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NOA449280

NOA449280 SL (A16003E)- In Vitro Absorption of NOA449280 Through Human Epidermis

Final Report

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

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STUDY COMPLETION DATE: October 5, 2009

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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, LLC

Company Agent: Tamara Murphy

Tamara Murphy
U. S. Product Registration Manager

8/20/2012
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 (JV2070) 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 R S Davies Date 5th October 09

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Thomas R. Hanley, M.S., DABT — 14 Aug 2012
Representative of Submitter/Sponsor — Date

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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
27 February 2009	Protocol	02 March 2009
04 March 2009	Cell assembly and assessment of membrane integrity	09 March 2009
04 March 2009	Dose preparation and radiolabel stock activity check	09 March 2009
05 March 2009	Liquid scintillation counting and addition of dose to donor	09 March 2009
11 March 2009	Cell processing	11 March 2009
12 March 2009	Sample dispatch	18 March 2009
17 March 2009	Thin layer chromatography	18 March 2009
16 June 2009	Draft report audit	17 June 2009
01 October 2009	Final report review	01 October 2009

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

02 March 2009	High performance liquid chromatography	09 March 2009

Facilities and process based procedures associated with this type of study were inspected in

Michael Howes ..... Date 5th October 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: 25 February 2009
Experimental start date: 4 March 2009
Experimental completion date: 8 June 2009

Deviations from the guidelines

None

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1.0 EXECUTIVE SUMMARY

1.1 Study Design

The absorption and distribution of NOA449280 from an SL (A16003E) formulation was measured *in vitro* through human epidermis conforming to the Regulatory Guidelines given in Section 2.2. The doses were applied as the formulation concentrate (200 g NOA449280/L) and as two aqueous spray dilutions (1/100 v/v and 1/400 v/v) of the formulation. The doses were applied to the epidermal membranes at a rate of 10 µL/cm^2 and left unoccluded for an exposure period of 24 hours.

The formulation concentrate was included to assess exposure to mixer/loaders. The spray strength dilutions used (1/100 v/v and 1/400 v/v) represented typical in-use concentrations. These applications were designed to simulate potential human dermal exposure to the formulation during normal use.

[^{14}C]-Radiolabelled NOA449280 was incorporated into the concentrate formulation and dilutions prior to application. The absorption process was followed by measuring radioactivity in samples of the receptor fluid (50% ethanol in water) taken at recorded intervals throughout the exposure period. The distribution of NOA449280 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 target dose levels had been achieved and that the dose preparations were homogeneous both prior to and following dosing.

TLC and HPLC analysis confirmed that the radiochemical purity of [^{14}C]-NOA449280 when formulated as a concentrate formulation and two aqueous spray dilutions (1/100 v/v and 1/400 v/v) was greater than 95% over at least 3 days which is a period of time longer than that used in this study.

1.2.2 NOA449280 Absorption

1.2.2.1 Formulation Concentrate

The fastest absorption occurred between 0-2 hours, during which time NOA449280 was absorbed at a rate of 0.009 µg/cm^2/h. Absorption was much slower between 2-24 hours (0.004 µg/cm^2/h) and between 0-24 hours the mean absorption rate was 0.005 µg/cm^2/h.

The amounts of NOA449280 absorbed at 6, 8 and 10 hours were 0.035, 0.039 and 0.052 µg/cm^2, respectively. These respective amounts expressed as percentages of the

applied dose were 0.002, 0.002 and 0.003%. The amount absorbed over the entire 24 hour exposure period was 0.114 μg/cm^2 (0.006% of the applied dose).

1.2.2.2 1/100 v/v Aqueous Spray Dilution

Between 0-12 hours, absorption of NOA449280 was 0.0003 μg/cm^2/h increasing to its fastest rate of 0.0005 μg/cm^2/h between 12-24 hours. Between 0-24 hours the mean absorption rate was 0.0004 μg/cm^2/h.

The amounts of NOA449280 absorbed at 6, 8 and 10 hours were 0.002, 0.003 and 0.003 μg/cm^2, respectively. These respective amounts expressed as percentages of the applied dose were 0.010, 0.013 and 0.016%. The amount absorbed over the entire 24 hour exposure period was 0.010 μg/cm^2 (0.048% of the applied dose).

1.2.2.3 1/400 v/v Aqueous Spray Dilution

Between 0-12 hours, absorption of NOA449280 was 0.0003 μg/cm^2/h increasing to its fastest rate of 0.0006 μg/cm^2/h between 12-24 hours. Between 0-24 hours the mean absorption rate was 0.0004 μg/cm^2/h.

The amounts of NOA449280 absorbed at 6, 8 and 10 hours were 0.001, 0.002 and 0.003 μg/cm^2, respectively. These respective amounts expressed as percentages of the applied dose were 0.027, 0.038 and 0.049%. The amount absorbed over the entire 24 hour exposure period was 0.011 μg/cm^2 (0.204% of the applied dose).

1.2.3 Mass Balance and NOA449280 Distribution

1.2.3.1 Formulation Concentrate

Mean recovery of the applied test material was 106%.

The majority of the applied dose, 106% 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 0.006%. This percentage equated to 0.114 μg/cm^2.

A total of 0.060% of the applied dose remained in the epidermal membrane following a 24 hour skin washing procedure. Of this total <0.044% was present in the outer layers of the *stratum corneum.*

1.2.3.2 1/100 v/v Aaqueous Spray Dilution

Mean recovery of the applied test material was 109%.

Skin washing 24 hours after application removed 109% of the applied dose.

The proportion of the applied dose present in receptor fluid following the total 24 hour exposure was 0.048%. In terms of actual amounts this percentage equated to 0.010 µg/cm^2.

A total of 0.314% of the applied dose remained in the epidermal membrane following a 24 hour skin washing procedure. Of this total <0.108% was present in the outer layers of the *stratum corneum*.

1.2.3.3 1/400 v/v Aqueous Spray Dilution

Mean recovery of the applied test material was 105%.

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 0.204%. In terms of actual amounts this percentage equated to 0.011 µg/cm^2.

A total of 1.21% of the applied dose remained in the epidermal membrane following a 24 hour skin washing procedure. Of this total <0.360% was present in the outer layers of the *stratum corneum*.

1.3 Conclusion

The results obtained in this study indicate that NOA449280 was absorbed through human epidermis at a slow rate from the A16003E SL concentrate formulation, and at extremely slow rates from the two aqueous spray dilutions (1/100 v/v and 1/400 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 epidermal membrane.

These data predict that during a typical working exposure interval of 10 hours to the concentrate formulation and two aqueous spray dilutions (1/100 v/v and 1/400 v/v), absorption of NOA449280 would be minimal.

2.0 INTRODUCTION

2.1 Purpose

The purpose of this study was to determine the *in vitro* absorption of NOA449280 through human skin over a 24 hour exposure period to aid the quantitative assessment of the risk arising from skin contact with an SL formulation containing a nominal 200 g NOA449280/L and two aqueous spray strength dilutions (1/100 v/v and 1/400 v/v) containing a nominal 2 and 0.5 g NOA449280/L, respectively. The distribution of NOA449280 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.

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), Med IC4, Keele University Science and Business Park, Keele, Staffordshire, 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 Substance

3.1.1 NOA449280

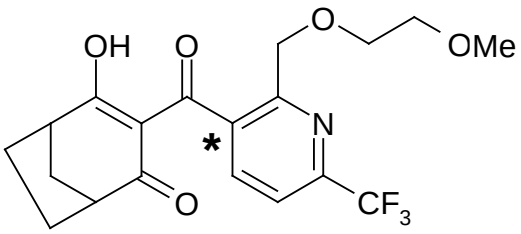
Name:	NOA449280 – analytical standard
DTL test substance reference number:	TS00069/001
Source:	Syngenta Crop Protection, Münchwilen AG, Breitenloh 5, CH-4333 Münchwilen, Switzerland
Batch reference:	AMS 1144/1
Analytical certificate reference number:	10291523
Molecular formula:	$C_{19}H_{20}F_3NO_5$
Molecular weight:	399.4
Colour:	White
Physical state:	Crystalline solid
Purity:	99.9%
Storage conditions:	Less than 30 ^{o}C
Expiry date:	End of May 2011

From information supplied by the Sponsor, the test substance was used within the expiry date. A certificate of analysis (reference number: 10291523, dated 13 June 2007) is presented in Appendix 1. The test substance characterisation was the responsibility of the Sponsor.

3.1.2 Radiolabelled Test Material

Name:	[Pyridinyl-3-^{14}C]-NOA449280
DTL test substance reference number:	TS00069/010
Source:	Syngenta Crop Protection, Inc. P.O. Box 18300, Greensboro, NC 27419-8300, USA
Batch reference:	RDR-V-59
Chemical purity:	96.8%
Radiochemical purity:	This material was provided with a certified radiochemical purity of 98.5%.
Specific activity:	99.3 µCi/mg (3.6741 MBq/mg)
Storage conditions:	Freezer (-20 ^{o}C)
Expiration Date:	31st August 2009

3.1.2.1 Structure of the Radiolabelled Test Substance



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

A certificate of analysis (reference number: RDR-V-59, dated 20 February 2009) is retained in the DTL Archives and presented in Appendix 2.

3.2 A16003E Blank Formulation

Name: DTL test substance reference number: Source: Batch no: Storage:	Blank formulation of A16003E TS00069/005 Syngenta Ltd, Jealott's Hill International Research Centre, Bracknell, Berkshire RG42 6EY, UK J8225/041 J8388/065 Ambient temperature

The blank formulation contained all the ingredients of the A16003E commercial formulation with the exception of the active ingredient NOA449280.

3.2.1 CIPAC D Water

Name:	CIPAC D water
DTL test substance reference number:	TS00069/009
Source: Bottle number:	Syngenta Ltd, Jealott's Hill International Research Centre, Bracknell, Berkshire RG42 6EY, UK 153605
Storage:	Ambient temperature

3.2.2 Reference Formulation

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

Formulation details:	NOA449280 SL (200)
DTL test substance reference number:	TS00069/008
Source:	Syngenta Ltd, Jealott's Hill International Research Centre, Bracknell, Berkshire RG42 6EY, UK
Design code:	A16003E
Proportion of NOA449280:	20% w/v
Storage:	Ambient temperature

3.3 Experimental Procedures

3.3.1 Radioactivity Content and Radiochemical Purity of the [^{14}C]-Radiolabelled NOA449280

The radiolabelled material was supplied dry. A stock solution was prepared by solubilising the dry material in ethanol and mixing thoroughly.

The radioactivity content of the [^{14}C]-radiolabelled NOA449280 (TS00069/010) stock solution was determined by analysing sub-samples of solvent dilutions by LSC.

The radiochemical purity of the radiolabelled test substance was not determined prior to formulation as the material was supplied with a current certificate of analysis (Appendix 2).

3.3.2 Dose Preparation

The doses were prepared, conforming to instructions supplied by the Sponsor to mimic the A16003E commercial 200 g NOA449280/L formulation and its two aqueous spray dilutions (1/100 v/v and 1/400 v/v).

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

3.3.2.1 Formulation Concentrate

Unlabelled NOA449280 (TS00069/001), actual weight 395 mg, was mixed with an appropriate volume of [^{14}C]-radiolabelled NOA449280 (TS00069/010) in ethanol, equivalent to an activity of 20.2 MBq (5.50 mg NOA449280). An additional 1.5 ml of ethanol was added to fully solubilise the test materials. The ethanol was removed using a stream of nitrogen gas and 1724 mg of the blank formulation (TS00069/005) added. The preparation was mixed thoroughly and stirred constantly in the dark until used for dosing.

3.3.2.2 1/100 v/v Aqueous Spray Dilution

An appropriate volume of [^{14}C]-radiolabelled NOA449280 (TS00069/010) in ethanol, equivalent to an activity of 14.5 MBq (3.96 mg NOA449280) was dispensed into an empty vial. The ethanol was removed using a stream of nitrogen gas and 17.4 mg of the blank formulation (TS00069/005) added. A weighed (1979 mg) amount of CIPAC D water (TS00069/009) was added and the preparation was mixed thoroughly and stirred constantly in the dark until used for dosing.

3.3.2.3 1/400 v/v Aqueous Spray Dilution

An appropriate volume of [^{14}C]-radiolabelled NOA449280 (TS00069/010) in ethanol, equivalent to an activity of 3.83 MBq (1.04 mg NOA449280) was dispensed into an empty vial. The ethanol was removed using a stream of nitrogen gas and 3.99 mg of the blank formulation (TS00069/005) added. A weighed (1998 mg) amount of CIPAC D water (TS00069/009) was added and the preparation was mixed thoroughly and stirred constantly in the dark until used for dosing.

3.3.3 Radioactivity Content of the Dose Preparations

The radioactivity content of each formulated [^{14}C]-NOA449280 preparation (Section 3.3.2) was determined by analysing sub-samples of solvent dilutions by LSC.

3.3.4 Homogeneity of the Dose Preparations

Homogeneity of each formulated [^{14}C]-radiolabelled NOA449280 preparation was confirmed by analysing replicate sub-samples of solvent dilutions by LSC prior to dosing and again after a period of time greater than that used in the study.

3.3.5 Radiochemical Purity and Stability

A sample of each formulated [^{14}C]-radiolabelled NOA449280 preparation, was analysed to determine the radiochemical purity of the formulated NOA449280, using the analytical chromatographic procedures shown in Section 3.3.6.

3.3.6 Analytical Techniques

Samples collected during this study were analysed by LSC.

The limit of quantitation using LSC for the concentrate and two spray dilutions (1/100 v/v and 1/400 v/v) in each of the study compartments expressed as µg/cm^2 and percentage of applied dose are shown in Appendix 9.

Liquid scintillation counting (LSC)	
Counting period:	6 minutes or to a 0.5% standard deviation of the count
Scintillation fluid:	Goldstar
Model of LSC:	Packard 3100 TR

Test materials were analysed by high performance HPLC and TLC.

Thin layer chromatography (TLC):
The radiochemical purity of [^{14}C]-radiolabelled test material was determined by TLC using silica gel plates (K6f) using the following solvent system: Toluene : Dioxane : Methanol : Ammonium Hydroxide (4 : 4 : 3 : 1 v/v) Radioactivity on the TLC plates was measured using a Packard Instant Imager. Unlabelled standard material were visualised under UV light at 254 nm.

High performance liquid chromatography (HPLC): Analysis was performed using the method detailed below.			
Equipment:	HPLC system = Agilent 1100		
Column:	ACE 5µm, C18, 15 cm x 4.6mm		
Mobile phase:	A: Aceonitrile, B: 0.1%v/v Formic acid		
Gradient:	Time (minutes)	Mobile phase A (%)	Mobile phase B (%)
	Initial	10	90
	17.0	90	10
	18.0	10	90
Flow rate:	1 mL/min		
Injection volume:	10-20 µL		
Column temperature:	20 ºC		
UV detector wavelength:	254 nm		
Radiochemical detector:	Packard Flow Scintillation Analyser (FSA) Model 525 (500TR Series)		

3.3.7 Human Epidermis 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 epidermis 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.

3.3.8 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. 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.

3.3.9 Measurement of Membrane Integrity

Membrane integrity was determined by measurement of the electrical resistance across the skin membrane. Human 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.

3.3.10 Selection of Cells and Dosing

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

The receptor chambers of the cells containing small magnetic stirrer bars were filled with a recorded volume of receptor fluid (50% ethanol in water) and placed in a water bath maintained at a temperature of 32 ºC ± 1 ºC.

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 development study (VV2077 - Davies D J, 2009).

A pre-treatment sample (500 µL) 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 as the formulation concentrate and as two aqueous spray strength dilutions and left unoccluded for the duration of the 24 hour exposure period.

Test substance	Application rate	Total dose applied
Formulation concentrate 200 g ai/L	10 µL/cm^2 ≡ 2000 µg ai/cm^2	25.4 µL
1/100 v/v Aqueous dilution 2 g ai/L	10 µL/cm^2 ≡ 20 µg ai/cm^2	25.4 µL
1/400 v/v Aqueous dilution 0.5 g ai/L	10 µL/cm^2 ≡ 5 µg ai/cm^2	25.4 µL

After dosing, the cells were placed in a water bath maintained at a temperature of 32 °C ± 1 °C.

3.3.11 Sampling of Receptor Fluid

For cells assigned to the 24 hour exposure period, 500 µL samples 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.

3.3.12 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 one sponge pre-wetted with 3%Teepol L® in water which was added to the wash sponges. The donor chamber was washed with ethanol followed by sonication, to ensure thorough extraction, and the sample analysed for [^{14}C]-NOA449280 by LSC.

The epidermal surface of the skin was decontaminated by gently swabbing the application site with natural sponges pre-wetted with 3% Teepol L®, and with further sponges pre-wetted with water. Decontamination was shown to be complete following assessment of residual radioactivity levels on the skin surface with a Geiger counter. The sponges were digested in Soluene 350® and made up to a recorded volume. A sample 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 individually in ethanol to extract any test material. The extracts were sequentially numbered and analysed by LSC.

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

3.4 Data Evaluation

Results of the analysis of the samples of receptor fluid collected in the study were expressed as amounts of NOA449280 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 calculation in Appendix 4.

Membranes with absorption profiles that indicated membrane damage during the course of the experiment have been excluded.

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 minor variations from values reported.

The absorbed (systemically available) dose is considered to be the NOA449280 detected in the receptor fluid. Material removed from the surface of the epidermis by the washing procedure is regarded as unabsorbed. NOA449280 recovered from the epidermis 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 epidermis, 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 Dose Levels Achieved and Homogeneity of the Dose Preparations

LSC analysis of the dose preparations confirmed that the dose levels achieved were 199, 1.98 and 0.519 g NOA449280/L for the concentrate formulation and two aqueous spray dilutions (1/100 v/v and 1/400 v/v, respectively).

The dose preparations were considered to be homogeneous and acceptable for use in these experiments. For the concentrate formulation, the LSC analysis of the dose preparations, immediately prior to application, gave a 2.74% mean deviation between the replicates. For the 1/100 v/v and 1/400 v/v aqueous spray dilutions, analysis gave mean deviations of 2.56 and 3.88% mean deviation between the replicates.

Analysis of the concentrate formulation eight days post preparation gave a 0.916% mean deviation between the replicates. For the 1/100 v/v spray dilution, analysis gave a 0.865% deviation between the replicates 3 days post preparation and the 1/400 v/v spray dilution, analysis gave a 2.18% deviation between the replicates 6 days post preparation.

4.1.2 Stability and Radiochemical Purity of NOA449280 in the Formulations

Radiochemical purity of the formulated [^{14}C]-NOA449280 by TLC and HPLC is shown in Appendix 10. Example chromatograms from TLC and HPLC analysis of the formulation are presented in Appendices 11-13.

NOA449280, when formulated as a concentrate formulation and 1/100 v/v and 1/400 v/v spray dilutions, was shown to be stable by TLC and HPLC for a period of time longer than

that used in the study. Radiochemical purities of greater than 95% were seen in all the dose preparations both prior to application and post dose preparation.

4.2 NOA449280 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 10h) and at 24 hours. The absorption profiles for NOA449280 from this formulation and spray strength dilutions are displayed in Figures 1 -3.

The absorption data for each individual cell are given in Appendix 5.

4.2.1 Formulation Concentrate

The fastest absorption occurred between 0-2 hours, where NOA449280 was absorbed at a rate of 0.009 $\mu g/cm^2/h$. Absorption was much slower between 2-24 hours (0.004 $\mu g/cm^2/h$) and between 0-24 hours the mean absorption rate was 0.005 $\mu g/cm^2/h$.

The amounts absorbed at 6, 8 and 10 hours were 0.035, 0.039 and 0.052 $\mu g/cm^2$, respectively. These respective amounts expressed as percentages of the applied dose were 0.002, 0.002 and 0.003%. The amount absorbed over the entire 24 hour exposure period was 0.114 $\mu g/cm^2$ (0.006% of the applied dose).

4.2.2 1/100 v/v Aqueous Spray Dilution

Between 0-12 hours, absorption of NOA449280 was 0.0003 $\mu g/cm^2/h$ increasing to its fastest rate of 0.0005 $\mu g/cm^2/h$ between 12-24 hours. Between 0-24 hours the mean absorption rate was 0.0004 $\mu g/cm^2/h$.

The amounts absorbed at 6, 8 and 10 hours were 0.002, 0.003 and 0.003 $\mu g/cm^2$, respectively. These respective amounts expressed as percentages of the applied dose were 0.010, 0.013 and 0.016%. The amount absorbed over the entire 24 hour exposure period was 0.010 $\mu g/cm^2$ (0.048% of the applied dose).

4.2.3 1/400 v/v Aqueous Spray Dilution

Between 0-12 hours, absorption of NOA449280 was 0.0003 $\mu g/cm^2/h$ increasing to its fastest rate of 0.0006 $\mu g/cm^2/h$ between 12-24 hours. Between 0-24 hours the mean absorption rate was 0.0004 $\mu g/cm^2/h$.

The amounts absorbed at 6, 8 and 10 hours were 0.001, 0.002 and 0.003 $\mu g/cm^2$, respectively. These respective amounts expressed as percentages of the applied dose were 0.027, 0.038 and 0.049%. The amount absorbed over the entire 24 hour exposure period was 0.011 $\mu g/cm^2$ (0.204% of the applied dose).

4.3 Mass Balance and NOA449280 Distribution

The data for distribution in the test system are presented in Table 2 in terms of μg/cm^2 and percentage of the applied dose. The individual mass balance data are given in Appendices 6-8.

4.3.1 Formulation Concentrate

Mean recovery of the applied test material was 106%.

The majority of the applied dose, 106% 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 0.006%. This percentage equated to 0.114 μg/cm^2.

A total of 0.060% of the applied dose remained in the epidermal membrane following a 24 hour skin washing procedure. Of this total <0.044% was present in the outer layers of the *stratum corneum*.

4.3.2 1/100 v/v Aqueous Spray Dilution

Mean recovery of the applied test material was 109%.

Skin washing 24 hours after application removed 109% of the applied dose.

The proportion of the applied dose present in receptor fluid following the total 24 hour exposure was 0.048%. In terms of actual amounts this percentage equated to 0.010 μg/cm^2.

A total of 0.314% of the applied dose remained in the epidermal membrane following a 24 hour skin washing procedure. Of this total <0.108% was present in the outer layers of the *stratum corneum*.

4.3.3 1/400 v/v Aqueous Spray Dilution

Mean recovery of the applied test material was 105%.

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 0.204%. In terms of actual amounts this percentage equated to 0.011 μg/cm^2.

A total of 1.21% of the applied dose remained in the epidermal membrane following a 24 hour skin washing procedure. Of this total <0.360% was present in the outer layers of the *stratum corneum*.

5.0 CONCLUSIONS

The results obtained in this study indicate that NOA449280 was absorbed through human epidermis at a slow rate from the A16003E SL concentrate formulation, and at an extremely slow rate from the two aqueous spray dilutions (1/100 v/v and 1/400 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 epidermal membrane.

These data predict that during a typical working exposure interval of 10 hours to the concentrate formulation and two aqueous spray dilutions (1/100 v/v and 1/400 v/v), absorption of NOA449280 would be minimal.

6.0 REFERENCES

Davies D J, Ward R J and Heylings J R (2004). Multi-species assessment of electrical resistance as a skin integrity marker for *in vitro* percutaneous absorption studies. Toxicology *In Vitro*, **18**, 351-358.

Davies D J, (2009). Evaluation of Study Techniques and Dose Methodology for Use with NOA449280 Dose Preparations. VV2077.

European Commission Guidance Document on Dermal Absorption. (2004). Sanco/222/2000 rev. 7 (19 March 2004).

OECD Test Guideline 428 (2004). Organisation for Economic Co-operation and Development. Skin Absorption: *In Vitro* Method. Organisation for Economic Co-operation and Development, Paris.

OECD Guidance Document No. 28 (2004). Organisation for Economic Co-operation and Development.: The Conduct of Skin Absorption Studies. Organisation for Economic Co-operation and Development, Paris.

Ramsey J D, Woollen B H, Auton T R, Batten P L, Leeser J E (1992). Pharmacokinetics of Fluazifop-Butyl in Human Volunteers. II: Dermal dosing. Human Exp Toxicol. **4**, 247-254.

Ramsey J D, Woollen B H, Auton T R and Scott R C (1994). The Predictive Accuracy of *In Vitro* Measurements for the Dermal Absorption of a Lipophilic Penetrant (Fluazifop-Butyl) through Rat and Human Skin. Fundam. Appl. Toxicol. **23,** 230-236.

TABLES SECTION

TABLE 1 Summary of NOA449280 Absorption through Human Epidermis

Application of Test Materials	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**
Concentrate Formulation					
(199 g NOA449280/L)	0-2	*0.009 ± 0.004	6	0.035	0.002
10 μLl/cm^2 (1994 μg ai/cm^2)	2-24	0.004 ± 0.002	8	0.039	0.002
Unoccluded	0-24	0.005 ± 0.002	10	0.052	0.003
Duration of exposure: 24h			24	0.114	0.006
n = 4			*LOQ*	*0.032*	*0.002*
1/100 v/v aqueous spray dilution					
(1.98 g NOA449280/L)	0-12	0.0003 ± 0.0001	6	0.002	0.010
10 μL/cm^2 (19.8 μg ai/cm^2)	12-24	*0.0005 ± 0.0001	8	0.003	0.013
Unoccluded	0-24	0.0004 ± 0.0001	10	0.003	0.016
Duration of exposure: 24h			24	0.010	0.048
n = 6			*LOQ*	*0.0005*	*0.002*
1/400 v/v aqueous spray dilution					
(0.519 g NOA449280/L)	0-12	0.0003 ± 0.00009	6	0.001	0.027
10 μL/cm^2 (5.19 μg ai/cm^2)	12-24	*0.0006 ± 0.0002	8	0.002	0.038
Unoccluded	0-24	0.0004 ± 0.0002	10	0.003	0.049
Duration of exposure: 24h			24	0.011	0.204
n = 6			*LOQ*	*0.0005*	*0.009*

*Fastest rate of absorption over the 24h time course.

TABLE 2 Summary of NOA449280 Distribution in the Test System

Concentrate formulation

Test Compartment n = 4	µg NOA449280 per cm²		% of applied dose	
	Mean	SEM	Mean	SEM
Donor chamber	*<1.51	1.16	*<0.076	0.058
Skin wash	2106	17.6	106	0.881
Stratum corneum	*<0.884	-	*<0.044	-
Remaining epidermis	0.329	0.109	0.016	0.005
Absorbed	0.114	0.052	0.006	0.003
Total recovered	2109	18.4	106	0.921

1/100 v/v aqueous spray diluton

Test Compartment n = 6	µg NOA449280 per cm²		% of applied dose	
	Mean	SEM	Mean	SEM
Donor chamber	*<0.004	-	*<0.020	-
Skin wash	21.5	0.193	109	0.975
Stratum corneum	*<0.021	0.002	*<0.108	0.010
Remaining epidermis	0.041	0.020	0.206	0.101
Absorbed	0.010	0.002	0.048	0.012
Total recovered	21.6	0.192	109	0.971

1/400 v/v aqueous spray dilution

Test Compartment n = 6	µg NOA449280 per cm²		% of applied dose	
	Mean	SEM	Mean	SEM
Donor chamber	0.039	0.014	0.752	0.272
Skin wash	5.34	0.075	103	1.45
Stratum corneum	*<0.019	0.0001	*<0.360	0.002
Remaining epidermis	0.044	0.012	0.845	0.229
Absorbed	0.011	0.004	0.204	0.074
Total recovered	5.45	0.074	105	1.43

Absorbed = amount in receptor fluid
Stratum corneum = amount in tape strips
Remaining epidermis = epidermal tissue remaining after tape stripping
*The LOQ values have been used as positive values in the calculation of the mean where values were <LOQ.

FIGURES SECTION

FIGURE 1 Profiles of NOA449280 Absorption from the Formulation Concentrate through Human Epidermis

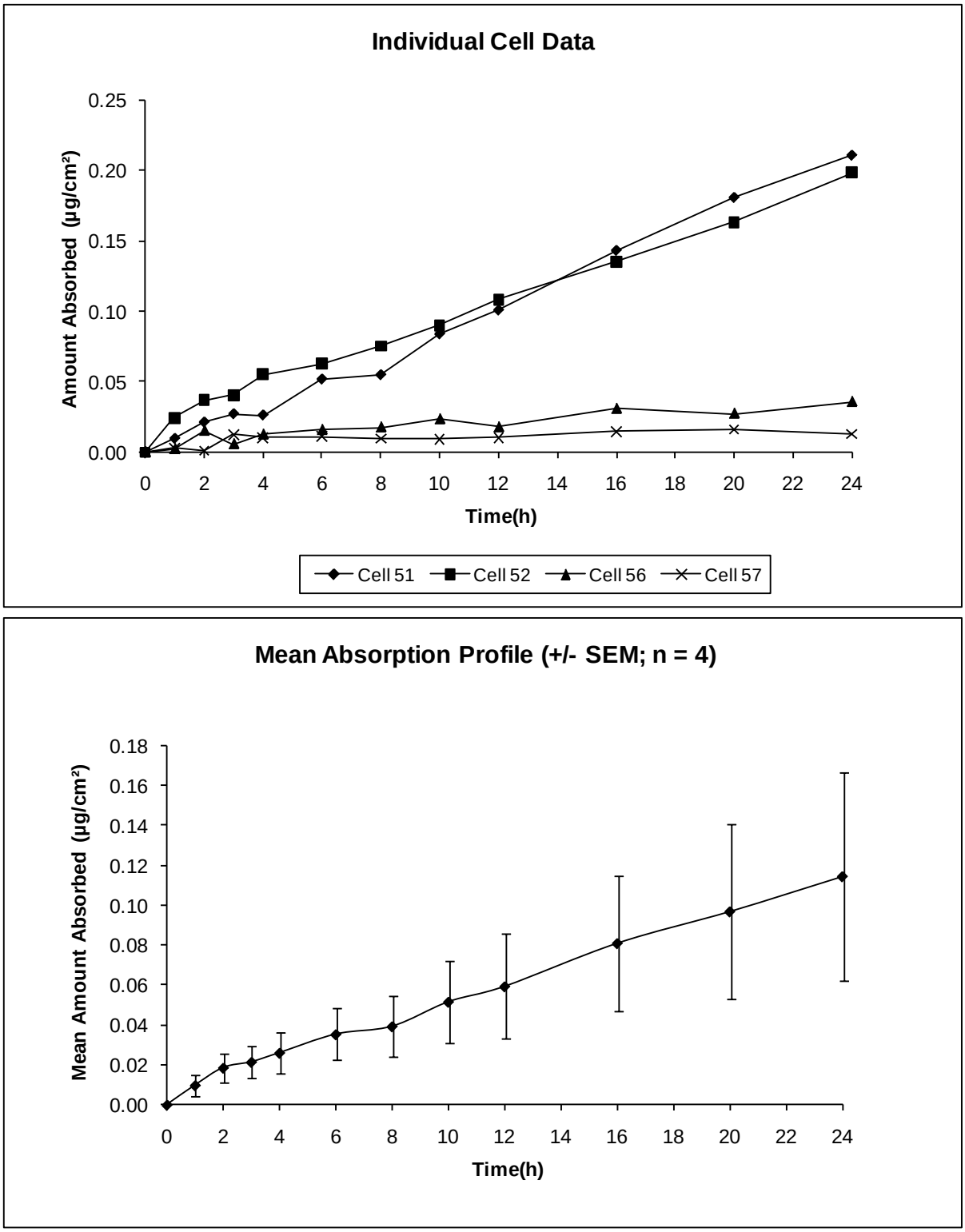
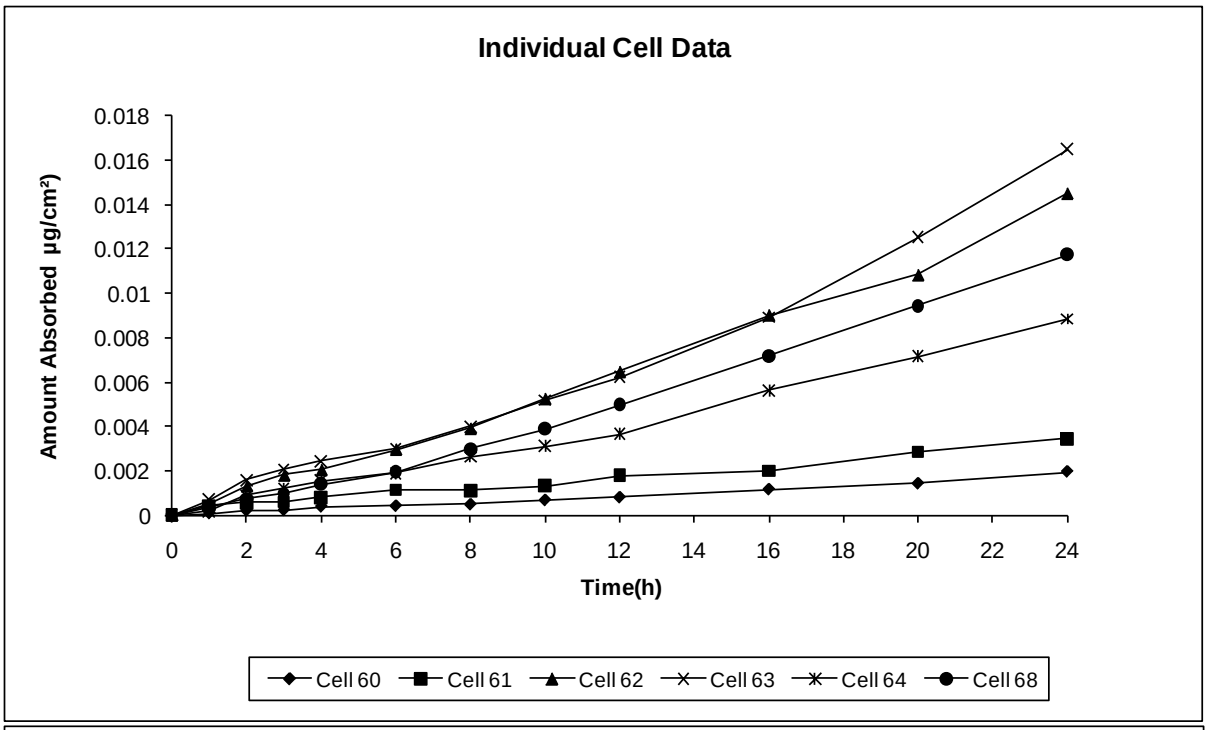


FIGURE 2 Profiles of NOA449280 Absorption from the 1/100 v/v Aqueous Spray Strength Dilution through Human Epidermis



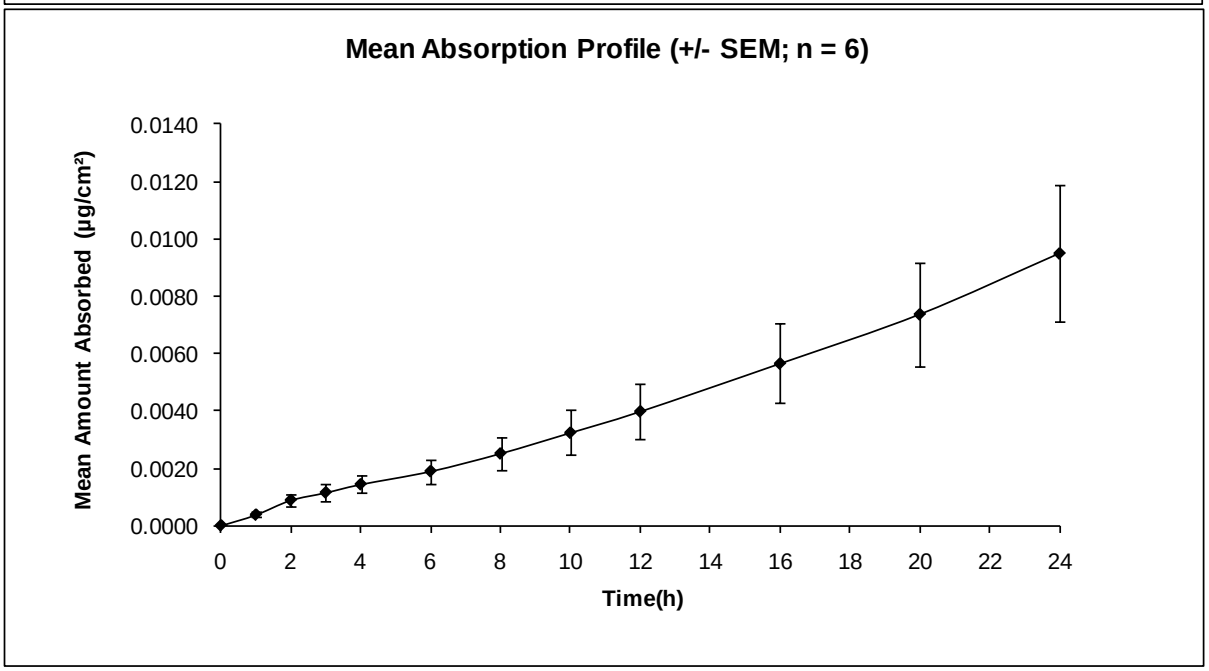
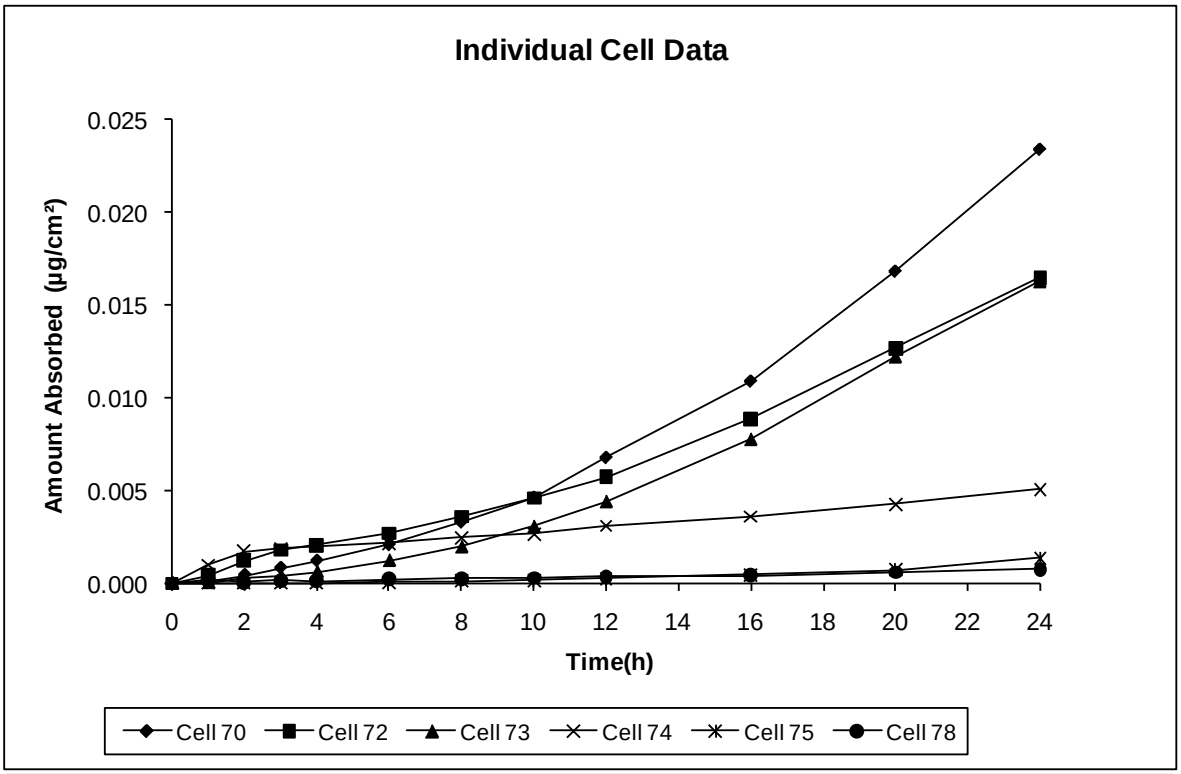
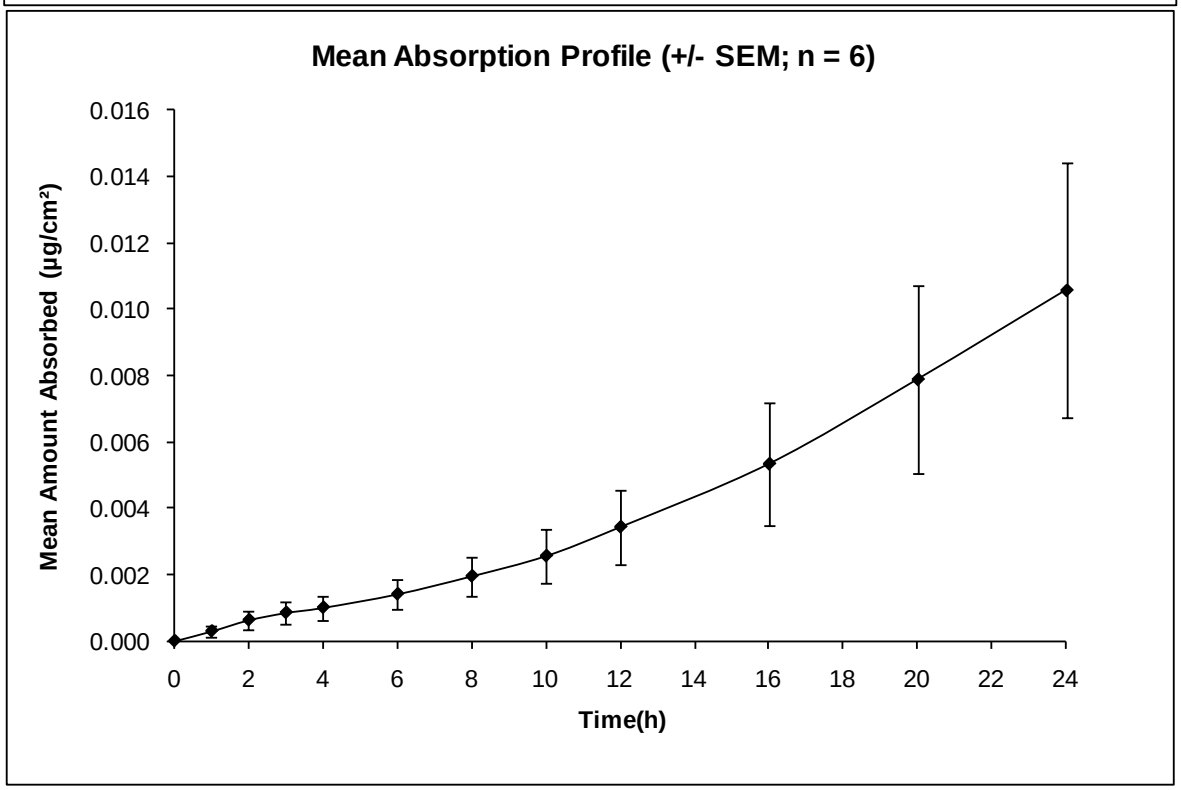


FIGURE 3 Profiles of NOA449280 Absorption from the 1/400 v/v Aqueous Spray Strength Dilution through Human Epidermis





APPENDICES SECTION

APPENDIX 1 Test Substance Information - Unlabelled NOA449280

TSC0069/001 1001

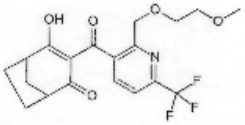
syngenta

GLP Testing Facility WMU
Analytical Development & Product Chemistry GS2131

Syngenta Crop Protection Münchwilen AG
Breitenloh 5
CH-4333 Münchwilen

Certificate of Analysis

NOA449280



AMS 1144/1 – Purity 99.9%

Batch Identification	**AMS 1144/1**
Product Code	**NOA449280**
Other Product Code(s)	---
ISO Common Name	NOA 449280
CA Reg. No.	352010-68-5
CA Index Name	bicyclo[3.2.1]oct-3-en-2-one, 4-hydroxy-3-[[2-[(2-methoxyethoxy)methyl]-6-(trifluoromethyl)-3-pyridinyl]carbonyl]-
IUPAC Name	4-hydroxy-3-[2-(2-methoxy-ethoxymethyl)-6-(trifluoromethyl)-pyridine-3-carbonyl]-bicyclo[3.2.1]oct-3-en-2-one
Molecular formula	$C_{19}H_{20}F_3NO_5$
Molecular mass	399.4
Chemical Analysis	
– **Identity***	**confirmed**
– **Content of NOA449280 ***	**99.9 %** (estimated error: ± 0.3 %)
Methodology used for Characterization / Reanalysis	HPLC, CGC and ^{1}H-NMR, Karl-Fischer titration, elemental analysis
Physical Analysis	
– **Appearance ***	**white crystalline solid**
Stability:	
– **Storage Temperature**	**< 30°C**
– **Reanalysis date**	**End of May 2011**

The stability of this test substance will be controlled by reanalysis of material held in the inventory at Syngenta Crop Protection Münchwilen AG at the appropriate time.

This Certificate of Analysis summarizes data which originates either from a single study or from several individual studies. Tests marked with an asterisk (*) have been conducted in compliance with GLP. Raw data, documentation, study plans, any amendments to study plans and reports pertaining to this/these study/studies are stored under the study number(s) referenced below within the archives of the GLP Testing Facility WMU at Syngenta Crop Protection Muenchwilen AG.

Characterisation: 108696 Reanalysis: 115507, 117655

Authorisation:

13. Jun. 2007 / P Kundel

Dr. P. Kundel
Analytical Development & Product Chemistry

10291523 Page 1 of 1

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

CERTIFICATE OF ANALYSIS

SYNGENTA CODE: **[PYRIDINYL-3-14C]-NOA 449280**

SYNONYMNS: **[PYRIDINYL-3-14C]-CSAA798499**

REFERENCE NUMBER (Batch Identification): **RDR-V-59**

STRUCTURE:

CHEMICAL PURITY: **96.8%**

RADIOCHEMICAL PURITY: **98.5%**

SPECIFIC ACTIVITY: **99.3 μCi/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: **August 31, 2009**

STUDY COMPLETION DATE: **February 20, 2009**

STUDY DIRECTOR: **William F. Helke**

SIGNATURE: William F. Helke

ANALYTICAL STANDARD CHARACTERIZATION REPORT

IDENTITY

- **COMPARISON TO AN AUTHENTIC STANDARD:**
  Reference: (data ref.: R09-10/3,4; test date: 2/19/09)

  Thin-Layer Chromatography Systems:

  Silica gel plate; Toluene : Dioxane : Methanol : Ammonium Hydroxide (4:4:3:1); Rf = 0.55
  Diol gel plate; Acetonitrile : n-Butanol : Ammonium Hydroxide (3:1:1); Rf = 0.78

- **SPECTRAL IDENTITY:**

MASS SPECTROSCOPY	:	Consistent with proposed structure. Reference: (data ref.: w0517, d3690; test date: 2/20/09)

PURITY

CHEMICAL PURITY – EXTERNAL STANDARD ANALYSIS BY HPLC	96.8% Reference: (data ref.: R09-10/1,2,3; test date: 2/19/09)
RADIOCHEMICAL PURITY – AREA DISTRIBUTION BY THIN-LAYER CHROMATOGRAPHY	98.5% Reference: (data ref.: R09-10/3,4; test date: 2/19/09)

SPECIFIC ACTIVITY

SPECIFIC ACTIVITY – EXTERNAL STANDARD ANALYSIS BY HPLC	99.3 μCi/mg Reference: (data ref.: R09-10/1,2,3; test date: 2/19/09)

APPENDIX 3 Dermal Technology Laboratory Ltd - Certificate of Good Laboratory Practice

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
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 4 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 4 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

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

Any calculated values below these LOQs were reported as the <LOQ value

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 5 Individual Absorption Rates of NOA449280 through Human Epidermis

Concentrate formulation

Cell No	Skin No	Absorption Rate (µg/cm^2/h)		
		0 - 2h	2 - 24h	0 - 24h
Cell 51	1109	0.011	0.009	0.009
Cell 52	1109	0.018	0.007	0.008
Cell 56	1106	0.008	0.001	0.001
Cell 57	1106	0.0005	0.0004	0.0005
Mean	-	0.009	0.004	0.005
SD	-	0.007	0.004	0.004
SEM	-	0.004	0.002	0.002
n	-	4	4	4

1/100 v/v spray strength dilution

Cell No	Skin No	Absorption Rate (µg/cm^2/h)		
		0 - 12h	12 - 24h	0 - 24h
Cell 60	1106	0.0001	0.0001	0.0001
Cell 61	1106	0.0001	0.0001	0.0001
Cell 62	1109	0.0005	0.0006	0.0006
Cell 63	1109	0.0005	0.0009	0.0006
Cell 64	1109	0.0003	0.0004	0.0004
Cell 68	1110C	0.0004	0.0006	0.0005
Mean	-	0.0003	0.0005	0.0004
SD	-	0.0002	0.0003	0.0002
SEM	-	0.0001	0.0001	0.0001
n	-	6	6	6

1/400 v/v spray strength dilution

Cell No	Skin No	Absorption Rate (µg/cm^2/h)		
		0 - 12h	12 - 24h	0 - 24h
Cell 70	1109	0.0005	0.0014	0.0009
Cell 72	1109	0.0005	0.0009	0.0007
Cell 73	1109	0.0004	0.0010	0.0007
Cell 74	1106	0.0002	0.0002	0.0002
Cell 75	1106	0.00002	0.00009	0.00005
Cell 78	1106	0.00002	0.00004	0.00003
Mean	-	0.0003	0.0006	0.0004
SD	-	0.0002	0.0006	0.0004
SEM	-	0.0001	0.0002	0.0002
n	-	6	6	6

APPENDIX 6 Individual Distribution of NOA449280 from the Concentrate Formulation in the Test System

Test Compartment	Amount of Dose Recovered (µg/cm²):				Mean µg/cm² Recovered	SEM
	Cell 51	Cell 52	Cell 56	Cell 57		
Donor chamber	1.00	*<0.094	0.024	4.92	#<1.51	1.16
Skin wash	2140	2089	2066	2131	2106	17.6
Stratum corneum	*<0.884	*<0.884	*<0.884	*<0.884	#<0.884	-
Epidermis	0.395	0.570	0.046	0.304	0.329	0.109
Absorbed	0.211	0.198	0.036	0.013	0.114	0.052
Total recovered	2143	2091	2067	2137	2109	18.4

Test Compartment	Percentage of Dose Recovered (%):				Mean % Recovered	SEM
	Cell 51	Cell 52	Cell 56	Cell 57		
Donor chamber	0.050	*<0.005	0.001	0.247	#<0.076	0.058
Skin wash	107	105	104	107	106	0.881
Stratum corneum	*<0.044	*<0.044	*<0.044	*<0.044	#<0.044	-
Epidermis	0.020	0.029	0.002	0.015	0.016	0.005
Absorbed	0.011	0.010	0.002	0.001	0.006	0.003
Total recovered	107	105	104	107	106	0.921

Absorbed = amount in receptor fluid
Stratum corneum = amount in tape strips
Remaining epidermis = epidermal tissue remaining after tape stripping
#The LOQ values have been used as positive values in the calculation of the mean where values were <LOQ.
*Where flagged, the data were below the LOQ. The LOQ value has been reported proceeded by <.

APPENDIX 7 Individual Distribution of NOA449280 from the 1/100 v/v Aqueous Spray Strength Dilution in the Test System

Test Compartment	Amount of Dose Recovered (µg/cm²):						Mean µg/cm² Recovered	SEM
	Cell 60	Cell 61	Cell 62	Cell 63	Cell 64	Cell 68		
Donor chamber	*<0.004	*<0.004	*<0.004	*<0.004	*<0.004	*<0.004	#<0.004	-
Skin wash	21.6	21.2	21.0	22.2	21.8	21.1	21.5	0.193
Stratum corneum	*<0.021	*<0.021	*<0.021	0.030	*<0.021	0.015	#<0.021	0.002
Epidermis	0.012	0.016	0.020	0.047	0.013	0.137	0.041	0.020
Absorbed	0.002	0.003	0.015	0.016	0.009	0.012	0.010	0.002
Total recovered	21.6	21.2	21.1	22.3	21.9	21.3	21.6	0.192

Test Compartment	Percentage of Dose Recovered (%):						Mean % Recovered	SEM
	Cell 60	Cell 61	Cell 62	Cell 63	Cell 64	Cell 68		
Donor chamber	*<0.020	*<0.020	*<0.020	*<0.020	*<0.020	*<0.020	#<0.020	-
Skin wash	109	107	106	112	111	107	109	0.975
Stratum corneum	*<0.105	*<0.105	*<0.105	0.152	*<0.105	0.075	#<0.108	0.010
Epidermis	0.059	0.083	0.099	0.235	0.068	0.693	0.206	0.101
Absorbed	0.010	0.018	0.073	0.083	0.045	0.059	0.048	0.012
Total recovered	109	107	107	113	111	108	109	0.971

Absorbed = amount in receptor fluid
Stratum corneum = amount in tape strips
Remaining epidermis = epidermal tissue remaining after tape stripping
#The LOQ values have been used as positive values in the calculation of the mean where values were <LOQ.
*Where flagged, the data were below the LOQ. The LOQ value has been reported proceeded by <.

APPENDIX 8 Individual Distribution of NOA449280 from the 1/400 v/v Aqueous Spray Strength Dilution in the Test System

Test Compartment	Amount of Dose Recovered (µg/cm²):						Mean µg/cm² Recovered	SEM
	Cell 70	Cell 72	Cell 73	Cell 74	Cell 75	Cell 78		
Donor chamber	0.008	0.039	0.014	0.087	0.075	0.012	0.039	0.014
Skin wash	5.16	5.23	5.53	5.55	5.14	5.41	5.34	0.075
Stratum corneum	*<0.019	*<0.019	0.019	*<0.019	*<0.019	*<0.019	#<0.019	0.0001
Epidermis	0.080	0.057	0.070	0.023	0.021	0.011	0.044	0.012
Absorbed	0.023	0.016	0.016	0.005	0.001	0.001	0.011	0.004
Total recovered	5.29	5.36	5.65	5.68	5.25	5.45	5.45	0.074

Test Compartment	Percentage of Dose Recovered (%):						Mean % Recovered	SEM
	Cell 70	Cell 72	Cell 73	Cell 74	Cell 75	Cell 78		
Donor chamber	0.148	0.750	0.265	1.67	1.45	0.225	0.752	0.272
Skin wash	99.4	101	107	107	99.0	104	103	1.45
Stratum corneum	*<0.358	*<0.358	0.372	*<0.358	*<0.358	*<0.358	#<0.360	0.002
Epidermis	1.55	1.11	1.35	0.448	0.401	0.216	0.845	0.229
Absorbed	0.451	0.318	0.314	0.098	0.027	0.015	0.204	0.074
Total recovered	102	103	109	110	101	105	105	1.43

Absorbed = amount in receptor fluid
Stratum corneum = amount in tape strips
Remaining epidermis = epidermal tissue remaining after tape stripping
#The LOQ values have been used as positive values in the calculation of the mean where values were <LOQ.
*Where flagged, the data were below the LOQ. The LOQ value has been reported proceeded by <.

APPENDIX 9 Limit of Quantitation Values

Concentrate formulation

Study compartment	µg/cm^2	% of applied dose
Receptor fluid	0.032	0.002
Donor chamber	0.094	0.005
Skin wash	0.748	0.038
Tape strips	0.177	0.009
Remaining epidermis	0.008	0.0004

1/100 v/v aqueous spray dilution

Study compartment	µg/cm^2	% of applied dose
Receptor fluid	0.0005	0.0024
Donor chamber	0.004	0.020
Skin wash	0.008	0.039
Tape strips	0.004	0.021
Remaining epidermis	0.0001	0.001

1/400 v/v aqueous spray dilution

Study compartment	µg/cm^2	% of applied dose
Receptor fluid	0.0005	0.009
Donor chamber	0.001	0.023
Skin wash	0.005	0.099
Tape strips	0.004	0.072
Remaining epidermis	0.0001	0.002

APPENDIX 10 Analysis of Dose Preparations

Formulation concentrate

Analysis – days post preparation		Analysed radiochemical purity
TLC	Day 2 (post exposure)	98.5%
	Day 8 (post exposure)	98.9%
HPLC	Day 2 (post exposure)	97.8%
	Day 9 (post exposure)	97.0%
	Day 21 (post exposure)	97.0%

1/100 Aqueous spray dilution

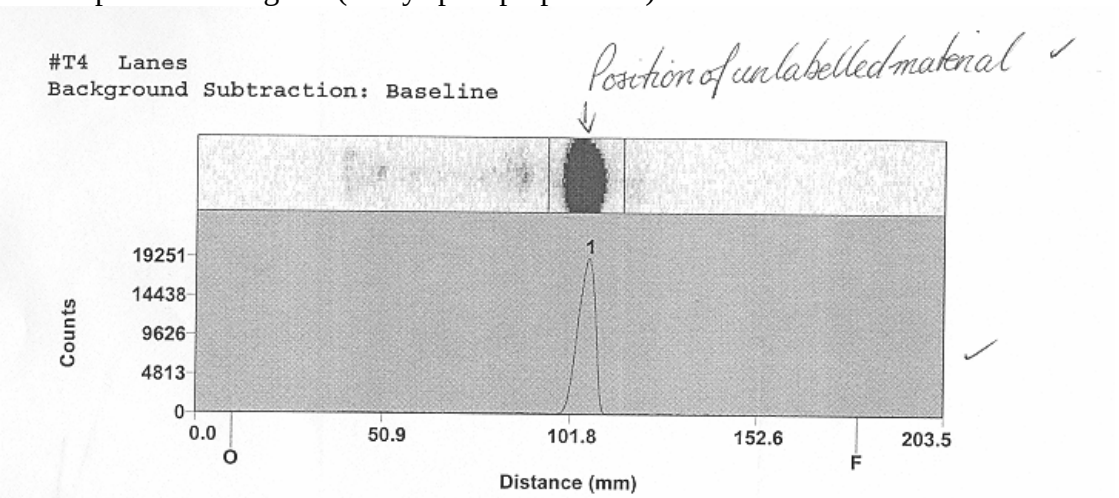
Analysis – days post preparation		Analysed radiochemical purity
TLC	Day 3 (post exposure)	98.5%
HPLC	Day 4 (post exposure)	96.2%
	Day 9 (post exposure)	98.5%
	Day 16 (post exposure)	98.4%

1/400 Aqueous spray dilution

Analysis – days post preparation		Analysed radiochemical purity
TLC	Day 2 (post exposure)	98.3%
	Day 7 (post exposure)	98.7%
HPLC	Day 1 (post exposure)	100%
	Day 2 (post exposure)	97.3%
	Day 6 (post exposure)	98.5%
	Day 13 (post exposure)	97.8%
	Day 14 (post exposure)	97.3%

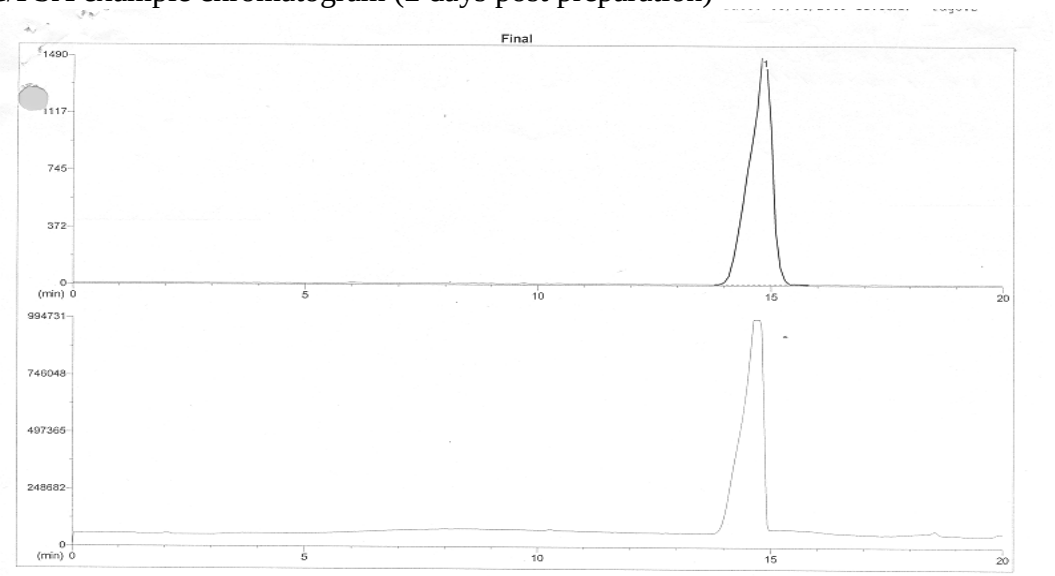
APPENDIX 11 Stability of [^{14}C]-NOA449280 in the Formulation Concentrate

TLC example chromatogram (2 days post preparation)



ID	Peak (mm)	Gross Counts	Baseline Subtract	Net Counts	Net CPM	Net % Total	Lane Rf	Label
Lane #1 A16003E CONC FORMULATION Origin=10.00 Front=180.00								
1-1	107.0	210,688	531	210,157	778.17	98.5	0.571	NOA449280
Lane		218,670	5,248	213,422	790.26	100.0		
Unres		7,982	4,717	3,265	12.09	1.5		

HPLC/FSA example chromatogram (2 days post preparation)

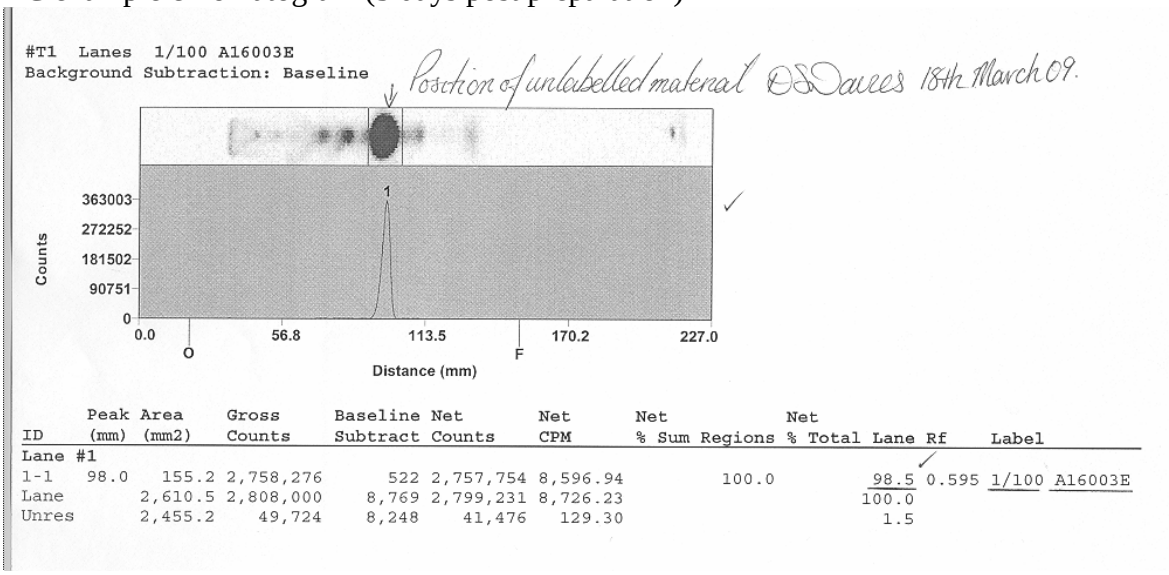


Peak Area Report (%Run) Channel 1 C-14 CTS

Name	Ret. Time (min.)	Pk#	Area	% Run	Conc.	Units
	14.80	1	8414	97.76	0.00	
Total Run Area:			8607	97.76		

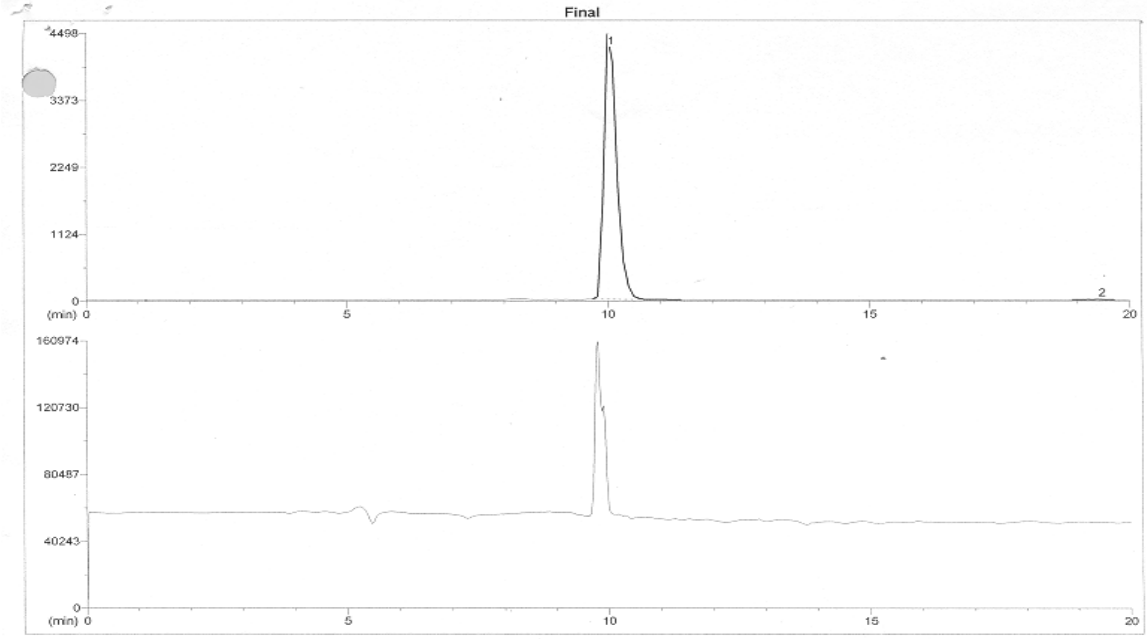
APPENDIX 12 Stability of [^{14}C]-NOA449280 in the 1/100 v/v Aqueous Spray Dilution

TLC example chromatogram (3 days post preparation)



ID	Peak Area (mm)	Peak Area (mm2)	Gross Counts	Baseline Subtract	Net Counts	Net CPM	Net % Sum Regions	Net % Total	Lane Rf	Label
Lane #1										
1-1	98.0	155.2	2,758,276	522	2,757,754	8,596.94	100.0	98.5	0.595	1/100 A16003E
Lane		2,610.5	2,808,000	8,769	2,799,231	8,726.23		100.0		
Unres		2,455.2	49,724	8,248	41,476	129.30		1.5		

HPLC/FSA example chromatogram (4 days post preparation)

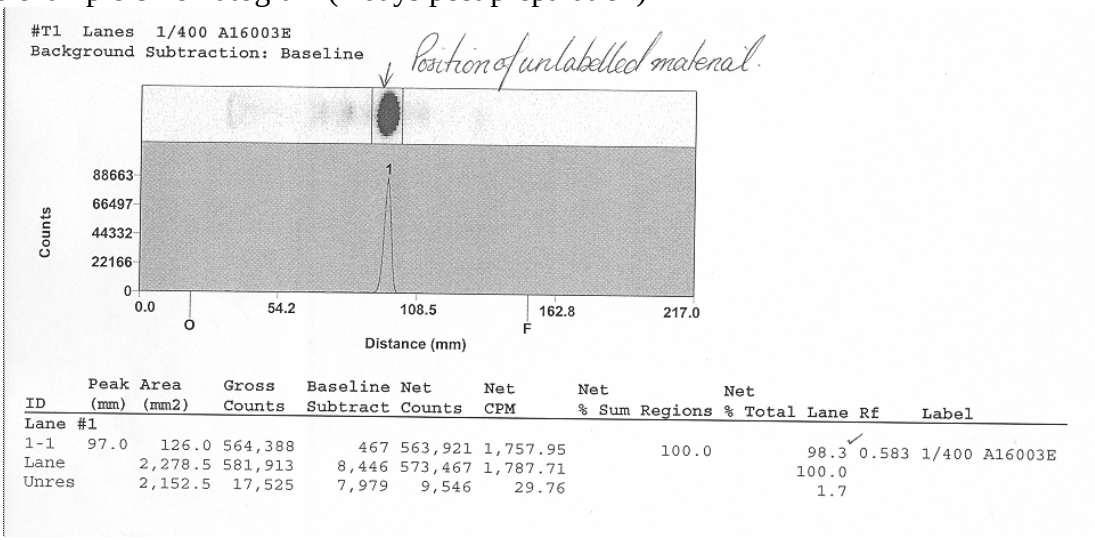


Peak Area Report (%Run) Channel 1

Name	Ret. Time (min.)	Pk#	C-14 CTS Area	% Run	Conc.	Units
	10.00	1	12916	96.21	0.00	
	19.40	2	27	0.20	0.00	
Total Run Area:			13425	96.41		

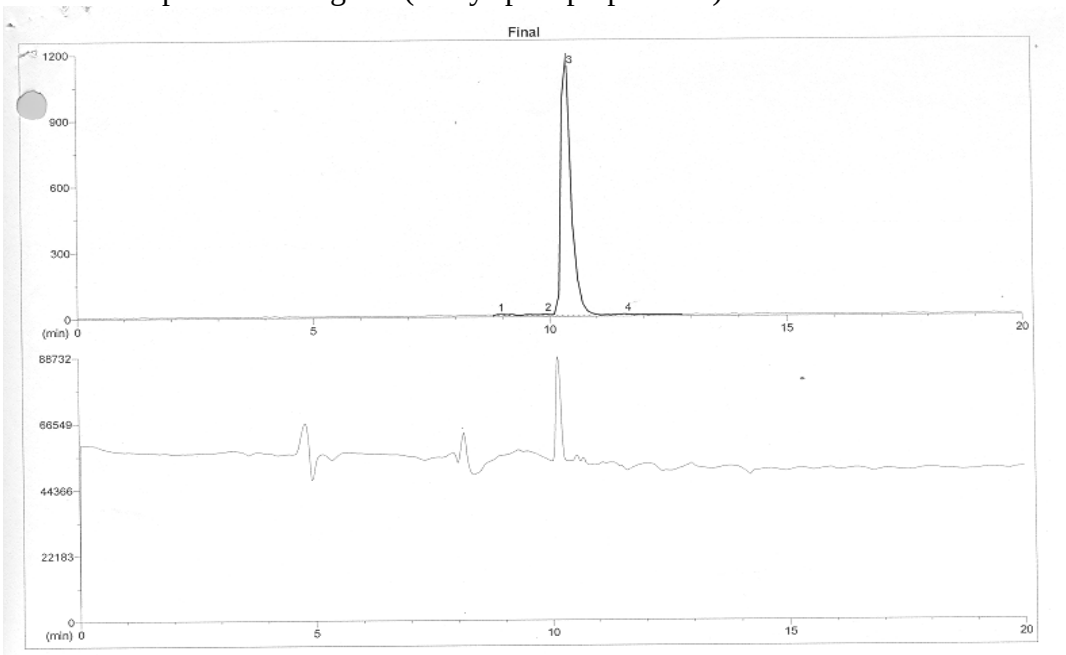
APPENDIX 13 Stability of [^{14}C]-NOA449280 in the 1/400 v/v Aqueous Spray Dilution

TLC example chromatogram (2 days post preparation)



ID	Peak Area (mm)	Peak Area (mm2)	Gross Counts	Baseline Net Subtract	Net Counts	Net CPM	Net % Sum Regions	Net % Total	Lane Rf	Label
Lane #1										
1-1	97.0	126.0	564,388	467	563,921	1,757.95	100.0	98.3	0.583	1/400 A16003E
Lane		2,278.5	581,913	8,446	573,467	1,787.71		100.0		
Unres		2,152.5	17,525	7,979	9,546	29.76		1.7		

HPLC/FSA example chromatogram (2 days post preparation)



Peak Area Report (%Pks) Channel 1

Name	Ret. Time (min.)	Pk#	Area	% Pks	Conc.	Units
	8.90	1	23	0.76	0.00	
	9.90	2	25	0.82	0.00	
	10.40	3	2963	97.28	0.00	
	11.60	4	35	1.15	0.00	

Total Peak Area: 3046
Total Run Area: 3252