QUANTIFYING THE TRANSFER OF OPTICAL BRIGHTENERS FROM FABRIC TO SKIN

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ABSTRACT

Quantifying the Transfer of Optical Brighteners from Fabric to Skin

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Many commercial textiles (clothing, linens, upholstery, furniture) are impregnated, or may be contaminated with, chemicals capable of penetrating the skin. Several researchers have detected high concentrations of metals, pesticides, flame-retardants, phthalates, and optical brighteners in child and adult clothing items. Other studies have demonstrated that chemicals in clothing can migrate into the body by measuring target compound metabolite concentrations in blood and urine. However, the fabric-to-skin pathway remains poorly quantified. The purpose of this study is to measure the rate at which a low volatility optical brightener might transfer from clothing to the skin and into the bloodstream. In this experimental study, [^{14}C]-7-hydroxycoumarin was applied to three types of fabrics in two fabric concentrations. Human cadaver skin was exposed to the loaded fabric *in vitro* for 24-hours. Migration of the radiolabeled compound was quantified by liquid scintillation counting (LSC). These data enable the calculation of flux to and through skin (pg/cm^2/hr) as well as the rates of mass transfer (cm/hr). Analyses suggest that dermal exposure to chemicals in textiles can contribute to the total body burden of water soluble, low volatility compounds. Quantification of transfer rates in the fabric-to-skin pathway can aid in the assessment of fabrics as a source for chronic low-dose exposure to potentially harmful chemicals.

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1.0 Introduction

1.1 Chemicals in Fabrics

Textile manufacturers have been impregnating fabrics with potentially harmful chemicals for decades. Upholsteries, linens, clothing, and protective uniforms regularly contain unlabeled quantities of heavy metals, pesticides, phthalates, antimicrobials, preservatives, parfums, musks, and dyes. In 1974, Schorr *et al*. tested 112 articles of clothing and found that—regardless of fabric composition—100% of their clothing samples contained formaldehyde, a carcinogenic preservative, at concentrations ranging from 1 to 3,517 parts per million (ppm). A few years later in 1977, Blum *et al*. reported that tris-2,3,-dibromopropylphosphate (*tris*-BP), a brominated flame-retardant associated with mutagenicity and nephrotoxicity, was being intentionally added to children's pajamas during manufacturing at levels reaching 200,000 ppm—20% of the material's weight. Although *tris*-BP was subsequently banned, evidence of harmful compounds in textiles

has not subsided. If anything, the research on chemicals found in consumer and protective clothing has only continued to grow.

Chen & Ding (2006) quantified optical brightening agents (OBAs), a group of ultraviolet (UV) light absorbing compounds that can cause contact dermatitis on sensitive skin, in unwashed infant clothing and blankets at levels up to 118 ppm. Bridgen *et al.* (2012) analyzed plastisol screenprints on a variety of retail apparel and found phthalates, a class of hormone-mimicking compounds linked to reproductive toxicity, at concentrations up to 376,000 ppm. The same study also reported concentrations of nonylphenol ethoxylates (NPEs)—nonionic surfactants associated with reproductive and developmental toxicity—as high as 45,000 ppm in designer-brand retail clothing. In the present year, Avagyan *et al.* (2015) found levels of benzothiazole, a known skin sensitizer, in retail-sourced clothing at levels up to 51 ppm.

In occupational settings, impregnated clothing worn for protective purposes creates a scenario for dermal exposure. Military uniforms are often impregnated with high levels (up to 1500 mg/m^2) of pesticides, such as permethrin and methyl-parathion, to protect soldiers from vector-borne diseases (Appel *et al*., 2008; Rossbach *et al.,* 2010). Similarly, thick protective clothing worn by firefighters is often impregnated with phthalates to give the fabric more flexibility. Alexander & Baxter (2014) found 4.5 ppm of di-(2-ethylhexyl) phthalate (DEHP) in the inner fabric layer of unused firefighting gloves, which after being worn firefighting, elevated to concentrations ranging from 30 and 1400 ppm due to migration of DEHP from the middle and outer layers of the gloves. Clearly, the plethora of research and reporting on such high chemical concentrations in both retail and protective apparel is an indicator that fabrics may be a significant source of chemical exposure.

As exemplified above, textiles are an intimate part of our daily lives. Fabrics of all types are found in nearly every indoor environment, and many, such as clothing, athletic apparel, bath towels, and bed linens, come into direct contact with large areas of bare skin for extended periods of time. Although the human skin is often considered a protective barrier, unbroken skin will absorb molecules from its ambient environment by way of passive diffusion (Weschler & Nazaroff, 2012). The outer-most layer of the skin (the stratum corneum) provides a stronger barrier to hydrophilic compounds than lipophilic compounds. However, hydrophilic compounds can still permeate unbroken skin and then easily pass into the vascular system. Skin, being the largest organ of the human body, has an average surface area of 1.1 m^2 for children aged 6-11 and 2.1 m^2 for adults aged 18 years and older (U.S. EPA, 2011). And while the cumulative skin-to-fabric contact time will vary widely among people and place, the U.S. Environmental Protection Agency's Exposure Factors Handbook reports that the average human covers 67% to 78% of their body with clothing in warm weather and 92% to 97% of their body in cold weather (U.S. EPA, 2011).

1.2 Evidence of Dermal Exposure from Fabrics

Several researchers have already demonstrated that dermal exposure to impregnated clothing can result in a quantifiable absorbed dose. Table 1 lists past investigations that are most applicable to this study. This collection of prior research encompasses a range of exposure scenarios, including non-occupational (at-home) exposure, occupational exposure (specifically in military, forestry, and agriculture), and accidental exposure (*i.e.*, chemical spills). As an example of non-occupational exposure due to intentionally impregnated clothing, Blum *et al.* (1978) collected urine samples from 8 children who wore pajamas containing *tris*-BP for 5 consecutive nights. Concentrations of the compound's metabolites measured in the urine led the authors to the conclusion that the children must have absorbed roughly 180 micrograms (μg) of *tris*-BP per day.

A case of non-occupational exposure resulting from the unintentional contamination of fabrics was reported by Armstrong *et al.* (1969), in which pentachlorophenol (PCP) poisoning resulted in multiple infant deaths. The poisonings were connected to the infants' cloth diapers that had been washed with laundry detergent containing the toxic PCP. A case for occupational exposure was demonstrated by Appel *et al*. (2008) and Rossbach *et al*. (2010), who showed that military personnel wearing uniforms impregnated with 1250-1500 mg/m^2 of permethrin for 8 hours each day were receiving a maximum daily dose of 5-6 μg/kilogram of body weight (kg BW)/day.

In addition to the compounds referenced in Table 1 (which tend to be semi-volatile or low-volatility compounds), research on the concentration of metallic nanoparticles in commercial textiles is emerging (Von Goetz *et al*., 2013; Rovira *et al.,* 2015). For instance, Von Goetz *et al.* (2013) measured dermal exposure *in vitro* under simulated sweating conditions when wearing designer-brand clothing with concentrations of silver (Ag) and titanium dioxide (TiO_2) at levels up to 183 ppm and 8,543 ppm, respectively. While this evidence—among others—suggests dermal absorption of nanoparticles from clothing is a potential source of exposure, this class of chemicals falls beyond the scope of this study. Hence, the studies performed by Von Goetz *et al*. (2013) and Rovira *et al.* (2015) are excluded from Table 1.

Table 1. Previous studies investigating the fabric-to-skin exposure pathway using human cadaver *in vitro* or human *in vivo* methodologies.

Reference	Study Type	Tested Fabric(s)	Compound(s)	MW g/mol	VP mmHg @ 25C	$K_{o/w}$
Moore et al. 2014	*in vitro*	Consumer clothing	Chlorpyrifos	350.6	$1.87x10^{-5}$	$5.0x10^{4}$
Salocks et al. 2014	*in vitro*	Cotton/Nylon	Methamphetamine-HCl	149.2	N/A	$1.2\text{-}1.7x10^{2}$**
Wester et al. 2005	*in vitro*	Cotton sheets	Malathion, Parathion	330.4 291.3	$1.78x10^{-4}$ $3.78x10^{-5}$	$5.62x10^{2}$ $6.76x10^{3}$
Obendorf et al. 2003	*in vitro*	Denim & Cotton	Methyl parathion	263.2	$0.97x10^{-5}$	$2.51x10^{3}$
Wester et al. 1996	*in vitro*	Cotton sheets	Glyphosate, Malathion	169.1 330.4	$7.5x10^{-8}$ $1.78x10^{-4}$	$3.16x10^{-4}$ $5.62x10^{2}$
Rossbach et al. 2014	*in vivo*	Forestry-worker clothing	Permethrin	391.3	$1.5x10^{-8}$	$1.26x10^{6}$
Rossbach et al. 2010	*in vivo*	Military uniforms	Permethrin	391.3	$1.5x10^{-8}$	$1.26x10^{6}$
Appel et al. 2008	*in vivo*	Military uniforms	Permethrin	391.3	$1.5x10^{-8}$	$1.26x10^{6}$
Snodgrass et al. 1992	*in vivo**	Cotton & Nylon/Cotton	Permethrin	391.3	$1.5x10^{-8}$	$1.26x10^{6}$
Clifford & Nies 1989	*in vivo*	Worker coveralls	Dichlorodiphenoltrichloroethane	354.5	$2.0x10^{-7}$	$8.13x10^{6}$
Blum et al. 1978	*in vivo*	Polyester pajamas	*tris*-BP	697.7	$1.9x10^{-4}$	$8.1x10^{9}$**
Armstrong et al. 1969	*in vivo*	Cloth diapers	Pentachlorophenol	266.3	$1.1x10^{-4}$	$1.4x10^{5}$

*Study used rabbits as test subjects rather than humans.
**Estimated by the U.S. EPA's EPISuite™

1.3 Opportunity for Research

Few of the published dermal exposure studies have produced data sufficient for use in comprehensive exposure assessments. In most cases, dermal absorption has been inappropriately measured and reported as a percent absorbed. Because fractional absorption is a function of the load delivered (and mass loadings used in human cadaver *in vitro* studies may be orders of magnitude higher than realistic loadings), results reported as percent absorbed may be grossly underestimating actual exposure (Kissel, 2011). Consequently, dermal exposure models used in risk assessment are oftentimes producing unreliable exposure assessments and are mischaracterizing the mass transfer of environmental toxicants.

In the Children's Total Exposure to Persistent Pesticides and Other Persistent Organic Pollutants (CTEPP) study, led by EPA's National Exposure Research Laboratory, a discrepancy was observed between the urinary biomarker level measured in children and the urinary estimates made based on levels measured in environmental media (Wilson *et al*., 2004; Morgan *et al*., 2008). However, only inhalation and dietary and non-dietary ingestion pathways were investigated, thus supporting the theory that a substantial amount of mass can be transferred into the body via the dermal pathway as well (Kissel, 2011; Bekö *et al.,* 2013).

In order to accurately describe the total human exposure to environmental chemicals, the dermal pathway must be better quantified and properly characterized in exposure assessments. Rather than relying on fractional absorption as a measure of dose, experimental studies are needed to determine compound specific mass transfer rates (in units of mass per area per time) in order to more realistically define the dermally absorbed dose. It is hypothesized that fabrics—primarily

clothing—may be a source (and sink) of environmental chemicals capable of migrating from the fabric to and through the skin.

1.4 Barriers to Quantifying Dermal Exposure from Fabrics

One of the greatest barriers to characterizing and quantifying dermal exposure to chemicals from consumer fabrics accurately is the undisclosed composition and concentration of compounds added to textiles by the manufacturer. Not only does this leave the general public unaware of their exposures, but the lack of information forces investigators to perform expensive and imprecise analytical techniques in order to understand a single exposure scenario.

Characterizing chemical concentration and composition in fabrics is additionally difficult because fabrics act as a replenishing reservoir for a multitude of volatile and semi-volatile compounds. Throughout their lifetime, fabrics are constantly absorbing and off-gassing compounds; making them a continuous vehicle for human exposure to environmental chemicals (Weschler & Nazaroff, 2012). Semi-volatile indoor and outdoor air pollutants, including toxicants from combustion, smoking, cleaning products, personal care products, and industrial materials are likely to be prominent in fabrics exposed to corresponding sources.

In contrast to environmental chemicals that inadvertently contaminate fabrics, some consumer products, such as laundry detergent, perfume, and protective finishing, are designed to intentionally deposit semi- and non-volatile compounds onto fabrics (Corea *et al*. 2006; Iamazaki & Atvars, 2006; Iamazaki & Atvars, 2007). Hence, the characterization of chemical exposure from fabrics in real-world scenarios becomes increasingly complicated.

1.5 Specific Aims of Research

In this study, the transfer of optical brightening agents from new and laundered clothing to the adjacent skin is of particular interest. OBAs are known constituents of most laundry detergents and stain removers, typically formulated to concentrations ranging from 0.5 to 7,200 μg/g of detergent (Shu & Ding, 2005). However, the amount of OBAs that actually sorb to fabrics during the washing process is largely unknown, with the best estimate being anywhere from 20-95% of the dispensed mass (Poiger *et al.*, 1997; Stoll & Giger, 1998). The wide range stems mostly from variability in the laundry mass to water volume ratio, OBA detergent concentration, detergent volume used, wash water quality and temperature, as well as the initial loading of OBAs in the fabric material itself.

The ultimate goal of this study is to narrow the existing data gap by quantifying the rate at which a non-volatile OBA will transfer from clothing into skin in a controlled environment. The direct measurement of chemical transfer allows for the empirical determination of flux to and through the skin (mass/area/time) and a corresponding fabric-to-skin mass transfer rate (k_F). This, in turn, enables the calculation of an average daily dose (ADD) for the tested compound under the defined exposure scenario. It is hypothesized that the fabric-to-skin pathway may be a significant route of exposure in both occupational and non-occupational settings.

2.0 Study Design & Methodology

The study design took a two-phase approach, described hereinafter as ‘Phase I’ and ‘Phase II’.

2.1 Phase I

Phase I sought to quantify the mass loading of OBAs in clothing before and after laundering treatments, and was designed to emulate a common real-world exposure scenario. Phase I was conducted in the Seventh Generation® laboratory located in Burlington, Vermont, under the supervision of Research Manager, Cara Bondi. Impact Analytical® (Midland, MI), a commercial analytical service provider, was hired to perform chemical analyses.

2.1.1 Materials

The Seventh Generation laboratory is equipped with a Whirlpool® Top Loader High Efficiency washing machine. Laundry detergent was obtained from Seventh Generation (Natural Liquid Laundry Detergent – Free & Clear, Lot #23-077A) and the Tinopal CBS-X® (distyryl biphenyl (DSBP); CAS# 38775-22-3) was provided by BASF Corporation® (Wyandotte, MI). Tinopal

CBS-X was selected for this study at the recommendation of Clement Choy, Research Director at Seventh Generation, due to it being one of the most widely used OBAs in commercial laundry detergents. Consumer fabrics tested in this experiment were purchased from retailers located in the Burlington Town Center Mall in Burlington, Vermont on the day of the experiment (November 11th 2014). Three different types of fabric were selected to test an association between fabric type and OBA absorption. Fabrics selected for testing were: 100% Egyptian cotton pillowcases from Pottery Barn® (white); 60% cotton and 40% polyester blend Women's sleep-shirt from Victoria's Secret® (white); 100% polyester Men's athletic jerseys from Jim's Sports® (white). Fabrics marketed as being "white" were selected in order to measure the manufactured OBA concentration in new, unwashed items. Methanol (Certified ACS 99.9%) was used as the extraction solvent, and sterilized vials were used to collect treated fabric samples. Both the solvent and vials were purchased from Fisher Scientific (Waltham, MA).

2.1.2 Methods

To test the effect of laundering on optical brightener concentration in consumer fabrics, samples of each fabric were collected after each of the following treatments: 1) unwashed; 2) washed once with no detergent; 3) washed once using detergent without optical brighteners; and 4) washed once with detergent containing a known amount of Tinopal CBS-X. Consumer fabrics were treated in the sequence outlined in Table 2. After the 4th (and final) treatment in a fabric series, the washing machine was run for two consecutive clean cycles to remove any residual optical brightener from the washing machine. The inside of the washer basin was visually inspected with UV-light to ensure removal.

Immediately following each laundering treatment, eight 1-in^2 sample pieces were cut from the fabric. Three of the eight samples were placed individually in an 11 mL vial containing 10 mL of methanol. The remaining five samples were weighed to calculate the average fabric mass. Background water samples were collected in the morning and evening on each day of the experiment (November 11th - 13th, 2014). Machine effluent water samples were collected directly from the washing machine's drainpipe during the final two minutes of each Spin cycle. All water samples were kept at 4°C until analysis.

Table 2. Phase I study design and fabric sampling plan.

			Laundering Treatment (in sequential order)			
			1st	*2nd*	*3rd*	*4th*
	Item Description	**Fabric Type**	0 wash cycles	1 wash cycle[a], no detergent	1 wash cycle[a], with detergent[b]	1 wash cycle[a], detergent w/ OBA[c]
1st	Pillowcases	100% cotton	x3	x3	x3	x3
2nd	Women's shirt	60% cotton/ 40% polyester	x3	x3	x3	x3
3rd	Men's shirt	100% polyester	x3	x3	x3	x3
		(N = 36)	**9**	**9**	**9**	**9**

[a] Wash cycle settings: Heavy Duty (52 minutes); Cold Water (average: 25°C); Light Load
[b] 1 fl. oz. of Seventh Generation Natural Liquid Laundry Detergent, Free & Clear
[c] 1 fl. oz. of Seventh Generation Natural Liquid Laundry, Free & Clear spiked with 0.5% wt/wt Tinopal CBS-X (delivering ~ 150 mg of Tinopal CBS-X per 1 fl. oz. detergent)

All fabric and water samples (n = 51; 36 fabric samples in methanol; 9 machine effluent water samples; 6 background water samples) were shipped on ice to the laboratory at Impact Analytical with the intention of being chemically analyzed for a suite of semi-volatile compounds, including Tinopal CBS-X. One background water sample was lost in transit.

2.1.3 Analysis

Technicians at Impact Analytical analyzed the 51 samples using gas-chromatography and mass spectrometry (GC-MS). A report on the GC-MS analysis was issued by Impact Analytical, providing an index of chromatographs and a table of the presence or absence of volatiles. The detailed description of laboratory protocols is included in the GC-MS is included in the report (Report Number R140688).

The samples were later re-analyzed using high-performance liquid chromatography (HPLC), a more suitable method for characterizing low-volatility compounds, such as Tinapol CBS-X.

2.2 Phase II

Phase II of the study aimed to measure the rate at which 7-hydroxycoumarin (an optical brightening agent common to the textile manufacturing industry) transfers from clothing to and through human skin in a controlled environment. This *in vitro* mass-transfer experiment was conducted at the University of Washington, in Seattle, Washington, under the advising of Dr. John C. Kissel, in affiliation with the Department of Environmental & Occupational Health Sciences in the School of Public Health.

2.2.1 Materials

[^{14}C]-7-hydroxycoumarin ([^{14}C]-7H) dissolved in ethanol as a solution was obtained from American Radiolabeled Chemicals, Inc. (St. Louis, MO). The manufacturer reported a specific activity of 56 mCi/mmol at the time of shipping. Split-thickness human cadaver skin samples were obtained from National Disease Research Interchange (NDRI) (Philadelphia, PA). The two skin samples received were from Caucasian males, aged 65 and 68 years old at time of death. Skin

samples were from the back and abdomen, respectively. The skin was stored at -20°C until use (1-3 weeks).

Fabric samples were obtained from used clothing purchased from Goodwill Industries® (Seattle, WA). To test the association between fabric type and chemical flux to and through the skin, three different fabric types were selected for the experiment. Non-white fabrics were selected in order to avoid background OBA interference, as adequate OBA application was confirmed using UV-light. The purchased items were a 100% cotton Men's T-Shirt (blue), a 60% cotton/40% polyester Women's T-Shirt (blue), and a 100% polyester Men's Athletic Shirt (red). Soluene-350 Tissue Solubilizer™ was used to dissolve skin samples. Scintillation cocktails Hionic-Fluor™, and Ultima Gold™ were used for non-aqueous and aqueous samples, respectively. Scintillation vials (40 mL) were used to capture all radioactive material at the end of experimental trials. Radioactivity in each vial was measured using an on-site LS 6000 Scintillation Counter (LSC) (Beckman Instruments Inc., Fullerton, CA).

2.2.2 Methods

Experimental trials were performed in an environmental chamber constructed in-house with materials sourced from Permegear® (Hellertown, PA). The chamber apparatus was specifically designed for *in vitro* dermal exposure studies using Permegear® designed Teflon™ diffusion cells (Figure 1). In general, diffusion cell experiments using a radiolabeled tracer compound are an accepted method for studying percutaneous penetration and similar methods have been used elsewhere (Hoang, 1992; Duff & Kissel, 1996; Romonchuk & Bunge, 2006; Odendorf *et al.*, 2013; Moore *et al.*, 2014; Salocks *et al.*, 2014; Peckham *et al*., 2015). The environmental chamber setup allowed for twelve cells to undergo exposure per experimental trial. The order in which samples

were run was randomized using the random number generator function in Excel™. Positive and negative controls (*i.e.* blanks) were used for each experimental trial.

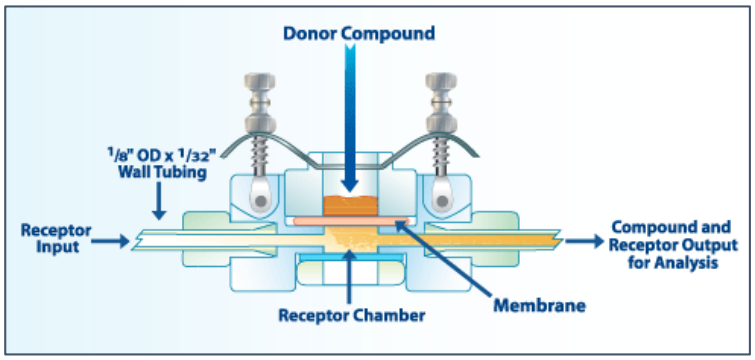

Figure 1. Cross-sectional view of Permagear ® In-Line Cell™
Image source: http://permegear.com/inline.htm

The experiments were designed to test a 3-way interaction between fabric type, fabric mass loading, and skin donor. Triplicate combinations of the three fabric types, two skin donors, and two mass loadings of [^{14}C]-7H yielded the sample size of 36 summarized in Table 3.

Table 3. Phase II study design and sampling plan.

		Fabric Type		
Skin Donor	**Mass Loading**	100% cotton	60% cotton/ 40% polyester	100% polyester
Donor 'A'	Low	x3	x3	x3
	High	x3	x3	x3
Donor 'B'	Low	x3	x3	x3
	High	x3	x3	x3
		Total N = 36		

Mass loadings were selected based on criteria of utility and ease of comparison. The low mass loading was targeted to ensure clearance above the LSC background. The high mass loading was to be one order of magnitude higher. Considering that the concentration of OBAs in unwashed clothing has been reported to range from 8.0 ng/g to 118 µg/g (Chen & Ding, 2006; Sh & Ding, 2009), the two target mass loadings were set to be 10 and 100 ng/0.64cm^{2a} (Table 4). These mass

loadings, estimated to produce concentrations of approximately 0.8 and 8.0 μg/g, respectively, were deemed realistic relative to real-world scenarios. Table 4 illustrates the conversations between fabric concentration and mass loading.

Table 4. Determination of Phase II mass loadings and fabric concentration by fabric type.

	Low			**High**		
	Cotton	*Blend*	*Polyester*	*Cotton*	*Blend*	*Polyester*
Fabric concentration (μg/g)	0.80	0.90	0.78	7.7	9.0	7.5
Fabric mass/area (mg/cm^2)	20.2	17.4	20.9	20.2	17.4	20.9
Mass loading (ng/cm^2)	16	16	16	156	157	157
Target mass per 0.64 cm^{2a} (ng)	10	10	10	100	100	100

[a] 0.64 cm^2 is the area of the diffusion cell aperture (*i.e.* the fabric-skin-receptor fluid interface)

Ethanol was used as the vehicle for delivering [^{14}C]-7H to the fabric. It was discovered, however, that each fabric type had unique wicking behaviors, so the concentration of [^{14}C]-7H in ethanol needed to be unique to each fabric type in order to meet the same target mass loading (Table 5). The volume of ethanol (in mL) that saturated a 0.64 cm^2 area on the center of each fabric type was determined experimentally using a fluorescent tracer solution. This approach would ensure—with reasonable confidence—that the [^{14}C]-7H delivered to the fabric samples would provide full coverage without spreading beyond the 0.64 cm^2 skin area exposed to receptor fluid (Figure 1). This required six unique [^{14}C]-7H in ethanol stock solutions and associated delivery volumes to allow for constant mass (10 or 100 ng) delivery to each fabric type (Table 5).

Table 5. [^{14}C]-7-hydroxycoumarin stock solution concentrations required for each fabric type to meet target mass loadings.

	Low			**High**		
	Cotton	*Blend*	*Polyester*	*Cotton*	*Blend*	*Polyester*
Stock solution solvent radioactivity (mCi/mL)	2.0E-04	2.2E-04	2.5E-04	1.8E-03	2.2E-03	2.3E-03
Stock solution mass (μg)	2.9	3.2	4.3	26	32	35
Stock solution concentration (ng/μL)	0.58	0.64	0.72	5.1	6.2	6.8
Delivery volume (μL)	20	17	15	20	17	15
Target mass (ng)	10	10	10	100	100	100
Stock concentration (ng/μL)	**0.50**	**0.59**	**0.67**	**5.0**	**5.9**	**6.7**

On the day preceding an experimental trial, skin samples were cut to 2 – 2.25 cm^2 for each diffusion cell, and heat separation of the skin was performed so that only the stratum corneum could be used as the membrane. The skin was set in the diffusion cell and allowed to condition in the environmental chamber for 20 to 24 hours while a receptor fluid was pumped through the cells at 10.1 uL/min. Temperature and relative humidity (RH) were controlled at 32°C and 40%, respectively. Preparation of fabric samples involved cutting out 4.4 cm^2 squares (the interior surface area of the diffusion cell base) from the clothing items.

After the chamber conditioning period, the [^{14}C]-7H solutions were dispensed to the center of the cotton, cotton-polyester blend, and polyester fabrics in 20 μL, 17 μL, and 15 μL aliquots, respectively, and allowed to dry completely under a fume hood for 60 minutes. The dry, loaded fabric samples were then placed on top of the skin membrane in the diffusion cells. Three positive controls (loaded fabric samples) were made for each diffusion cell. Positive controls were put into

scintillation cocktail immediately after the 60-minute drying period, and stored until the end of the trial.

The exposure period ran for 24 hours. During which, a receptor fluid (RF) consisting of deionized (DI) water, phosphate buffer solution (0.138 M NaCl, 0.0027 M KCl, pH 7.4; Sigma-Aldrich P3813) and bovine serum albumin (Sigma-Aldrich A2153)—intended to emulate human blood—was pumped through the underbelly of the diffusion cell at a flow rate of 25 μL/min as done in similar *in vitro* methodologies (Peckham *et al.,* 2015). The receptor fluid from each diffusion cell was collected in vials inside the environmental chamber.

Following the exposure period, diffusion cells were disassembled and sample components were collected in scintillation vials. Cotton swabs were used to recover any [^{14}C]-7H that may have migrated to the donor chamber surface, the diffusion cell base, or the external surface of the skin (see Appendix 7.A: *Table 3A. Wipe Protocol Details*). Tweezers used to transfer fabric and skin samples to the scintillation vials were rinsed in the Hionic Fluor™ to recover any mass lost during the transfer. Fabric samples that were observed to have leaked or become wet during the exposure period were reported as failed cells and removed from the dataset. Following the solublization of the skin samples, all vials were run through a Liquid Scintillation Counter (LSC) to count the radioactivity present in each vial.

A detailed account of the materials, methods, and lessons learned from Phase II is documented in the Standard Operating Procedure (SOP) included here as Appendix 7.A.

2.2.3 Analysis

Radioactivity contained in each vial was counted by the LSC and recorded as disintegrations per minute (dpm). Vials that were counted at a quench value of 170.0 or above and 100.00 or below were considered unreliable (according to manufacturer guidelines) and the corresponding sample as removed from the dataset.

Raw data were transcribed into Excel™ and converted into mass using the specific activity and molecular weight of [^{14}C]-7H. A mass balance was calculated for each sample using the equation:

$$Mass\ Balance\ (\%) = \frac{F + D + W + CB + S + RF + T1 + T2}{M} \times 100$$

Where:

M = Mass loaded at the start of the trial (ng)
F = Mass remaining in the fabric after the trial (ng)
D = Mass recovered from the donor chamber swabs (ng)
W = Mass recovered from skin surface swabs (ng)
CB = Mass recovered from cell base swabs (ng)
S = Mass recovered in solubilized stratum corneum (ng)
RF = Mass recovered from receptor fluid (ng)
T1 and T2 = Mass recovered from tweezers used during breakdown (ng)

Fluxes (J) to and through the skin were calculated for each sample using the respective equations:

$$J_{skin} = \frac{S}{A \times T} \quad \text{and} \quad J_{blood} = \frac{RF}{A \times T}$$

Where:

J_{skin} = Flux to the skin (ng/cm^2/hr)
J_{blood} = Flux through skin into the receptor fluid (ng/cm^2/hr)
A = Area of the fabric and skin interface exposed to receptor fluid (0.64 cm^2)
T = Exposure time (hours)

The average flux was then computed for each test combination. Interactions between the fabric type, skin donor, and mass loading on flux to and through skin were analyzed using 2- and 3-way ANOVA regression models in STATA™.

Fabric-to-skin and fabric-to-blood mass transfer rates were calculated using the respective equations:

$$k_S = \frac{J_{skin}}{\frac{C}{FW/FT}} \quad \text{and} \quad k_{RF} = \frac{J_{blood}}{\frac{C}{FW/FT}}$$

Where:

k_S = Fabric-to-skin mass transfer rate (cm/hr)
k_{RF} = Fabric-to-blood mass transfer rate (cm/hr)
FW = Fabric weight (g/cm^2)
FT = Fabric thickness (cm)
C = Concentration in fabric (μg/g)

The overall fabric-to-skin and blood mass transfer rate ($k_{Foverall}$) would then be defined as:

$$k_{Foverall} = \frac{1}{\frac{1}{k_S} + \frac{1}{k_{RF}}}$$

The *Dermal Exposure Assessment: Summary of EPA Approaches* (U.S. EPA, 2007) provides guidance on calculating average daily dose (ADD) and daily absorbed dose (DAD). However, neither model considers textiles as a potential source of exposure. In order to incorporate fabric-matrix effects, the model was adjusted to include the fabric weight (FW) and fabric thickness (FT).

The average daily dose (μg/kg/day) of a chemical absorbed from clothing would therefore use the following equation:

$$Dose = \left(\left(\frac{FW}{FT}\right) \times C \times k_{RF}\right) \times \left(\frac{SA}{BW}\right) X\ EF$$

Where:

SA = Surface area of the skin in contact with fabric (cm^2)
BW = Body weight (kg)
EF = Exposure frequency (hr/day)

Empirical measurement of flux to the receptor fluid allows for dose to be estimated using the a more simplified equation:

$$Dose = J_{blood} \times \left(\frac{SA}{BW}\right) \times EF$$

3.0 Results

3.1 Phase I

The report issued by Impact Analytical® (#R140688T) provided tables and chromatographs of volatiles detected in each sample by GC-MS. Neither Tinopal CBS-X nor any other OBA were detected by the GC-MS. Most samples did not show the presence of volatiles above background and no difference in volatile presence/absence was observed across the laundering treatments. Only fabric samples from the 100% polyester Men's T-shirt reported the presence of phthalates. Dimethyl terephthalate was detected in all of the polyester fabric samples, regardless of laundering treatment.

Impact Analytical re-analyzed the samples using HPLC and was provided a standard for Tinopal CBS-X in order to quantify the mass in each sample—as opposed to mere presence/absence. After running the Tinopal CBS-X standard through the HPLC, Impact Analytical verbally reported a detection limit of 10 ppm and that all samples were below the detection limit and no signal in the

MS was observed. Signals were observed in the UV monitor, but no report was issued. Results from Phase I were deemed inconclusive.

3.2 Phase II

The Phase II experimental trials ran for a period of three weeks. Each week, 12 diffusion cells underwent the *in vitro* dermal exposure trial. Of the 36 samples, two failed due to receptor fluid leaking through the skin membrane onto fabric. All results reported below are from the final dataset (n = 34).

3.2.1 Mass Balance

Table 6 shows the average mass balances of the replicated samples from each test combination. The average mass recovered from each diffusion cell component of the experimental trial is reported in nanograms (ng) of [^{14}C]-7H. Across all 34 samples, the mean mass balance was 94%, with a range of 82% to 106%. The initial mass loading was taken to be the average of mass loadings measured in the corresponding positive controls. It is important to note that the measured mass loadings were lower than the targeted mass loading (Table 5).

Table 6. Average mass balances for each test combination.

	n	M	D	W	CB	F	S	RF	Mass Balance (%)
High Mass Load	18	74	0.23	0.62	0.02	64	3.3	1.2	93
Cotton	6	73	0.04	0.14	0.02	64	0.97	0.78	90
Blend	6	76	0.03	0.21	0.01	69	3.8	0.28	98
Polyester	6	73	0.64	1.5	0.03	57	5.2	2.4	92
Low Mass Load	16	9.0	0.02	0.07	0.07	7.5	0.60	0.16	94
Cotton	6	9.9	0.003	0.03	0.11	8.5	0.38	0.20	93
Blend	5	8.6	0.01	0.01	0.10	7.5	0.57	0.10	96
Polyester	5	8.2	0.06	0.18	0.01	6.4	0.91	0.18	94

M = mass loaded at the start of the trial; D = mass recovered from the donor chamber swabs; W = mass recovered from skin surface swabs; CB = mass recovered from cell base swabs; F = mass remaining in the fabric after the trial, S = mass recovered in solubilized stratum corneum; RF = mass recovered from receptor fluid. Note: T1 and T2 recovered negligible amounts of [^{14}C]-7H and are therefore not shown above.

3.2.2 *Analyses of Variance (ANOVA) Results*

A 3-way ANOVA analysis was run on the full dataset (n = 34) to examine the effects of skin donor, fabric type, and mass loading on flux. No significant interaction was found between skin donor and either flux to skin ($F(1, 22) = 0.53$, $p = 0.47$), or flux to receptor fluid ($F(1,22) = 1.3$, $p = 0.26$). Therefore, the two skin types were pooled for the remaining analysis.

A two-way ANOVA was run on the dataset of 34 to examine the effects of mass loading and fabric type on flux. No significant interactions between fabric type and flux to skin ($F(2, 28) = 2.2$, $p = 0.14$), or flux to receptor fluid ($F(2, 28) = 2.7$, $p = 0.09$) were observed. However, a significant effect ($p < 0.05$) was found between mass loading and flux to skin ($F(1, 28) = 7.9$, $p = 0.009$) as well as flux to receptor fluid ($F(1, 28) = 6.3$, $p = 0.02$). Raw ANOVA outputs from STATA™ are included as Appendix 7.B.

3.2.3 Flux Into and Through Skin

The data reveal that 7-hydroxycoumarin is capable of migrating out of fabric material into and through the skin after 24 hours of direct skin-to-fabric contact. Tables 7 and 8 show mean and median results stratified by mass loading and fabric type. Table 7 reports flux (pg/cm^2/hr) in terms of gross dose, combining the mass transferred into the skin and into the receptor fluid. Table 8 examines flux (pg/cm^2/hr) as the actual absorbed dose, representing only the mass recovered in the receptor fluid.

Table 7. Distribution of 7-hydroxycoumarin flux from fabric to and through skin (pg/cm^2/hr).

	n	**Median**	**Mean**	**CV[a]**
High Mass Loading	18	138	217	1.20
Cotton	6	55.4	63.6	0.73
Blend	6	163	247	1.12
Polyester	6	198	341	0.95
Low Mass Loading	16	22.9	39.6	1.04
Cotton	6	21.5	24.7	0.90
Blend	5	38.3	37.4	1.09
Polyester	5	24.1	59.6	0.95

[a] *Coefficient of variation (standard deviation/mean)*

Table 8. Distribution of 7-hydroxycoumarin flux from fabric through skin (pg/cm^2/hr).

	n	**Median**	**Mean**	**CV[a]**
High Mass Loading	18	11.2	76.0	1.51
Cotton	6	15	51.0	1.37
Blend	6	6	18.6	1.65
Polyester	6	18	133	1.22
Low Mass Loading	16	9.5	10.5	0.61
Cotton	6	14	12.8	0.50
Blend	5	5.8	6.6	0.70
Polyester	5	8.3	11.3	0.50

[a] *Coefficient of variation (standard deviation/mean)*

The full range of values calculated for fluxes to and through skin are displayed in Figures 2 and 3 in units of ng/cm^2/hr. Median values are demarcated by the centerline inside each boxplot. The inner box area defines the 25 – 75% quartile ranges. The dots represent outliers in the dataset.

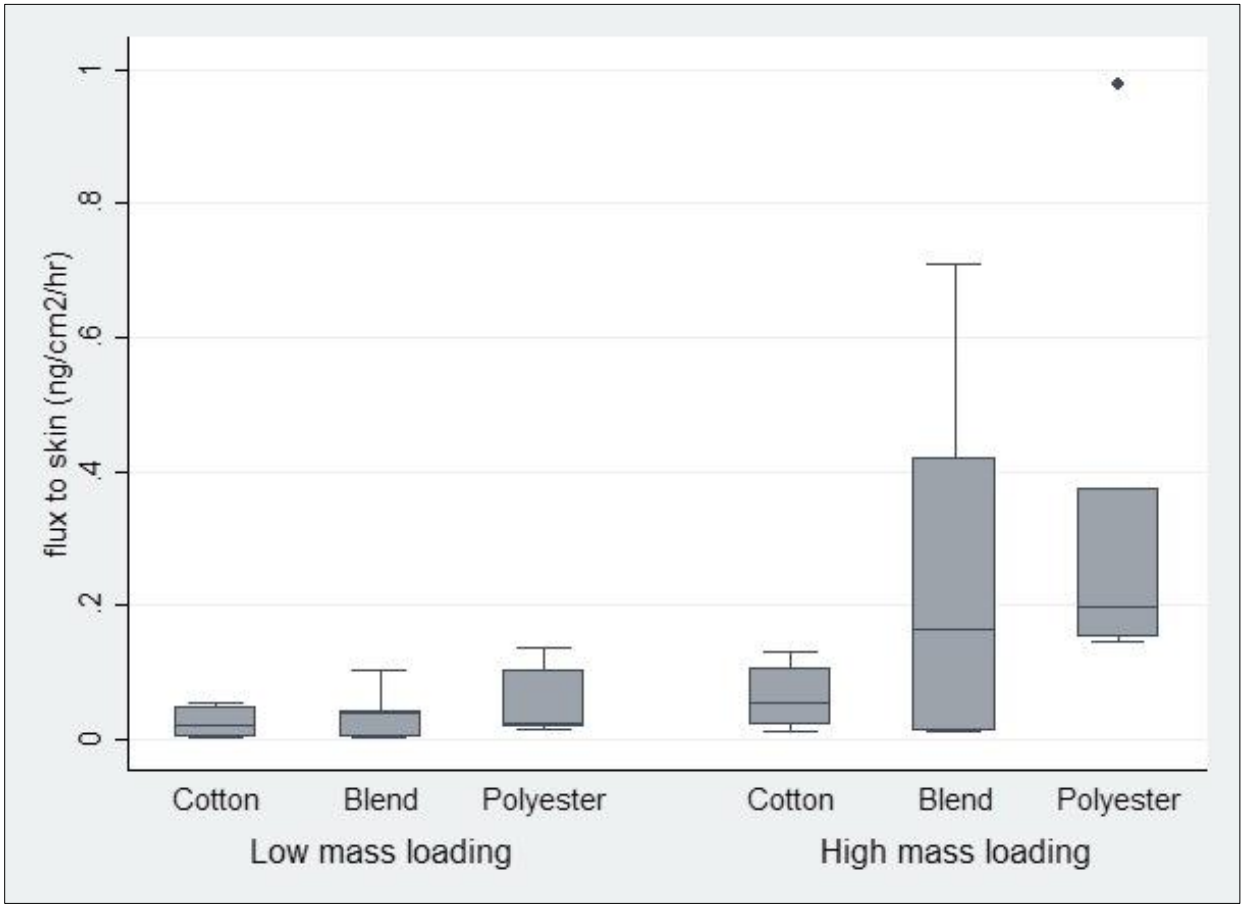

Figure 2. Plot of flux to skin (ng/cm^2/hr).

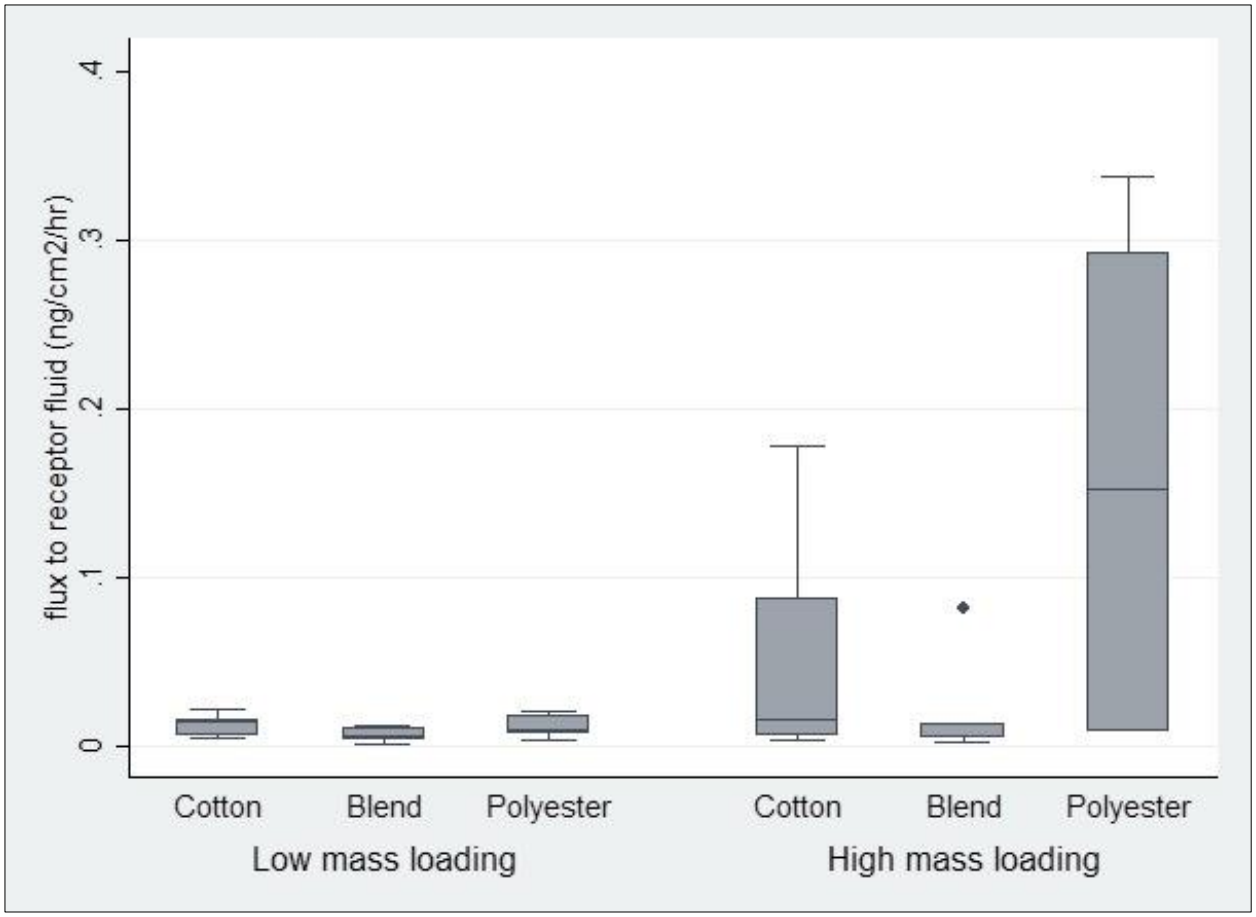

Figure 3. Plot of flux to blood (receptor fluid) (ng/cm^2/hr).

3.2.4 Mass Transfer Rates

Fabric-to-skin and fabric-to-blood mass transfer rates were calculated using the measured flux to skin and receptor fluid, respectively, assuming steady-state conditions. Table 9 breaks down the median mass transfer rates within each test combination. Fabric-to-skin mass transfer rates (k_S) represent the rate at which [^{14}C]-7H migrated from within the fabric matrix to the surface of the skin and penetrated into the stratum corneum. The fabric-to-blood mass transfer rates (k_{RF}) represent the rate at which [^{14}C]-7H was able to migrate from within the fabric matrix through the skin barrier and into the subcutaneous fluid (Table 9). Under the experimental conditions, the

fabric and skin were expected to be in direct physical contact for the duration of the 24 hour exposure period. Because of the very low vapor pressure of [^{14}C]-7H, it is most likely that transfer would occur due to physical contact between fabric and skin rather than [^{14}C]-7H volatilizing and moving through any miniscule air gap existing between the fabric and skin.

Table 9. Median mass transfer rates calculated from flux.

		Median Mass Transfer Rates (cm/hr)	
	n	K_{RF}	k_S
High Mass Loading	18	**3.8E-07**	**4.8E-06**
Cotton	6	5.3E-07	1.9E-06
Blend	6	2.0E-07	5.5E-06
Polyester	6	5.3E-06	6.9E-06
Low Mass Loading	16	**2.9E-06**	**6.7E-06**
Cotton	6	3.6E-06	5.5E-06
Blend	5	1.7E-06	1.1E-05
Polyester	5	2.6E-06	7.5E-06
Total:	**34**	**1.7E-06**	**5.5E-06**

The three fabric types tested were of comparable weights (0.02 g/cm^2) and thickness (0.1 cm), so a uniform fabric density of 0.20 g/cm^3 was applied to the mass transfer rate equation. Fabric concentrations of [^{14}C]-7H were calculated by converting the positive control measurements of mass loading into units of µg/g.

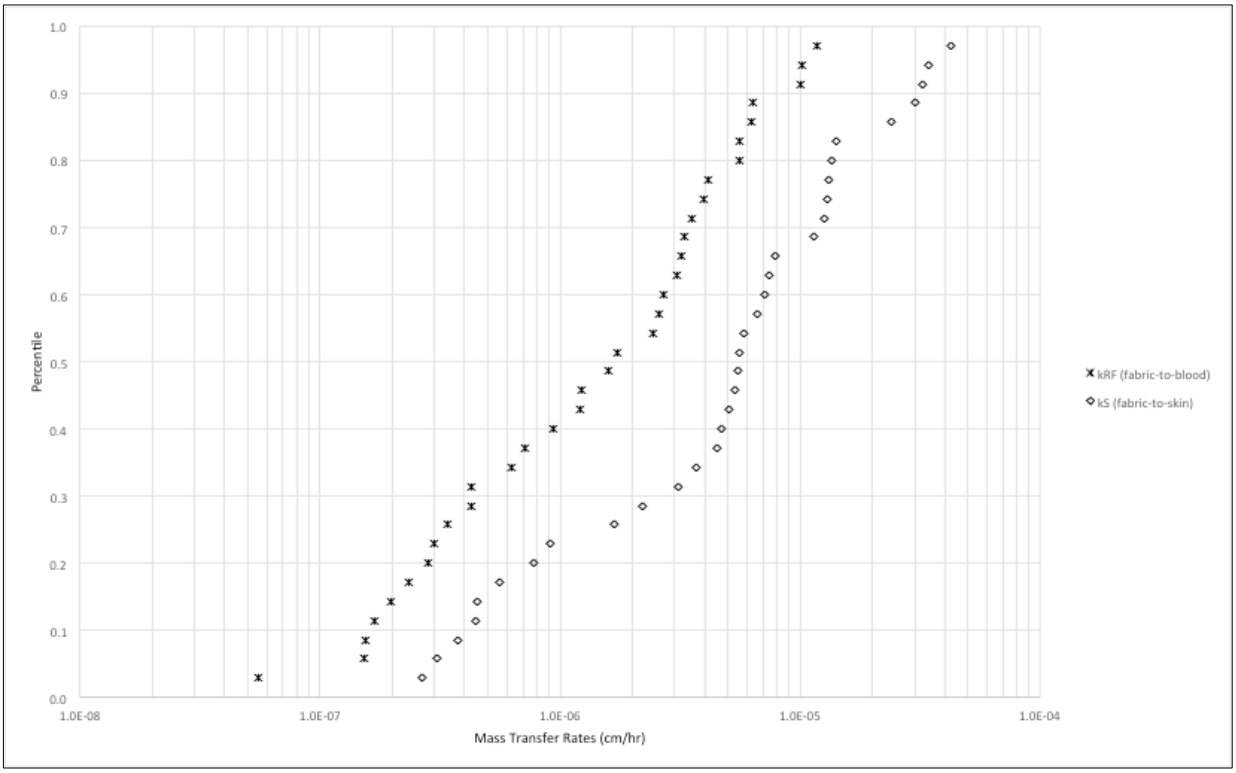

Figure 4. Cumulative frequency graph of calculated mass transfer rates (k_{RF} and k_S).

Figure 4 depicts the cumulative frequencies of mass transfer rates to and through the skin that were observed across the full eligible dataset (n = 34). Across the entire dataset, mass transfer rates from the fabric into the receptor fluid ranged from 5.6 x 10^{-8} to 1.2 x 10^{-5} cm/hr, with the median being 1.7 x 10^{-6} cm/hr. Mass transfer rates from the fabric to the skin ranged from 2.7 x 10^{-7} to 4.2 x 10^{-5} cm/hr, with the median being 5.5 x 10^{-6} cm/hr. As expected, it was observed that mass transfer from dry fabric to the skin occurs more rapidly than transfer from dry fabric into subcutaneous fluid.

3.2.5 Estimation of Daily Dose

Absorbed dose was calculated from the flux to receptor fluid (J_{blood}) results. Table 10 summarizes the daily absorbed dose estimations for infants, children, and adults in µg/day given the experimental conditions. Per U.S. EPA's *Exposure Factors Handbook, Chapter 7: Dermal*

Exposure Factors, people cover 62-97% of their body in clothing depending on seasonal weather conditions and temperature. The EPA also provides population averages for adult, child, and infant skin surface area to body weight ratios (m^2/kg) (U.S. EPA, 2011). Average body weights are assumed to be 80 kg for adults, 48 kg for children, and 8 kg for toddlers and infants (U.S. EPA, 2011). The dose calculations in Table 10 assume that skin-to-fabric contact remains constant throughout the 24-hour exposure frequency (as skin contact with fabrics is assumed to be virtually constant) with 70% of the body's surface area in direct contact with the loaded fabric.

Table 10. Daily absorbed doses (µg/day) of 7-hydroxycoumarin for infants, children, and adults extrapolated from the *in vitro* dermal exposure scenario.

	Adult (18+)[b]		**Child (2-17)[c]**		**Infant (< 2)[d]**	
Mass Loading	*High*	*Low*	*High*	*Low*	*High*	*Low*
Mean	24	4.0	21	3.6	5.3	0.9
Median	3.6	3.6	3.3	3.2	0.8	0.8
CV[a]	1.7	0.6	1.7	0.6	1.7	0.6
Minimum	0.6	0.5	0.6	0.5	0.1	0.1
Maximum	127	8.1	114	7.3	28	1.8

[a] *CV = coefficient of variation*
[b] *Assumes average body weight of 80 kg, per recommendation of U.S. EPA Exposure Factors Handbook, 2011*
[c] *Assumes average body weight of 48 kg, per recommendation of U.S. EPA Exposure Factors Handbook, 2011*
[d] *Assumes average body weight of 8 kg, per recommendation of U.S. EPA Exposure Factors Handbook, 2011*

Little to no variation in daily absorbed dose median values was observed across mass loadings. Under the Phase II exposure scenario, adults over the age of 18 are expected to receive an absorbed dose of 3.6 µg/day. Children ages 2-17 would absorb a dose of 3.2-3.3 µg/day, and infants under 2-years-old would likely absorb 0.8 µg/day (Table 10).

Table 11 uses flux to skin (J_{skin}) results to calculate an average daily exposed dose under the same model assumptions. As OBAs, such as 7-hydroxycoumarin, are potential skin sensitizers it is pertinent to quantify the dose delivered to the skin surface in addition to the dose likely to be absorbed by the vascular system.

Table 11. Descriptive statistics of daily dose (µg/day) of 7-hydroxycoumarin delivered to the skin for infants, children, and adults extrapolated from the *in vitro* dermal exposure scenario.

	Adult (18+)[b]		**Child (2-17)[c]**		**Infant (< 2)[d]**	
Mass Loading	*High*	*Low*	*High*	*Low*	*High*	*Low*
Mean	65	17	15	58	15	3.8
Median	49	15	14	44	11	3.5
CV[a]	1.1	5.7	6	1.1	1.1	5.7
Minimum	4.2	0.3	0.3	3.8	1.0	0.1
Maximum	267	368	329	239	61	84

4.0 Discussion of Results

4.1 Limitations to the in vitro *Study Design*

Although the study design was intended to reflect a real-world exposure scenario, there are several limitations in comparing an *in vitro* model to the actual human experience. For instance, the *in vitro* exposure model described above applied a known chemical in known quantities to three fabrics with similar densities. A single exposure period was observed under constant, stable environmental conditions. Only a small area of cadaver skin from two parts of the body was tested in the experiment. Contact time was continuous and pressure applied to the fabric was light and uniform. Together, the above conditions create a very over-simplified exposure scenario. As discussed in previous sections, fabrics can be impregnated with multiple chemicals in unknown quantities, and people interact with fabrics in numerous ways throughout a single day under fluctuating conditions.

4.2 Confounding Factors of Dermal Absorption

There may be several confounding factors to dermal absorption that were not investigated in this study. Fabric attributes—such as the weight, thickness, tightness of weave, and chemical treatment—have been shown to have an affect on mass transfer rates and flux to the skin (Snodgrass *et al.,* 1992; Obendorf *et al.,* 2003). Evaluating the influence of said properties was attempted in Phase I of the study, but inconclusive results rendered the analysis invalid. Under the experimental conditions of Phase II no statistically significant variation in flux was found among fabric type (cotton, polyester, and a cotton/polyester blend)—however it is likely that the non-significance is due to all tested fabric materials being of similar weight and thickness.

Skin conditions, such as moisture, temperature, pH, part of body, size and quantity of hair follicles, are also known to effect dermal absorption (Chang & Riviere, 1991; Von Goetz *et al.,* 2013; Pan *et al.,* 2014; Salocks *et al.,* 2014) yet the two skin donors used in the *in vitro* experiment did not create statistically significant variation in flux. This may be a consequence of using previously frozen, minimally-viable cadaver skin. Skin conditions were not formally evaluated in this experiment, nor were they included as a variable in the experimental trials. Therefore, the influence of various skin conditions on dermal absorption could not be investigated.

Finally, human activity such as physical movement, sweating, pressure, and friction, can further influence dermal absorption and mass transfer rates from fabric to skin (Yang & Li, 1993). However, consideration of these conditions were beyond the scope of this study.

4.3 The State of Flux

The *in vitro* model reported here assumes steady state diffusion—in which flux does not depend on time. In this study, the steady state assumption is thought to be realistic for several reasons. The first being that the radiolabeled compound, 7-hydroxycoumarin, is expected to achieve steady state diffusion relatively quickly (in roughly 4 hours) according to the methodology in the EPA's *Risk Assessment Guidance for Superfund, Appendix A34-A35* (U.S. EPA, 2004). Secondly, the mass balance measurements show that most of the chemical mass had remained on the fabric after 24 hours. Therefore, the available dose was not exhausted and the steady state assumption is further supported.

Depending on the compound, it is possible for *in vitro* experiments and the real-world to be in either a steady or non-steady state, hence the results from this study (as well as the assumptions made) can be compared to the real-world. In the real-world, fabrics can act as replenishing reservoirs for semi- and non-volatile compounds, and the available mass (or chemical load) in fabric may not actually diminish with time or use. In addition to the implanted additives and resins, fabrics can retain molecules derived from indoor and outdoor air pollution, hazardous work sites, household chemicals, wood and tobacco smoke, as well as a variety of additional sources. While the *in vitro* model provides a finite load of mass in the fabric available for transfer, humans may actually be exposed to an infinitely replenishing load of chemicals.

The mass of chemical loaded to the fabric was the only variable in this study found to have a significant influence on flux. However, fluxes were not affected proportionally to the mass loading. To investigate why this might be, maximum flux (J_{Max})—in units of ng/cm^2/hr—was

estimated from 7-hydroxycoumarin's skin permeability coefficient (K_p), given by the Potts-Guy Equation (Potts, 1992; U.S. EPA, 2007):

$$\log K_p = -2.8 + \log K_{o/w} - 0.0056\,(MW)$$

Where:

$K_{o/w}$ = octanol/water partitioning coefficient
MW = molecular weight (g/mol)

The Potts-Guy equation predicts the K_p for 7-hydroxycoumarin to be $9x10^{-3}$ cm/hr. J_{Max} was then estimated by multiplying the K_p value by the compound's water solubility. Because the water solubility of 7-hydroxycoumarin ranges from 1 to 10 g/L (depending on water temperature), a J_{Max} of 940 to 9,400 ng/cm^2/hr was predicted. Since the J_{Max} is much higher than any flux observed in this study, it is presumed that the fabric was not saturated and could not be the reason for the lack of proportional flux increase. The proportionality observed between flux-to-skin from fabric with low mass loadings and fabrics with high mass loadings was most likely affected by an uneven distribution (or incomplete coverage) of chemical mass on the fabric. This would suggest that a higher chemical loading would have lead to higher fluxes and higher doses.

4.4 Comparison of Results

Table 12 compares the results from this study with others that have investigated the fabric-to-skin pathway as a dermal exposure route for semi-volatile and low-volatility compounds. Studies presented in Table 12 represent other empirical evidence that dermal absorption of chemicals from fabrics can result in measurable—if not detrimental—doses. It also highlights the utility of flux and mass transfer rates as means to estimate average dermal dose rather than fractional absorption.

Additional studies (not listed in Table 12) have also attempted to quantify absorbed dose for other types of compounds found in clothing. For instance, Von Goetz *et al.* (2013) estimated a maximum absorbed dose of 1,300 μg/day in males and 600 μg/day in females who wore a shirt and pants containing up to 183 μg/g silver nanoparticles for 24 hours.

4.5 Significance of the Results

The *in vitro* dermal exposure experiment reported here reveals that 7-hydroxycoumarin, a water-soluble, low-volatility optical brightening agent, is capable of migrating out of dry fabric materials, penetrating the stratum corneum, and entering the bloodstream. This empirical evidence demonstrates that quantification of chemical transfer across from a dry fabric matrix into and through the human skin membrane is possible and reproducible. Utility of flux and mass transfer rates in the estimation of dose is a seemingly apparent improvement to the current dermal exposure assessment paradigm.

Results from this study indicate that physical contact with fabrics containing 6.0 ppm of OBA can result in a mass transfer of up to 200 – 300 pg OBA to 1 cm^2 of skin per hour (Tables 7 and 8). Of that, it was observed that 9 – 10 $pg/cm^2/hr$ were absorbed into the subcutaneous fluid, demonstrating that the unbroken skin layer does not prevent hydrophilic chemicals from passively diffusing across its membrane. In some aspects, this scenario would not be unusual in the real-world context. OBA concentrations applied in this study (actual fabric concentrations ranged from 0.6 – 6.0 μg/g) are well within the range others have found in retail-sourced, unlaundered clothing (Chen & Ding, 2006; Shu & Ding, 2009).

Table 12. Comparison of present study results to other measured and estimated fabric-to-skin transfer rates for semi-volatile compounds.

Reference	Study Type	Tested Fabric(s)	Compound	Mass Loading ($\mu g/cm^2$)	Exposure Period	Flux ($\mu g/cm^2/hr$)	k_F [b] (cm/hr)	Absorbed Dose ($\mu g/day$)	% Absorbed
Marks et al. 2015	*in vitro*	Cotton, Polyester, Cotton/Poly	7-hydroxycoumarin	0.016 0.16	24 hrs	$1.4x10^{-6}$ – $3.4x10^{-4}$ [a]	$5.6x10^{-8}$ – $1.2x10^{-5}$	0.6 – 127	1.0 – 5.6
Moore et al. 2014	*in vitro*	Consumer clothing	Chlorpyrifos	500	4 hrs	-	-	-	1.0
Salocks et al. 2014	*in vitro*	Cotton/Nylon	Methamphetamine-HCl	1.34	4 hrs	0.005 – 0.017 ($\mu g/hr$)	-	-	1.3
Obendorf et al. 2003	*in vitro*	Denim	Methyl parathion	3,155	8 hrs	-	-	-	1.4 – 2.6
Wester et al. 1996	*in vitro*	Cotton sheets	Glyphosate	-	24 hrs	0.05 – 0.5 ($\mu g/hr$)	$5.2x10^{-6}$ – $5.0x10^{-5}$	-	0.7 – 0.08
Rossbach et al. 2014	*in vivo*	Forestry-worker clothing	Permethrin	1250 – 1500 mg/m^2	8 hrs / 3 days	-	-	497	-
Rossbach et al. 2010	*in vivo*	Military uniforms	Permethrin	0.13	8 hrs / 28-48 days	-	-	< 400	1.0 – 2.0
Appel et al. 2008	*in vivo*	Military uniforms	Permethrin	1.25	8 hrs/ 14 - 28 days	-	-	265-329	-
Snodgrass et al. 1992	*in vivo**	Cotton & Nylon/Cotton	Permethrin	1.25	24 hrs / 7 days	-	-	-	0.5 – 1.8
Blum et al. 1978	*in vivo*	Polyester pajamas	*tris*-BP	-	8 hrs / 5 days	-	-	180	-

* *Animal (rabbit) study*

[a] *Minimum and maximum values of flux through skin (absorbed)*

[b] *Assumes steady state diffusion*

Dermal exposure to OBAs can cause mild to severe allergic reactions, including contact dermatitis, in certain populations (Hausen & Berger, 1989; Pan *et al.,* 2014). Specifically, Pan *et al.* (2014) showed that dermal exposure to 7-hydroxycoumarin significantly elevates transepidermal water loss (TEWL) and skin surface pH, thus damaging the protective barrier performance of the skin. Similar to the results reported here, Pan *et al.* (2014) observed when 7-hydroxycoumarin was delivered directly to mouse and porcine skin in a volatile solvent, a substantial flux can occur. Pan *et al.* (2014) measured fluxes of 107.5±16.5 nmol/cm^2/hr (~17,334 ng/cm^2/hr) in mouse skin and 13.7±1.9 nmol/cm^2/hr (~2219 ng/cm^2/hr) in porcine skin.

Although investigating the toxicological responses from dermal exposure to chemicals in clothing was beyond the scope of this study, others have demonstrated that the fabric-to-skin pathway can lead to an array of adverse health effects. Multiple cases of textile dermatitis from dyes and allergic reactions caused by formaldehyde in clothing have been reported (Osmundsen, 1969; Fowler *et al.*, 1992; Corea *et al*., 2006; Vocanson *et al*., 2007). From cases sited previously in which traces of PCP on cloth diapers killed several infants, and contaminated clothing caused multiple episodes of pesticide poisoning, it is evident that severe adverse health outcomes can result from dermal exposure to fabrics. Given the large surface area of the human body and the long exposure time typically experienced from wearing clothing and sleeping on linens, it is not surprising that the transferred mass could accumulate rather quickly. Even at very low mass transfer rates, chronic dermal exposure could lead to a large lifetime dose. As evident by the aforementioned cases, health outcomes from dermal exposure to fabrics appear to depend on the absorbed dose, the toxicological properties of the chemical, and the susceptibility of the individual.

5.0 Conclusion

Dermal absorption is a commonly underestimated—and oftentimes unaccounted for—route of exposure in most risk assessment models. This *in vitro* model provides a reasonably good method for measuring compound-specific flux and mass transfer from fabric to skin for predicting dose, however a single set of empirically-derived mass transfer rates is not enough to predict population level dermal exposure to chemicals from clothing. Further research is needed in order to understand fully the dermal exposure acquired from long-term contact with 'everyday' fabrics. In order to better understand and characterize chemical flux and mass transfer rates from clothing through skin, a comprehensive data bank of compound- and textile-specific flux and mass transfer rates will need to be developed. Further information is also needed on the sorption capacity of fabrics, the properties and concentrations of compounds in clothing material, as well as the effect of suspected confounding factors or effect modifiers, such as sweating, skin moisture and pH, and friction. In order to provide accurate description of the total human exposure to environmental

chemicals, there must be a commitment to the rigorous quantification of the fabric-to-skin pathway.

In lieu of immediately available information, it is expected that removing sources of toxic or irritating chemicals likely to sorb to clothing would mitigate the chemical loading of indoor fabrics. Other protective actions, such as repeated laundering of new fabrics and avoidance of impregnated textiles, are suggested to minimize contact with chemically laden fabrics and reduce flux from clothing to skin. However, pursuing the suggested areas of further research would elucidate the most effective actions for mitigating exposure to potentially harmful chemicals in the fabric-to-skin pathway.

6.0 List of References

Alexander BM & Baxter CS (2015) Plasticizer Contamination of Firefighter Personal Protective Clothing – A Potential Factor in Increased Health Risks in Firefighters. *Journal of Occupational and Environmental Hygiene*, 11(5): D43-D48.

Appel KE, Gundert-Remy U, Fischer H, *et al*. (2008) Risk assessment of Bundeswehr (German Federal Armed Forces) permethrin-impregnated battle dress uniforms (BDU). *International Journal of Hygiene and Environmental Health*, 211: 88-104.

Armstrong RW, Eichner ER, Klein DE, Barthel WF, Bennett JV *et al*. (1969) Pentachlorophenol poisoning in a nursery for newborn infants. II. Epidemiologic and toxicologic studies *Journal of Pediatrics*, 75(2): 317-325.

Avagyan R, Luongo G, Thorsén G, Östman C (2015) Benzothiazole, benzotriazole, and their derivates in clothing textiles--a potential source of environmental pollutants and human exposure. *Environ Sci Pollut Res Int.*, 22(8): 5842-9.

Bekö G, Weschler CJ, Langer S, Callesen M, Toftum J, *et al*. (2013) Children's Phthalate Intakes and Resultant Cumulative Exposures Estimated from Urine Compared with Estimates from Dust Ingestion, Inhalation and Dermal Absorption in Their Homes and Daycare Centers. *PLoS ONE:* 8(4).

Blum A (2007) The Fire Retardant Dilemma. *Letters in Science*, 318: 194.

Blum A, Gold MD, Ames BN, *et al*. (1978) Children Absorb Tris-BP Flame Retardant from Sleepwear: Urine Contains the Mutagenic Metabolite, 2,3-Dibromopropanol. *Science*, 201(4360): 1020-1023.

Brigden K, Hetherington S, Wang M, Santillo D, Johnston P (2014) Hazardous chemicals in branded luxury textile products on sale during 2013. Greenpeace Research Laboratories Technical Report 01/2014.

Canonica S, Kramer JB, Reiss D, Gygax H (1997) Photoisomerization Kinetics of Stilbene-Type Fluorescent Whitening Agents. *Environmental Science & Technology*, 31: 1754-1760.

Chang SK & Riviere JE (1991) Percutaneous Absorption of Parathion in Vitro in Porcine Skin: Effects of Dose, Temperature, Humidity, and Perfusate Composition on Absorptive Flux. *Fundamental and Applied Toxicology*, 17,494-504.

Chen HC & Ding WH (2006) Hot-water and solid-phase extraction of fluorescent whitening agents in paper materials and infant clothes followed by unequivocal determination with ion-pair chromatography-tandem mass spectrometry. *Journal of Chromatography A*, 1108: 202–207.

Clifford NJ & Nies AS (1989) Organophosphate poisoning from wearing a laundered uniform previously contaminated with parathion. *JAMA*, 262(21): 3035-3036.

Corea NV, Basketter DA, Clapp C, *et al*. (2006) Fragrance allergy: Assessing the risk from washed fabrics. Contact Dermatitis. 55: 48–53 [from Unilever]

Correia P, Cruz A, Santos L, Alves A (2013) Human dermal exposure to galaxolide from personal care products. *International Journal of Cosmetic Science*, 35, 299-309.

Duff RM & Kissel JC (1996) Effect of Soil Loading on Dermal Absorption Efficiency from Contaminated Soils. *J Toxicology and Environmental Health*, 48: 93-106.

Elsisi AE, Carter DE, & Sipes IG (1989) Dermal Absorption of Phthalate Diesters in Rats. *Fundamental and Applied Toxicology*, 12: 70-77.

Environmental Working Group (2014) Research: Consumer Products. Accessed May 7, 2014. http://www.ewg.org/research

Fitzgerald RH & Manley-Harris M (2005) Laundering Protocols for Chlorpyrifos Residue Removal from Pest Control Operators' Overalls. *Bull. Environ. Contam. Toxicol*., 75:94–101

Ford RA, Hawkins DR, Schwarzenbach R & Api AM (1999) The systemic exposure to the polycyclic musks, AHTN and HHCB, under conditions of use as fragrance ingredients: evidence of lack of complete absorption from a skin reservoir. *Toxicol. Lett.*,111(1–2), 133–142.

Fowler JF, Skinner SM, Belsito DV (1992) Allergic contact dermatitis from formaldehyde resins in permanent press clothing: An underdiagnosed cause of generalized dermatitis. *J Am Acad Dermatol*, 27:962-8.

Ganz CR, Schulze J, Stensby PS, Lyman FL, Macek K (1975) Accumulation and elimination studies of four detergent fluorescent whitening agents in bluegill (Lepomis macrochirus). *Environmental Science & Technology*, 9: 738-744.

Gong M, Zhang Y, Weschler CJ (2014) Predicting dermal absorption of gas-phase chemicals: transient model development, evaluation, and application. *Indoor Air*. 24: 292–306.

Hausen BM & Berger M (1989) The sensitizing capacity of coumarins (III). *Contact Dermatitis*, 21: 141-147.

Hoang KT (1992) Dermal Exposure Assessment: Principles and Applications. U.S. Environmental Protection Agency, Office of Health and Environmental Assessment, Washington, DC, EPA/600/8-91/011B.

Iamazaki ET & Atvars TD (2006) Role of Surfactants in the Sorption of the Whitening Agent Tinopal-CBS onto Viscose Fibers: A Fluorescence Spectroscopy Study. *Langmuir*, 22: 9866-73.

Iamazaki ET & Atvars TD (2007) Sorption of a Fluorescent Whitening Agent (Tinopal CBS) onto Modified Cellulose Fibers in the Presence of Surfactants and Salt. *Langmuir*, 23: 12886-92.

Kajiwara N & Takigami H (2013) Emission behavior of hexabromocyclododecanes and polybrominated diphenyl ethers from flame-retardant-treated textiles: *Environ Sci Process Impacts*, 15:1957-63.

Kissel, JC (2011) The mismeasurement of dermal absorption. *Journal of Exposure Science and Environmental Epidemiology*. 21, 302-309.

Korinth G, Schaller KH, Bader M, Bartsch R, Göen T, Rossbach B, Drexler H (2012) Comparison of experimentally determined and mathematically predicted percutaneous penetration rates of chemicals. *Arch Toxicology*, 86:423–430.

Kramer J B, Canonica S, Hoigne J, Kaschig J. Degradation of Fluorescent Whitening Agents in Sunlit Natural Waters. *Environmental Science & Technology*, 1996: 30: 2227-2234.

Laughlin JM, Easley CB, Gold RE, Tupy DR (1981) Methyl parathion transfer from contaminated fabrics to subsequent laundry and laundry equipment. *Bull. Environ. Contam. Toxicol.*, 27: 518-523.

Luongo G, Thorsén G, & Östman C (2014) Quinolines in clothing textiles—a source of human exposure and wastewater pollution? *Anal. Bioanal. Chem.*, 406: 2747–2756.

Moore CA, Wilkinson SC, Blain PG, Dunn M, Aust GA, Williams FM (2014) Use of a human skin in vitro model to investigate the influence of ‘every-day’ clothing and skin surface decontamination on the percutaneous penetration of organophosphates. *Toxicology Letters*, 229: 257–264.

Morgan M, Sheldon L, Thomas K, Egeghy P, Croghan C, Jones P, Chuang J, Wilson N (2008) Adult and children’s exposure to 2,4-d from multiple sources and pathways. J*ournal of Exposure Science and Environmental Epidemiology*, 1–9.

Obendorf SK, Csiszar E, Maneefuangfoo D, Borsa J (2003) Kinetic Transport of Pesticide from Contaminated Fabric Through a Model Skin. Arch. Environ. *Contam. Toxicol.* 45, 283–288.

Osmundsen PE (1969) Contact dermatitis due to an optical whitener in washing products. *British Journal of Dermatology*, 81: 799-803.

Pan TL, Wang PW, Aljuffali IA, Leue YL, Hung Y, Fang JY (2014) Coumarin derivatives, but not coumarin itself, cause skin irritation via topical delivery. *Toxicology Letters*, 226: 173–181.

Pan TL, Wang PW, Aljuffali IA, Hung Y, Lin CF, Fang JY (2014) Dermal toxicity elicited by phthalates: Evaluation of skin absorption, immunohistology, and functional proteomics. *Food and Chemical Toxicology*, 65: 105–114.

Peckham TK, Shirai JH, Bunge AL, Lowney YW, Ruby MV, Kissel JC. Dermal absorption of benzo[a]pyrene into human skin from soil: Effect of artificial weathering, concentration and exposure duration. *Master's Thesis*. University of Washington, Seattle, 2015.

Poiger T, Field J A, Field T M, Siegrist H, Giger W. Behavior of fluorescent whitening agents during sewage treatment. *Water Research* 1998: 32: 1939-1947.

Potts RO (1992) Predicting skin permeability. *Pharmaceutical Research*. 9(5): 663-669.

Romonchuk WJ & Bunge AL (2006) Permeation of 4-Cyanophenol and Methyl Paraben from Powder and Saturated Aqueous Solution through Silicone Rubber Membranes and Human Skin, *Journal of Pharmaceutical Sciences*, 95(11): 2526-2533.

Rossbach B, Appel KE, Mross KG, Letzel S (2010) Uptake of permethrin from impregnated clothing. *Toxicology Letters,* 192: 50–55.

Rowe HD (2006) Detergents, clothing and the consumer with sensitive skin. *International Journal of Consumer Studies,* 30: 369-377.

Rovira J, Nadal M, Schuhmacher M, Domingo JL (2015) Human exposure to trace elements through the skin by direct contact with clothing: Risk assessment. *Environmental Research*, 140: 308–316.

Salocks CB, Hui X, Lamel S, Hafeez F, Qiao P, Sanborn JR, et al. (2014) Dermal exposure to methamphetamine hydrochloride contaminated residential surfaces II. Skin surface contact and dermal transfer relationship. *Food and Chemical Toxicology*, 66:1–6.

Schettler T (2006) Human exposure to phthalates via consumer products. *International Journal of Andrology*. 29: 134-139.

Schorr WF; Keran E; Plotka E. 1974. The Quantitative Analysis of American Clothing for Free Formaldehyde and its Relevance in Clinical Practice. *Arch Dermatol.,* 110(1):73-76.

Senthilkumaran S, SathyaPrabhu K, Balamurugan N, Thirumalaikolundusubramanian P (2012) Deadly Drop—Cholinergic Syndrome From Wearing a Laundered Uniform. *Pediatr Emer Care,* 28: 57Y58.

Shu WC & Ding WH. 2009. Determination of Fluorescent Whitening Agents in Infant Clothes and Paper Materials by Ion-Pair Chromatography and Fluorescence Detection. *Journal of the Chinese Chemical Society*. 56(4).

Singer BC, Revzan KL, Hotchi T, Hodgson AT, Brown NJ (2004) Sorption of organic gases in a furnished room. Atmospheric Environment. 38, 2483–2494.

Snodgrass HL (1992) Permethrin transfer from treated cloth to the skin surface: Potential for exposure in humans. *Journal of Toxicology and Environmental Health,* 35(2): 91-105.

St. John LE (1976) Studies of possible absorption of a flame retardant from treated fabrics worn by rats and humans. *Bulletin of Environmental Contamination and Toxicology,* 15(2): 192-197.

Stana K, Pohar C, Ribitsch V (1995) Adsorption of whitening agents on cellulose fibers — Monitored by streaming potential measurements, calorimetry and fluorescence. *Colloid & Polymer Science,* 273: 1174-1178.

Steinmann AC (2009) Fragranced consumer products and undisclosed ingredients. *Environmental Impact Assessment Review* 29. 32–38.

Stoll, J. M. A., & Giger, W. (1998). Mass balance for detergent-derived fluorescent whitening agents in surface waters of Switzerland. *Water Research*, 32(7), 2041-2050.

U.S. Environmental Protection Agency (EPA). (2004) Risk Assessment Guidance for Superfund (RAGS), Volume I: Human Health Evaluation Manual (Part E, Supplemental Guidance for Dermal Risk Assessment) Interim. U.S. Environmental Protection Agency, Washington, DC, EPA/540/R/99/005. Online at http://www.epa.gov/oswer/riskassessment/ragse/index.htm

U.S. Environmental Protection Agency (EPA). (2007) Dermal Exposure Assessment: A Summary of EPA Approaches. U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-07/040F.

U.S. Environmental Protection Agency (EPA) (2011) Exposure Factors Handbook: Chapter 7 – Dermal Exposure Factors, 2011 Edition. National Center for Environmental Assessment, Washington, DC; EPA/600/R-09/052F. Available from the National Technical Information Service, Springfield, VA, and online at http://www.epa.gov/ncea/efh

Vocanson M, Valeyrie M, Rozieres A, Hennino A, Floch F, Gard A, Nicolas JF (2007). Lack of evidence for allergenic properties of coumarin in a fragrance allergy mouse model. *Contact Dermatitis*, 57: 361–364.

Von Goetz N, Lorenz C, Windle L, et al (2013) Migration of Ag- and TiO2- (Nano)particles from Textiles into Artificial Sweat under Physical Stress: Experiments and Exposure Modeling. *Environmental Science Technology*, 47: 9979-9987.

Weschler CJ & Nazaroff WW (2012) Review Article: SVOC exposure indoors: A fresh look at dermal pathways. *Indoor Air*, 22: 356-377.

Wester RC, Quan D, Maibach HI (1996) In vitro percutaneous absorption of model compounds Glyphosate and Malathion from cotton fabric into and through human skin. Food and Chemical *Toxicology*, 34: 731-735.

Wester RC, Quan D, Maibach HI. Percutaneous absorption of hazardous chemicals from fabric into and through human skin. In *Percutaneous Absorption*, 4th Ed., Taylor & Francis Group (2005); Boca Raton, FL; Chapter 22, pgs 303-310

Wijnhoven S, Kooi M, Biesebeek JD (2010) Consumer exposure to chemicals in indoor environment: A specific focus on chemicals from textile products. Prepared for: National Institute for Public Health and the Environment. Prepared by: RIVM, Centre for Substances and Integrated Risk Assessment. Report 320104010/2010.

Wilson NK, Chuang JC, Iachan R, Lyu C, Gordon SM, Morgan MK, *et al*. (2004) Design and sampling methodology for a large study of preschool children's aggregate exposures to persistent organic pollutants in their everyday environments. *J Expo. Anal. Environ. Epid.*,14: 260–274.

Yang Y & Li S (1993) Frictional transition of pesticides from protective clothing. *Arch. Environ. Contam. Toxicol.*, 25(2): 279-84.

7.0 Appendices A & B

APPENDIX 7.A

Standard Operating Procedure: Operation of Dermal Chamber for the Quantification of Radiolabeled Chemical Flux from Loaded Fabric to Human Cadaver Skin

7.A.1 Objective

To develop an appropriate and consistent manner to determine the average flux of chemical contaminants in loaded fabric across human epidermis using flow-through diffusion cells.

7.A.2 Materials

- Radiolabeled stock solution (*7-hydroxycourmin*)
- TraceClean® vials
- Dermal chamber
- Receptor fluid solution
- Fume hood
- Small glass beakers (10 ml, 25 ml, 100 ml)
- Scalpel
- Sterile tweezers
- Sterile metal trays
- ASTM Type I deionized water
- Pipettor
- Pipettes (5 ml, 10 ml)
- Micropipettor (5-40uL)
- Disposable Kimwipes
- 40mL Scintillation Vials (12 per run)
- Test tubes (13 per run)
- Scintillation cocktails:
    - Hionic-Fluor®
    - PerkinElmer Inc.
    - Ultima Gold®
- Mettler balance
- Liquid Scintillation Counter (Beckman LS 6000)
- Thermometer
- Hot plate
- Hot bath sonicator

7.A.3 Methodology

This SOP describes the protocol for an *in vitro* investigation of dermal absorption of radiolabeled chemical from loaded fabric using human cadaver skin and flow-through diffusion cells. Radioactivity is measured using a liquid scintillation counter. The radiolabeled chemical used here is 7-hydroxycoumarin (14-C). 7-hydroxycoumarin is a hydrophilic, non-volatile optical brightening agent.

This example applies three test fabric types (100% cotton, 100% polyester, 60/40% cotton/polyester), each loaded with a low and high mass loading of the radiolabeled compound, and an uniform exposure period of 24-hours. Two different skin sources (donors) were obtained.

This protocol is intended to be adaptable for other exposure scenarios and chemicals. Additional radiolabeled chemicals, test matrices, mass loadings, and exposure durations can be used in future studies.

7.A.3.1 Assembly of chamber apparatus

See Figure A-1 for main components and general set up of the dermal chamber apparatus within a laboratory fume hood:

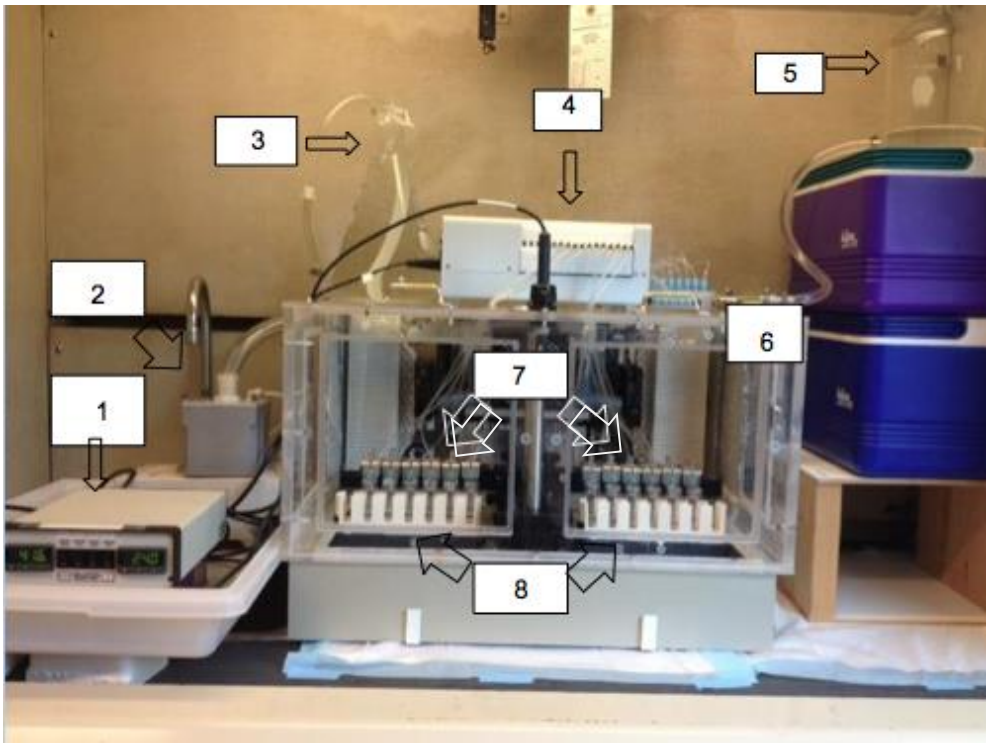

Figure A-1. Experimental apparatus for evaluating percutaneous absorption of chemical from matrix: (1) Controller (heat, humidity, etc.); (2) humidifier; (3) humidifier reservoir; (4) peristaltic pump; (5) receptor fluid reservoir; (6) dermal chamber; (7) diffusion cell racks with diffusion cells connected to tubing; (8) scintillation vial racks containing receptor fluid scintillation vials.

7.A.3.2 Labeling and weighing of vials

Vials and test tubes were labeled and dated as illustrated in Table A-1.

All empty 40 mL receptor fluid collection vials (#-R), skin solubilization test tubes (#-Sol) including a blank test tube, and solubilized skin aliquot scintillation vials (#-S) were weighed and masses recorded. Unloaded fabric samples were also weighed prior to trial. Table A-2 details the in-lab pre-trial preparation protocol.

Table A-1. Example List of vials/test tubes needed per run

ID	Description	Cell 1	Cell 2	Cell 3	Cell 4	Cell 5	Cell 6	Cell 7	Cell 8	Cell 9	Cell 10	Cell 11	Cell 12
#-T1*	tweezer rinse after fabric placement pre-run	1-T1	2-T1	3-T1	4-T1	5-T1	6-T1	7-T1	8-T1	9-T1	10-T1	11-T1	12-T1
#-D*	donor chamber wipe (2 swabs w/ acetone)	1-D	2-D	3-D	4-D	5-D	6-D	7-D	8-D	9-D	10-D	11-D	12-D
#-F*	fabric square with tweezer rinse	1-F	2-F	3-F	4-F	5-F	6-F	7-F	8-F	9-F	10-F	11-F	12-F
#-W1*	skin surface wipe 1 (water w/ 10% soap)	1-W1	2-W1	3-W1	4-W1	5-W1	6-W1	7-W1	8-W1	9-W1	10-W1	11-W1	12-W1
#-W2*	skin surface wipe 2 (water only)	1-W2	2-W2	3-W2	4-W2	5-W2	6-W2	7-W2	8-W2	9-W2	10-W2	11-W2	12-W2
#-W3*	skin surface wipe 3 (water only)	1-W3	2-W3	3-W3	4-W3	5-W3	6-W3	7-W3	8-W3	9-W3	10-W3	11-W3	12-W3
#-T2*	tweezer rinse after skin removal	1-T2	2-T2	3-T2	4-T2	5-T2	6-T2	7-T2	8-T2	9-T2	10-T2	11-T2	12-T2
#-C*	cell base swab	1-C	2-C	3-C	4-C	5-C	6-C	7-C	8-C	9-C	10-C	11-C	12-C
#-S**	solubilized skin aliquot	1-S	2-S	3-S	4-S	5-S	6-S	7-S	8-S	9-S	10-S	11-S	12-S
#-Ra*	receptor fluid aliquot	1-Ra	2-Ra	3-Ra	4-Ra	5-Ra	6-Ra	7-Ra	8-Ra	9-Ra	10-Ra	11-Ra	12-Ra
#-R	receptor fluid (40 mL)	1-R	2-R	3-R	4-R	5-R	6-R	7-R	8-R	9-R	10-R	11-R	12-R
#-Sol**	solubilized skin (test tube)	1-Sol	2-Sol	3-Sol	4-Sol	5-Sol	6-Sol	7-Sol	8-Sol	9-Sol	10-Sol	11-Sol	12-Sol
[Fabric-Conc]*	fabric positive controls 1	C-L1	B-L1	S-L1	C-H1	B-H1	S-H1						
[Fabric-Conc]*	fabric positive controls 2	C-L2	B-L2	S-L2	C-H2	B-H2	S-H2						
[Fabric-Conc]*	fabric positive controls 3	C-L3	B-L3	S-L3	C-H3	B-H3	S-H3						
[RM]*	[radiolabeled material solution]	[RM]1	[RM]2	[RM]3									

**20 mL scintillation vials*

***additional vial(s) required for blanks*

Table A-2. Pre-experimental trial preparation instructions

Vial ID	Vial Description	Instructions	Sequence
[all]		Label with ID and Date	Pre-Run Prep
S	skin aliquots	Weigh and record on mass sheet (including a blank)	Pre-Run Prep
R	receptor fluid	Weigh and record on mass sheet	Pre-Run Prep
Sol	skin solubilization test tubes	Weigh and record on mass sheet (including a blank)	Pre-Run Prep
F	fabric samples	Fill with 10mL of Hionic-Fluor®	Pre-Run Prep
[RM]	radiolabeled material solution	Take 3 aliquots of each concentration of radiolabeled solution. Refrigerate until LSC run.	Day of loading
n/a	unexposed fabric samples	Cut & weigh a matching fabric square (20 x 20 mm) for each fabric type used in trial. Then place on metal tray next to its fabric square match. Use micropipettor to add identical aliquot of radiolabeled solution to each pair. Set in hood and allow to dry completely.	Day of loading
n/a	fabric samples	Cut & weigh fabric squares (20 x 20 mm) of the fabric types dedicated by randomization	Day of loading

7.A.3.3 Preparation of receptor fluid

A 2L Erlenmeyer flask was filled with 1 liter of ASTM Type I deionized water, one packet of phosphate-buffered saline (PBS; 0.138 M NaCl, 0.0027 M KCl, pH 7.4; Sigma-Aldrich product id#: P3813) and 40 grams of bovine serum albumin (BSA; Sigma-Aldrich product id#: A2153). Solution was mixed on stir plate and magnetic stir bar for two hours. After mixing was complete, solution was run through a Corning 500 mL Bottle Top Filter (.45 µm CA, low protein binding, with 45 mm neck) into a 1L pyrex media bottle using a vacuum hose (Figure A-2).

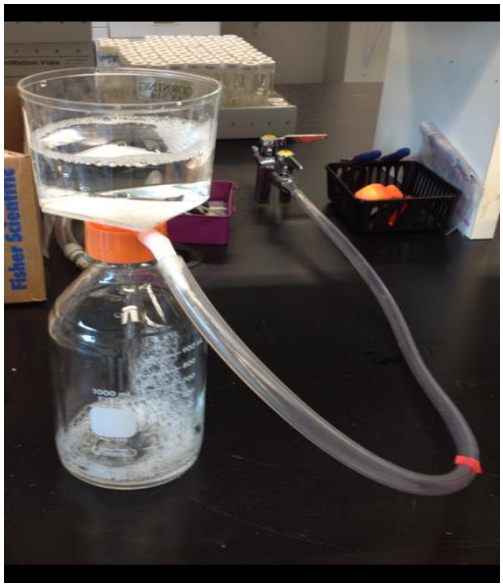

Figure A-2. Filtration of receptor fluid.

7.A.3.4 Preparation of skin samples and pre-exposure apparatus assembly

80 mL of DI water was heated in a 100 ml beaker to 60ºC. Frozen skin from donor(s) was cut using a scalpel or scissors into pieces ~2 cm x ~2 cm each (Figure A-3).

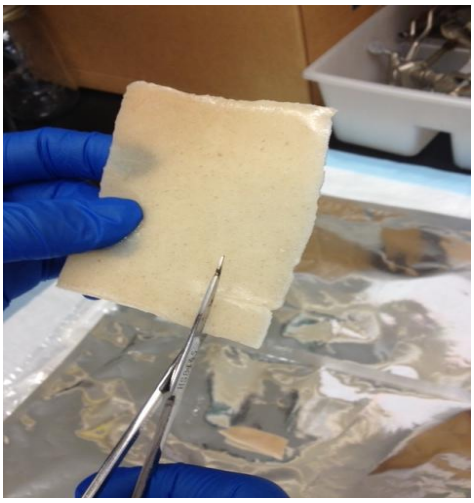

Figure A-3. Skin cutting.

Figure A-4. Skin separation.

NOTE: Skin anomalies were minimized (i.e. left out moles, acne, age spots, etc.)

One at a time, each skin piece was placed into the 60ºC DI water for one minute. Skin was then removed from heated bath and placed on metal tray. Using two tweezers, the dermis was carefully separated from the epidermis (Figure A-4). The epidermis layer consisted of the stratum corneum and some viable epidermis.

The epidermis (stratum corneum) was floated stratum corneum side up into a deep tray filled with DI water. The diffusion cell was immersed in the water and positioned beneath the floating epidermis. With the cell chamber aperture directly below the skin, the cell was lifted out of the water and all water was removed (Figure A-5).

The donor chamber was carefully placed on top of the skin, and checked to ensure a good seal was made with the separated skin. The donor chamber was fixed in place using metal clamps and metal screws tightened so that the clip placed light adequate pressure atop donor chamber.

Figure A-5. Placement of skin on cell base.

In-line cells were connected to proper pump channel tubing and place cell on rack accordingly. Tangling of the tubing was avoided by rotating the inlet tubing from the pump counter-clockwise a few times before inserting into threading and tightening clockwise. Bubbles beneath the skin were removed by performing the following steps:

1) using one hand, point spout of inline cell straight up
2) turn on pump (scintillation vials should be in place to catch outgoing fluid from other cells)
3) press and release "MAX CAL" button repeatedly to push the bubble(s) out of the outlet.

Once the bubbles were removed, a paper towel or kimwipe was used to wipe dry the diffusion cell before returning them to the rack. Once all 12 cells were assembled, the heater and humidifier were turned on and the pump was set at a flow rate of 10.1 ul/min. All pertinent data/information (including humidity/temperature) was recorded in data collection worksheets. The skin was allowed to condition in the chamber for 15-24 hours prior to beginning the experimental trial.

7.A.3.5 Preparation for experimental trial

Solutions of radiolabeled material were prepared in house.

A scalpel was used to cut fabric into samples pieces 20x20 mm (Figure A-6). Keeping track of which fabric piece will be placed in which cell, weights of each fabric sample were recorded. *NOTE: fabric types and skin donor were randomized by random number generation for each run.*

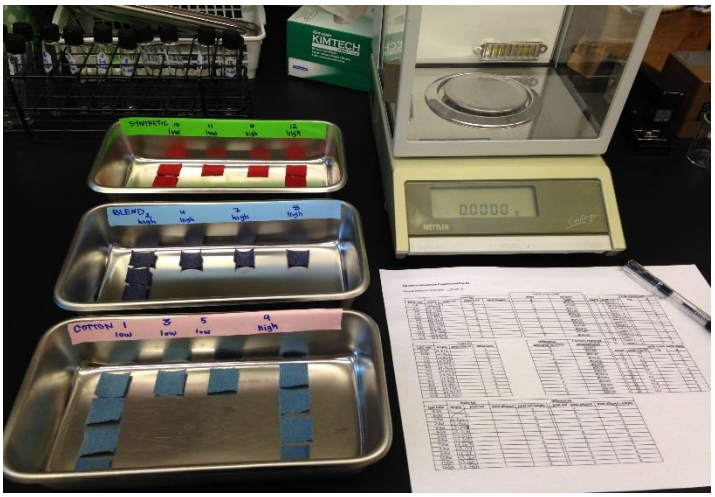

Figure A-6. Preparation of fabric samples prior to run.

A micropipettor was used to load an aliqout of radiolabeled solution on to fabric samples (sample ID: #-F and #-PF-1,2,3) under fume hood (Figure A-7). The stock solution was dropped on the center of fabric sample (where contact with receptor fluid is expected) and allowed to dry completely under fume hood for at least 60 minutes or until the ethanol had fully evaporated.

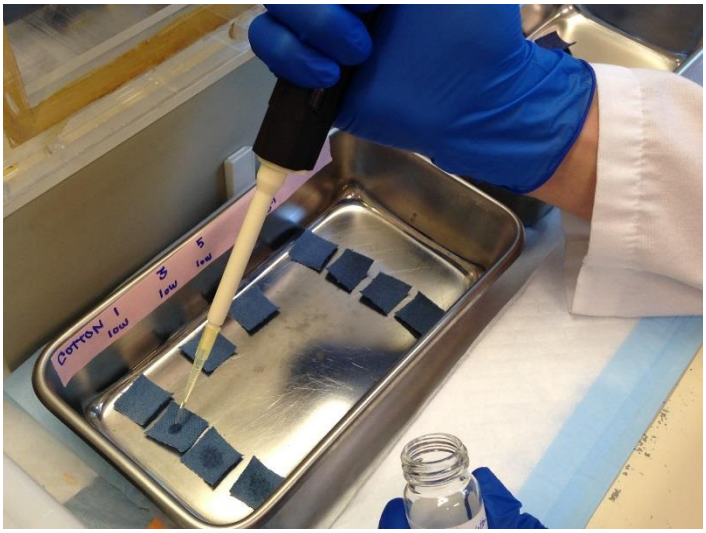

Figure A-7. Loading radiolabeled compound to fabric sample.

Positive controls [Fabric-Concentration-#] were placed in vials containing 10 ml of Hionic-Fluor using sterile tweezers. Tweezers were dunked in the Hionic-Fluor to ensure capture of residual radiolabeled compound that make have transferred to the tweezers. Controls were stored in a safe place until the LSC run.

7.A.3.6 Loading the environmental chamber and commencing exposure

With the pump turned off, the flask connected to humidifier was filled with DI water and kept full throughout the exposure period. One at a time, the diffusion cells were removed from the rack with tubing still attached. Metal clamps and donor chamber were removed from the cell without disconnecting tubing or disturbing the skin. With sterilized tweezers, the loaded fabric square designated to Cell 1 from the metal tray was placed gently on top of the skin membrane (Figure A-8), ensuring fabric was lying flat (Figure A-9). The side of the fabric facing up was the side loaded with the radiolabeled compound. After fabric was placed on top of the skin, the tweezers were rinsed in scintillation cocktail Hionic-Fluor (vials #-T1) to capture any loss of radioactive material.

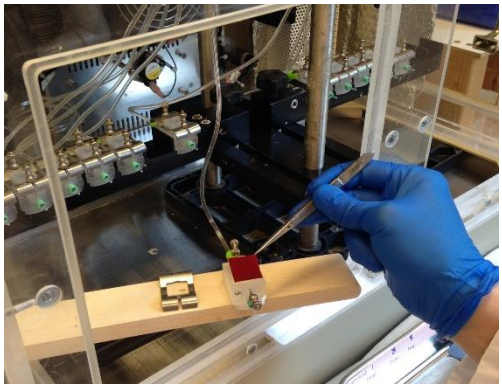

Figure A-8. Transferring fabric to cell.

Figure A-9. Fabric placed on skin.

The donor chamber and metal clamps were replaced and the loaded cell was reposition in the rack (Figure A-10).

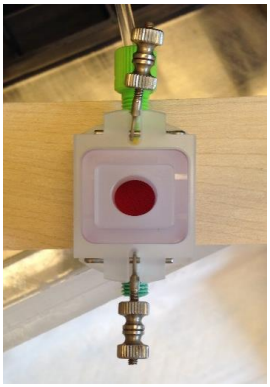

Figure A-10. Loaded cell.

Uncapped receptor fluid collection vials (40 mL was barely enough to capture fluid in a 24-hour exposure period) were secured beneath the corresponding diffusion cells as pictured in Figure A-11.

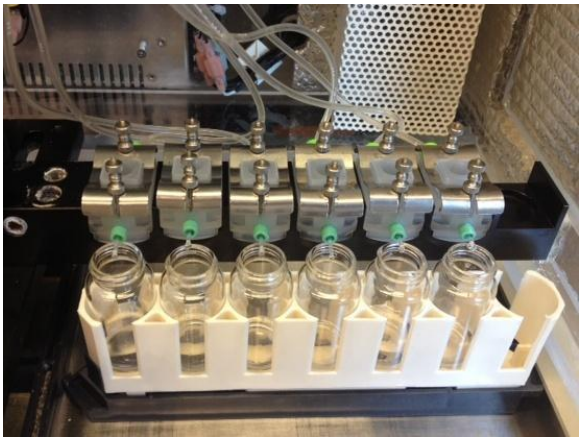

Figure A-11. Receptor fluid collection vials.

The pump was set to 25 ul/min and turned ON. All pertinent data/information (start time, humidity, temperature, etc.) was recorded in a datasheet. Each cell was observed for 10-15 minutes to assure no blockage of fluid, leaking, or other problems with flow of receptor fluid.

7.A.3.7 Apparatus break down

After 24 hours the pump was turned off and the exposure period was stopped. The 40 mL receptor fluid collection vials were removed from the chamber and recapped. Once cool, masses of each receptor fluid vial were recorded. One at a time, each cell was removed from the rack, and metal clamps were put in a tray dedicated for materials that may have come in contact with radioactive compound.

The cotton tip of a sterile Q-tip was dipped into DI water with 10% soap solution. The wet Q-tip was used to wipe the surfaces of the donor chamber that may have be exposed to the radiolabeled compound. After the wipe, the very end of the Q-tip (excluding as much wood as possible) was clipped off into the vial labeled #-D (Figure A-12). A second Q-tip was dipped into a beaker containing DI water only and the wiping procedure of donor chamber was repeated twice.

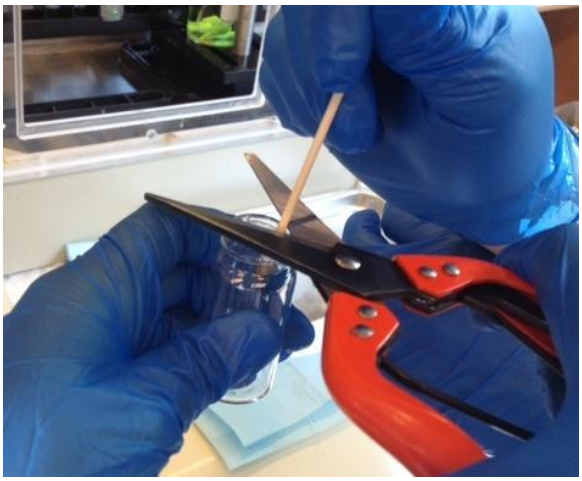

Figure A-12. Collection of Q-tip swab into vial.

Sterile tweezers were then used to carefully lift fabric off of skin and transfer it into the #-F vial, pre-filled with 10 mL of Hionic-Fluor. The tweezer tip was rinsed in the scintillation cocktail to capture any radioactive residue.

Three Q-tips were used to collect skin surface wipes as detailed in Table A-3 (Figure A-13).

NOTE: It was discovered that solids present in the scintillation vials interfere with the scintillation counting. After exposed fabric samples had been soaking in the cocktail for at least three weeks, 5 mL aliquots were taken from the #-F vials and put into clean scintillation vials. 5 mL of Hionic-Fluor was added to the aliquot and vials were re-run in the LSC. Absence of the fabric yielding more accurate and precise counts.

Table A-3. Wipe Protocol Details

Vial	Vial description	Swab protocol
#-D	donor chamber swab (x2)	1^{sr} swab: Q-tip dip in DI water with 10% soap 2nd swab: Q-tip dip in DI water
#-W1	Skin surface wipe (x1)	1 swab: Q-tip dip in DI water with 10% soap
#-W2	Skin surface wipe (x2)	2 swabs: Q-tip dipped in DI water
#-C	cell base swab (x2)	1^{sr} swab: Q-tip dip in DI water with 10% soap 2nd swab: Q-tip dip in DI water

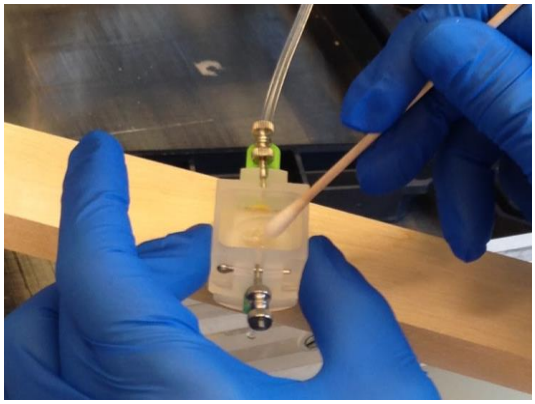

Figure A-13. Skin wipe.

Sterile tweezers were used to transfer the skin from cell base into a pre-labeled and pre-weighed test tube #-Sol (Figure A-14).

Figure A-14. Transfer of skin to test tube.

The cell base was wiped with Q-tips as detailed in Table A-3 and the cotton tips were clipped into the #-C vial.

7.A.3.8 Post-experiment processing of vials

2 ml of Soluene 350 was added to each test tube containing skin samples, including skin blank, and let to sit overnight. The next morning, the test tubes of skin samples were positioned in a hot-bath sonicator filled with DI water (heated to 65°C) for 2 hours (Figure A-15).

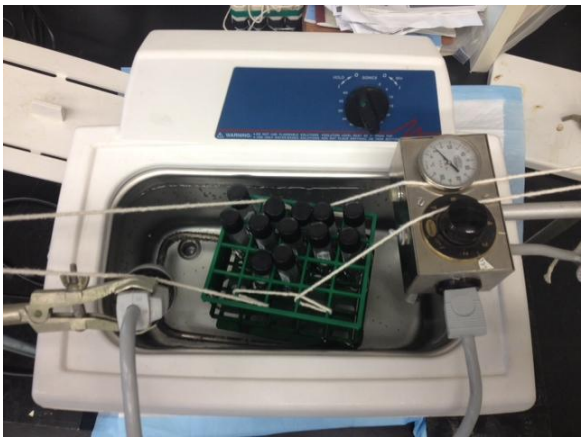

Figure A-15. Skin solubilizing in hot bath sonicator.

When the solubilization was complete, test tubes were removed from sonicator water bath and allowed to cool to room temperature (~10-15 min). When cooled, the post-solubilization masses of the test tubes were recorded. The contents were then transferred into appropriate #-S vials and the masses of the emptied #-Sol test tubes were recorded. Post-aliquot masses of #-S vials were also recorded prior to adding 10ml of Hionic-Fluor to each #-S vial.

Ensuring that all #-R vials had been weighed and masses recorded, a 5 ml aliquot of receptor fluid was taken from each #-R vial and added to its corresponding #-Ra vial. The post-aliquot masses of the #-R vials were recorded prior to the addition of 12 ml of Ultima Gold. Table A-4 compiles the post-experiment processing of samples.

Table A-4. Scintillation cocktail details

Vial ID	Vial description	Cocktail	Cocktail volume (ml)
Controls & Blanks	Fabric positive controls & stock solution controls	Hionic-Fluor	10
F	Fabric exposed to skin	Hionic-Fluor	10
TI	tweezer swish (pre-run)	Hionic-Fluor	10
T2	tweezer swish (post-run)	Hionic-Fluor	10
D	donor chamber wipe (x2)	Hionic-Fluor	10
W1	skin swab 1 - 10% soap	Hionic-Fluor	10
W2	skin swab 2 - DI water	Hionic-Fluor	10
W3	skin swab 3 - DI water	Hionic-Fluor	10
C	cell base wipe (x2)	Hionic-Fluor	10
S	skin aliquot	Hionic-Fluor	10
Ra	receptor fluid aliquots	Ultima Gold	12

7.A.3.9 Liquid Scintillation Counting

Scintillation vials were placed in the Liquid Scintillation Counter (LSC) in a recorded order. The LSC was set to count vials in triplicate at 2.5 minutes/vial. The quench limits (normal range of the H#) were 112.07-145.96. Vials counted at or above an H# of 170.0 and at or below an H# of 100.0 showed high variability in the dpm counts and were therefore considered inconclusive and removed from the dataset.

APPENDIX 7.B

STATA™ Outputs from ANOVAs

```
. mean fluxtoskin, over(relativemassload)

Mean estimation                     Number of obs    =        34

            0: relativemassload = 0
            1: relativemassload = 1
```

Over	Mean	Std. Err.	[95% Conf.	Interval]
fluxtoskin				
0	.0395625	.0102628	.0186827	.0604423
1	.2170556	.0613052	.0923291	.341782

```
. mean fluxtorf, over(relativemassload)

Mean estimation                     Number of obs    =        34

            0: relativemassload = 0
            1: relativemassload = 1
```

Over	Mean	Std. Err.	[95% Conf.	Interval]
fluxtorf				
0	.0105625	.0016201	.0072664	.0138586
1	.0761111	.0271092	.0209571	.1312652

```
anova  fluxtorf skindonor##fabrictype##relativemassload
```

Number of obs =	34	R-squared	=	0.4900	
Root MSE	= .077923	Adj R-squared	=	0.2349	

Source	Partial SS	df	MS	F	Prob > F
Model	.128323624	11	.011665784	1.92	0.0927
skindonor	.008012333	1	.008012333	1.32	0.2630
fabrictype	.030686137	2	.015343068	2.53	0.1028
skindonor#fabrictype	.007238917	2	.003619459	0.60	0.5596
relativem~d	.035280232	1	.035280232	5.81	0.0247
skindonor#relativem~d	.004373564	1	.004373564	0.72	0.4052
fabrictype#relativem~d	.026912282	2	.013456141	2.22	0.1328
skindonor#fabrictype# relativem~d	.005553667	2	.002776833	0.46	0.6389
Residual	.133583007	22	.006071955		
Total	.261906631	33	.007936565		

```
anova  fluxtoskin skindonor##fabrictype##relativemassload
```

Number of obs =	34	R-squared	=	0.4846	
Root MSE	= .183818	Adj R-squared	=	0.2268	

Source	Partial SS	df	MS	F	Prob > F
Model	.698820618	11	.063529147	1.88	0.1000
skindonor	.017984773	1	.017984773	0.53	0.4734
fabrictype	.150142813	2	.075071407	2.22	0.1322
skindonor#fabrictype	.043399646	2	.021699823	0.64	0.5357
relativem~d	.248561075	1	.248561075	7.36	0.0127
skindonor#relativem~d	.062440005	1	.062440005	1.85	0.1878
fabrictype#relativem~d	.082732214	2	.041366107	1.22	0.3132
skindonor#fabrictype# relativem~d	.057815148	2	.028907574	0.86	0.4387
Residual	.743361809	22	.033789173		
Total	1.44218243	33	.043702498		

```
anova fluxtoskin relativemassload##fabrictype
```

	Number of obs =	34	R-squared	=	0.3526
	Root MSE =	.182601	Adj R-squared	=	0.2370

Source	Partial SS	df	MS	F	Prob > F
Model	.508573227	5	.101714645	3.05	0.0254
relativem~d	.262847102	1	.262847102	7.88	0.0090
fabrictype	.14325303	2	.071626515	2.15	0.1355
relativem~d#fabrictype	.089396214	2	.044698107	1.34	0.2780
Residual	.9336092	28	.033343186		
Total	1.44218243	33	.043702498		

```
anova fluxtorf fabrictype##relativemassload
```

	Number of obs =	34	R-squared	=	0.3845
	Root MSE =	.075876	Adj R-squared	=	0.2746

Source	Partial SS	df	MS	F	Prob > F
Model	.100707457	5	.020141491	3.50	0.0140
fabrictype	.030859562	2	.015429781	2.68	0.0861
relativem~d	.036420386	1	.036420386	6.33	0.0179
fabrictype#relativem~d	.027957634	2	.013978817	2.43	0.1066
Residual	.161199174	28	.005757113		
Total	.261906631	33	.007936565		