutions thereof, a mixture of industrial chemicals in a solvent, etc.).

Infinite dose: amount of test preparation applied to the skin where a maximum absorption rate of the test substance is achieved and maintained.

Lag time: derived from a graph of cumulative absorbed dose and time. Intercept of the tangent of the linear part of the absorption profile with the x-axis.

Penetration enhancer: adjuvant, which facilitates penetration of the test substance through skin.

Appendix 9 (Continued)

Percentage absorption: the mass of test substance absorbed (over a given time period) divided by the mass of test substance applied multiplied by 100.

Permeability coefficient (Kp): a value, in units of cm/h, that represents the rate at which a chemical penetrates the skin. This is calculated from the flux divided by the applied concentration.

Steady-state: the part of an absorption profile where the absorption rate remains constant.

Test substance: a single chemical entity whose penetration characteristics are under investigation.

Test preparation: actual material that is applied to the skin. Usually the test preparation will be the 'in-use' preparation that reflects actual use conditions; alternatively it may be a mixture of the test substance in a carrier or solvent to facilitate application to the skin.

Unabsorbed dose: represents that washed from the skin surface after exposure and any present on the non-occlusive cover, including any dose shown to volatilise from the skin during exposure.