U.S. ARMY PUBLIC HEALTH CENTER
8252 Blackhawk Road, Aberdeen Proving Ground, Maryland 21010-5403

Toxicity Report No. S.0055513-18, May 2018
Toxicology Directorate

In Vitro Dermal Absorption Proficiency Demonstration, May 2018

Prepared by Emily May Lent

Toxicology Directorate
Toxicity Evaluation Division
Army Public Health Center

ARIMS designation: 500C Toxicity Test

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Army Public Health Center
Toxicology Directorate (MCHB-PH-TEV)
8252 Blackhawk Road, Building 5158
Aberdeen Proving Ground, MD 21010-5403

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Office of the Surgeon General (DASG-HCZ)
ATTN: MAJ Thomas Rezentes
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The dermal absorption of the three reference substances, benzoic acid, caffeine, and testosterone, was tested as a demonstration of technical proficiency. Reference substances were tested under infinite-dose conditions using the EpiDerm™ (EPI-606-X) RHE model and results compared to published validation studies. Permeation rates were in good general agreement with the results reported in the validation study. The performing lab is therefore technically proficient in performing the in vitro dermal absorption assay and may routinely use this assay for in vitro prediction of skin absorption.

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Protocol No. 99-IV18-05-01
In Vitro Dermal Absorption Proficiency Demonstration, May 2018

Data Requirement

OECD Guidelines for the Testing of Chemicals, Section 4, Test No. 428: Skin Absorption: *In Vitro* Method

Authors

Emily May Lent

Study Completed On

February 2019

Performing Laboratory

Army Public Health Center
Toxicology Directorate (MCHB-PH-TEV)
Building 5158
8252 Blackhawk Road
Aberdeen Proving Ground, MD 21010-5403

Laboratory Project ID

Protocol No. 99-IV18-05-01

Good Laboratory Practice Compliance Statement

The study described in this report was conducted in compliance with Title 40, Code of Federal Regulations (CFR), Part 792, Good Laboratory Practice Standards, except for the following:

1. Manufacturer reported purity of the neat compounds was not verified analytically. This is not considered to have affected the outcome of the study as certificates of analyses were provided with each compound.

Submitted By:

Study Director:

LENT.EMILY.MAY.1296114371 Digitally signed by LENT.EMILY.MAY.1296114371 Date: 2019.02.08 14:04:33 -05'00'

_____ _____

EMILY MAY LENT Date
Toxicologist
Toxicity Evaluation Division (TEV)

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PROTOCOL NO. 99-IV18-05-01
IN VITRO DERMAL ABSORPTION PROFICIENCY DEMONSTRATION
MAY 2018

1 Summary

1.1 Purpose

In vitro dermal absorption methods measure the diffusion of chemicals into and across skin to a fluid reservoir. The test substance is applied to the surface of a skin sample separating the two chambers of a diffusion cell. The test substance remains on the skin for a specified time, under specified conditions and absorption of the test substance over time is measured by analysis of the receptor fluid. *In vitro* methods can be used as a screen for comparing delivery of chemicals into and through skin from different formulations and can also provide useful models for the assessment of percutaneous absorption in humans [1-3]. The following study was conducted to demonstrate performance and reliability of the test system in the performing laboratory.

1.2 Conclusions

The dermal absorption of the three reference substances recommended by OECD, benzoic acid, caffeine, and testosterone, was tested as a demonstration of technical proficiency of the laboratory prior to routine use of this test method. Reference substances were tested under infinite-dose conditions using the EpiDerm™ (EPI-606-X) RhE model and results compared to published validation studies. Permeation rates for the three reference substances were in good general agreement with the results reported in the validation study. The slightly higher permeation rates may be due to slightly higher receptor medium temperatures in this study. The performing lab is therefore technically proficient in performing the *in vitro* dermal absorption assay and may routinely use this assay for *in vitro* prediction of skin absorption.

2 References

See Appendix A for a listing of references.

3 Authority

This study was sponsored by the U.S. Army Medical Command, Office of the Surgeon General and identified as WBS element S.0055513.

4 Background

Exposure to a chemical can be by oral, inhalation, or dermal routes; however, in an occupational and many environmental and consumer settings, the latter two can be major contributors to exposure. Inhalation exposures to some chemicals have decreased as a result of reduced occupational exposure limits (OELs) leading to improved control technologies, potentially increasing the relative contribution of dermal exposure. For certain chemicals and in certain scenarios, dermal exposure may be greater than respiratory exposure, and intoxications due to skin exposure have been documented. Assessment of dermal absorption is an important aspect of the overall risk assessment of chemicals coming in contact with the skin. Potential exposures can occur during manufacturing processes, transport, and the end use of products. In addition, since most laboratory animal testing is by oral administration, the extent of dermal absorption needs to be determined to perform a risk assessment via route-to-route extrapolation [2, 4].

Dermal absorption has historically been assessed in laboratory animals, however, *in vitro* methods have been used for many years. The *in vitro* test system can use excised skin from several mammalian species, including humans. In most cases, these studies are intended to predict skin absorption in humans. Therefore, use of human skin is most relevant. However, for regulatory reasons and due to the limited availability of excised human skin for experimental purposes, alternative models are often used. Rat skin is often used because the majority of regulatory toxicity studies, including *in vivo* dermal absorption studies, are conducted in this species. Pig skin is used because of its similarity to human skin in terms of its morphology and permeability characteristics, making it a practical alternative [1, 3]. There are, however, considerable differences in stratum corneum thickness, hydration, and lipid composition across body sites in humans and laboratory animals, contributing to differences in skin absorption across body sites, variability in studies with excised skin, and potential differences between *in vitro* and *in vivo* studies [5]. In recent years, organotypic models have become more useful for investigators, and today, reconstructed human epidermis (RhE) models are commercially available (e.g., EpiDerm, EpiSkin, SkinEthic). They are well described with respect to tissue architecture and lipid composition [6, 7], and demonstrated sufficient correlation with animal models in validation studies [8, 9]. Thus, the RhE models, EPISKIN, EpiDerm and SkinEthic are appropriate alternatives to human and pig skin for the *in vitro* assessment of dermal absorption [8, 9].

The *in vitro* method has several advantages including that it can be used with skin from humans and other species, multiple replicate measurements can be made, live animals are not used, intended use scenarios can be studied, solids and granules can be tested, the impact of skin damage on absorption can be assessed without ethical issues, and non-radio-labelled test substances can be tested. Direct comparisons between *in vitro* and *in vivo* methods indicate that this test system provides qualitatively similar data that is useful in comparing delivery of chemicals into and through skin and can provide useful models for the assessment of risk due to dermal absorption in humans [2, 3].

To demonstrate the performance and reliability of the test system in the performing laboratory, the results for relevant reference chemicals should be available and in agreement with published literature for the method used [1, 2]. The present study was conducted as a technical proficiency demonstration.

5 Materials and Methods

5.1 Materials

5.1.1.1 Test Substances

The three relevant reference chemicals listed in the OECD Guideline (Table 1) were purchased from Sigma Aldrich (St. Louis, MO).

Table 1. Reference Chemicals

Substance	CASRN	MW	Log K_{ow}	Donor Conc. (%); µg/cm^2	Donor Medium	Receptor Medium
Benzoic Acid	65-85-0	122.1	1.83	0.1; 282.9	DPBS	DPBS
Caffeine	58-08-2	194.2	0.01	0.1; 282.9	DPBS	DPBS
Testosterone	58-22-0	288.4	3.32	0.004; 11.32	DPBS + 2% Igepal	DPBS

DPBS: Dulbeco's phosphate buffered saline; CASRN: Chemical Abstract Service Registry Number; MW: molecular weight; Log Kow: Log of octanol-water partition coefficient

5.1.1.2 Test System, Controls, and Reagents

The reconstructed human epidermal model EpiDerm™ was acquired from MatTek (EPI-606X, MatTek, Ashland). The EpiDerm™ tissues are shipped as kits, containing 6 tissues on shipping agarose together with culture media – Dulbeco's Modified Eagle's Medium (DMEM) based, Dulbeco's Phosphate Buffered Saline (DPBS), and 6-well plates. Additional DPBS without calcium, magnesium, or phenol red was purchased from Gibco, Inc. (a subsidiary of ThermoFisher, Waltham, MA) and Krebs-Ringer bicarbonate solution was purchased from Fisher Scientific (Hanover Park, IL). The testosterone enzyme-linked immunosorbent assay (ELISA) kit was purchased from ALPCO (Salem, NH). All test systems, reagents, and chemicals were stored according to the manufacturer's instructions.

5.2 Quality Assurance

5.2.1.1 Quality Control of Test System

The EpiDerm™ System is manufactured according to defined quality assurance procedures. All biological components of the epidermis and the culture medium are tested by the manufacturer for viral, bacterial, fungal, and mycoplasma contamination. MatTek determines the effective time for 50% viability (ET-50 value) following exposure to Triton X-100 (1%) for each EpiDerm™ lot. The ET-50 must fall within the range of the EpiDerm historical database of 4.77 – 8.72 hours. If tissue lots fail quality control (QC)

or sterility testing, the manufacturer notifies the customer. All of the tissue lots used in this proficiency demonstration passed QC and sterility testing.

5.2.2 Quality Compliance

The APHC Quality Systems and Regulatory Compliance Office audited critical study phases. Appendix B provides the dates of these audits, the phases audited, and the dates the results were reported to the Study Director (SD) and Management.

5.3 Study Personnel

Appendix C lists the names of individuals contributing to the study performance.

5.4 Methods

5.4.1 Preparation of Test Substances

5.4.1.1 Benzoic Acid

A 0.1% (w/v) donor solution was prepared by dissolving 0.1009 g of benzoic acid (lot# MKCC9722) in DPBS (lot#1924302) in a 100 ml volumetric flask. Solution was stored at 4°C overnight.

5.4.1.2 Caffeine

A 0.1% (w/v) donor solution was prepared by dissolving 0.1004 g of caffeine (lot#BCBS9512V) in DPBS (lot#1924302) in a 100 ml volumetric flask. Solution was stored at 4°C overnight.

A second caffeine donor solution was prepared with testosterone as an internal standard (ISD). A 0.1% (w/v) caffeine solution with 0.004% testosterone ISD was prepared by pipetting 400 µl of testosterone stock solution (see below) and 0.0993 g of caffeine in a 100 ml volumetric flask and bringing to volume with DPBS.

5.4.1.3 Testosterone

To prepare a testosterone donor solution with a final concentration of 0.004% (w/v), a stock solution (10 mg/ml) was prepared by dissolving 0.0999 g of testosterone (lot# SLBV0956) in ethanol (Sigma lot#020K3658). To aid in the dissolution of this lipophilic compound, octylphenoxypolyethoxyethanol (Igepal CA-360; Sigma lot# MKBX3662V) was added to the donor solution. A DPBS donor solution with 2% Igepal was prepared by adding 10 ml Igepal to 490 ml DPBS and gently inverting to mix. The testosterone donor solution was then prepared by pipetting 400 µl of testosterone stock solution into a 100 ml volumetric flask and bringing to volume with DPBS with 2% Igepal solution.

5.4.2 EPI-606-X Dermal Absorption Test

5.4.2.1 Experimental Design

All reference substances were tested using an infinite-dose and were sampled 6 times over a 6-hour period. Benzoic acid was tested in 6 replicates of RhE in a single run. Caffeine was tested in two runs, one run of 6 replicates of caffeine alone and one run of 3 replicates of caffeine alone and 3 replicates with caffeine with testosterone as ISD. Testosterone was tested in two runs, one run of 6 replicates of testosterone with 2% Igepal and one run with 3 replicates of testosterone as an internal standard (without Igepal).

5.4.2.2 Day of Receipt

Upon receipt of assay kit, all components were stored according to the manufacturer's instructions. The EpiDerm tissues were maintained in the original packaging and stored at 4±2°C.

5.4.2.3 Day of Testing

Prior to use each day, the receptor chambers of all Franz cells (water jacketed, 12 ml, 15 mm orafice) were filled approximately ¾ full with DPBS and the dermal absorption system (Logan Instruments FDC-6/VTC-300, Somerset, NJ) was allowed to equilibrate to 37±0.1°C. The EpiDerm samples were removed from the tissue culture inserts by inverting the insert on lab bench paper wetted with DPBS and cutting the tissue and underlying membrane from the insert using a sharp scalpel. The resultant disc was then placed stratum corneum side up (membrane side down) on the top of the receptor chamber of the Franz cell. The donor chamber was tightly clamped on top of the EpiDerm disc and the receptor chamber was filled to volume taking care to remove all air bubbles. Tissues were then allowed to equilibrate for 30±5 minutes prior to dosing. Tissues were visually inspected for integrity. Tissues were rejected if there was moisture/receptor media present on the tissue surface or defects were apparent. The donor solution was then pipetted (0.5 ml) onto the stratum corneum in the donor chamber. All donor solutions were brought to room temperature prior to use. Both the donor chamber and the sampling arm were covered with parafilm. The receiver solution was continuously stirred (500-600 rpm) using a Teflon-coated magnetic stir bar.

The receiver solution was sampled at 0.5, 1, 2, 3, 4, and 6 hours (±2 minutes) by withdrawing a fixed volume (800, 1600, or 1000 µl) from each receptor chamber via the sampling arm using an 18 gauge blunt end stainless steel pipetting needle and syringe. The sampled receiver solution was replaced with fresh DPBS. The donor solution was sampled prior to dosing and at the conclusion of the exposure period to verify concentration and ensure the concentration remained constant throughout the exposure. Samples were stored at approximately -30±°C prior to analysis.

5.4.3 Receiver Fluid Analysis

5.4.3.1 Benzoic Acid and Caffeine

Benzoic acid and caffeine were analyzed by UV spectrophotometry. Stock solutions (200 µg/ml) of benzoic acid and caffeine were prepared by weighing 0.0020 g of each into 10 ml volumetric flasks, dissolving in DPBS and bringing to volume in DPBS. Calibration standards were prepared by serially diluting the stock solutions. Benzoic

acid was initially diluted by pipetting 0.5 ml of stock solution into 4.5 ml of DPBS to achieve a 20 µg/ml solution. This solution was then serially diluted 1:1 for concentrations of 10, 5, 2.5, 1.25, 0.625, 0.3125, and 0.15625 µg/ml. Caffeine was initially diluted by pipetting 0.25 ml of stock solution into 4.75 ml of DPBS to achieve a 10 µg/ml solution. This was then serially diluted 1:1 for concentrations of 10, 5, 2.5, 1.25, 0.625, 0.3125, and 0.15625 µg/ml. Two replicate 200 µl aliquots of calibration standards and receiver fluid were pipetted into UV transparent 96-well plates and the absorbance measured at 230 nm and 273 nm for benzoic acid and caffeine, respectively, using a Molecular Devices SpectraMax3 plate reader.

5.4.3.2 Testosterone

Testosterone was analyzed, according to the manufacturer's instructions, using an ELISA kit (ALPCO, Salem, NH). Briefly, working solutions of the testosterone-horse radish peroxidase (HRP) conjugate and wash buffer were prepared by diluting 240 µl HRP in 12 ml assay buffer and diluting 50 ml of wash buffer in 450 ml deionized water, respectively. Calibrator, control, and samples (50 µL of each) were pipetted into correspondingly labelled wells in duplicate. Conjugate working solution (100 µL) was pipetted into each well and the plate was incubated for 1 hour at room temperature while shaking on a plate shaker (approximately 200 rpm). All wells were then washed 3 times with 300 µL of diluted wash buffer. The 3,3',5,5'-Tetramethylbenzidine (TMB) substrate (150 µL) was pipetted into each well at timed intervals and the plate incubated on a plate shaker for 10–15 minutes at room temperature. Stop solution (50 µL) was pipetted into each well at the same timed intervals used previously. The absorbance was read at 450 nm using a Molecular Devices SpectraMax3 plate reader.

5.5 Data Calculations, Analyses, and Interpretation

Experimental data generated during the course of this study were recorded by hand and tabulated, summarized, and/or analyzed using Microsoft® Excel and GraphPad Prism.

5.5.1 Receiver Fluid Concentration

5.5.1.1 Benzoic Acid and Caffeine

Mean optical density of each calibration standard and unknown was calculated. A linear regression analysis was conducted on the calibration data and concentrations of unknowns were determined from the calibration curve.

5.5.1.2 Testosterone

Mean optical density of each calibrator and unknown was calculated. The calibrator concentrations were log transformed and a 5-parameter curve was fit to the data. Concentrations of unknowns were determined from the calibration curve and the result obtained multiplied by the dilution factor as needed.

5.5.2 Total Flux, Permeability Coefficient, and Lag Time

Receiver fluid concentrations were multiplied by the receptor chamber volume to determine the amount of test substance in the receptor chamber at each sampling point. The result was added to the amount of test substance removed in the previous sampling as determined by multiplying the volume of receiver fluid removed by the receiver fluid concentration. The receiver fluid data were plotted as the cumulative amount of test substance in the receptor chamber as a function of time. The permeability coefficient was calculated from the following equation.

$J_T = A\ P\ \Delta C$
Where:
J_T is the total flux at steady state (µg/hour)
A is the area of the membrane, 1.8 cm^2
P is the effective permeability coefficient (cm/hr)
ΔC is the concentration differential between the donor and receptor chambers, taken as the initial donor solution concentration (µg/cm^3)

Flux at steady state, J_T, was estimated based the following equation.

$J_T = V\ dC/dt$

Where:
V is the volume of the receptor chamber, 12 ml
dC/dt is the rate of change in concentration in the receptor fluid at steady state
Flux at steady state, J_T, was estimated as the slope of the linear regression analysis of the linear portion of the cumulative penetration versus time plot. Lag time (t_L) was determined by extrapolating the steady-state curves to the x-axis (i.e., determining the x-intercept) (see Appendix D).

Data are presented as the mean, standard deviation, and coefficient of variation (CV%) of replicate runs for each test substance.

6 Results and Discussion

6.1 Benzoic Acid

The permeation rate for benzoic acid observed in this study (K_p $0.76x10^{-6}$cm/s) exceeded the rates observed in the validation study [8, 9] for the EpiDerm model by approximately 5-fold (Table 2). The permeation rate was, however, similar to that reported for EPISKIN in the validation study. Additionally, the results from the current study fall within the range of values reported for EpiDerm due to the high variability in the benzoic acid results in the validation study. The permeation at 6 hours (13.2%) was similar to that reported for EpiDerm in the validation study (16.3%) and benzoic acid had no lag-time in either study. Benzoic acid was judged to be a poor reference chemical in the validation study due to the high variability. Additionally, benzoic acid decreased significantly in analyzed samples in the validation study, a problem that was not due to stability but may have been due to microbial contamination.

6.2 Caffeine

Data from the first run with caffeine was discarded due to the observation of large air bubbles under the tissues. Data from the second run (Table 2) were in good general agreement with the data for EpiDerm in the validation study [8, 9]. The permeation rate in this study (K_p 1.29x10^{-6}cm/s) was about 4-fold higher than that observed in the validation study, however, the data ranges overlap. Permeation at 6 hours (8.7%) was approximately 2-fold higher than in the validation study (3.96%). The lag-time, however, was approximately 4-fold longer in the current study (1.15 hours) than in the validation study (0.35 hours). The current study was conducted at a higher temperature (37°C vs. 33.5°C) which may account for the higher permeation rate. Dermal absorption studies are normally conducted with skin temperatures of 32°C [1, 2] which is achieved by maintaining the receptor solutions at 35-37°C [10]. The receptor fluid temperature used in the current study, therefore, is consistent with standard practice and the guideline.

6.3 Testosterone

The permeation rate observed for testosterone in this study (K_p 3.9x10^{-6}cm/s) closely approximated that found in the validation study [8, 9] for EpiDerm (2.78x10^{-6}cm/s) (Table 2). Similarly, the permeation at 6 hours (28.4%) was similar to that observed in the validation study (20.05%) and no lag-time was observed in either study.

The mean permeability coefficient (K_p), lag-time, and permeation at 6 hours for each test substance can be found in Table 2. Data for each run, from individual replicate tissues can be found in Appendix D and calculations of permeation and lag time in Appendix E.

Table 2. EpiDerm (EPI-606-X) dermal absorption assay results. Mean permeability coefficient (Kp), lag-time, and permeation (%) at 6 hours for reference chemicals.

Test Substance	n	K_p (10^{-6} cm/s) mean ± SD	K_p CV (%)	Lag time (hours) mean ± SD	Lag time CV (%)	Permeation (%) mean ± SD	Permeation CV (%)
Benzoic Acid	6	0.76 ± 0.1	13.16	-7.89 ± 1.9	24.08	13.2 ± 1.0	7.58
Caffeine	6	1.29 ± 0.3	23.26	1.15 ± 0.13	11.30	8.7 ± 2.3	26.44
Testosterone	3	3.88 ± 0.5	12.89	-0.35 ± 0.22	62.86	28.4 ± 4.4	15.49

SD: standard deviation; CV: coefficient of variation; Kp: permeation coefficient; n: number of replicates

7 Conclusions

The dermal absorption of the three reference substances recommended by OECD, benzoic acid, caffeine, and testosterone, was tested as a demonstration of technical proficiency of the laboratory prior to routine use of this test method. Reference substances were tested under infinite-dose conditions using the EpiDerm™ (EPI-606-X) RhE model and results compared to published validation studies. Permeation rates for

the three reference substances were in good general agreement with the results reported in the validation study. The slightly higher permeation rates may be due to slightly higher receptor medium temperatures in this study. The performing lab is therefore technically proficient in performing the *in vitro* dermal absorption assay and may routinely use this assay for *in vitro* prediction of skin absorption.

8 Point of Contact

Questions pertaining to this report should be referred to Emily May Lent at DSN 584-3980, commercial 410-436-3980, or by e-mail: usarmy.apg.medcom-aphc.mbx.tox-info@mail.mil.

Prepared By:

LENT.EMILY.MAY.1296114371 Digitally signed by LENT.EMILY.MAY.1296114371 Date: 2019.02.08 14:04:54 -05'00'

EMILY MAY LENT — Date
Toxicologist
Toxicity Evaluation Division (TEV)

Approved By:

ONEILL.ARTHUR.J.III.1299508443 Digitally signed by ONEILL.ARTHUR.J.III.1299508443 Date: 2019.02.12 11:42:46 -05'00'

ARTHUR J. O'NEILL — Date
Division Chief, TEV

JOHNSON.MARK.STEVEN.1229380717 Digitally signed by JOHNSON.MARK.STEVEN.1229380717 Date: 2019.02.13 08:36:38 -05'00'

MARK S. JOHNSON — Date
Director, Toxicology

Appendix A

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Appendix B

QUALITY ASSURANCE STATEMENT

For: Toxicology Study No. S.0055513-18, Protocol No. 99-iv18-05-01, entitled "*In vitro* dermal absorption proficiency demonstration, May 2018", the following Good Laboratory Practice Standard Inspections were conducted:

Study Specific Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to Management/SD
Type Protocol Good Laboratory Practice Standard Review	03/21/2018	03/21/2018
Study Raw Data Good Laboratory Practice Standard Review	10/18/2018	10/30/2018
Final Study Good Laboratory Practice Standard Report Review	10/18/2018	10/30/2018

Note 1: *All findings were made known to the Study Director and the Program Manager at the time of the audit/inspection. If there were no findings during the inspection, the inspection was reported to Management and the Study Director on the date shown in the table.*
Note 2: *This report has been audited by the Quality Assurance Unit (QSARC), and is considered to be an accurate account of the data generated and of the procedures conducted.*
Note 3: *In addition to the study specific critical phase inspections listed here, general facility and process based inspection not specifically related to this study are done monthly or annually in accordance with QA Standard Operating Procedure.*

Michael P. Kefauver
Good Laboratory Practice Standards
Quality Assurance Specialist, QSARC

28 FEB 2019
Date

Appendix C

Archives and Study Personnel

C-1 Archives

All raw data, documentation, records, protocol, and a copy of the final report generated as a result of this study will be archived in room 1026, building E-2100, APHC, for a minimum of ten (10) years following submission of the final report to the Sponsor. If the report is used to support a regulatory action, it shall, along with all supporting data, be retained indefinitely.

Some ancillary records pertaining to this study, such as instrument maintenance logs will not be archived until those logbooks have been completed. Once complete they will be archived in room 1026, building E-2100, APHC.

C-2 Personnel

Management: Dr. Mark S. Johnson, Director, Toxicology; MAJ Jarod Hanson, Executive Officer, Toxicology; Mr. Arthur J. O'Neill, Chief, Toxicity Evaluation Division (TEV); Dr. Michael J. Quinn, Chief, Health Effects Research Division (HEF).

Study Director: Dr. Emily May Lent, Toxicologist, TEV.

Quality Assurance: Michael P. Kefauver, Quality Assurance Specialist, Quality Systems and Regulatory Compliance Office.

Archivist: Lee C.B. Crouse, Biologist, TEV.

Appendix D

Dermal Absorption Data

Toxicity Report No. S.0055513-18, May 2018

Exp. No.:	2		
Tissue Lot No.:	28355		
Date:	17-May-18		
Operator:	Emily Lent		
Benzoic Acid 1 mg/ml			mean
Blanks:	0.00004	0.000	0.000000

Time	Tissue	Raw Data		Blank Corrected		Mean of Aliquots	Calc. Conc. In aliquot
ug/ml		Aliquot 1	Aliquot 2	Aliquot 1	Aliquot 2		
20	std curve	1.254	1.219	1.25	1.22	1.237	
10	std curve	0.630	0.607	0.63	0.61	0.618	
5	std curve	0.307	0.304	0.31	0.30	0.305	
2.5	std curve	0.159	0.163	0.16	0.16	0.161	
1.25	std curve	0.082	0.078	0.08	0.08	0.080	
0.625	std curve	0.039	0.041	0.04	0.04	0.040	
0.3125	std curve	0.018	0.020	0.02	0.02	0.019	
0.15625	std curve	0.009	0.013	0.01	0.01	0.011	
0.5	1	0.140	0.143	0.14	0.14	0.142	2.27
	2	0.133	0.131	0.13	0.13	0.132	2.11
	3	0.143	0.142	0.14	0.14	0.143	2.29
	4	0.165	0.159	0.17	0.16	0.162	2.60
	5	0.151	0.142	0.15	0.14	0.147	2.35
	6	0.170	0.173	0.17	0.17	0.171	2.75
1	1	0.174	0.184	0.17	0.18	0.179	2.87
	2	0.187	0.180	0.19	0.18	0.183	2.95
	3	0.218	0.232	0.22	0.23	0.225	3.63
	4	0.199	0.235	0.20	0.23	0.217	3.49
	5	0.238	0.251	0.24	0.25	0.244	3.94
	6	0.226	0.238	0.23	0.24	0.232	3.74
2	1	0.199	0.201	0.20	0.20	0.200	3.21
	2	0.238	0.254	0.24	0.25	0.246	3.96
	3	0.189	0.189	0.19	0.19	0.189	3.04
	4	0.212	0.210	0.21	0.21	0.211	3.40
	5	0.299	0.293	0.30	0.29	0.296	4.78
	6	0.232	0.227	0.23	0.23	0.230	3.70
3	1	0.236	0.234	0.24	0.23	0.235	3.79
	2	0.288	0.296	0.29	0.30	0.292	4.71
	3	0.223	0.225	0.22	0.22	0.224	3.61
	4	0.235	0.237	0.24	0.24	0.236	3.80
	5	0.269	0.281	0.27	0.28	0.275	4.44

	6	0.269	0.266	0.27	0.27	0.267	4.31
4	1	0.273	0.273	0.27	0.27	0.273	4.40
	2	0.310	0.306	0.31	0.31	0.308	4.97
	3	0.255	0.251	0.26	0.25	0.253	4.08
	4	0.269	0.270	0.27	0.27	0.270	4.35
	5	0.289	0.295	0.29	0.29	0.292	4.71
	6	0.295	0.311	0.30	0.31	0.303	4.89
6	1	0.303	0.296	0.30	0.30	0.299	4.83
	2	0.362	0.360	0.36	0.36	0.361	5.83
	3	0.315	0.313	0.31	0.31	0.314	5.07
	4	0.305	0.314	0.30	0.31	0.309	4.99
	5	0.343	0.341	0.34	0.34	0.342	5.51
	6	0.343	0.344	0.34	0.34	0.343	5.54
	start	6.905	6.960	6.91	6.96	6.933	1.64
donor	1	6.549	6.888	6.55	6.89	6.718	1.43
	2	6.029	6.794	6.03	6.79	6.412	1.14
	3	6.402	6.132	6.40	6.13	6.267	1.00
	4	6.729	6.737	6.73	6.74	6.733	1.45
	5	6.462	6.321	6.46	6.32	6.392	1.12
	6	6.302	6.308	6.30	6.31	6.305	1.03

Toxicity Report No. S.0055513-18, May 2018

Exp. No.:	1						
Tissue Lot No.:	28355						
Date:	16-May-18						
Operator:	Emily Lent						
							mean
Blanks:	0.00030	0.000					0.000000
Caffeine 1 mg/ml							

Time ug/ml	Tissue	Raw Data		Blank Corrected		Mean of Aliquots	Calc. Conc. In aliquot
		Aliquot 1	Aliquot 2	Aliquot 1	Aliquot 2		
10	std curve	0.557	0.549	0.56	0.55	0.553	
5	std curve	0.300	0.313	0.30	0.31	0.307	
2.5	std curve	0.171	0.163	0.17	0.16	0.167	
1.25	std curve	0.107	0.107	0.11	0.11	0.107	
0.625	std curve	0.078	0.077	0.08	0.08	0.077	
0.3125	std curve	0.053	0.052	0.05	0.05	0.053	
0.15625	std curve	0.018	0.017	0.02	0.02	0.017	
0.5	1	0.035	0.036	0.04	0.04	0.036	0.03
	2	0.023	0.023	0.02	0.02	0.023	-0.21
	3	0.034	0.038	0.03	0.04	0.036	0.04
	4	0.032	0.038	0.03	0.04	0.035	0.02
	5	0.041	0.036	0.04	0.04	0.038	0.08
	6	0.051	0.052	0.05	0.05	0.051	0.33
1	1	0.056	0.055	0.06	0.05	0.055	0.40
	2	0.040	0.037	0.04	0.04	0.039	0.09
	3	0.041	0.040	0.04	0.04	0.041	0.13
	4	0.027	0.027	0.03	0.03	0.027	-0.13
	5	0.036	0.026	0.04	0.03	0.031	-0.06
	6	0.045	0.076	0.05	0.08	0.061	0.51
2	1	0.047	0.049	0.05	0.05	0.048	0.27
	2	0.035	0.036	0.03	0.04	0.035	0.03
	3	0.057	0.057	0.06	0.06	0.057	0.43
	4	0.041	0.042	0.04	0.04	0.041	0.14
	5	0.025	0.027	0.02	0.03	0.026	-0.15
	6	0.036	0.036	0.04	0.04	0.036	0.03
3	1	0.040	0.044	0.04	0.04	0.042	0.15
	2	0.025	0.030	0.02	0.03	0.028	-0.12
	3	0.048	0.061	0.05	0.06	0.054	0.39
	4	0.042	0.041	0.04	0.04	0.041	0.14
	5	0.037	0.033	0.04	0.03	0.035	0.01
	6	0.049	0.044	0.05	0.04	0.046	0.24
4	1	0.045	0.051	0.05	0.05	0.048	0.27
	2	0.026	0.028	0.03	0.03	0.027	-0.13

	3	0.038	0.044	0.04	0.04	0.041	0.13
	4	0.039	0.046	0.04	0.05	0.043	0.16
	5	0.041	0.044	0.04	0.04	0.042	0.16
	6	0.044	0.043	0.04	0.04	0.044	0.18
6	1	0.054	0.062	0.05	0.06	0.058	0.45
	2	0.030	0.027	0.03	0.03	0.028	-0.11
	3	0.035	0.033	0.03	0.03	0.034	0.00
	4	0.029	0.024	0.03	0.02	0.026	-0.15
	5	0.040	0.036	0.04	0.04	0.038	0.07
	6	0.034	0.034	0.03	0.03	0.034	0.00
	start	5.891	5.981	5.89	5.98	5.936	0.97
donor	1	6.543	6.537	6.54	6.54	6.540	1.41
	2	6.313	6.600	6.31	6.60	6.457	1.35
	3	6.281	6.499	6.28	6.50	6.390	1.30
	4	7.342	7.002	7.34	7.00	7.172	1.86
	5	11.723		11.72		11.723	5.13
	6	6.456	7.064	6.46	7.06	6.760	1.57

Toxicity Report No. S.0055513-18, May 2018

Exp. No.:	3		
Tissue Lot No.:	28366		
Date:	24-May-18		
Operator:	Emily Lent		
Caffeine 1 mg/ml Blanks:	-0.00005	0.000	mean 0.000000

Time	Tissue	Raw Data		Blank Corrected		Mean of Aliquots	Calc. Conc. In aliquot
ug/ml		Aliquot 1	Aliquot 2	Aliquot 1	Aliquot 2		
10	std curve	0.359	0.361	0.36	0.36	0.360	
5	std curve	0.231	0.230	0.23	0.23	0.230	
2.5	std curve	0.152	0.153	0.15	0.15	0.152	
1.25	std curve	0.115	0.112	0.11	0.11	0.113	
0.625	std curve	0.101	0.100	0.10	0.10	0.101	
0.3125	std curve	0.075	0.073	0.07	0.07	0.074	
0.15625	std curve	0.019	0.019	0.02	0.02	0.019	
0.25	1	0.022	0.022	0.02	0.02	0.022	-1.28
	2	0.016	0.016	0.02	0.02	0.016	-1.48
	3	0.023	0.022	0.02	0.02	0.023	-1.27
0.5	1	0.025	0.023	0.02	0.02	0.024	-1.23
	2	0.019	0.018	0.02	0.02	0.018	-1.41
	3	0.027	0.026	0.03	0.03	0.026	-1.15
0.75	1	0.024	0.023	0.02	0.02	0.023	-1.25
	2	0.018	0.018	0.02	0.02	0.018	-1.42
	3	0.022	0.049	0.02	0.05	0.035	-0.86
1	1	0.027	0.026	0.03	0.03	0.026	-1.15
	2	0.016	0.016	0.02	0.02	0.016	-1.49
	3	0.025	0.025	0.03	0.03	0.025	-1.19
2	1	0.072	0.072	0.07	0.07	0.072	0.32
	2	0.054	0.053	0.05	0.05	0.054	-0.27
	3	0.071	0.074	0.07	0.07	0.072	0.33
3	1	0.112	0.114	0.11	0.11	0.113	1.63
	2	0.076	0.078	0.08	0.08	0.077	0.49
	3	0.109	0.112	0.11	0.11	0.110	1.55
4	1	0.141	0.141	0.14	0.14	0.141	2.55
	2	0.103	0.102	0.10	0.10	0.103	1.31
	3	0.135	0.136	0.14	0.14	0.136	2.37
6	1	0.208	0.210	0.21	0.21	0.209	4.75
	2	0.151	0.154	0.15	0.15	0.153	2.92
	3	0.211	0.206	0.21	0.21	0.208	4.72

donor	1	3.961	3.961	3.96	3.96	3.961	74.66
	2	3.961		3.96		3.961	74.66
	3	3.961		3.96		3.961	74.66
	4	3.961		3.96		3.961	74.66
	5	0.000	0.000	0.00	0.00	0.000	-0.65
	6	0.000	0.000	0.00	0.00	0.000	-0.65

Time	Tissue	Raw Data		Blank Corrected		Mean of Aliquots	Calc. Conc. In aliquot
ug/ml		Aliquot 1	Aliquot 2	Aliquot 1	Aliquot 2		
10	std curve	0.376	0.374	0.38	0.37	0.375	
5	std curve	0.249	0.240	0.25	0.24	0.244	
2.5	std curve	0.165	0.161	0.17	0.16	0.163	
1.25	std curve	0.124	0.120	0.12	0.12	0.122	
0.625	std curve	0.112	0.112	0.11	0.11	0.112	
0.3125	std curve	0.081	0.081	0.08	0.08	0.081	
0.15625	std curve	0.019	0.019	0.02	0.02	0.019	
0.25	1	0.016	0.019	0.02	0.02	0.018	-1.60
	2	0.020	0.022	0.02	0.02	0.021	-1.50
	3	0.020	0.022	0.02	0.02	0.021	-1.50
0.5	1	0.023	0.027	0.02	0.03	0.025	-1.38
	2	0.022	0.023	0.02	0.02	0.023	-1.45
	3	0.027	0.023	0.03	0.02	0.025	-1.37
0.75	1	0.020	0.019	0.02	0.02	0.020	-1.54
	2	0.021	0.024	0.02	0.02	0.022	-1.46
	3	0.019	0.018	0.02	0.02	0.019	-1.57
1	1	0.026	0.018	0.03	0.02	0.022	-1.46
	2	0.020	0.023	0.02	0.02	0.021	-1.49
	3	0.023	0.022	0.02	0.02	0.022	-1.46
2	1	0.064	0.064	0.06	0.06	0.064	-0.15
	2	0.056	0.056	0.06	0.06	0.056	-0.41
	3	0.062	0.062	0.06	0.06	0.062	-0.22
3	1	0.165	0.105	0.16	0.10	0.135	2.06
	2	0.081	0.083	0.08	0.08	0.082	0.41
	3	0.094	0.095	0.09	0.09	0.095	0.80
4	1	0.130	0.132	0.13	0.13	0.131	1.95
	2	0.106	0.107	0.11	0.11	0.107	1.18
	3	0.116	0.117	0.12	0.12	0.116	1.48
6	1	0.196	0.198	0.20	0.20	0.197	4.00
	2	0.142	0.147	0.14	0.15	0.145	2.37
	3	0.167	0.168	0.17	0.17	0.167	3.08
donor	1	3.962		3.96		3.962	-0.44
	2	3.962		3.96		3.962	-0.44
	3	3.962		3.96		3.962	-0.44
	1	0.000		0.00		0.000	-2.15
	2	0.000	0.000	0.00	0.00	0.000	-2.15
	3	0.000	0.000	0.00	0.00	0.000	-2.15

Toxicity Report No. S.0055513-18, May 2018

Exp. No.:	3
Tissue Lot No.:	28366
Date:	24-May-18
Operator:	Emily Lent
Testosterone (40 mg/ml) with caffeine (1 mg/ml)	
Blanks:	mean 0.000000

Time ng/ml	Tissue	Raw Data Aliquot 1	Raw Data Aliquot 2	Blank Corrected Aliquot 1	Blank Corrected Aliquot 2	Mean of Aliquots	Curve Calc Conc. (ng/ml)	with dil factor (ng/ml)
0	std curve	2.015	1.995	2.02	2.00	2.005		
0.08	std curve	1.499	1.480	1.50	1.48	1.490		
0.42	std curve	0.921	0.889	0.92	0.89	0.905		
1.67	std curve	0.370	0.421	0.37	0.42	0.395		
5	std curve	0.216	0.206	0.22	0.21	0.211		
16.7	std curve	0.091	0.088	0.09	0.09	0.090		
0.25	1	0.078	0.076	0.08	0.08	0.077	30.79	30.79
	2	0.094	0.086	0.09	0.09	0.090	20.40	20.40
	3	0.075	0.073	0.07	0.07	0.074	34.79	34.79
0.5	1	0.065	0.064	0.07	0.06	0.064	60.18	60.18
	2	0.073	0.072	0.07	0.07	0.073	36.35	36.35
	3	0.074	0.068	0.07	0.07	0.071	39.91	39.91
0.75	1	0.064	0.061	0.06	0.06	0.062	70.15	70.15
	2	0.070	0.065	0.07	0.07	0.068	46.71	46.71
	3	0.063	0.058	0.06	0.06	0.060	83.89	83.89
1	1	0.067	0.068	0.07	0.07	0.067	49.50	49.50
	2	0.061	0.061	0.06	0.06	0.061	76.42	76.42
	3	0.066	0.063	0.07	0.06	0.065	56.16	56.16
2	1	0.049	0.046	0.05	0.05	0.048		
	2	0.050	0.045	0.05	0.05	0.048		
	3	0.045	0.043	0.05	0.04	0.044		
3	1	0.045	0.042	0.05	0.04	0.044		
	2	0.044	0.043	0.04	0.04	0.043		
	3	0.045	0.044	0.05	0.04	0.044		
4	1	0.048	0.045	0.05	0.05	0.047		
	2	0.048	0.045	0.05	0.05	0.047		
	3	0.052	0.046	0.05	0.05	0.049		
6	1	0.044	0.044	0.04	0.04	0.044		
	2	0.042	0.044	0.04	0.04	0.043		
	3	0.047	0.044	0.05	0.04	0.045		

0.5	1	0.166	0.148	0.17		0.166	6.41	64.13
	2	0.191	0.187	0.19		0.191	5.14	51.42
	3	0.165	0.162	0.16		0.165	6.48	64.76
0.75	1	0.169	0.157	0.17		0.169	6.23	62.32
	2	0.164	0.164	0.16	0.16	0.164	6.54	65.39
	3	0.156	0.146	0.16	0.15	0.151	7.49	74.86
1	1	0.159	0.152	0.16	0.15	0.155	7.17	71.69
	2	0.151	0.161	0.15	0.16	0.156	7.09	70.94
	3	0.163	0.159	0.16	0.16	0.161	6.74	67.38
2	1	0.082	0.083	0.08	0.08	0.082	25.79	257.94
	2	0.089	0.079	0.09	0.08	0.084	24.21	242.06
	3	0.083	0.103	0.08	0.10	0.093	18.89	188.93
3	1	0.081	0.072	0.08	0.07	0.076	32.02	320.22
	2	0.080	0.070	0.08	0.07	0.075	33.35	333.49
	3	0.073	0.069	0.07	0.07	0.071	39.91	399.07
6	1	0.068	0.067	0.07	0.07	0.067	49.50	494.95
	2	0.069	0.068	0.07	0.07	0.069	44.21	442.06
	3	0.077	0.072	0.08	0.07	0.075	33.35	333.49

Appendix E

Dermal Absorption Calculations

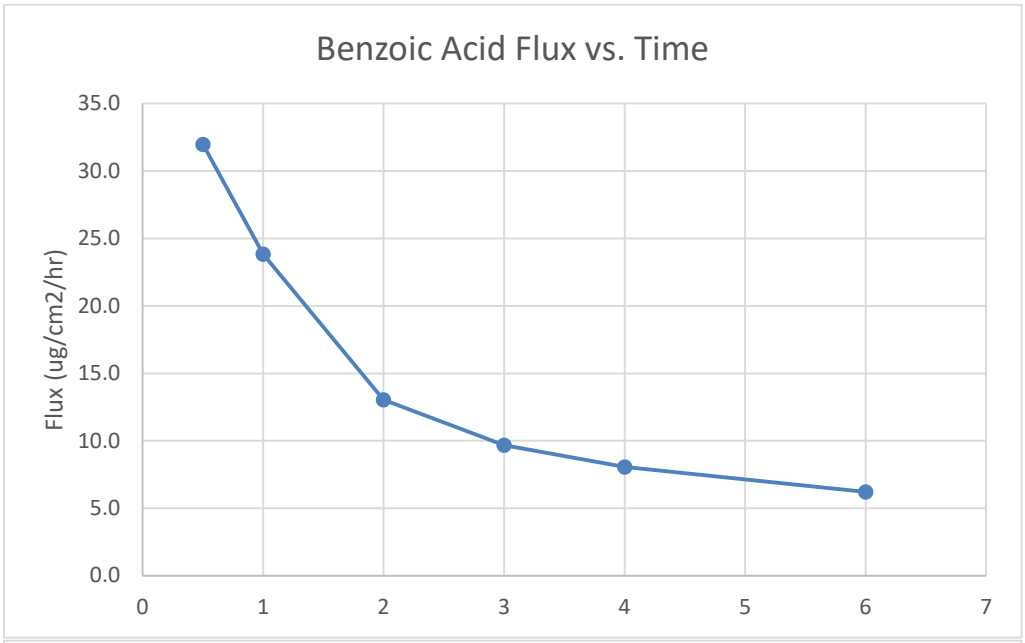
Toxicity Report No. S.0055513-18, May 2018

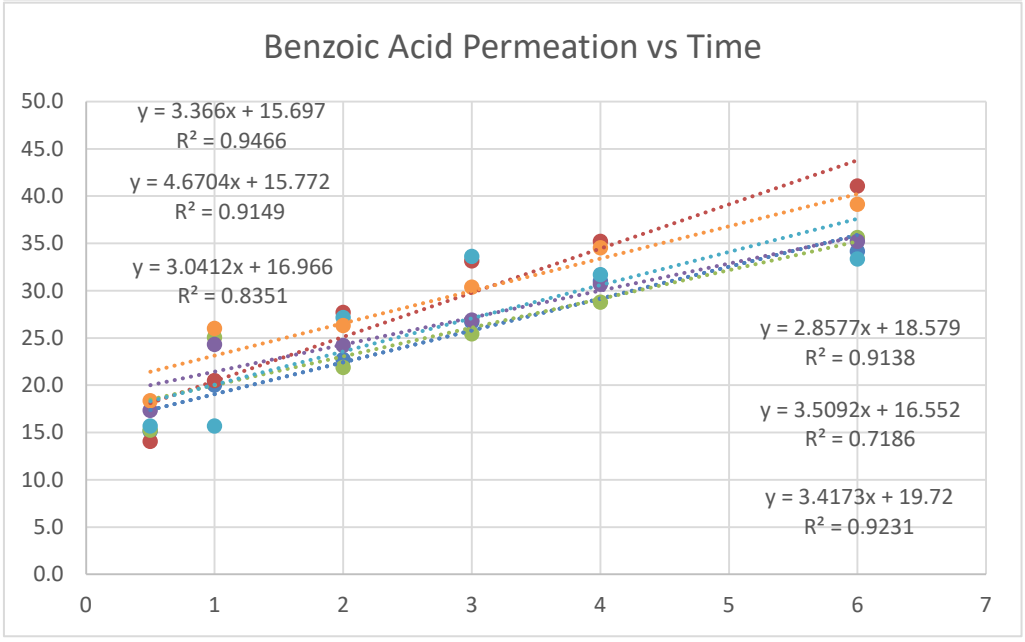
Exp. No.:	2
Tissue Lot No.:	28355
Date:	17-May-18
Operator:	Emily Lent

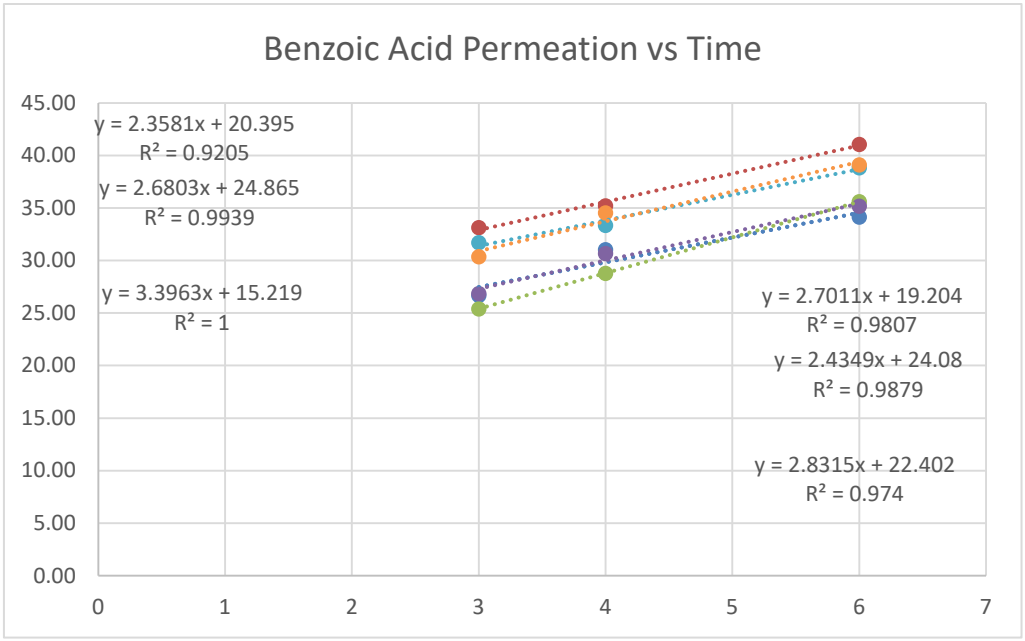
Benzoic Acid 1 mg/ml

Sample Collection Time	Tissue	Measured Conc. (ug/ml)	Amnt in cell (ug)	sampled amnt removed	Amnt incl sample removed	Permeation (ug/cm2)	Flux (ug/cm2/hr)
0.5	1	2.27	27.26	1.59	27.26	15.14	30.28
	2	2.11	25.32	1.48	25.32	14.07	28.14
	3	2.29	27.45	1.60	27.45	15.25	30.50
	4	2.60	31.25	1.82	31.25	17.36	34.72
	5	2.35	28.26	1.65	28.26	15.70	31.40
	6	2.75	33.06	1.93	33.06	18.37	36.73
Mean		**2.40**	**28.77**	**1.68**	**28.77**	**15.98**	**31.96**
STDEV		**0.24**	**2.85**	**0.17**	**2.85**	**1.59**	**3.17**
%CV		**9.92**	**9.92**	**9.92**	**9.92**	**9.92**	**9.92**
1	1	2.87	34.47	2.30	36.06	20.03	20.03
	2	2.95	35.41	2.36	36.89	20.49	20.49
	3	3.63	43.50	2.90	45.10	25.06	25.06
	4	3.49	41.94	2.80	43.76	24.31	24.31
	5	3.94	47.24	3.15	48.89	27.16	27.16
	6	3.74	44.88	2.99	46.80	26.00	26.00
Mean		**3.44**	**41.24**	**2.75**	**42.92**	**23.84**	**23.84**
STDEV		**0.43**	**5.19**	**0.35**	**5.29**	**2.94**	**2.94**
%CV		**12.59**	**12.59**	**12.59**	**12.32**	**12.32**	**12.32**
2	1	3.21	38.57	2.57	40.87	22.71	11.35
	2	3.96	47.49	3.17	49.85	27.70	13.85
	3	3.04	36.45	2.43	39.35	21.86	10.93
	4	3.40	40.81	2.72	43.60	24.22	12.11
	5	4.78	57.34	3.82	60.49	33.61	16.80
	6	3.70	44.38	2.96	47.37	26.32	13.16
Mean		**3.68**	**44.18**	**2.95**	**46.92**	**26.07**	**13.03**
STDEV		**0.63**	**7.57**	**0.50**	**7.72**	**4.29**	**2.14**
%CV		**17.15**	**17.15**	**17.15**	**16.45**	**16.45**	**16.45**
3	1	3.79	45.43	3.03	48.00	26.67	8.89
	2	4.71	56.50	3.77	59.67	33.15	11.05
	3	3.61	43.32	2.89	45.75	25.42	8.47
	4	3.80	45.65	3.04	48.37	26.87	8.96
	5	4.44	53.23	3.55	57.05	31.70	10.57
	6	4.31	51.69	3.45	54.65	30.36	10.12

Mean		**4.11**	**49.30**	**3.29**	**52.25**	**29.03**	**9.68**
STDEV		**0.44**	**5.24**	**0.35**	**5.64**	**3.13**	**1.04**
%CV		**10.62**	**10.62**	**10.62**	**10.80**	**10.80**	**10.80**
4	1	4.40	52.82	3.52	55.85	31.03	7.76
	2	4.97	59.64	3.98	63.40	35.22	8.81
	3	4.08	48.94	3.26	51.83	28.79	7.20
	4	4.35	52.15	3.48	55.20	30.67	7.67
	5	4.71	56.49	3.77	60.04	33.35	8.34
	6	4.89	58.71	3.91	62.15	34.53	8.63
Mean		**4.57**	**54.79**	**3.65**	**58.08**	**32.27**	**8.07**
STDEV		**0.35**	**4.17**	**0.28**	**4.50**	**2.50**	**0.62**
%CV		**7.60**	**7.60**	**7.60**	**7.74**	**7.74**	**7.74**
6	1	4.83	57.94	3.86	61.46	34.14	5.69
	2	5.83	69.95	4.66	73.92	41.07	6.84
	3	5.07	60.82	4.05	64.08	35.60	5.93
	4	4.99	59.87	3.99	63.35	35.19	5.87
	5	5.51	66.15	4.41	69.92	38.84	6.47
	6	5.54	66.51	4.43	70.42	39.12	6.52
Mean		**5.29**	**63.54**	**4.24**	**67.19**	**37.33**	**6.22**
STDEV		**0.39**	**4.67**	**0.31**	**4.91**	**2.73**	**0.45**
%CV		**7.35**	**7.35**	**7.35**	**7.31**	**7.31**	**7.31**


Benzoic Acid Flux vs. Time
Flux (ug/cm2/hr)
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
0
1
2
3
4
5
6
7


Benzoic Acid Permeation vs Time
y = 3.366x + 15.697
R² = 0.9466
y = 4.6704x + 15.772
R² = 0.9149
y = 3.0412x + 16.966
R² = 0.8351
y = 2.8577x + 18.579
R² = 0.9138
y = 3.5092x + 16.552
R² = 0.7186
y = 3.4173x + 19.72
R² = 0.9231
50.0
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
0
1
2
3
4
5
6
7


Benzoic Acid Permeation vs Time
y = 2.3581x + 20.395
R² = 0.9205
y = 2.6803x + 24.865
R² = 0.9939
y = 3.3963x + 15.219
R² = 1
y = 2.7011x + 19.204
R² = 0.9807
y = 2.4349x + 24.08
R² = 0.9879
y = 2.8315x + 22.402
R² = 0.974
45.00
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
0
1
2
3
4
5
6
7

Calculation of benzoic acid permeation with all 6 time points

tissue	*slope*	*donor conc*	*Kp (cm/hr)*	*Lag time (Hr)*
1	3.366	1000	0.00337	-4.66
2	4.670	1000	0.00467	-3.38
3	3.041	1000	0.00304	-5.58
4	2.858	1000	0.00286	-6.50
5	3.509	1000	0.00351	-4.72
6	3.417	1000	0.00342	-5.77
		mean	**0.00348**	**-5.10**
		SD	**0.00063**	**1.09**
		mean	**3.48E-03**	
		SD	**6.35E-04**	
		mean	9.66E-07	cm/s
		SD	1.76E-07	

Calculation of benzoic acid permeation with linear end of curve – last 3 time points

tissue	*slope*	*donor conc*	*Kp (cm/hr)*	*Lag time (Hr)*
1	2.358	1000	0.00236	-8.65
2	2.680	1000	0.00268	-9.28
3	3.396	1000	0.00340	-4.48
4	2.701	1000	0.00270	-7.11
5	2.435	1000	0.00243	-9.89
6	2.832	1000	0.00283	-7.91
		mean	**0.00273**	**-7.89**
		SD	**0.00037**	**1.94**
		mean	**2.73E-03**	
		SD	**3.70E-04**	
		mean	7.59E-07	cm/s
		SD	1.03E-07	

Toxicity Report No. S.0055513-18, May 2018

Exp. No.:	3
Tissue Lot No.:	28366
Date:	24-May-18
Operator:	Emily Lent
Caffeine 1 mg/ml	

Time	Tissue	Calc. Conc. In aliquot	Amnt in cell (ug)	sampled amnt removed	Amnt incl sample removed	Permeation (ug/cm2)	Flux (ug/cm2/hr)
0.25	1	0.00	0.00	0.00	0.00	0.00	0.00
	2	0.00	0.00	0.00	0.00	0.00	0.00
	3	0.00	0.00	0.00	0.00	0.00	0.00
Mean		**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
STDEV		**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
%CV							
0.5	1	0.00	0.00	0.00	0.00	0.00	0.00
	2	0.00	0.00	0.00	0.00	0.00	0.00
	3	0.00	0.00	0.00	0.00	0.00	0.00
Mean		**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
STDEV		**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
%CV							
0.75	1	0.00	0.00	0.00	0.00	0.00	0.00
	2	0.00	0.00	0.00	0.00	0.00	0.00
	3	0.00	0.00	0.00	0.00	0.00	0.00
Mean		**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
STDEV		**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
%CV							
1	1	0.00	0.00	0.00	0.00	0.00	0.00
	2	0.00	0.00	0.00	0.00	0.00	0.00
	3	0.00	0.00	0.00	0.00	0.00	0.00
Mean		**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
STDEV		**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
%CV							
2	1	0.32	3.87	0.32	3.87	2.15	1.08
	2	0.00	0.00	0.00	0.00	0.00	0.00
	3	0.33	3.99	0.33	3.99	2.22	1.11
Mean		**0.22**	**2.62**	**0.22**	**2.62**	**1.46**	**0.73**
STDEV		**0.19**	**2.27**	**0.19**	**2.27**	**1.26**	**0.63**
%CV		**86.63**	**86.63**	**86.63**	**86.63**	**86.63**	**86.63**
3	1	1.63	19.61	1.63	19.93	10.89	3.63
	2	0.49	5.86	0.49	5.86	3.26	1.09
	3	1.55	18.62	1.55	18.95	10.34	3.45
Mean		**1.22**	**14.70**	**1.22**	**14.92**	**8.16**	**2.72**
STDEV		**0.64**	**7.66**	**0.64**	**7.85**	**4.26**	**1.42**
%CV		**52.15**	**52.15**	**52.15**	**52.65**	**52.15**	**52.15**

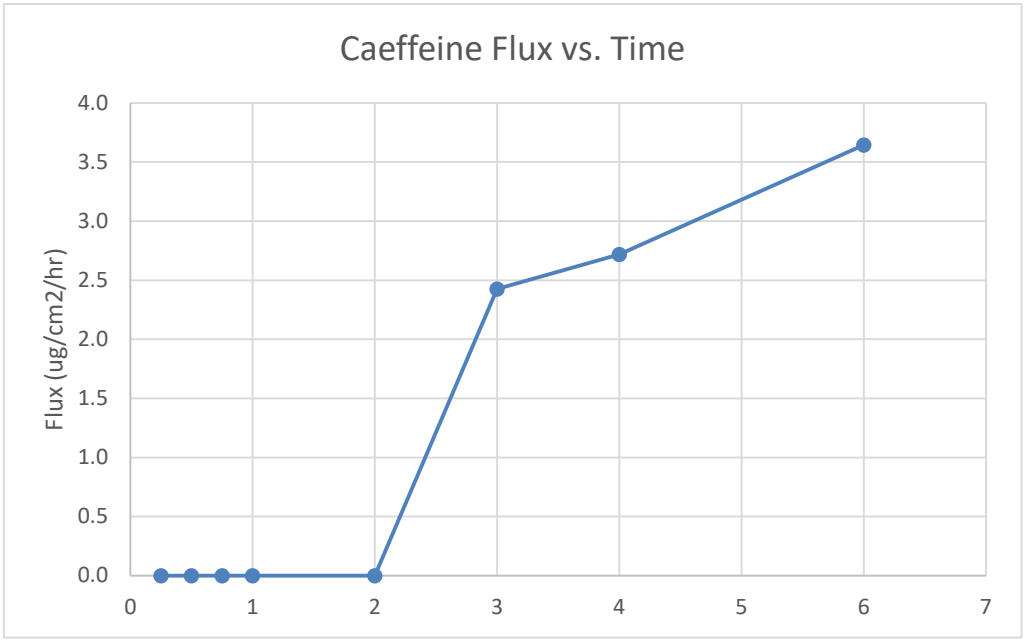
4	1	2.55	30.58	2.55	32.21	16.99	4.25
	2	1.31	15.72	1.31	16.20	8.73	2.18
	3	2.37	28.45	2.37	30.00	15.81	3.95
Mean		**2.08**	**24.92**	**2.08**	**26.14**	**13.84**	**3.46**
STDEV		**0.67**	**8.04**	**0.67**	**8.68**	**4.47**	**1.12**
%CV		**32.26**	**32.26**	**32.26**	**33.19**	**32.26**	**32.26**
6	1	4.75	56.98	4.75	59.53	31.66	5.28
	2	2.92	35.05	2.92	36.36	19.47	3.25
	3	4.72	56.59	4.72	58.96	31.44	5.24
Mean		**4.13**	**49.54**	**4.13**	**51.62**	**27.52**	**4.59**
STDEV		**1.05**	**12.55**	**1.05**	**13.22**	**6.97**	**1.16**
%CV		**25.33**	**25.33**	**25.33**	**25.60**	**25.33**	**25.33**

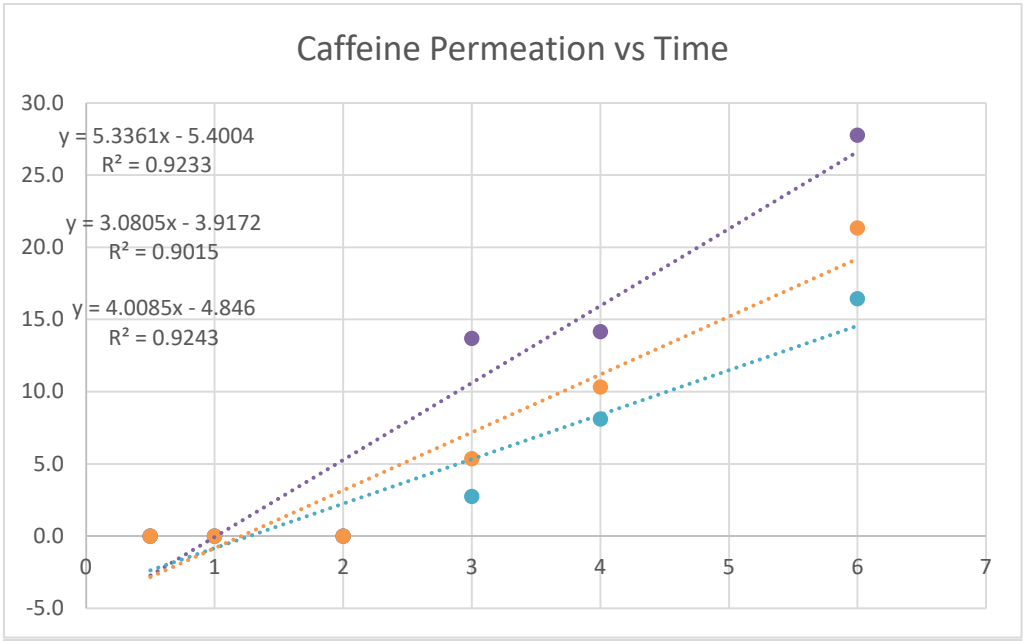
Toxicity Report No. S.0055513-18, May 2018

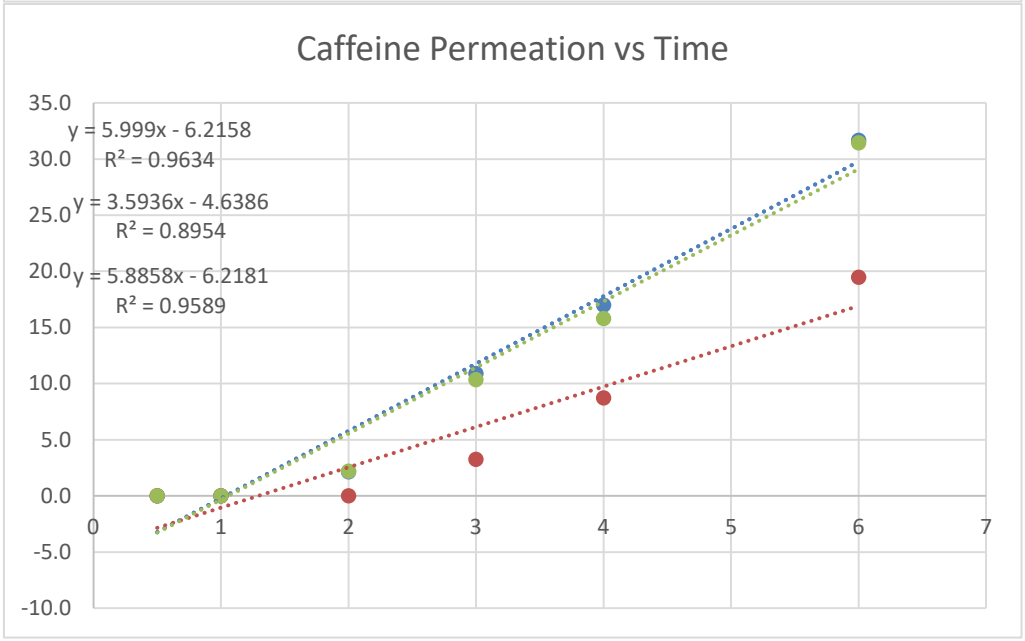
Exp. No.:	3
Tissue Lot No.:	28366
Date:	24-May-18
Operator:	Emily Lent
Caffeine 1 mg/ml	

Time	Tissue	Calc. Conc. In aliquot	Amnt in cell (ug)	sampled amnt removed	Amnt incl sample removal (ug)	Permeation (ug/cm2)	Flux (ug/cm2/hr)
0.25	4	0.00	0.00	0.00	0.00	0.00	0.00
	5	0.00	0.00	0.00	0.00	0.00	0.00
	6	0.00	0.00	0.00	0.00	0.00	0.00
Mean		**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
STDEV		**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
%CV							
0.5	4	0.00	0.00	0.00	0.00	0.00	0.00
	5	0.00	0.00	0.00	0.00	0.00	0.00
	6	0.00	0.00	0.00	0.00	0.00	0.00
Mean		**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
STDEV		**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
%CV							
0.75	4	0.00	0.00	0.00	0.00	0.00	0.00
	5	0.00	0.00	0.00	0.00	0.00	0.00
	6	0.00	0.00	0.00	0.00	0.00	0.00
Mean		**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
STDEV		**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
%CV							
1	4	0.00	0.00	0.00	0.00	0.00	0.00
	5	0.00	0.00	0.00	0.00	0.00	0.00
	6	0.00	0.00	0.00	0.00	0.00	0.00
Mean		**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
STDEV		**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
%CV							
2	4	0.00	0.00	0.00	0.00	0.00	0.00
	5	0.00	0.00	0.00	0.00	0.00	0.00
	6	0.00	0.00	0.00	0.00	0.00	0.00
Mean		**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
STDEV		**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
%CV							
3	4	2.06	24.68	2.06	24.68	13.71	4.57
	5	0.41	4.95	0.41	4.95	2.75	0.92
	6	0.80	9.66	0.80	9.66	5.36	1.79

Mean		**1.09**	**13.09**	**1.09**	**13.09**	**7.27**	**2.42**
STDEV		**0.86**	**10.30**	**0.86**	**10.30**	**5.72**	**1.91**
%CV		**78.68**	**78.68**	**78.68**	**78.68**	**78.68**	**78.68**
4	4	1.95	23.44	1.95	25.49	14.16	3.54
	5	1.18	14.21	1.18	14.63	8.13	2.03
	6	1.48	17.81	1.48	18.62	10.34	2.59
Mean		**1.54**	**18.49**	**1.54**	**19.58**	**10.88**	**2.72**
STDEV		**0.39**	**4.65**	**0.39**	**5.50**	**3.05**	**0.76**
%CV		**25.15**	**25.15**	**25.15**	**28.08**	**28.08**	**28.08**
6	4	4.00	48.04	4.00	49.99	27.77	4.63
	5	2.37	28.43	2.37	29.61	16.45	2.74
	6	3.08	36.96	3.08	38.44	21.36	3.56
Mean		**3.15**	**37.81**	**3.15**	**39.35**	**21.86**	**3.64**
STDEV		**0.82**	**9.83**	**0.82**	**10.22**	**5.68**	**0.95**
%CV		**26.01**	**26.01**	**26.01**	**25.98**	**25.98**	**25.98**


Caeffeine Flux vs. Time
Flux (ug/cm2/hr)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
6
7


Caffeine Permeation vs Time
y = 5.3361x - 5.4004
R² = 0.9233
y = 3.0805x - 3.9172
R² = 0.9015
y = 4.0085x - 4.846
R² = 0.9243
30.0
25.0
20.0
15.0
10.0
5.0
0.0
-5.0
0
1
2
3
4
5
6
7


Caffeine Permeation vs Time
y = 5.999x - 6.2158
R² = 0.9634
y = 3.5936x - 4.6386
R² = 0.8954
y = 5.8858x - 6.2181
R² = 0.9589
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
-5.0
-10.0
0
1
2
3
4
5
6
7

Calculation of caffeine permeation

tissue	*slope*	*donor conc*	*Kp (cm/hr)*	*Lag time (Hr)*
1	5.999	1000	0.00600	1.04
2	3.594	1000	0.00359	1.29
3	5.886	1000	0.00589	1.06
4	5.336	1000	0.00534	1.01
5	3.081	1000	0.00308	1.27
6	4.009	1000	0.00401	1.21
		mean	**0.00465**	**1.15**
		SD	**0.00125**	**0.13**
		mean	**4.65E-03**	
		SD	**1.25E-03**	
		mean	1.29E-06	cm/s
		SD	3.47E-07	

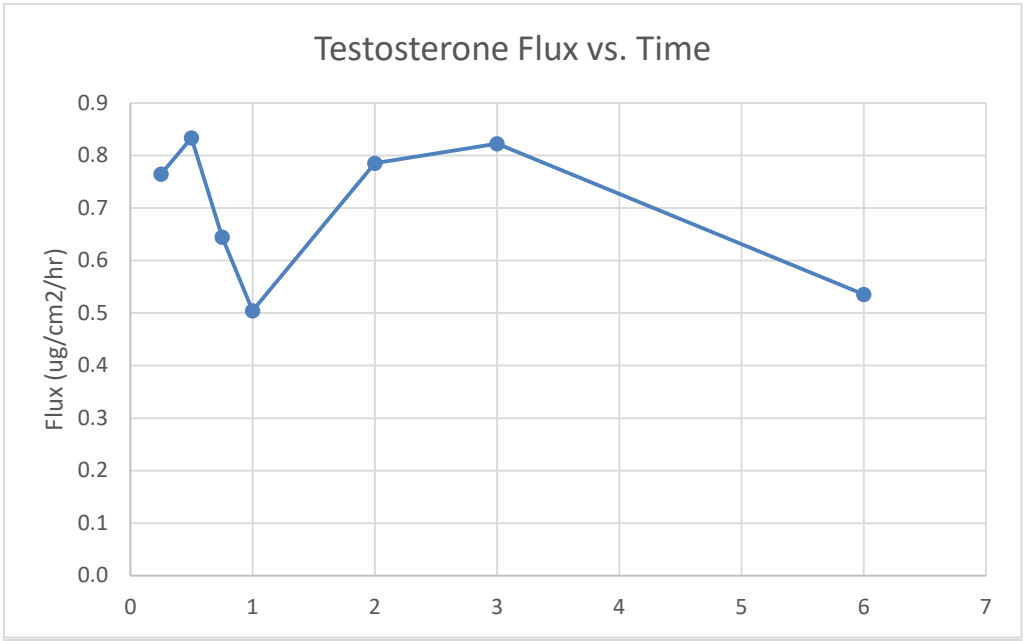
Toxicity Report No. S.0055513-18, May 2018

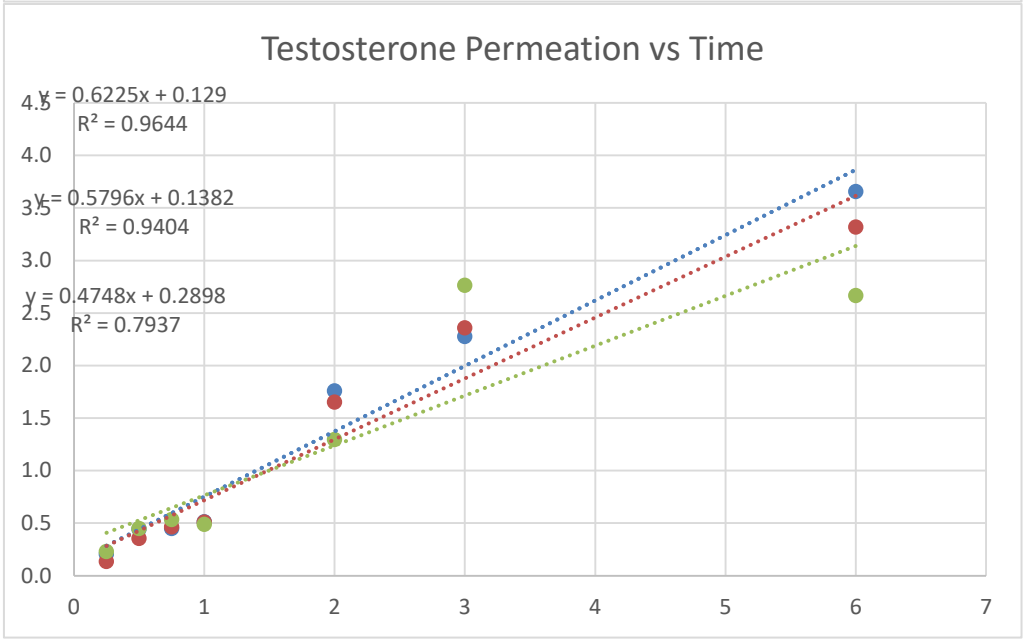
Exp. No.:	3
Tissue Lot No.:	28366
Date:	24-May-18
Operator:	Emily Lent

Testosterone (40 mg/ml) with Caffeine (1 mg/ml)

Time	Tissue	Measured Conc. (ng/ml)	Amnt in cell (ug)	sampled amnt removed (ug)	Amnt incl sample removed (ug)	Permeation (ug/cm2)	Flux (ug/cm2/hr)
0.25	1	30.79	0.37	0.03	0.37	0.21	0.82
	2	20.40	0.24	0.02	0.24	0.14	0.54
	3	34.79	0.42	0.03	0.42	0.23	0.93
Mean		**28.66**	**0.34**	**0.03**	**0.34**	**0.19**	**0.76**
STDEV		**7.43**	**0.09**	**0.01**	**0.09**	**0.05**	**0.20**
%CV		**25.92**	**25.92**	**25.92**	**25.92**	**25.92**	**25.92**
0.5	1	64.13	0.77	0.06	0.80	0.44	0.89
	2	51.42	0.62	0.05	0.64	0.35	0.71
	3	64.76	0.78	0.06	0.81	0.45	0.90
Mean		**60.10**	**0.72**	**0.06**	**0.75**	**0.42**	**0.83**
STDEV		**7.53**	**0.09**	**0.01**	**0.10**	**0.05**	**0.11**
%CV		**12.52**	**12.52**	**12.52**	**13.01**	**13.01**	**13.01**
0.75	1	62.32	0.75	0.06	0.81	0.45	0.60
	2	65.39	0.78	0.07	0.84	0.46	0.62
	3	74.86	0.90	0.07	0.96	0.54	0.71
Mean		**67.52**	**0.81**	**0.07**	**0.87**	**0.48**	**0.64**
STDEV		**6.54**	**0.08**	**0.01**	**0.08**	**0.05**	**0.06**
%CV		**9.68**	**9.68**	**9.68**	**9.33**	**9.33**	**9.33**
1	1	71.69	0.86	0.07	0.92	0.51	0.51
	2	70.94	0.85	0.07	0.92	0.51	0.51
	3	67.38	0.81	0.07	0.88	0.49	0.49
Mean		**70.00**	**0.84**	**0.07**	**0.91**	**0.50**	**0.50**
STDEV		**2.30**	**0.03**	**0.00**	**0.02**	**0.01**	**0.01**
%CV		**3.29**	**3.29**	**3.29**	**2.33**	**2.33**	**2.33**
2	1	257.94	3.10	0.26	3.17	1.76	0.88
	2	242.06	2.90	0.24	2.98	1.65	0.83
	3	188.93	2.27	0.19	2.33	1.30	0.65
Mean		**229.64**	**2.76**	**0.23**	**2.83**	**1.57**	**0.78**
STDEV		**36.14**	**0.43**	**0.04**	**0.44**	**0.24**	**0.12**
%CV		**15.74**	**15.74**	**15.74**	**15.43**	**15.43**	**15.43**
3	1	320.22	3.84	0.32	4.10	2.28	0.76

	2	333.49	4.00	0.33	4.24	2.36	0.79
	3	399.07	4.79	0.40	4.98	2.77	0.92
Mean		**350.92**	**4.21**	**0.35**	**4.44**	**2.47**	**0.82**
STDEV		**42.22**	**0.51**	**0.04**	**0.47**	**0.26**	**0.09**
%CV		**12.03**	**12.03**	**12.03**	**10.60**	**10.60**	**10.60**
4	1						
	2						
	3						
Mean							
STDEV							
%CV							
6	1	494.95	5.94	0.49	6.58	3.66	0.61
	2	442.06	5.30	0.44	5.97	3.32	0.55
	3	333.49	4.00	0.33	4.80	2.67	0.44
Mean		**423.50**	**5.08**	**0.42**	**5.78**	**3.21**	**0.54**
STDEV		**82.32**	**0.99**	**0.08**	**0.90**	**0.50**	**0.08**
%CV		**19.44**	**19.44**	**19.44**	**15.64**	**15.64**	**15.64**


Testosterone Flux vs. Time
Flux (ug/cm2/hr)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
1
2
3
4
5
6
7


Testosterone Permeation vs Time
y = 0.6225x + 0.129
R² = 0.9644
y = 0.5796x + 0.1382
R² = 0.9404
y = 0.4748x + 0.2898
R² = 0.7937
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
6
7

Calculation of testosterone permeation

tissue	*slope*	*donor conc*	*Kp (cm/hr)*	*Lag time (Hr)*
1	0.623	40	0.01556	-0.21
2	0.580	40	0.01449	-0.24
3	0.475	40	0.01187	-0.61
4				
5				
6				
		mean	**0.01397**	**-0.35**
		SD	**0.00190**	**0.22**
		mean	**1.40E-02**	
		SD	**1.90E-03**	
		mean	3.88E-06	cm/s
		SD	5.28E-07	