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SYN524464

SYN524464 FS (A16148C) – *In Vitro* Absorption Through Human Epidermal Membranes Using [Pyrazole-5-^{14}C]-SYN524464

Final Report

DATA REQUIREMENT(S): OECD 428 (2004)
Supplemental to EPA OPPTS 870.7600 (1998)

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STUDY COMPLETION DATE: November 18, 2009

PERFORMING LABORATORY: Dermal Technology Laboratory Ltd.
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SUBMITTER/SPONSOR: Syngenta Crop Protection, Inc.
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VOLUME 1 OF 1 OF STUDY

STATEMENT OF DATA CONFIDENTIALITY CLAIMS

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS
UNDER SPECIFIED FIFRA PROVISIONS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Section 10(d)(1)(A) (discloses manufacturing or quality control processes), (B) (discloses the details of methods for testing, detecting or measuring the quantity of any deliberately added inert ingredient of a pesticide), or (C) (discloses the identity or percentage quantity of any deliberately added inert ingredient of a pesticide).

Company
Syngenta Crop Protection, Inc.

Company Agent: Adora Clark, Ph.D.

Adora Clark — March 1, 2010

U. S. Product Registration Manager — Date

Syngenta is the owner of this study. Syngenta has submitted this material to the United States Environmental Protection Agency specifically under the provisions contained in FIFRA as amended and, hereby, consents to use and disclosure of this material by EPA according to FIFRA. Notwithstanding the wording of our marking TRADE SECRET, this marking, by itself, conveys no supplemental claims of confidentiality under FIFRA Sections 10(a) or 10(b) (addressing protection of trade secrets and commercial and financial information). In submitting this material to EPA according to method and format requirements contained in PR Notice 86-5, we do not waive any protection or right involving this material that would have been claimed by the company if this material had not been submitted to the EPA, nor do we waive any protection or right provided under FIFRA Section 3 (concerning data exclusivity and data compensation) or FIFRA Section 10(g) (prohibiting disclosure to foreign and multinational pesticide companies or their agents).

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

I, the undersigned, declare that the objectives laid down in the protocol were achieved and that the data generated are valid. The report fully and accurately reflects the procedures used and the raw data generated in this study.

The study (JV2052) was conducted in compliance with the UK Principles of Good Laboratory Practice (The United Kingdom GLP Regulations 1999, Statutory Instrument No. 3106 as amended 2004, Statutory Instrument No. 994). These Principles are in accordance with the OECD Principles of Good Laboratory Practice, revised 1997 (ENV/MC/CHEM(98)17), which is acceptable to U.S. EPA FIFRA (40 CFR part 160) Good Laboratory Practice Standards.

Study Director D S Davies Date 18th November 09

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Richard C Peffer 1 March 2010

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FLAGGING STATEMENT

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QUALITY ASSURANCE STATEMENT

In accordance with Dermal Technology Laboratory Ltd. policy and QA procedures for Good Laboratory Practice, this report has been audited and the conduct of this study has been inspected as follows:

Date	Audit/Inspection	Date of QA Report
01 June 2009	Protocol	03 June 2009
08 June 2009	Protocol amendment 1	08 June 2009
26 June 2009	Thin layer chromatography	30 June 2009
28 August 2009	Draft report	04 September 2009
30 October 2009	Draft report	05 November 2009
13 November 2009	Final report	13 November 2009
18 November 2009	Final re-issued report review	18 November 2009

In addition, inspections associated with this type of study were made as follows:

07 April 2009	Cell assembly and assessment of membrane integrity	08 April 2009
08 April 2009	Dose preparation	15 April 2009
08 April 2009	Addition of dose to donor	15 April 2009
08 & 14 April 2009	Liquid scintillation counting	15 April 2009
09 April 2009	Skin/cell processing	14 April 2009
23 June 2009	Sample dispatch	23 June 2009
26 June 2009	High performance liquid chromatography	30 June 2009

Facilities and process based procedures associated with this type of study were inspected in accordance with QA Standard Operating Procedures.

So far as can be reasonably established, the methods described and the results given in the final report accurately reflect the raw data produced during the study, JV2052.

Michael Howes

Date 18th Nov 2009

Michael Howes
(Quality Assurance Team,
Dermal Technology Laboratory Ltd)

GENERAL INFORMATION

Contributors

The following contributed to this report in the capacities indicated:

Name	Title
D J Davies	Study Director
Prof. J R Heylings	Study Reviewer
A Majid	Analyst
B K Jones	Syngenta Study Manager

Study dates

Study initiation date: 29 May 2009.
Experimental start date: 29 May 2009.
Experimental completion date: 12 November 2009.

Deviations from the guidelines

None.

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1.0 EXECUTIVE SUMMARY

1.1 Study Design

The absorption and distribution of SYN524464 from an FS formulation concentrate (A16148C) was measured *in vitro* through human epidermal membranes conforming to the Regulatory Guidelines given in Section 2.2. The doses were applied as the formulation concentrate (nominally 500 g SYN524464/L) and as a two aqueous end use dilutions (1/20 and 1/200 v/v) of the formulation. The doses were applied to the epidermal membranes at rates of 10 µL/cm^2. The applications were left unoccluded for an exposure period of 24 hours.

The formulation concentrate was included to assess exposure when handling the concentrated product. The aqueous dilutions used (1/20 and 1/200 v/v) represented typical end-use concentrations. These applications were designed to simulate potential human dermal exposure to the formulation during normal use.

[^{14}C]-Radiolabelled SYN524464 was incorporated into dose preparations that were equivalent to the formulation concentrate and 1/20 and 1/200 aqueous dilutions thereof prior to application. The absorption process was followed by taking samples of the receptor fluid (50% ethanol in water) at recorded intervals throughout the exposure period. The distribution of SYN524464 within the test system and a 24 hour absorption profile were determined. All samples were analysed by liquid scintillation counting (LSC).

1.2 Results

1.2.1 Analysis of the [^{14}C]-dose preparations

LSC analysis of the dose preparations confirmed that the dose preparations were homogeneous both prior to and following dosing.

TLC and HPLC analysis confirmed that the radiochemical purity of [^{14}C]-SYN524464 when assessed as a formulation concentrate and two aqueous end-use dilutions (1/20 v/v and 1/200 v/v) was greater than 95% for a period of time longer than that used in this study.

Some of the HPLC data used to support the statement of radiochemical purity were obtained from Quotient Bioresearch Laboratories (Read H, 2009).

1.2.2 Absorption

1.2.2.1 Formulation concentrate

After a short lag phase of about 2 hours, absorption was linear over the remaining exposure period of 2-24 hours. The absorption rate between 2-24 hours was 0.011 µg/cm^2/h.

The amounts of SYN524464 absorbed at 6, 8 and 10 hours were 0.054, 0.059 and 0.083 µg/cm^2, respectively. These respective amounts expressed as percentages of the

applied dose were 0.001, 0.001 and 0.002%. The amount absorbed over the entire 24 hour exposure period was 0.247 μg/cm^2 (0.005% of the applied dose).

1.2.2.2 1/20 v/v Aqueous end-use dilution

Absorption of SYN524464 was linear over the entire 24 hour exposure period. The absorption rate between 0-24 hours was 0.005 μg/cm^2/h.

The amounts of SYN524464 absorbed at 6, 8 and 10 hours were 0.024, 0.034 and 0.042 μg/cm^2, respectively. These respective amounts expressed as percentages of the applied dose were 0.009, 0.013 and 0.017%. The amount absorbed over the entire 24 hour exposure period was 0.109 μg/cm^2 (0.043% of the applied dose).

1.2.2.3 1/200 v/v Aqueous end-use dilution

Absorption of SYN524464 was linear over the entire 24 hour exposure period. The absorption rate between 0-24 hours was 0.004 μg/cm^2/h.

The amounts of SYN524464 absorbed at 6, 8 and 10 hours were 0.029, 0.036 and 0.043 μg/cm^2, respectively. These respective amounts expressed as percentages of the applied dose were 0.115, 0.144 and 0.173%. The amount absorbed over the entire 24 hour exposure period was 0.090 μg/cm^2 (0.360% of the applied dose).

1.2.3 Mass balance and SYN524464 distribution

1.2.3.1 Formulation concentrate

Mean recovery of the applied test material was 101%.

The majority of the applied dose, 100% was found in the skin wash, 24 hours after application.

The proportion of the applied dose present in receptor fluid following the total 24 hour exposure was 0.005%. This percentage equated to 0.247 μg/cm^2.

A total of <0.120% of the applied dose remained in the epidermal membrane following a 24 hour skin washing procedure. Of this total, <0.055% was present in the outer layers of the *stratum corneum* with only 0.065% of the applied dose present in the remaining epidermal tissue.

The mean proportions of the applied dose present in tape strips 1 and 2 were <0.017% and 0.013%, respectively.

1.2.3.2 1/20 v/v Aqueous end-use dilution

Mean recovery of the applied test material was 104%.

Skin washing 24 hours after application removed 103% of the applied dose.

The proportion of the applied dose present in receptor fluid following the total 24 hour exposure was 0.043%. In terms of actual amounts this percentage equated to 0.109 µg/cm^2.

A total of 0.996% of the applied dose remained in the epidermal membrane following a 24 hour skin washing procedure. Of this total, 0.488% was present in the outer layers of the *stratum corneum* with only 0.508% of the applied dose present in the remaining epidermal tissue.

The mean proportions of the applied dose present in tape strips 1 and 2 were 0.136% and 0.120%, respectively.

1.2.3.3 1/200 v/v Aqueous end-use dilution

Mean recovery of the applied test material was 107%.

Skin washing 24 hours after application removed 106% of the applied dose.

The proportion of the applied dose present in receptor fluid following the total 24 hour exposure was 0.360%. In terms of actual amounts this percentage equated to 0.090 µg/cm^2.

A total of <0.331% of the applied dose remained in the epidermal membrane following a 24 hour skin washing procedure. Of this total, <0.120% was present in the outer layers of the *stratum corneum* with only 0.211% of the applied dose present in the remaining epidermal tissue.

The mean proportions of the applied dose present in tape strips 1 and 2 were <0.028% and <0.022%, respectively.

1.3 Conclusion

The results obtained in this study indicate that SYN524464 was absorbed through human epidermal membranes at a slow rate from the FS formulation concentrate (A16148C), and at progressively slower rates from the two aqueous end-use dilutions (1/20 v/v and 1/200 v/v).

Irrespective of dose, virtually all of the applied dose remained on the skin surface after a 24 hour exposure period and was readily removed by gentle skin washing. Very low proportions of the dose were associated with the *stratum corneum* and the remaining epidermal membrane.

These data predict that following human dermal exposure to the formulation concentrate and two aqueous end-use dilutions (1/20 v/v and 1/200 v/v), absorption of SYN524464 from formulation A16148C would be minimal.

The percentage absorption values to be used in risk assessment calculations are the 6 hour values because this time represents a typical working day. These 6 hour absorption values for the formulation concentrate, the 1/20 and the 1/200 end-use dilutions are 0.001%, 0.009% and 0.115%, respectively.

2.0 INTRODUCTION

2.1 Purpose

The purpose of this study was to determine the *in vitro* absorption of SYN524464 through human epidermal membranes over a 24 hour exposure period to aid the quantitative assessment of the risk arising from skin contact with a flowable suspension (FS) formulation concentrate (A16148C) containing a nominal 500 g SYN524464/L and two aqueous end-use dilutions (1/20 and 1/200 v/v) containing a nominal 25 and 2.5 g SYN524464/L. The distribution of SYN524464 within the test system following the 24 hour exposure was also determined.

2.2 Regulatory Guidelines and Guidance Documents

1) OECD Test Guideline 428 (2004). Skin Absorption: *In Vitro* Method.

2) OECD (Guidance Document No. 28 (2004). The Conduct of Skin Absorption Studies.

3) European Commission Guidance Document on Dermal Absorption (2004).

2.3 Justification for Selection of the Test System

This is a validated system for determining dermal absorption across human skin *in vitro* and its reliability has been demonstrated (OECD 428, 2004). Reliability of results obtained in the reported study was demonstrated by the methods specified in the Guidance documents in Section 2.2.

2.4 Dose Level Selection

The application rates and exposure conditions used in this study were designed to simulate predicted normal human exposure to the test material and were requested by the Sponsor. The concentrated formulation A16148C has an approximate concentration of 500 g SYN524464/L. The 1/20 and 1/200 dilutions reflect representative end-use dilutions.

2.5 Data Storage

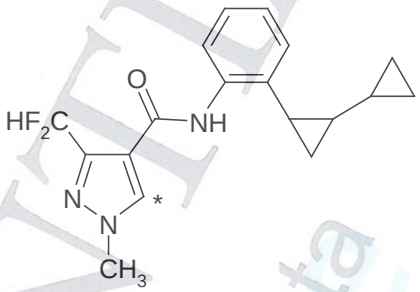
An original report, the study protocol and all raw data pertaining to this study will be retained in the Archives, Dermal Technology Laboratory (DTL) Ltd, Med IC4, Keele University Science and Business Park, Keele, Staffordshire, ST5 5NL, UK for a minimum of one year from the date of issue of the final report. At the end of this period, the Sponsor will be contacted regarding the future fate of the archived materials.

3.0 MATERIALS AND METHODS

3.1 Test and Control Substances

The Sponsor provided pre-mixes of the active ingredient, comprising radiolabelled and unlabelled SYN524464 (Section 3.2) of the appropriate specific activities, for the preparation of the three dose levels (formulation concentrate and 1/20 and 1/200 v/v dilutions).

3.1.1 Structure of the radiolabelled test substance



SYN524464

* denotes the position of [^{14}C]-labelled atoms.

SYN524464 is comprised of a mixture of a trans isomer (SYN508210) and a cis isomer (SYN508211). The ratios of isomers for each premix, described below, were designed to closely approximate the final commercial product specification.

3.2 [^{14}C]-Radiolabelled SYN524464 Pre-mixes

3.2.1 Formulation concentrate pre-mix

Name:	[Pyrazole-5-^{14}C]-SYN524464
Isomer ratio:	86.8% SYN508210 : 13.2% SYN508211
Lot number:	RDR-V-10
Source:	Syngenta Crop Protection, Inc. P.O. Box 18300, Greensboro, NC 27419-8300, USA
DTL test substance reference number:	TS00055/011
Chemical purity:	>99.9% (by HPLC 5th December 2008)
Radiochemical purity:	96.5% (by HPLC 5th December 2008)
Specific activity:	0.010 MBq/mg
Storage conditions:	Frozen (at least -70 °C)
Expiration Date:	30th June 2009

From information supplied by the Sponsor, the test substance was used within the expiry date. A certificate of analysis (reference number: RDR-V-10, dated 5th December 2008) is presented in Appendix 1. The test substance characterisation was the responsibility of the Sponsor.

3.2.2 1/20 v/v Aqueous dilution pre-mix

Name:	[Pyrazole-5-^{14}C]-SYN524464
Isomer ratio:	86.9% SYN508210 : 13.1 % SYN508211
Lot number:	RDR-V-11
Source:	Syngenta Crop Protection, Inc. P.O. Box 18300, Greensboro, NC 27419-8300, USA
DTL test substance reference number:	TS00055/012
Chemical purity:	>99.9% (by HPLC 5th December 2008)
Radiochemical purity:	98.7% (by TLC 5th December 2008)
Specific activity:	0.2035 MBq/mg
Storage conditions:	Frozen (at least -70 °C)
Expiration Date:	30th June 2009

From information supplied by the Sponsor, the test substance was used within the expiry date. A certificate of analysis (reference number: RDR-V-11, dated 5th December 2008) is presented in Appendix 2. The test substance characterisation was the responsibility of the Sponsor.

3.2.3 1/200 v/v Aqueous dilution pre-mix

Name:	[Pyrazole-5-^{14}C]-SYN524464
Isomer ratio:	85.8% SYN508210 : 14.2 % SYN508211
Lot number:	RDR-V-12
Source:	Syngenta Crop Protection, Inc. P.O. Box 18300, Greensboro, NC 27419-8300, USA
DTL test substance reference number:	TS00055/013
Chemical purity:	98.3% (by HPLC 5th December 2008)
Radiochemical purity:	98.8% (by TLC 5th December 2008)
Specific activity:	2.031 MBq/mg
Storage conditions:	Frozen (at least -70 °C)
Expiration Date:	30th June 2009

From information supplied by the Sponsor, the test substance was used within the expiry date. A certificate of analysis (reference number: RDR-V-12, dated 5th December 2008) is

presented in Appendix 3. The test substance characterisation was the responsibility of the Sponsor.

3.3 SYN524464 FS (500)-C - Remaining Formulation Components

3.3.1 Component A – Formulation Mill-base

Name:	SYN524464 FS (500)-C formulation blank – component A
Source:	Syngenta Crop Protection, Inc. P.O. Box 18300, Greensboro, NC 27419-8300, USA
DTL test substance reference number:	TS00055/002
Lot number:	1467-178-1
Storage:	Ambient temperature

3.3.2 Component B – Other formulation components

Name:	SYN524464 FS (500)-C formulation blank – component B
Source:	Syngenta Crop Protection, Inc. P.O. Box 18300, Greensboro, NC 27419-8300, USA
DTL test substance reference number:	TS00055/003
Lot number:	1467-178-2
Storage:	Ambient temperature

3.3.3 2% Xanthan gum pregel component - Thickener gel

Name:	SYN524464 FS (500)-C formulation blank – 2% xanthan gum pregel component
Source:	Syngenta Crop Protection, Inc. P.O. Box 18300, Greensboro, NC 27419-8300, USA
DTL test substance reference number:	TS00055/004
Lot number:	1467-77-3
Storage:	Ambient temperature

3.3.4 CIPAC D water

Name:	CIPAC D water (342 ppm hardness)
Source:	Syngenta Crop Protection, Inc. P.O. Box 18300, Greensboro, NC 27419-8300, USA
DTL test substance reference number:	TS00055/005
Lot number:	10082008
Storage:	Ambient temperature

3.4 Analytical Standard Material

As the premixes of radiolabelled test substance were supplied at the required specific activity values, this unlabelled test material was used exclusively as a reference compound.

Name:	SYN524464
Isomer ratio:	85.8:13.8 ± 0.3% mixture of the trans and cis isomers, SYN508210 and SYN508211
Chemical name (IUPAC):	3-Difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid (2-bicyclopropyl-2-yl-phenyl)-amide
Chemical name (CAS):	1H-Pyrazole-4-carboxamide, N-[2-[1,1'-bicyclopropyl]-2-ylphenyl]-3-(difluoromethyl)-1-methyl
CAS registry number:	874967-67-6 mixture of 599197-38-3 (trans isomer) and 599194-51-1 (cis isomer)
Molecular formula:	$C_{18}H_{19}F_2N_3O$
Molecular weight:	331.4
Batch identification:	AMS 1238/1
Source:	Syngenta Crop Protection, Münchwilen AG, Breitenloh 5, CH-4333 Münchwilen
Colour:	White
Physical state:	Powder
DTL test substance reference number:	TS00055/010
Purity:	99.6%
Expiration date:	End of December 2010
Storage conditions:	Less than 30 ^{o}C

From information supplied by the Sponsor, the analytical standard was used within the expiry date. The analytical standard characterisation was the responsibility of the Sponsor.

3.5 Reference Formulation

This material was for reference purposes only and was not used for experimental purposes.

Name:	A16148C full commercial formulation
Source:	Syngenta Crop Protection, Inc. P.O. Box 18300, Greensboro, NC 27419-8300, USA
DTL test substance reference number:	TS00055/006
Lot number:	1425-137C
Storage:	Ambient temperature

3.6 Experimental Procedures

3.6.1 Radioactivity content and radiochemical purity of the [^{14}C]-radiolabelled SYN524464 pre-mixes

The radiolabelled materials were supplied dry. As part of preliminary investigations, stock solutions of the powdered [^{14}C]-SYN524464 pre-mixes were made by diluting the pre-mixes with a recorded volume of acetonitrile. The radioactivity content of the [^{14}C]-radiolabelled pre-mixes (TS00055/011, TS00055/012 and TS00055/013) was determined by analysing sub-samples of solvent dilutions by LSC (VV2059, Davies D J 2009).

The [^{14}C]-SYN524464 pre-mixes were supplied with current certificates of analyses (Appendices 1-3) detailing radiochemical purity values and expiry dates. The purity of the [^{14}C]-SYN524464 pre-mixes was not determined as part of these investigations. However, the radiochemical purity and stability of the [^{14}C]-SYN524464 in each dose formulation was verified (Section 3.6.6).

3.6.2 Dose preparation

The doses were prepared using methodology determined as part of preliminary investigations (VV2059, Davies D J 2009) with these test materials. The intention was to mimic the commercial 500 g SYN524464/L formulation and its two aqueous end-use dilutions (1/20 and 1/200 v/v).

The doses were prepared as close to the time of application as was practicable.

3.6.2.1 Formulation concentrate

A volume (36.2 mL) of the stock [^{14}C]-radiolabelled test material (TS00055/011) in acetonitrile was diluted with 100 µL of pentane. The solvent was evaporated using a stream of nitrogen gas and further drying, and recrystallisation, was achieved by snap freezing the test materials over dry ice for 15 minutes followed by freeze drying for at least 24 hours.

A 4346 mg weight of the mill-base (TS00055/002) was added followed by 513 mg of water (TS00055/005). Following the addition of ninety ca 3 mm glass beads, the preparation was mixed thoroughly using a combination of sonication and shaking. To achieve a preparation with the appropriate sized particles (Section 3.6.3), a combination of milling (frequency of 30 Hertz (cycles/second) using a ball mill (Retsch mixer mill type MM200)) and sonication was used. An aliquot of the mixture was taken for particle size assessment (Section 3.6.3). The remaining formulation components were then added in the following order; 2871 mg Component B (TS00055/003) and 594 mg thickener gel (TS00055/004). The preparation was thoroughly mixed by milling.

The dose preparation was stirred constantly and stored in the dark at room temperature prior to use.

3.6.2.2 1/20 v/v Aqueous end-use dilution

A volume (2.71 mL) of the stock [^{14}C]-radiolabelled test material (TS00055/012) in acetonitrile was diluted with 50 µL of pentane. The solvent was evaporated using a stream of nitrogen gas and further drying, and recrystallisation, was achieved by snap freezing the test materials over dry ice for 15 minutes followed by freeze drying for at least 24 hours.

A 179 mg weight of the mill-base (TS00055/002) was added followed by 4198 mg of water (TS00055/005). Following the addition of sixty ca 3 mm glass beads, the preparation was mixed thoroughly using a combination of sonication and shaking. To achieve a preparation with the appropriate sized particles (Section 3.6.3), a combination of milling (frequency of 30 Hertz (cycles/second) using a ball mill (Retsch mixer mill type MM200)) and sonication was used. An aliquot of the mixture was taken for particle size assessment (Section 3.6.3). The remaining formulation components were then added in the following order; 123 mg Component B (TS00055/003), 25.5 mg thickener gel (TS00055/004) and 5725 mg of the remaining water (TS00055/005). The preparation was thoroughly mixed by milling.

The dose preparation was stirred constantly and stored in the dark at room temperature prior to use.

3.6.2.3 1/200 v/v Aqueous end-use dilution

A volume (1.50 mL) of the stock [^{14}C]-radiolabelled test material (TS00055/013) in acetonitrile was diluted with 50 µL of pentane. The solvent was evaporated using a stream of nitrogen gas and further drying, and recrystallisation, was achieved by snap freezing the test materials over dry ice for 15 minutes followed by freeze drying for at least 24 hours.

A 21.5 mg weight of the mill-base (TS00055/002) was added followed by 5041 mg of water (TS00055/005). Following the addition of sixty 3 mm glass beads, the preparation was mixed thoroughly using a combination of sonication and shaking. To achieve a preparation with the appropriate sized particles (Section 3.6.3), a combination of milling (frequency of 30 Hertz (cycles/second) using a ball mill (Retsch mixer mill type MM200)) and sonication was used. An aliquot of the mixture was taken for particle size assessment (Section 3.6.3). The remaining formulation components were then added in the following order; 17.2 mg

Component B (TS00055/003), 5.85 mg thickener gel (TS00055/004) and 7187 mg of the remaining water (TS00055/005). The preparation was thoroughly mixed by milling.

The dose preparation was stirred constantly and stored in the dark at room temperature prior to use.

3.6.3 Particle size assessment

Prior to the addition of the other formulation components, a 25 µL aliquot of the preparation was diluted in 500 µL (formulation concentrate) or 225 µL (1/20 and 1/200 v/v end-use dilutions) 15% polypropylene glycol and mixed thoroughly. Particle size measurement was performed in duplicate using a validated particle sizing procedure, based upon the measurement of particles on microscope slides fitted with a graticule, to ensure that the optimum particle size of *circa* 10-12 µm had been achieved.

3.6.4 Radioactivity content of the dose preparations

The radioactivity content of each formulated [^{14}C]-SYN524464 preparation (Section 3.6.2) was determined by analysing replicate sub-samples of solvent dilutions by LSC.

3.6.5 Homogeneity of the dose preparations

Homogeneity of each formulated [^{14}C]-radiolabelled SYN524464 preparation was confirmed by analysing replicate sub-samples of solvent dilutions by LSC prior, during and post dosing.

3.6.6 Radiochemical purity and stability

A sample of each formulated [^{14}C]-radiolabelled SYN524464 preparation, was analysed to determine the radiochemical purity of the formulated SYN524464, using the analytical chromatographic procedures shown in Section 3.6.7.

3.6.7 Analytical techniques

Samples collected during this study were analysed by liquid scintillation counting (LSC), high performance chromatography (HPLC) and thin layer chromatography (TLC).

The limit of quantitation using LSC for the concentrate and two end-use dilutions (1/20 v/v and 1/200 v/v) in each of the study compartments is expressed as µg/cm^2 and percentage of applied dose. These are shown in Appendix 10. The criteria for setting LOQ values are given in Appendix 5.

LSC	
Counting period:	6 minutes or to a 0.5% standard deviation of the count (0.5 minutes for time critical quick checks)
Scintillation fluid:	Goldstar (Meridian Biotechnologies Ltd, 5 West Street, Epsom, Surrey, KT18 7RL, UK,)
Model of LSC:	Packard 3100 TR

TLC:
The radiochemical purity of [^{14}C]-radiolabelled test material was determined by TLC using silica gel plates (K6f) using the following solvent system: Acetone : Hexane (40 : 60 v/v, normal phase plate) Radioactivity on the TLC plates was measured using a Packard Instant Imager. Unlabelled standard material were visualised under UV light at 254 nm.

Equipment:	HPLC system - Agilent 1100
Column:	Reverse Phase (RP). ACE 3 µ - C18, 15 cm x 3 mm ID, 100Å. Supplier: Hichrom Ltd.
Solvent A:	35:65% v/v, acetonitrile/purified water
Flow rate:	0.5 mL/min
Detector wavelength:	UV absorbance at 245 nm
Injection volume:	20 - 100µL
Flow Scintillation Analyser:	Packard 525
Total run time:	20-25 minutes
Radiochemical detector:	Packard Flow Scintillation Analyzer (FSA) Model 500TR

3.6.8 Supply of dose preparations for synchronised *in vivo* dermal absorption studies

Immediately after preparation and radiochemical analysis, each of the three doses was divided into two portions, one of which was used in this study. The second portion of each dose was sent, on the afternoon of dose preparation, to Quotient Bioresearch Laboratories for a synchronised *in vivo* dermal absorption study in the rat. Each of the three dose preparations was applied to rats on the morning following its preparation (Read H, 2009).

3.6.9 Human epidermal membrane preparation

Human skin samples were obtained from a tissue bank. The skin samples were immersed in water at 60 °C for 40-45 seconds and the epidermal membrane teased away from the dermis.

Each membrane was given an identifying number and stored frozen, at approximately -20 °C, on aluminium foil until required for use.

3.6.10 Assembly of diffusion cells

The type of static glass diffusion cell used in this study has an exposed membrane area of 2.54 cm^2 and a volume of approximately 4.5 mL. A diagram of the static glass diffusion cell used in this study is displayed in OECD guideline 428, (2004). Discs of approximately 3.3 cm diameter of prepared skin membrane were mounted, dermal side down, in diffusion cells held together with individually numbered clamps and placed in a water bath maintained at 32 °C ± 1 °C.

3.6.11 Measurement of membrane integrity

Membrane integrity was determined by measurement of the electrical resistance across the skin membrane. Membranes with a measured resistance of <10 kΩ (Davies *et al*, 2004) were regarded as having a lower integrity than normal and not used for exposure to the test materials.

3.6.12 Selection of cells and dosing

Cells were selected such that each application was represented by six intact membranes from at least two different donors.

The receptor chambers of the cells containing small magnetic stirrer bars were filled with a recorded volume of receptor fluid (50% ethanol in water). The cells were not placed in the water bath at this stage.

The 50% ethanol in water receptor fluid was selected to ensure that the test substance could freely partition into the receptor fluid from the skin membrane and never reached a concentration that would limit its diffusion. The suitability of this receptor fluid was determined in a method validation study (VV2059, Davies D J 2009). The highest concentration of SYN524464 being applied in this study was the 500 g SYN524464/L formulation concentrate. With a total application rate of 25.4 µL and an average receptor fluid volume of 4.5 mL a total concentration of 2.82 mg SYN524464/mL of receptor fluid would be expected if there was 100% penetration of the test material through the epidermal membranes. Unlabelled SYN524464 was shown to be soluble in the receptor fluid (50% ethanol in water) at a concentration of 2.91 mg/mL, which exceeds the maximum possible concentration attainable in this *in vitro* dermal absorption study.

A pre-treatment sample (0.5 mL) was taken from each receptor chamber for analysis by LSC. An equal volume of fresh receptor fluid was added to each receptor chamber to replace the volume removed.

The formulation was applied to the skin membranes using a positive displacement pipette as the formulation concentrate and as two end-use dilutions (1/20 and 1/200 v/v). The weight of dose formulation applied to the membranes was recorded and the applications were left unoccluded for the duration of the 24 hour exposure period.

Test substance	Application rate	Total dose applied
Formulation concentrate 500 g ai/L	10 µL/cm^2 ≡ 5000 µg ai/cm^2	25.4 µL
1/20 v/v aqueous dilution 25 g ai/L	10 µL/cm^2 ≡ 250 µg ai/cm^2	25.4 µL
1/200 v/v aqueous dilution 2.5 g ai/L	10 µL/cm^2 ≡ 25 µg ai/cm^2	25.4 µL

3.6.13 Sampling of receptor fluid

Samples (0.5 mL) of receptor fluid were taken using an autosampler 1, 2, 3, 4, 6, 8, 10, 12, 16, 20 and 24 hours after application for analysis by LSC.

The volume of fluid in the receptor chamber was maintained by the replacement of a volume of receptor fluid, equal to the sample volume immediately after each sample was taken.

3.6.14 Measurement of mass balance

After the final receptor fluid sample had been taken at the end of the exposure period, the remaining fluid in the receptor chamber was discarded and the chamber rinsed with fresh receptor fluid which was also discarded.

The donor chamber was carefully removed and the underside wiped with a single sponge pre-wetted with a solution of 3% Teepol L® in water which was added to the wash sponges (below). The donor chamber was washed with acetonitrile and the sample analysed for [^{14}C]-SYN524464 by LSC.

The epidermal surface of the skin was gently washed by swabbing the application site with natural sponges pre-wetted with a solution of 3% Teepol L® in water. Following assessment of radioactivity levels on the skin surface with a Geiger counter, sponges pre-wetted with water, were used to further swab the surface. The sponges were digested in a tissue digestant (Soluene 350®) and made up to a recorded volume. A sample of the digest was taken for analysis.

The surface of the skin was allowed to dry naturally.

To assess penetration through human *stratum corneum*, successive layers of the skin surface were removed by the repeated application of adhesive tape (e.g. Scotch 3M Magic Tape,

1.9 cm wide), to a maximum of 5 strips (Ramsey *et al*, 1994). A strip of adhesive tape was pressed onto the skin surface and then carefully peeled off to remove the *stratum corneum*. The adhesive strips were soaked in acetonitrile to extract any test material. The 5 strips were extracted individually. The extracts were sequentially numbered and analysed by LSC.

In one case, it was not possible to take the full 5 tape strips as the epidermal membrane began to tear, therefore, tape stripping was discontinued. The last tape strip for this diffusion cell was digested/extracted with the remaining epidermal membrane, so as not to underestimate residues in the remaining epidermal membrane compartment.

The remaining epidermal membrane was carefully removed from the receptor chamber, digested in Soluene 350® and analysed by LSC.

3.7 Data Evaluation

Results of the analysis of the samples of receptor fluid collected in the study were expressed as amounts of SYN524464 in the receptor solution in terms of µg/cm^2. The amounts absorbed, rates of absorption (µg/cm^2/h) and 'percentage of dose absorbed' were determined using the calculations in Appendix 5.

The reported tables, figures and appendices containing absorption and distribution data show the exact number of cells used for reporting. Membranes with absorption profiles that indicated membrane damage during the course of the experiment have been excluded from the reported tables, figures and appendices.

The results of the mass balance and distribution determinations are expressed in terms of amount absorbed and 'percentage of applied dose'.

Tables and appendices presented in the report are computer generated. The group mean and individual data are rounded appropriately for inclusion in the report. As a consequence, calculation of group mean, standard deviation and standard error data from the individual data in the tables may yield a minor variation in the value reported.

The absorbed (systemically available) dose is considered to be the SYN524464 detected in the receptor fluid. Material removed from the surface of the epidermal membrane by the washing procedure is regarded as unabsorbed. SYN524464 recovered from the epidermal membrane at the end of the exposure is also considered to be unabsorbed, although it is recognised that a proportion of this material may be absorbed beyond the duration of the exposure investigated in this study. *In vivo*, the majority of the dose in the epidermal membrane, especially that recovered from the *stratum corneum* (i.e. that found on the tape strips), would eventually be lost by desquamation (Ramsey *et al*, 1992).

4.0 RESULTS AND DISCUSSION

4.1 Analysis of the [^{14}C]-Dose Preparations

4.1.1 Particle size assessment

The optimum particle size range, based on the normal manufacture process for this product, was between 10-12 µm.

The mean particle size achieved for the formulation concentrate was 10.6 µm. For the 1/20 and 1/200 v/v end-use dilutions, the mean particle sizes achieved were 10.7 and 9.30 µm, respectively. The particle size of the formulated test materials was therefore considered to be acceptable for the purposes of this study.

4.1.2 Dose levels achieved and homogeneity of the dose preparations

LSC analysis of the dose preparations confirmed that the dose levels achieved were 510, 25.6 and 2.50 g SYN524464/L for the formulation concentrate and two end-use dilutions (1/20 v/v and 1/200 v/v), respectively.

The dose preparations were considered to be homogeneous and acceptable for use in these experiments (Appendix 12). For the formulation concentrate, the LSC analysis of the dose preparations, immediately prior to application, gave a % RSD value of 1.26% and for the 1/20 v/v and 1/200 v/v end-use dilutions, analysis gave % RSD values of 1.42 and 0.682%, respectively.

LSC analysis of the formulation concentrate 2 days post preparation gave a % RSD value of 3.21%. For the 1/20 v/v end-use dilution, analysis 3 days post preparation gave a % RSD value of 1.81% deviation and the 1/200 v/v end-use dilution, analysis 17 days post preparation, gave a % RSD value of 1.42%.

4.1.3 Stability and radiochemical purity of SYN524464 in the formulations

SYN524464, when formulated as a formulation concentrate and 1/20 v/v and 1/200 v/v end-use dilutions, was shown to be stable by TLC and HPLC (Appendices 13-14) for a period of time longer than that used in the study. Radiochemical purities of greater than 95% (Appendix 11) were seen in all the dose preparations both prior to application and post dose preparation.

Some of the HPLC data used to support the statement of radiochemical purity were obtained from Quotient Bioresearch Laboratories (Read H, 2009).

4.2 SYN524464 Absorption

The results obtained in this study are summarised in Table 1, where data are presented both in terms of absorption rate and in terms of amount and percentage of the dose applied during periods representing typical working days (6, 8 and 10 hours) and at 24 hours. The

absorption profiles for SYN524464 from this formulation and end-use dilutions are displayed in Figures 1 -3.

The absorption data for each individual cell are given in Appendix 6.

4.2.1 Formulation concentrate

After a short lag phase of about 2 hours, absorption was linear over the remaining exposure period of 2-24 hour exposure period. The absorption rate between 2-24 hours was 0.011 μg/cm^2/h.

The amounts of SYN524464 absorbed at 6, 8 and 10 hours were 0.054, 0.059 and 0.083 μg/cm^2, respectively. These respective amounts expressed as percentages of the applied dose were 0.001, 0.001 and 0.002%. The amount absorbed over the entire 24 hour exposure period was 0.247 μg/cm^2 (0.005% of the applied dose).

4.2.1.1 1/20 v/v Aqueous end-use dilution

Absorption of SYN524464 was linear over the entire 24 hour exposure period. The absorption rate between 0-24 hours was 0.005 μg/cm^2/h.

The amounts of SYN524464 absorbed at 6, 8 and 10 hours were 0.024, 0.034 and 0.042 μg/cm^2, respectively. These respective amounts expressed as percentages of the applied dose were 0.009, 0.013 and 0.017%. The amount absorbed over the entire 24 hour exposure period was 0.109 μg/cm^2 (0.043% of the applied dose).

4.2.1.2 1/200 v/v Aqueous end-use dilution

Absorption of SYN524464 was linear over the entire 24 hour exposure period. The absorption rate between 0-24 hours was 0.004 μg/cm^2/h.

The amounts of SYN524464 absorbed at 6, 8 and 10 hours were 0.029, 0.036 and 0.043 μg/cm^2, respectively. These respective amounts expressed as percentages of the applied dose were 0.115, 0.144 and 0.173%. The amount absorbed over the entire 24 hour exposure period was 0.090 μg/cm^2 (0.360% of the applied dose).

4.3 Mass Balance and SYN524464 Distribution

The data for distribution in the test system are presented in Table 2 in terms of percentage of the applied dose. The individual mass balance data are given in Appendices 7-9.

4.3.1 Formulation concentrate

Mean recovery of the applied test material was 101%.

The majority of the applied dose, 100% was found in the skin wash 24 hours after application.

The proportion of the applied dose present in receptor fluid following the total 24 hour exposure was 0.005%. This percentage equated to 0.247 μg/cm^2.

A total of <0.120% of the applied dose remained in the epidermal membrane following a 24 hour skin washing procedure. Of this total <0.055% was present in the outer layers of the *stratum corneum* (tape strips 1-5) with only 0.065% of the applied dose present in the remaining epidermal tissue.

The mean proportions of the applied dose present in tape strips 1 and 2 were <0.017% and 0.013%, respectively.

4.3.2 1/20 v/v Aqueous end-use dilution

Mean recovery of the applied test material was 104%.

Skin washing 24 hours after application removed 103% of the applied dose.

The proportion of the applied dose present in receptor fluid following the total 24 hour exposure was 0.043%. In terms of actual amounts this percentage equated to 0.109 μg/cm^2.

A total of 0.996% of the applied dose remained in the epidermal membrane following a 24 hour skin washing procedure. Of this total 0.488% was present in the outer layers of the *stratum corneum* with only 0.508% of the applied dose present in the remaining epidermal tissue.

The mean proportions of the applied dose present in tape strips 1 and 2 were 0.136% and 0.120%, respectively.

4.3.3 1/200 v/v Aqueous end-use dilution

Mean recovery of the applied test material was 107%.

Skin washing 24 hours after application removed 106% of the applied dose.

The proportion of the applied dose present in receptor fluid following the total 24 hour exposure was 0.360%. In terms of actual amounts this percentage equated to 0.090 μg/cm^2.

A total of <0.331% of the applied dose remained in the epidermal membrane following a 24 hour skin washing procedure. Of this total <0.120% was present in the outer layers of the *stratum corneum* with only 0.211% of the applied dose present in the remaining epidermal tissue.

The mean proportions of the applied dose present in tape strips 1 and 2 were <0.028% and <0.022%, respectively.

5.0 CONCLUSION

The results obtained in this study indicate that SYN524464 was absorbed through human epidermal membranes at a slow rate from the FS formulation concentrate (A16148C), and at progressively slower rates from the two aqueous end-use dilutions (1/20 v/v and 1/200 v/v).

Irrespective of dose, virtually all of the applied dose remained on the skin surface after a 24 hour exposure period and was readily removed by gentle skin washing. Very low proportions of the dose were associated with the *stratum corneum* and the remaining epidermal membrane.

These data predict that following human dermal exposure to the formulation concentrate and two aqueous end-use dilutions (1/20 v/v and 1/200 v/v), absorption of SYN524464 from formulation A16148C would be minimal.

The percentage absorption values to be used in risk assessment calculations are the 6 hour values because this time represents a typical working day. These 6 hour absorption values for the formulation concentrate, the 1/20 and the 1/200 end-use dilutions are 0.001%, 0.009% and 0.115%, respectively.

6.0 REFERENCES

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TABLES SECTION

TABLE 1 Summary of SYN524464 Absorption Through Human Epidermal Membranes

Application of Test Materials and Actual Concentration of Dose Preparation	Mean Absorption Rates		Mean Amount and Percentage of Dose Absorbed		
	Time period (h)	Absorption rate ($\mu g/cm^2/h \pm SEM$)	Time (h)	Amount ($\mu g/cm^2$)	Percentage absorbed
Formulation Concentrate					
(510 g SYN524464/L)			6	0.054*	0.001*
10 $\mu L/cm^2$ (5099 μg ai/cm^2)	2-24	0.011 ± 0.004	8	0.059*	0.001*
Unoccluded			10	0.083*	0.002*
Duration of exposure: 24h			24	0.247	0.005
n = 5			*LOQ*	*0.193*	*0.004*
1/20 v/v aqueous end-use dilution					
(25.6 g SYN524464/L)			6	0.024	0.009
10 $\mu L/cm^2$ (256 μg ai/cm^2)	0-24	0.005 ± 0.001	8	0.034	0.013
Unoccluded			10	0.042	0.017
Duration of exposure: 24h			24	0.109	0.043
n = 4			*LOQ*	*0.008*	*0.003*
1/200 v/v aqueous end-use dilution					
(2.50 g SYN524464/L)			6	0.029	0.115
10 $\mu L/cm^2$ (25.0 μg ai/cm^2)	0-24	0.004 ± 0.001	8	0.036	0.144
Unoccluded			10	0.043	0.173
Duration of exposure: 24h			24	0.090	0.360
n = 6			*LOQ*	*0.0007*	*0.003*

*Where indicated the values were below the LOQ however for the purposes of calculating amounts and percentages absorbed these values have been used as positive values in order not to distort the data or absorption profiles.

TABLE 2 Summary of SYN524464 Distribution in the Test System at 24 Hours

Formulation concentrate

Test Compartment n = 5	µg SYN524464 per cm² Mean	SEM	% of applied dose Mean	SEM
Donor chamber	<4.84*	4.32*	<0.095*	0.085*
Skin wash	5119	25.4	100	0.497
Stratum corneum (tape strips 1-5)	<2.80*	1.10*	<0.055*	0.021*
Remaining epidermal membranes	3.33	1.60	0.065	0.031
Absorbed	0.247	0.088	0.005	0.002
Total recovered	5130*	25.6*	101*	0.502*
Tape strip 1	<0.891*	0.415*	<0.017*	0.008*
Tape strip 2	<0.666*	0.398*	<0.013*	0.008*

1/20 v/v Aqueous end-use dilution

Test Compartment n = 4	µg SYN524464 per cm² Mean	SEM	% of applied dose Mean	SEM
Donor chamber	<0.010*	0.005*	<0.004*	0.002*
Skin wash	262	9.93	103	3.89
Stratum corneum (tape strips 1-5)	1.25	0.627	0.488	0.246
Remaining epidermal membranes	1.30	0.809	0.508	0.317
Absorbed	0.109	0.026	0.043	0.010
Total recovered	265*	9.60*	104*	3.76*
Tape strip 1	0.348	0.126	0.136	0.049
Tape strip 2	0.308	0.162	0.120	0.063

1/200 v/v Aqueous end-use dilution

Test Compartment n = 6	µg SYN524464 per cm² Mean	SEM	% of applied dose Mean	SEM
Donor chamber	<0.001*	0.0004*	<0.005*	0.002*
Skin wash	26.4	0.268	106	1.07
Stratum corneum (tape strips 1-5)	<0.030*	0.002*	<0.120*	0.010*
Remaining epidermal membranes	0.053	0.020	0.211	0.081
Absorbed	0.090	0.013	0.360	0.051
Total recovered	26.6*	0.257*	107*	1.03*
Tape strip 1	<0.007	0.001*	<0.028*	0.003*
Tape strip 2	<0.006	0.001*	<0.022*	0.004*

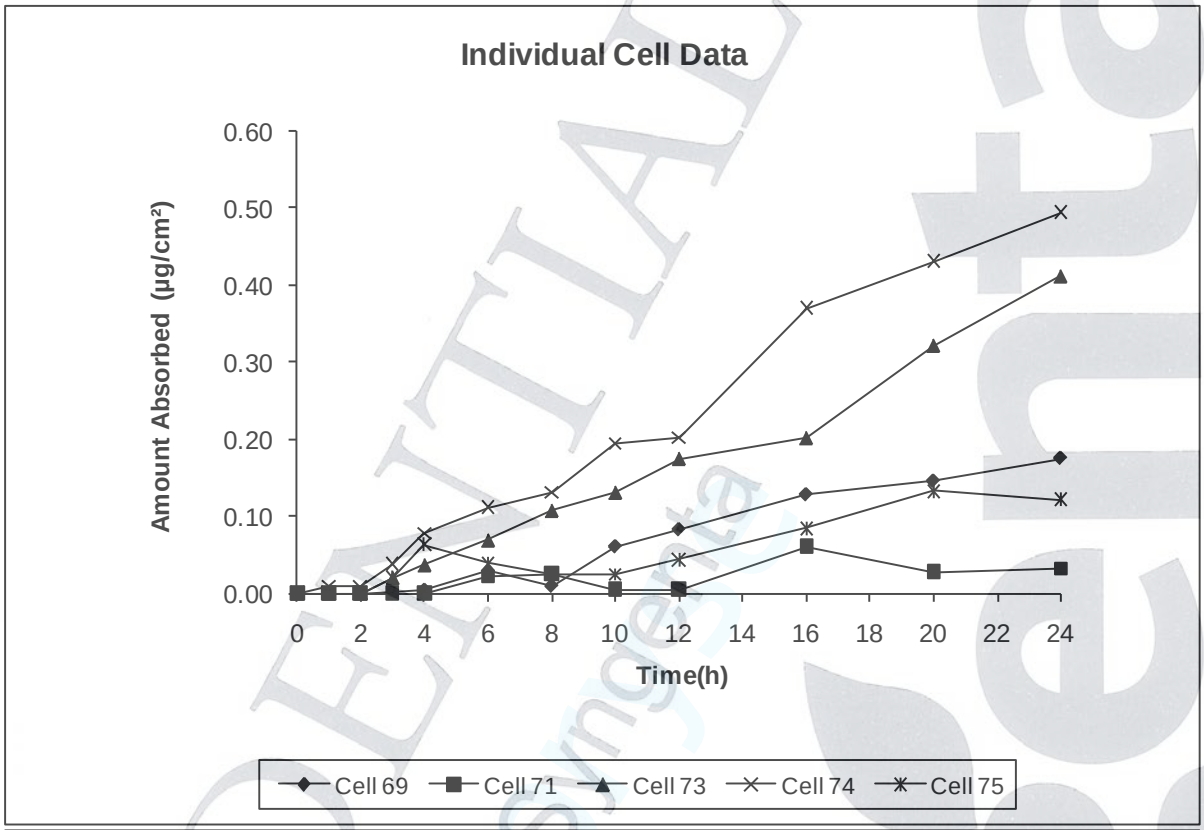
*The LOQ values have been used as positive values in the calculation of the means, SEM's or totals where values were <LOQ. These values have been reported preceded with a <.

Key to terminology used in table above

Stratum corneum = amount in tape strips; Remaining epidermal membranes = epidermal tissue remaining after tape stripping; Absorbed = amount in receptor fluid

FIGURES SECTION

FIGURE 1 Profiles of SYN524464 Absorption from the Formulation Concentrate Through Human Epidermal Membranes



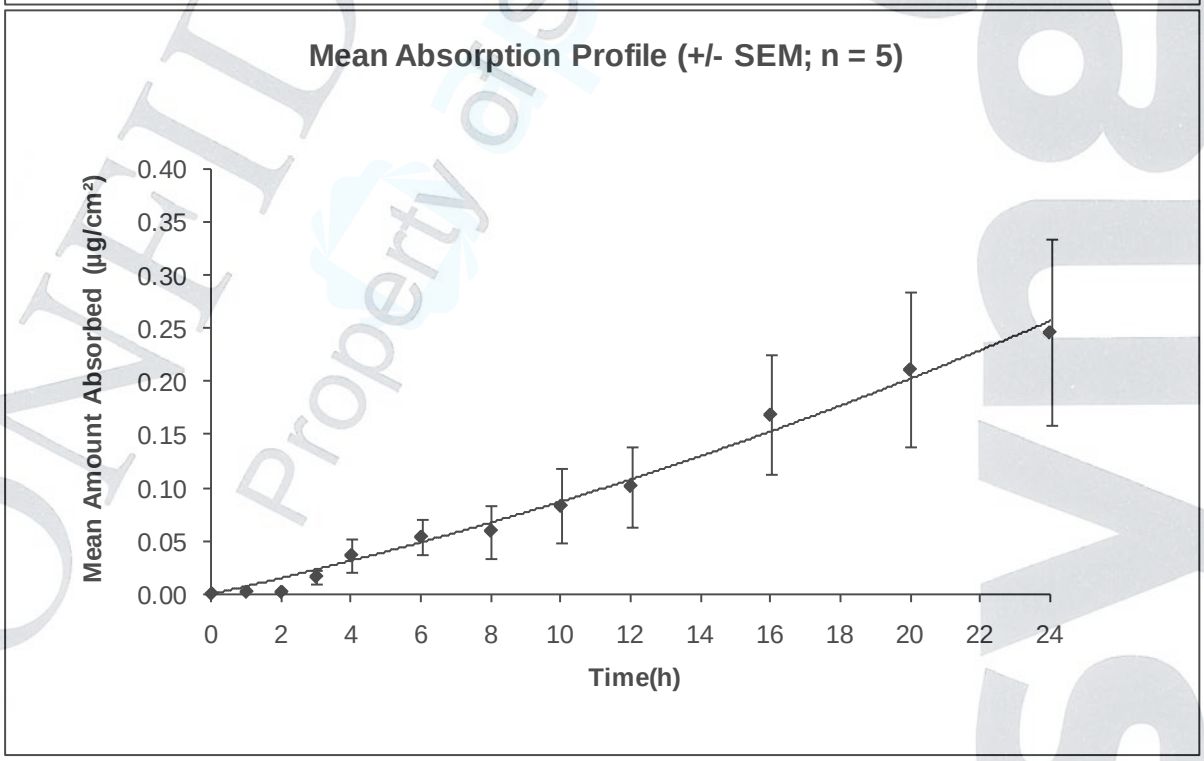
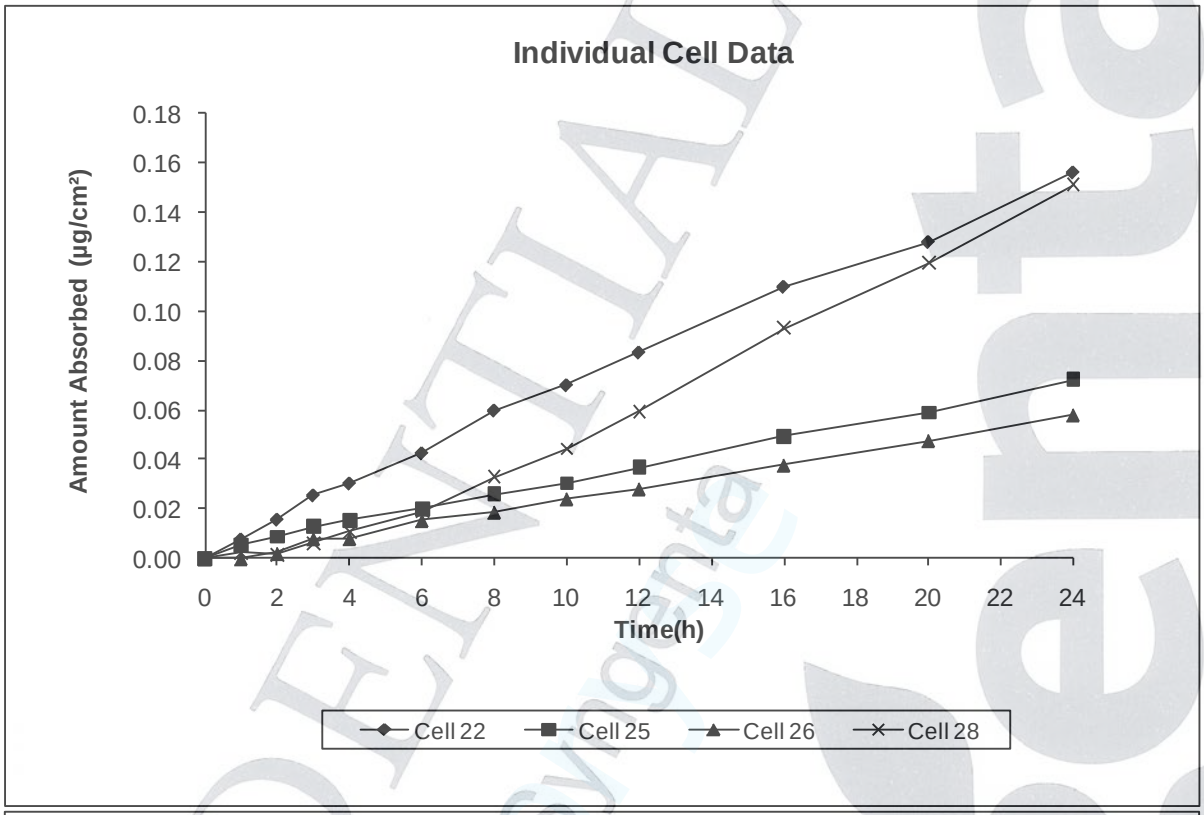


FIGURE 2 Profiles of SYN524464 Absorption from the 1/20 v/v Aqueous End-use Dilution Through Human Epidermal Membranes



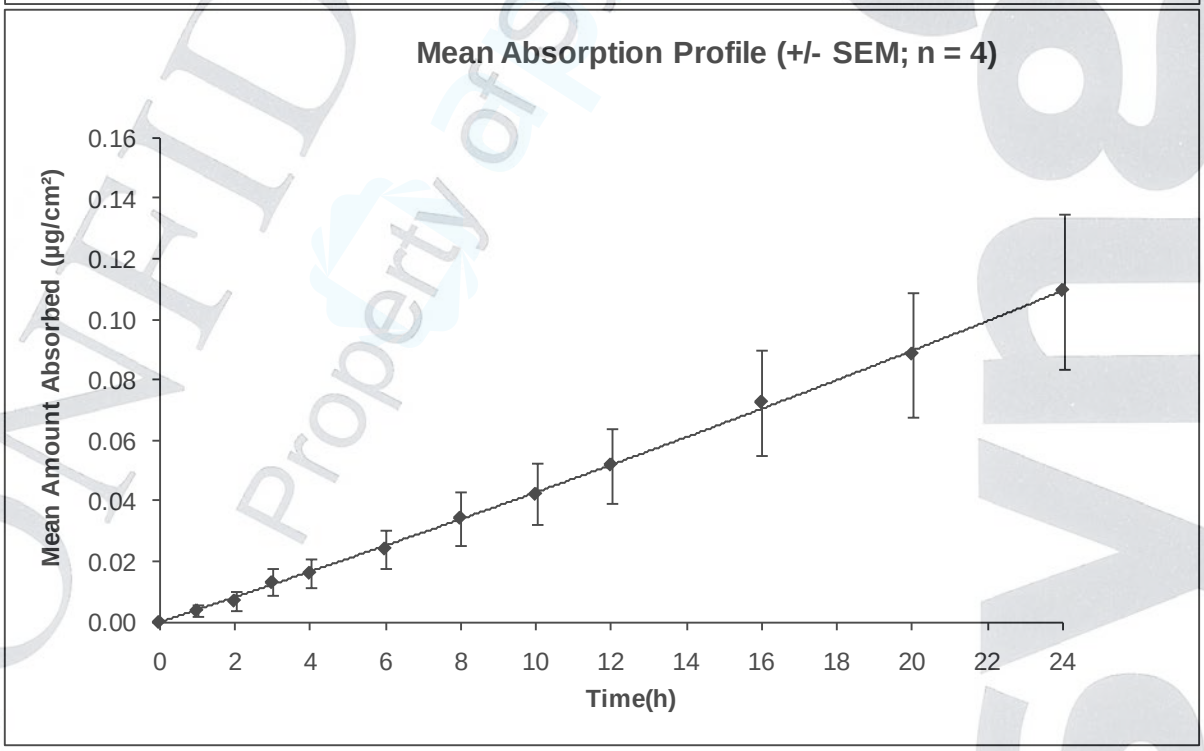
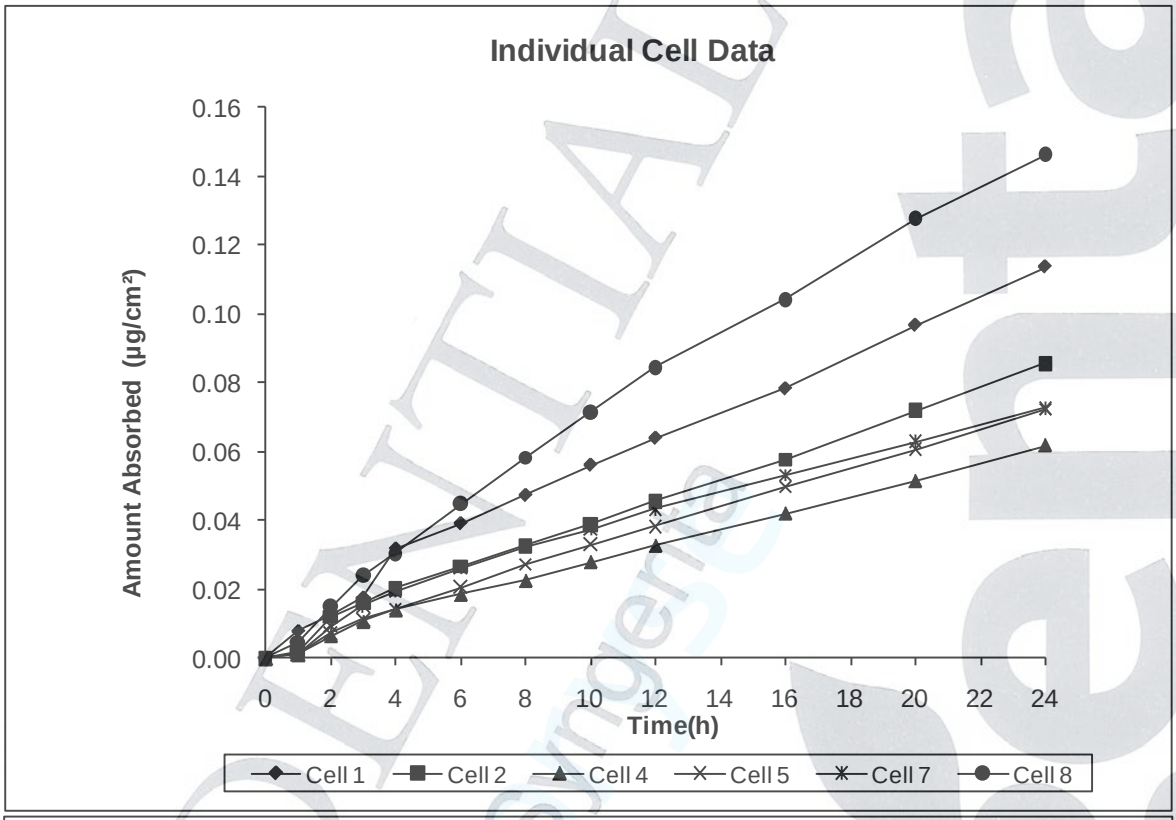
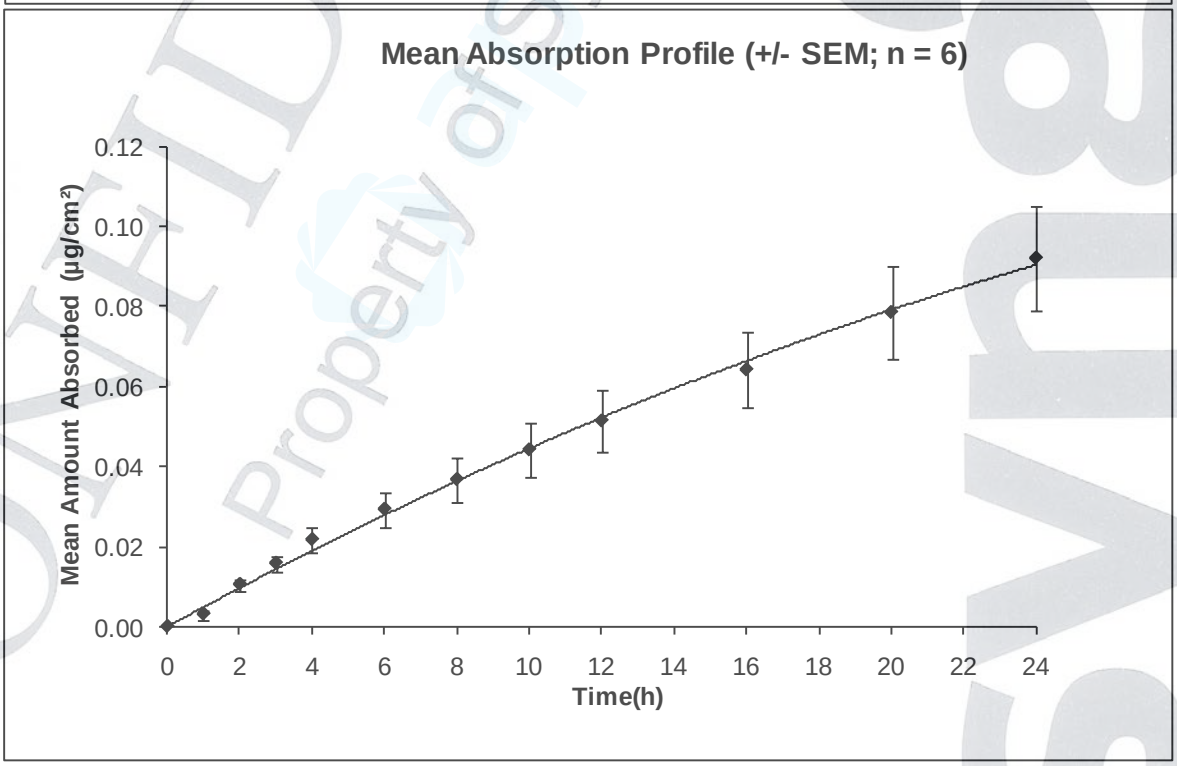


FIGURE 3 Profiles of SYN524464 Absorption from the 1/200 v/v Aqueous End-use Dilution Through Human Epidermal Membranes





APPENDICES SECTION

APPENDIX 1 Test Substance Information – [^{14}C]-Radiolabelled SYN524464 Formulation Concentrate Pre-mix

TS000555/011/001

SYNGENTA CROP PROTECTION, INC.
ES AMERICAS / LOGISTICS AND SUPPORT
GREENSBORO, NORTH CAROLINA, USA

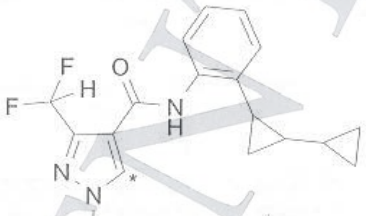
CERTIFICATE OF ANALYSIS

SYNGENTA CODE: [PYRAZOLYL-5-14C]-SYN 524464

SYNONYMNS: [PYRAZOLYL-5-14C]-CSCC231431

REFERENCE NUMBER (Batch Identification): RDR-V-10

STRUCTURE:



CHEMICAL PURITY: >99.9%

RADIOCHEMICAL PURITY: 96.5%

ISOMER RATIO: 86.8% SYN 508210 : 13.2% SYN 508211

SPECIFIC ACTIVITY: 0.27 µCi/mg (0.010 MBq/mg)

STATEMENT OF GLP COMPLIANCE:

The characterization study described in this Certificate of Analysis was conducted in compliance with EPA Good Laboratory Practice Standards; U.S.A., 40 CFR Part 160, August 17, 1989. Data obtained in conjunction with this characterization study have been archived at Syngenta Crop Protection Inc., Greensboro, NC.

STORAGE CONDITIONS: Freezer

EXPIRATION DATE: June 30, 2009

STUDY COMPLETION DATE: December 5, 2008

STUDY DIRECTOR: William F. Helke

SIGNATURE: William F. Helke

TS00055/011/001

ANALYTICAL STANDARD
CHARACTERIZATION REPORT

IDENTITY

- COMPARISON TO AN AUTHENTIC STANDARD:

  High Performance Liquid Chromatography Analysis
  Reference: (data ref.: R08-50/5,6; test date: 12/3/08)

- SPECTRAL IDENTITY:

MASS SPECTROSCOPY	:	Consistent with proposed structure. Reference: (data ref.: w0453, w0456; test date: 12/4/08)

PURITY

ISOMER RATIO – EXTERNAL STANDARD ANALYSIS BY HPLC	:	86.8% SYN 508210 : 13.2% SYN 508211 Reference: (data ref.: R08-50/1,2,3,4; test date: 12/3/08)
CHEMICAL PURITY – EXTERNAL STANDARD ANALYSIS BY HPLC		>99.9% Reference: (data ref.: R08-50/1,2,3,4; test date: 12/3/08)
RADIOCHEMICAL PURITY – AREA DISTRIBUTION BY HPLC		96.5% Reference: (data ref.: R08-50/5,6; test date: 12/3/08)

SPECIFIC ACTIVITY

SPECIFIC ACTIVITY – EXTERNAL STANDARD ANALYSIS BY HPLC		0.27 µCi/mg Reference: (data ref.: R08-50/1,2,3,4; test date: 12/3/08)

APPENDIX 2 Test Substance Information – [^{14}C]-Radiolabelled SYN524464 1/20 v/v Aqueous End-use Dilution Pre-mix

TS00055/012/2008
001
JV 10/12/08

SYNGENTA CROP PROTECTION, INC.
ES AMERICAS / LOGISTICS AND SUPPORT
GREENSBORO, NORTH CAROLINA, USA

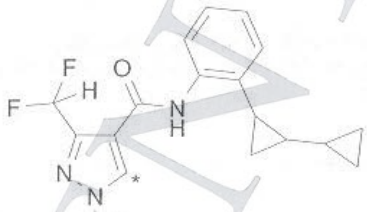
CERTIFICATE OF ANALYSIS

SYNGENTA CODE: [PYRAZOLYL-5-14C]-SYN 524464

SYNONYMNS: [PYRAZOLYL-5-14C]-CSCC231431

REFERENCE NUMBER (Batch Identification): RDR-V-11

STRUCTURE:



CHEMICAL PURITY: >99.9%

RADIOCHEMICAL PURITY: 98.7%

ISOMER RATIO: 86.9% SYN 508210 : 13.1% SYN 508211

SPECIFIC ACTIVITY: 5.5 μCi/mg (0.2035 MBq/mg)

STATEMENT OF GLP COMPLIANCE:

The characterization study described in this Certificate of Analysis was conducted in compliance with EPA Good Laboratory Practice Standards; U.S.A., 40 CFR Part 160, August 17, 1989. Data obtained in conjunction with this characterization study have been archived at Syngenta Crop Protection Inc., Greensboro, NC.

STORAGE CONDITIONS: Freezer

EXPIRATION DATE: June 30, 2009

STUDY COMPLETION DATE: December 5, 2008

STUDY DIRECTOR: William F. Helke

SIGNATURE: William F. Helke

ANALYTICAL STANDARD
CHARACTERIZATION REPORT TS00055/012/001

IDENTITY

- **COMPARISON TO AN AUTHENTIC STANDARD:**
  Reference: (data ref.: R08-51/5,6; test date: 12/3/08)

  Thin-Layer Chromatography Systems:

  Silica gel plate; Hexane : ethyl Acetate (1:1); Rf = 0.30
  C8 gel plate; Acetonitrile : Methanol : Water (6:1:3); Rf = 0.56

- **SPECTRAL IDENTITY:**

MASS SPECTROSCOPY	:	Consistent with proposed structure. Reference: (data ref.: w0454, w0457; test date: 12/4/08)

PURITY

ISOMER RATIO – EXTERNAL STANDARD ANALYSIS BY HPLC	:	86.9% SYN 508210 : 13.1% SYN 508211 Reference: (data ref.: R08-51/1,2,3,4; test date: 12/3/08)
CHEMICAL PURITY – EXTERNAL STANDARD ANALYSIS BY HPLC		>99.9% Reference: (data ref.: R08-51/1,2,3,4; test date: 12/3/08)
RADIOCHEMICAL PURITY – AREA DISTRIBUTION BY THIN-LAYER CHROMATOGRAPHY		98.7% Reference: (data ref.: R08-51/5,6; test date: 12/3/08)

SPECIFIC ACTIVITY

SPECIFIC ACTIVITY – EXTERNAL STANDARD ANALYSIS BY HPLC		5.5 µCi/mg Reference: (data ref.: R08-51/1,2,3,4; test date: 12/3/08)

APPENDIX 3 Test Substance Information – [^{14}C]-Radiolabelled SYN524464 1/200 v/v Aqueous End-use Dilution Pre-mix

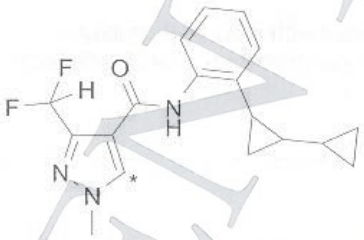
TS00055/013/001

SYNGENTA CROP PROTECTION, INC.
ES AMERICAS / LOGISTICS AND SUPPORT
GREENSBORO, NORTH CAROLINA, USA

CERTIFICATE OF ANALYSIS

SYNGENTA CODE:	[PYRAZOLYL-5-14C]-SYN 524464
SYNONYMNS:	[PYRAZOLYL-5-14C]-CSCC231431
REFERENCE NUMBER (Batch Identification):	RDR-V-12

STRUCTURE:



CHEMICAL PURITY:	98.3%
RADIOCHEMICAL PURITY:	98.8%
ISOMER RATIO:	85.8% SYN 508210 : 14.2% SYN 508211
SPECIFIC ACTIVITY:	54.9 μCi/mg (2.031 MBq/mg)

STATEMENT OF GLP COMPLIANCE:

The characterization study described in this Certificate of Analysis was conducted in compliance with EPA Good Laboratory Practice Standards; U.S.A., 40 CFR Part 160, August 17, 1989. Data obtained in conjunction with this characterization study have been archived at Syngenta Crop Protection Inc., Greensboro, NC.

STORAGE CONDITIONS:	Freezer
EXPIRATION DATE:	June 30, 2009
STUDY COMPLETION DATE:	December 5, 2008
STUDY DIRECTOR:	William F. Helke
SIGNATURE:	William F. Helke

ANALYTICAL STANDARD
CHARACTERIZATION REPORT

TS00055/013/001

IDENTITY

- COMPARISON TO AN AUTHENTIC STANDARD:
  Reference: (data ref.: R08-52/5,6; test date: 12/3/08)

  Thin-Layer Chromatography Systems:

  Silica gel plate; Hexane : ethyl Acetate (1:1); Rf = 0.30
  C8 gel plate; Acetonitrile : Methanol : Water (6:1:3); Rf = 0.56

- SPECTRAL IDENTITY:

MASS SPECTROSCOPY	:	Consistent with proposed structure. Reference: (data ref.: w0455, w0458; test date: 12/4/08)

PURITY

ISOMER RATIO – EXTERNAL STANDARD ANALYSIS BY HPLC	:	85.8% SYN 508210 : 14.2% SYN 508211 Reference: (data ref.: R08-52/1,2,3,4; test date: 12/3/08)
CHEMICAL PURITY – EXTERNAL STANDARD ANALYSIS BY HPLC		98.3% Reference: (data ref.: R08-52/1,2,3,4; test date: 12/3/08)
RADIOCHEMICAL PURITY – AREA DISTRIBUTION BY THIN-LAYER CHROMATOGRAPHY		98.8% Reference: (data ref.: R08-52/5,6; test date: 12/3/08)

SPECIFIC ACTIVITY

SPECIFIC ACTIVITY – EXTERNAL STANDARD ANALYSIS BY HPLC		54.9 µCi/mg Reference: (data ref.: R08-52/1,2,3,4; test date: 12/3/08)

APPENDIX 4 Dermal Technology Laboratory Ltd - Certificate of Good Laboratory Practice

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
Dermal Technology Laboratory Ltd. **IC4, Keele Science and Business Park** **Keele University** **Staffordshire** **ST5 5NL**	**Analytical Chemistry** ***in vitro* Dermal** **Penetration Studies**

DATE OF INSPECTION

24th July 2007

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

At the time of inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

13/8/07

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

MHRA

APPENDIX 5 Calculations

1. Absorption data

A validated computer program calculates absorption data using the following equations:

Equation 1

Receptor volume (mL) = V
Sample replacement volume (mL) = v

Concentrations (μg/mL) at sampling times (h) t_1, t_2, t_3 etc. are c_1, c_2, c_3 etc.

The total amount of penetrant having passed through the epidermal membrane into the receptor fluid, corrected for sample volume removed (c_1v, c_2v, c_3v etc.) at sampling times t_1, t_2, t_3 etc. are:

$t_1 = c_1V$
$t_2 = c_2V + c_1v$
$t_3 = c_3V + c_1v + c_2v$
$t_4 = c_4V + c_1v + c_2v + c_3v$
etc.

The amount of test penetrant at each time point is divided by the area of epidermal membrane ($2.54cm^2$) and the results plotted as amount of penetrant absorbed ($\mu g/cm^2$) versus time (h). The slope of this absorption profile between given time points gives the average rate of absorption of the penetrant per cm^2 of the skin ($\mu g/cm^2/h$) during that period.

The mean 'percent of dose absorbed' for any given time point is:

Equation 2

$$\% \text{ Absorbed} = \frac{\text{Mean amount absorbed } (\mu g/cm^2)}{\text{Mean amount applied } (\mu g/cm^2)} \times 100$$

APPENDIX 5 Calculations (Continued)

2. Limits of quantitation (LOQ) for absorption/penetration data

The LOQ for amount absorbed (µg/cm^2) at any given time point was calculated by inserting the value for the analytical LOQ (µg/mL) into Equation 1 at the required time point.

To calculate the absorption rate LOQ (µg/cm^2/h) for any given period during the exposure, the LOQ for amount absorbed (µg/cm^2) at the last time point in that period was calculated as above and divided by the time difference (h) between the first and last time points of the period.

e.g. the absorption rate LOQ for a period x to y, where y is the later time:

Equation 3

$$\text{LOQ } (\mu g/cm^2/h) = \frac{\text{LOQ at y } (\mu g/cm^2)}{\text{y-x (h)}}$$

3. Calculation of LOQ's for mass balance and absorption data

Sample and receptor fluid LOQ's are calculated from the background (or pre-sample in the case of receptor fluid) cpm/dpm values and expressed as µg/cm^2 and % of the applied dose (Appendix 10).

APPENDIX 6 Individual Absorption Rates of SYN524464 Through Human Epidermal Membranes

Formulation concentrate

Cell No	Skin No	Absorption Rate (µg/cm^2/h)
		2-24h
69	1127k	0.009
71	1124a	0.002
73	1099	0.018
74	1099	0.023
75	1127k	0.005
Mean	-	0.011
SD	-	0.009
SEM	-	0.004
n	-	5

1/20 v/v Aqueous end-use dilution

Cell No	Skin No	Absorption Rate (µg/cm^2/h)
		0-24h
22	1099	0.006
25	1127k	0.003
26	1124AIII	0.002
28	1124AIII	0.006
Mean	-	0.005
SD	-	0.002
SEM	-	0.001
n	-	4

1/200 v/v Aqueous end-use dilution

Cell No	Skin No	Absorption Rate (µg/cm^2/h)
		0-24h
1	1127E	0.005
2	1127E	0.003
4	1128I	0.003
5	1128I	0.003
7	1128I	0.003
8	1128I	0.006
Mean	-	0.004
SD	-	0.001
SEM	-	0.001
n	-	6

Some of the values used to calculate the absorption rate were below the LOQ however these values have been used as positive values in order not to distort the data or absorption profiles.

APPENDIX 7 Individual Distribution of SYN524464 from the Formulation Concentrate in the Test System at 24 Hours

Test Compartment	Amount of Dose Recovered (µg/cm²):					Mean µg/cm² Recovered	SEM
	Cell 69	Cell 71	Cell 73	Cell 74	Cell 75		
Donor chamber	22.1	<0.732*	0.028	<0.683*	<0.673*	<4.84*	4.32*
Skin wash	5116	5204	5118	5045	5109	5119	25.4
Stratum corneum	<1.51*	<1.38*	<1.87*	<2.08*	7.15	<2.80*	1.10*
Remaining epidermal membranes	2.82	2.91	0.613	0.883	9.43	3.33	1.60
Absorbed	0.175	0.032	0.411	0.494	0.121	0.247	0.088
Total recovered	5143*	5209*	5121*	5049*	5127*	5130*	25.6*

Test Compartment	Percentage of Dose Recovered (%):					Mean % Recovered	SEM
	Cell 69	Cell 71	Cell 73	Cell 74	Cell 75		
Donor chamber	0.434	<0.014*	0.001	<0.013*	<0.013*	<0.095*	0.085*
Skin wash	100	102	100	98.9	100	100	0.497
Stratum corneum	<0.030*	<0.027*	<0.037*	<0.041*	0.140	<0.055*	0.021*
Remaining epidermal membranes	0.055	0.057	0.012	0.017	0.185	0.065	0.031
Absorbed	0.003	0.001	0.008	0.010	0.002	0.005	0.002
Total recovered	101*	102*	100*	99.0*	101*	101*	0.502*

*The LOQ values have been used as positive values in the calculation of the means, SEM's or totals where values were <LOQ. These values have been reported preceded with a <.

Key to terminology used in table above

Stratum corneum = amount in tape strips; Remaining epidermal membranes = epidermal tissue remaining after tape stripping; Absorbed = amount in receptor fluid

APPENDIX 8 Individual Distribution of SYN524464 from the 1/20 v/v End-use Dilution in the Test System at 24 Hours

Test Compartment	Amount of Dose Recovered (µg/cm²):				Mean µg/cm² Recovered	SEM
	Cell 22	Cell 25	Cell 26	Cell 28		
Donor chamber	0.005	0.004	<0.024*	0.009	<0.010*	0.005*
Skin wash	286	240	269	255	262	9.93
Stratum corneum	0.068	0.548	1.45	2.92	1.25	0.627
Remaining epidermal membranes	0.080	0.404	1.06	3.65	1.30	0.809
Absorbed	0.156	0.072	0.058	0.151	0.109	0.026
Total recovered	286	241	272*	261	265*	9.60*

Test Compartment	Percentage of Dose Recovered (%):				Mean % Recovered	SEM
	Cell 22	Cell 25	Cell 26	Cell 28		
Donor chamber	0.002	0.002	<0.009*	0.003	<0.004*	0.002*
Skin wash	112	93.8	105	99.6	103	3.89
Stratum corneum	0.027	0.215	0.567	1.14	0.488	0.246
Remaining epidermal membranes	0.031	0.158	0.415	1.43	0.508	0.317
Absorbed	0.061	0.028	0.023	0.059	0.043	0.010
Total recovered	112	94.2	106*	102	104*	3.76*

*The LOQ values have been used as positive values in the calculation of the means, SEM's or totals where values were <LOQ. These values have been reported preceded with a <.

Key to terminology used in table above

Stratum corneum = amount in tape strips; Remaining epidermal membranes = epidermal tissue remaining after tape stripping; Absorbed = amount in receptor fluid

APPENDIX 9 Individual Distribution of SYN524464 from the 1/200 v/v End-use Dilution in the Test System at 24 Hours

Test Compartment	Amount of Dose Recovered (µg/cm²):						Mean µg/cm² Recovered	SEM
	Cell 1	Cell 2	Cell 4	Cell 5	Cell 7	Cell 8		
Donor chamber	<0.003*	0.0004	0.001	0.001	<0.003*	0.001	<0.001*	0.0004*
Skin wash	26.4	25.7	26.2	27.5	26.7	25.9	26.4	0.268
Stratum corneum	<0.036*	<0.020*	<0.026*	<0.033*	<0.033*	<0.031*	<0.030*	0.002*
Remaining epidermal membranes	0.020	0.010	0.094	0.016	0.043	0.132	0.053	0.020
Absorbed	0.111	0.084	0.060	0.071	0.071	0.143	0.090	0.013
Total recovered	26.6*	25.8*	26.4*	27.7*	26.9*	26.2*	26.6*	0.257*

Test Compartment	Percentage of Dose Recovered (%):						Mean % Recovered	SEM
	Cell 1	Cell 2	Cell 4	Cell 5	Cell 7	Cell 8		
Donor chamber	<0.010*	0.001	0.003	0.003	<0.010*	0.003	<0.005*	0.002*
Skin wash	106	103	105	110	107	104	106	1.07
Stratum corneum	<0.142*	<0.080*	<0.104*	<0.134*	<0.133*	<0.126*	<0.120*	0.010*
Remaining epidermal membranes	0.081	0.042	0.376	0.063	0.171	0.530	0.211	0.081
Absorbed	0.445	0.335	0.242	0.283	0.285	0.573	0.360	0.051
Total recovered	107*	103*	106*	111*	108*	105*	107*	1.03*

*The LOQ values have been used as positive values in the calculation of the means, SEM's or totals where values were <LOQ. These values have been reported preceded with a <.

Key to terminology used in table above

Stratum corneum = amount in tape strips; Remaining epidermal membranes = epidermal tissue remaining after tape stripping; Absorbed = amount in receptor fluid

APPENDIX 10 Limit of Quantitation Values

Formulation concentrate

Study compartment	µg/cm^2	% of applied dose
Receptor fluid	0.193	0.004
Donor chamber	0.681	0.013
Skin wash	1.70	0.033
Tape strips	0.414	0.008
Remaining epidermal membranes	0.021	0.0004

1/20 v/v end-use dilution

Study compartment	µg/cm^2	% of applied dose
Receptor fluid	0.008	0.003
Donor chamber	0.023	0.009
Skin wash	0.065	0.025
Tape strips	0.026	0.010
Remaining epidermal membranes	0.001	0.001

1/200 v/v end-use dilution

Study compartment	µg/cm^2	% of applied dose
Receptor fluid	0.0007	0.003
Donor chamber	0.003	0.010
Skin wash	0.021	0.082
Tape strips	0.007	0.027
Remaining epidermal membranes	0.0001	0.0004

Footnote:
The criteria for setting LOQ values are given in Appendix 5.

APPENDIX 11 Analysis of Dose Preparations – Radiochemical Purity

Formulation concentrate

Analysis – days post preparation		Analysed radiochemical purity
TLC	Day 0	98.6%
	Day 2	98.9%
	Day 3	98.5%
HPLC	Day 4*	98.3%

1/20 v/v End-use dilution

Analysis – days post preparation		Analysed radiochemical purity
TLC	Day 0	98.9%
	Day 1	98.3%
	Day 2	98.4%
	Day 3	98.6%
	Day 4	98.3%
HPLC	Day 1	100%
	Day 4	100%
	Day 22*	96.5%

1/200 v/v End-use dilution

Analysis – days post preparation		Analysed radiochemical purity
TLC	Day 0	97.9%
	Day 3	97.8%
	Day 4	98.3%
HPLC	Day 0	100%
	Day 1	97.2%
	Day 1*	97.7%
	Day 2	100%

*Additional radiochemical purity values provided by Quotient Bioresearch Laboratories (Read H, 2009).

APPENDIX 12 Analysis of Dose Preparations - Homogeneity

Formulation concentrate

Analysis – days post preparation		Mean dpm	SD	% RSD
LSC	Day 0	66449	835	1.26
	Day 1	67361	885	1.31
	Day 1	67525	556	0.823
	Day 2	66954	2147	3.21

1/20 v/v End-use dilution

Analysis – days post preparation		Mean dpm	SD	% RSD
LSC	Day 0	77690	1103	1.42
	Day 1	19398	438	2.26
	Day 1	20034	328	1.64
	Day 2	20000	441	2.21
	Day 3	20276	367	1.81
	Day 4	20132	186	0.926

1/200 v/v End-use dilution

Analysis – days post preparation		Mean dpm	SD	% RSD
LSC	Day 0	78793	537	0.682
	Day 1	79481	707	0.889
	Day 1	80505	708	0.879
	Day 1	81044	844	1.04
	Day 17	40784	578	1.42

Footnote:

The range of mean dpm values displayed above are due to the dilution volumes and amounts taken for LSC.

APPENDIX 13 Example TLC Chromatogram

#T1 Lanes SYN524464 (A16148C) 1/20 v/v
Background Subtraction: Baseline

Position of unlabelled material

ID	Gross Counts	Baseline Subtract	Net Counts	Net CPM	% Total	Lane Label
Lane #1						
1-1	134,709	586	134,123	558.84	98.6	SYN524464 (A16148C)
Lane	144,102	8,101	136,001	566.67	100.0	
Unres	9,393	7,514	1,879	7.82	1.4	

APPENDIX 14 Example HPLC Chromatogram