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DERMAL PENETRATION POTENTIAL OF PERFLUOROOCTANOIC ACID (PFOA) IN HUMAN AND MOUSE SKIN

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Recent data, using a murine model, have indicated that dermal exposure to perfluorooctanoic acid (PFOA) induces immune modulation, suggesting that this may be an important route of PFOA exposure. To investigate the dermal penetration potential of PFOA, serum concentrations were analyzed in mice following topical application. Statistically significant and dose-responsive increases in serum PFOA concentrations were identified. In vitro dermal penetration studies also demonstrated that PFOA permeates both mouse and human skin. Investigation into the mechanisms mediating PFOA penetration demonstrated that dermal absorption was strongly dependent upon the ionization status of PFOA. In addition, PFOA solid, but not 1% PFOA/acetone solution, was identified as corrosive using a cultured epidermis in vitro model. Despite its corrosive potential, expression of inflammatory cytokines in the skin of topically exposed mice was not altered. These data suggest that PFOA is dermally absorbed and that under certain conditions the skin may be a significant route of exposure.

Perfluorooctanoic acid (PFOA) is an organic fluorochemical once widely used in the manufacturing of flame retardants, firefighting foams, surfactants, wax and gloss finish enhancers, and carpet and fabric protectants (Kennedy et al. 2004). High stability and extremely low surface tension of PFOA, which lend it usefulness in consumer and industrial applications, also result in its environmental persistence (Kennedy et al. 2004). PFOA has an estimated hydrolytic half-life of more than 97 yr, allowing for continued environmental bioaccumulation, and has been found in various animal species, including mink, bald eagle, tuna, dolphins, other aquatic organisms, and whales (Calafat et al. 2006; Olsen et al. 2007). In humans, PFOA has a half life of approximately 4.5 yr and was detected in blood of both occupationally exposed individuals and the general population (Olsen et al. 2007). Due to potential health concerns associated with PFOA bioaccumulation, there is increased interest in understanding the biological effects resulting from PFOA exposure in humans and experimental animal models. Although the use of PFOA has been greatly reduced over the past 10 years and is expected to be eliminated completely by 2015, this chemical will remain a health concern due to its persistence in household goods and commercial products manufactured before production was reduced (Betts 2007).

Much of the concern regarding PFOA-mediated health effects focused specifically on occupationally exposed individuals due to increased levels of PFOA present in their blood (Olsen et al. 2007). Using animal models, the

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health effects associated with PFOA exposure have been extensively evaluated. Numerous reports demonstrated PFOA-induced organ (liver, thymus, spleen) and systemic (body weight) toxicity following oral exposure (Betts 2007; Kudo and Kawashima 2003). Other adverse effects commonly reported in experimental animals exposed to PFOA include carcinogenicity, hepatomegaly and hepatocyte proliferation, hormone disruption, and a myriad of developmental effects, including neonatal mortality (DeWitt et al. 2009a). PFOA is also immunosuppressive following oral exposure in murine models. A reduction in thymocyte and splenocyte populations, altered T lymphocyte populations, and an inhibition in T-cell-dependent immunoglobulin (Ig) M and IgG antibody responses have been documented (Dewitt et al. 2008; 2009a; 2009b; Yang et al. 2001; 2002). Our laboratory previously showed these same immunotoxic effects following dermal exposure to PFOA (Anderson et al. 2009). In addition to its inhibitory effects, it was also demonstrated that dermal exposure to PFOA, although not allergenic by itself, enhances ovalbumin (OVA)-induced IgE production and airway hypersensitivity in a murine model (Fairley et al. 2007). These data suggest that PFOA has the potential to augment IgE-mediated responses induced by other environmental and occupational allergens. Consistent with this hypothesis, a significantly greater prevalence of self-reported cases of chronic bronchitis and asthma was documented in individuals residing in a community with prolonged exposure to PFOA in their drinking water as compared to the general population (Anderson-Mahoney et al. 2008).

Despite the potential for dermal exposure during the manufacturing process, as well as in commercial products, such as firefighting foams and carpet and fabric protectants, most research has focused on oral and inhalation routes of PFOA exposure. Other than our recent findings and a previous report of occupational dermal absorption in Chemolite workers (U.S. EPA 2002), most investigations ignored dermal PFOA exposure due to the assumption that it is not well absorbed by the skin (U.S. EPA 2002). While dermal absorption of the ammonium salt of PFOA, ammonium perfluorooctanoate (APFO), previously have been examined by Fasano et al. (2005), to our knowledge, no studies have been published in the peer-reviewed literature that thoroughly investigate the potential for PFOA dermal absorption. Since the potential for PFOA dermal exposure exists not only in occupationally exposed adults but also in adult consumers and children, it is important to fully understand the potential for dermal penetration and the risk of exposure to PFOA through the dermal route.

Understanding and modeling the disposition of PFOA in contact with skin requires reliable values of relevant physicochemical properties. Unfortunately, physicochemical properties of PFOA that affect skin permeation and transdermal flux are elusive. A recent review (Rayne and Forest 2009) highlights problems with measurements of octanol–water partition coefficient, aqueous solubility, and acid dissociation constant. For example, a water solubility of 3.4 mg/ml has been measured (Shinoda et al. 1972), but the chemical property estimator SPARC (release 4.5.1522, http://archemcalc.com/sparc; accessed July 15, 2011) estimates PFOA solubility to be only 0.105 mg/ml. Likewise, an acid dissociation constant of 3.8 has been measured (Burns et al. 2008), while SPARC estimates the value to be –0.21. PFOA forms multiple layers in octanol/water, making an experimental determination of the partition coefficient (log K_{ow}) problematic. On the other hand, octanol/water partitioning of the fully ionized compound has been measured (Ping et al. 2009). Table 1 presents measured (where available) or estimated values for some physicochemical properties of PFOA.

In the present study, the issue of dermal PFOA absorption is addressed and the mechanisms of PFOA penetration, in both human and mouse skin, are highlighted. Serum PFOA levels were analyzed in mice following topical exposure, and in vitro dermal penetrations studies were performed to quantify the extent and time course of absorption in both human and mouse skin. To investigate

TABLE 1. Physicochemical Properties of PFOA

CAS	Formula	Structure	MW	log K_{ow}[a]	log D[b]	pK_a[c]	S_w[d](mg/ml)	P_{vap}[e](Pa)
335-67-1	$C_8HF_{15}O_2$		414.1	4.7	1.76	3.8	3.4	18

Note. CAS: Chemical Abstract Society registry number; log K_{ow}: base 10 logarithm of octanol/water partition coefficient; log D: base 10 logarithm of the octanol/water distribution coefficient; pK_a: acid dissociation constant; S_w: water solubility; P_{vap}: vapor pressure.

[a]Average of estimated values reported in Arp et al. (2006).

[b]Measured, fully ionized state (Ping et al. 2009).

[c]Measured (Burns et al. 2008).

[d]Measured, 20°C (Shinoda et al. 1972).

[e]Measured, 35.4°C (Barton et al. 2008).

the mechanisms mediating dermal absorption, the role of ionization state, potential for skin corrosivity, and inflammatory cytokine expression were explored following PFOA exposure. Understanding the exposure routes relevant to PFOA toxicity will aid in establishing more effective guidelines for personal protective device usage and engineering controls that will help reduce occupational exposure, as well as provide insight into potential mechanisms of action.

METHODS

Chemicals

Perfluorooctanoic acid (PFOA purity >96%, CAS number 335-67-1) was purchased from Sigma Aldrich. [Carbonyl-^{14}C]-PFOA was custom synthesized by Perkin Elmer. The specific activity was 59 mCi/mmol and the reported radiochemical purity was 99.5%.

Animals

Female BALB/*c* mice (17–20 g), purchased from Taconic, were used for these studies. This mouse strain was previously used in our laboratory to evaluate the immunotoxicity of PFOA (Anderson et al. 2009; Fairley et al. 2007). Upon arrival, the animals were allowed to acclimate for a minimum of 5 d. Each shipment of animals was randomly assigned to treatment group, weighed, and individually identified via tail marking using a permanent marker or tattoo. A preliminary analysis of variance on body weights was performed to ensure a homogeneous distribution of animals across treatment groups. The animals were housed at a maximum of 5 per cage in ventilated plastic shoebox cages with hardwood chip bedding and NIH-31 modified 6% irradiated rodent diet (Harlan Teklad), and tap water was provided from water bottles ad libitum. The temperature in the animal facility was maintained between 20 and 22°C and the relative humidity between 36 and 57%. The light/dark cycle was maintained on 12-h intervals. All animal experiments were performed in the AAALAC-accredited National Institute for Occupational Safety and Health (NIOSH) animal facility in accordance with an animal protocol approved by the Institutional Animal Care and Use Committee.

Serum PFOA Concentration

BALB/*c* mice ($n = 5$) were topically exposed to acetone or PFOA (0.5–2% in acetone) on the dorsal surface of each ear (1 cm^2), 25 µl per ear once a day for 4 consecutive days, and mice were euthanized by CO_2 inhalation 24 h after the last exposure. Blood samples were collected via cardiac puncture and sera were separated by centrifugation (10 min at 10,000 × g) and frozen at –20°C for subsequent serum analysis. Serum analysis was performed using on-line solid-phase extraction coupled to isotope dilution high-performance liquid

chromatography–tandem mass spectrometry as previously described by Kuklenyik et al. (2005). The limit of detection for PFOA was 0.1 ng/ml. Data were first tested for homogeneity using the Bartlett's chi-squared test. All data were homogeneous; therefore, a one-way analysis of variance (ANOVA) was conducted. If the ANOVA showed significance at $p < .05$ or less, Dunnett's multiple range *t*-test was used to compare treatment groups with the control group. Linear trend analysis was performed to determine whether PFOA had exposure concentration-related effects for the specified endpoints. Statistical analysis was performed using Graph Pad Prism version 5.0 (San Diego, CA).

In Vivo Evaluation of Inflammation

To evaluate the inflammatory response induced by PFOA, ears and dorsum skin of topically exposed BALB/*c* mice ($n = 5$) were analyzed for mRNA expression. Real-time polymerase chain reaction (RT-PCR) has previously been demonstrated as an effective method for determining levels of cytokine production in the skin (Sabourin et al. 2000). The inflammatory cytokines assessed included interleukin (IL)-1α, IL-1β, IL-6, IL-18, IL-33, tumor necrosis factor (TNF)-α, transforming growth factor (TGF)-β, granulocyte–macrophage colony-stimulating factor (GM-CSF), Ccl2, and Ccl5. Each mouse was exposed to vehicle (acetone) or PFOA (1%) topically on the dorsal surface of each ear (25 μl per ear) and on the shaved back (50 μl). Animals were euthanized by CO_2 inhalation 1, 3, 12, 24, or 48 h after exposure. The ears (2 per animal; 1 cm × 1 cm) and dorsum skin (2 cm × 2 cm, delineated as the shaved area) from each animal were collected and homogenized in liquid nitrogen using a mortar and pestle. TRI Reagent (Molecular Research Center) was added (800 μl) to each sample and RNA was isolated from the cells according to the TRIzol protocol with QIAGEN cleanup according to the manufacturers' instructions. The concentration of RNA was determined using an ND-1000 spectrophotometer (Thermo Scientific Nanodrop). RNA (2 μg) was reverse transcribed using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) as directed by the manufacturer. Relative fold changes in gene expression were assessed using RT-PCR on a 7500 Fast Real-Time PCR system (Applied Biosystems) using Taqman PCR Master Mix (Applied Biosystems) as specified in the manufacturer's protocol. RT-PCR data were collected and expressed as relative fold increase over control, calculated by the following formula: $2^{\Delta\Delta Ct} = \Delta Ct_{Sample} - \Delta Ct_{Control}$, where $\Delta Ct = Ct_{Target} - Ct_{GAPDH}$, where Ct is the cycle threshold as defined by manufacturer's instructions. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the endogenous control.

In Vitro Dermal Penetration Studies

Buffer HEPES-buffered Hanks balanced salt solution (HBSS) consisted of 5.96 g HEPES, 0.32 g $NaHCO_3$, and 0.05 g gentamicin sulfate added to 1 L of Hanks balanced salt solution. The buffer was titrated to a pH of 7.4 with NaOH and degassed by heating to 40°C and stirring under laboratory vacuum for approximately 15 min.

Skin Samples Full-thickness, shaved abdominal mouse skin was obtained from female BALB/*c* mice following CO_2 euthanasia. Mouse skin was used for analysis on the day of collection. Caucasian female skin samples from surgical procedures were obtained from the West Virginia University Skin Bank. The skin bank maintains approval for all collections, but the specific use of this tissue for these studies was deemed "not human research" and hence not subject to approval. Both full-thickness and heat-separated epidermal membranes were used. For the latter, skin was submersed in 60°C HEPES-buffered HBSS for 45 s and the epidermis was then teased from the dermis using cotton swabs. Human skin was stored frozen (–85°C on gauze pads saturated with HBSS plus 10% glycerol) and used within 6 mo of storage. Both mouse and human full-thickness dermis was cleaned of underlying fat prior to use. Skin samples were prescreened by examination under a 20×

dissecting microscope and rejected if obvious flaws (holes) were present.

Diffusion Cells Flow-through diffusion cells (PermeGear, Inc.) were used for these studies. Exposed skin surface was 0.64 cm^2 and flow was set at 2 ml/h (approximately 8 receptor volumes/h) to maintain sink conditions. A heater/recirculator was used to maintain the receptor compartments at 37°C, which maintained the skin surface temperature at 32°C. A fraction collector (Isco Retreiver IV) and an indexed controller (PermeGear, Inc.) allowed the collection of 2-h samples over the exposure period of 24 h. Skin discs were mounted on diffusion cells and allowed to equilibrate overnight prior to PFOA exposures.

Dosing Solutions and Exposures

^{14}C-PFOA was added to unlabeled PFOA to achieve a desired radiochemical concentration of 2–5 μCi/ml. PFOA solutions were mixed using acetone, water, or 0.1 *M* citrate buffer (pH 5.5) as solutes for different exposures, as described subsequently.

Finite-Dose Exposures Initial studies sought to quantify the in vitro dermal PFOA penetration following finite-dose exposures that were the same as those used for the in vivo mouse studies. Both mouse (full-thickness) and human (full-thickness and heat-separated epidermis) samples were used. For mouse studies, one skin sample from each of nine individual mice was used. For human studies, skin samples were obtained from a single donor, with seven replicates each for full-thickness and heat-separated epidermal exposures. Each skin sample was exposed to 50 μl solution of 0.5 mg PFOA in acetone (1%). Donor cells were left open to the ambient air and acetone vehicle appeared to evaporate within 5 min. At the conclusion of the 24-h exposures, skin surfaces were rinsed 3× with methanol and then dried with cotton swabs. All rinses and swabs were collected in scintillation vials. The skin samples were then removed from the diffusion cells and dissolved in 1–2 ml Soluene 350 (Perkin Elmer).

Infinite-Dose Exposures These studies were undertaken using heat-separated human epidermal membranes to obtain steady-state permeability measurements and to examine the role of PFOA ionization state and donor solution pH on the dermal absorption of PFOA. Three dosing solutions were used: 3000 μg/ml PFOA in H_2O; 3000 μg/ml PFOA in 0.1 *M* citrate buffer (pH 5.5); and 14 μg/ml PFOA in water. The pH values of the dosing solutions were measured and are reported later, in Table 3. With a pH of 2.25, PFOA at 3000 μg/ml in water is expected to exist primarily in its un-ionized form. At an identical concentration in a buffered (pH 5.5) solution, PFOA will be primarily ionized, as will low concentrations in water (pH 5.01). The 3000-μg/ml concentration was used as this is close to a reported solubility of PFOA but below the critical micelle concentration (Shinoda et al. 1972). These formulations appeared to be clear solutions with no visible residue; however, based on the uncertainty in the aqueous solubility of PFOA, it may be that the higher concentration formed micelles. The 14-μg/ml concentration was selected as this was the lowest concentration possible to maintain radiochemical concentration of 2 μCi/ml, given the specific activity of the ^{14}C-PFOA.

For each of the 24-h infinite-dose exposures, multiple replicates from 3 individual skin donors were used. Epidermal samples were dosed with approximately 500 μl donor solution and donor cells were covered to inhibit evaporation. Donor solutions were changed periodically to maintain "infinite dose" conditions.

Model-Based Estimates of Permeability Coefficients

Model-based estimates of skin permeability coefficients were obtained from the NIOSH Skin Permeability Calculator (http://www.cdc.gov/niosh/topics/skin/skinPermCalc.html). Average values of the three models available on the calculator were taken and reported later in Table 3 along with the measured k_p values. As inputs, the log K_{ow} value of 4.7 reported

in Table 1 was used for pH 5.5 and pH 5.01 donors on the assumption that PFOA is essentially all ionized under these conditions. The log *D* value of 1.76 was used for pH 2.25 donor, on the assumption that PFOA is essentially un-ionized under this acidic condition.

Data Analysis

PFOA amounts in receptor, skin surface, and skin samples were quantified using liquid scintillation counting. The time course of penetration (mass of PFOA collected in receptor fluid) was determined over the experimental duration (24 h). For finite-dose exposures, the total penetrated amount is reported as percent applied dose. Amounts of PFOA detected in skin surface wipes and in skin samples are also reported as percent applied dose. The total absorbable amount is considered to be the sum of the total penetrated amount plus the amount in skin samples. For infinite-dose exposures, permeability coefficients (k_p) were calculated as described previously (Frasch and Barbero 2005). One-way analyses of variance (ANOVAs) were performed using SigmaPlot 11.0 (Systat Software, Inc.) on k_p and τ values for the three exposures. Because the measurements failed the Shapiro–Wilk test for normality, a Kruskal–Wallis ANOVA on ranks was performed to assess differences in median values among the treatment groups. Pairwise comparisons were subsequently performed using Dunn's method. The criterion for significance was set at $p < .01$.

EpiDerm Skin Corrosivity Test

The corrosivity of PFOA was determined using the EpiDerm human reconstructed epidermis model (MatTek Corp., Ashland, MA) following the company's standard protocol. Briefly, tissues (0.64 cm^2) were preincubated (37°C, 5% CO_2) in culture medium for 1 h prior to dosing. Four EpiDerm tissues were then exposed either to approximately 50 mg solid PFOA with 50 µl H_2O added to wet the material, or to 50 µl 1% (w/v) PFOA in acetone. The addition of a small amount of water to a solid chemical is in accordance with the standard protocol. Negative (H_2O) and positive (8 *N* KOH) controls were performed, with 50 µl added to each of 4 tissues. Exposures were 3 min (2 tissues per treatment) and 1 h (2 tissues per treatment). After thorough rinsing, tissues were transferred to 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay buffer and incubated for 3 h (37°C, 5% CO_2). After rinsing with PBS and drying, tissues were immersed overnight in isopropanol at room temperature to extract formazan dye. The optical density of the extracted formazan was determined spectrophotometrically at 570 nm, and cell viability was calculated as a percent of the mean of the negative control tissues. In accordance with the recommendations of the manufacturer, the test substance is classified as "corrosive" to skin if the viability after a 3-min exposure is <50%, or if the viability after a 3-min exposure is ≥50% and viability after a 1-h exposure is <15%. If viability after a 3-min exposure is ≥50% and if viability after a 1-h exposure is ≥15%, then the test substance is classified as "noncorrosive."

RESULTS

Increased Serum PFOA Levels Following Dermal Exposure

To evaluate the extent by which PFOA is dermally absorbed, serum levels were analyzed following dermal exposure in BALB/c mice (Figure 1). Statistically significant

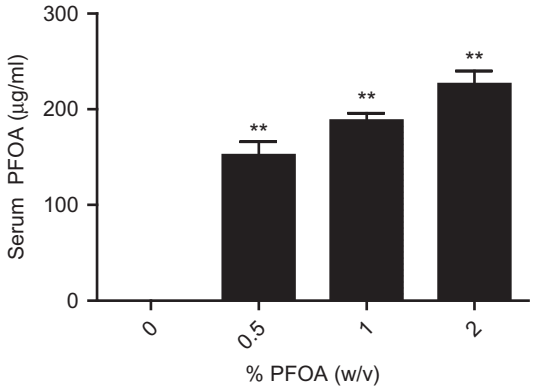

FIGURE 1. Serum concentrations of PFOA following dermal exposure. BALB/*c* mice were dermally exposed to increasing concentrations of PFOA for 4 d. Bars represent the group mean ± SE. Double asterisk indicates significant $p \leq 0.01$ as compared to the vehicle control; $n = 5$ mice per exposure group.

dose-responsive (linear trend test) increases in serum PFOA levels were identified following dermal exposure to PFOA. Serum PFOA levels in BALB/*c* mice ranged from 152 ± 14 μg/ml in the low concentration exposure group (0.5% PFOA) to 226 ± 14 μg/ml in the high exposure group (2% PFOA).

In Vitro Dermal Penetration of PFOA in Both Human and Mouse Skin

In vitro dermal penetration studies were subsequently performed to quantify the extent and time course of PFOA absorption. Figure 2 shows the time course of PFOA absorption through mouse and human skin following finite-dose exposures, while Table 2 summarizes the results of these studies. Approximately 39% of the applied PFOA dose penetrated mouse skin. In addition, 11% of the applied dose was found in the skin, making a total absorbable amount of approximately 50% of the applied dose. Because human skin is a more relevant model for use in dermal risk assessment, the same in vitro system was used to evaluate and compare the dermal penetration potential of PFOA in full-thickness and heat-separated epidermal human skin samples to that of the mouse skin samples. Approximately 24% of the applied PFOA dose penetrated both the full-thickness skin and epidermis samples after a 24-h exposure. An additional 45.6 ± 5.2% and 23.2 ± 7.3% of the applied dose was found within the full thickness skin and epidermis samples, respectively. Consequently, the total absorbable amount of PFOA for these samples was approximately 69% and 48% of the applied dose, respectively.

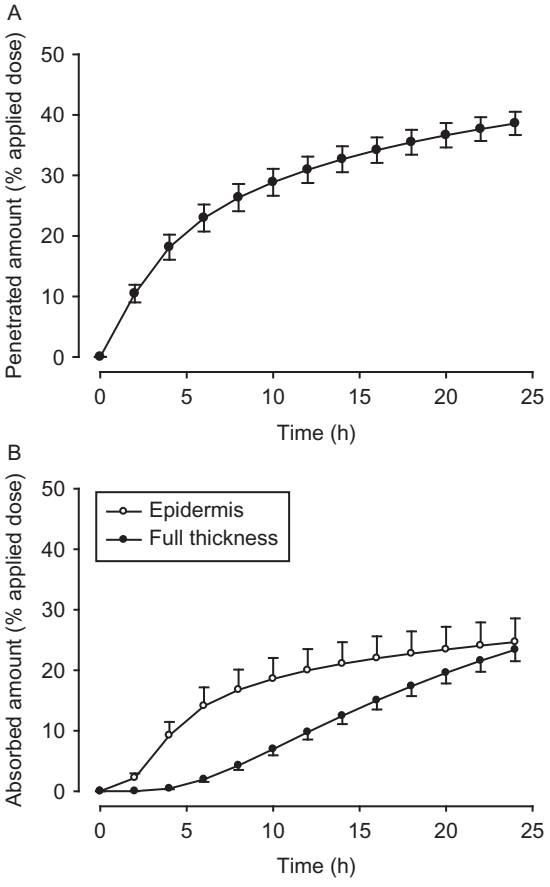

FIGURE 2. Time course of PFOA in vitro dermal penetration. Penetration profiles of (A) full-thickness mouse skin ($n = 9$ BALB/*c* mice) and (B) human full-thickness skin and heat-separated epidermis ($n = 6$ or 7 samples each from 1 donor). Unoccluded skin samples were exposed to 0.5 mg PFOA in 50 μl acetone. Shown are means ± SE.

TABLE 2. Summary of Finite Dose PFOA Skin Permeation Studies

Parameter	Mouse ($n = 9$)	Human	
		Full thickness ($n = 7$)	Epidermis ($n = 6$)
Surface residue (% applied dose)	37.2 ± 1.9	12.7 ± 0.8	21.4 ± 4.1
Skin (% applied dose)	11.4 ± 2.1	45.6 ± 1.9	23.2 ± 3.0
Penetration (% applied dose)	38.6 ± 1.9	23.4 ± 1.9	24.7 ± 3.9

Note. Values are means ± SEM. Applied dose = 0.5 mg PFOA in 50 μl acetone.

PFOA Ionization State and Dermal Absorption

The effect of PFOA ionization state on dermal penetration was explored and results are presented in Table 3. The median permeability coefficient of the free acid (3000 μg/ml in H_2O, 5.5×10^{-2} cm/h) is more than 1000-fold greater than that of the charged species (3000 μg/ml in a buffered (pH 5.5) solution, 4.4×10^{-5} cm/h). Low concentrations of PFOA in water (14 μg/ml, 5.8×10^{-5} cm/h) were also found to be similar to the permeability of high concentrations (3000 μg/ml, 4.4×10^{-5}

TABLE 3. Skin Permeability Coefficients (k_p) Following Infinite-Dose PFOA Exposures, and Model-Based Estimates

PFOA dosing solution	pH	k_p (cm/h), Measured			k_p (cm/h), Estimated
		Median	25%	75%	
3000 μg/ml in H_2O ($n = 8$)	2.25	5.5×10^{-2}(!*)	5.2×10^{-2}	5.7×10^{-2}	1.3×10^{-2}
3000 μg/ml in buffer ($n = 10$)	5.50	4.4×10^{-5} (!)	1.2×10^{-5}	6.0×10^{-5}	8.8×10^{-5}
14 μg/ml in H_2O ($n = 8$)	5.01	5.8×10^{-5} (*)	1.1×10^{-5}	1.5×10^{-3}	8.8×10^{-5}

Note. !,*: Pairs of measured values with identical symbols are significantly different ($p < .05$). 25%, 75%: Indicate quartiles of measured quantity.

cm/h) buffered at pH 5.5. At low concentration the buffering capacity of the stratum corneum is likely to be strong enough to ensure that PFOA exists in its ionized form. That is, it is likely that the PFOA is dissociated under these conditions.

Epidermal Corrosivity of PFOA

As a result of its acidic nature, corrosivity was also evaluated as a potential mechanism mediating PFOA dermal absorption. When applied as a neat solid, with the addition of a small amount of H_2O added to wet the material and assure even coverage, PFOA was classified as a corrosive compound according to the results of EpiDerm studies (Table 4). Following a 1-h exposure to 50 mg PFOA solid, cell viability was determined to be 14% of the negative (water-exposed) control sample. Based on the manufacturers' guidelines, this classifies PFOA as a corrosive compound. Conversely, based on this test, a 1% solution of PFOA in acetone was classified as noncorrosive (18%). No marked changes in viability were observed following a 3-min exposure to PFOA solid or solution. Raw optical density values for H_2O-treated negative control indicate that there was no tissue deterioration over the 1-h exposure duration.

In Vivo Inflammatory Response Induced by Dermal Exposure to PFOA

To investigate the effect of PFOA on cytokine expression in the skin of dermally treated animals, mRNA was analyzed in ear and dorsum skin following exposure to 1% PFOA at 1, 3, 12, 24, or 48 h. Of the cytokines analyzed, mRNA expression was detected (Ct values < 37) in ear skin samples at all time points tested in both vehicle control and PFOA-exposed mice. Inflammatory cytokine mRNA expression was detected only at 1 and 3 h in back skin samples. PFOA, however, did not significantly modulate the expression of any cytokine analyzed when compared to acetone control groups (data not shown).

TABLE 4. EpiDerm Cell Viability Following PFOA Exposures

Treatment	Exposure duration	
	3 min	1 h
PFOA (solid)	109	14
PFOA (1% in acetone)	122	18
8 *N* KOH	22	9
EpiDerm corrosive classification	<50%	<15%

Note. Expressed as percent viability compared to negative (H_2O-treated) control. Optical densities of negative controls were 1.654 (3 min) and 1.681 (1 h).

DISCUSSION

Increasing concerns for PFOA-related human health effects have been prompted by its bioaccumulative and environmentally persistent tendencies. Although there is the potential for absorption of this chemical into and penetration through the skin, this exposure route has not been thoroughly investigated. The current studies suggest that PFOA permeates both mouse and human skin. Using concentrations of PFOA previously reported in our lab to induce immunotoxic effects, 4 d of dermal exposure was shown to result in significant increases in serum PFOA levels (Anderson et al. 2009; Fairley et al.

2007). Similar serum PFOA levels were noted between mice dermally exposed to PFOA in the present study and mice orally exposed in previously described literature. The average serum concentrations of PFOA in C57BL/6N mice exposed to 7.5 mg PFOA/kg body weight (bw) per day for 15 d (total administered dose: 112.5 mg/kg bw PFOA) by gavage were previously reported to measure 84 ± 10 μg/ml (Dewitt et al. 2008), while the mean concentration in the serum of BALB/*c* mice following 4 d of dermal exposure to 1% PFOA (total administered dose 100 mg/kg bw PFOA) was 188 ± 16 μg/ml. These experimental concentrations are approximately 15- to 30-fold higher than those measured in the serum of the highest occupationally exposed human populations (mean PFOA concentrations = 6.44 ppm [range 0.1–81.3 ppm] at Cottage Grove, MN, 3M processing plant) (Kudo and Kawashima 2003). Since the animals used in the present study were also used for immunotoxicological analysis, mice were not individually housed or restrained following chemical application to minimize the immunosuppressive effects of stress-induced factors, such as cortisol. It is possible that grooming may have resulted in additional oral uptake of PFOA indirectly, thereby contributing to a portion of the documented serum levels. Potential PFOA uptake by grooming may explain why a twofold increase in PFOA dosage did not result in a twofold increase in serum PFOA concentrations. However the potential for dermal absorption is supported by the findings from in vitro studies.

Penetration of PFOA was observed in both mouse (39%) and human (24%) skin samples. In addition, if one considers the amount of chemical within the skin to be absorbable, then the total absorbable amount of PFOA was 50% in full-thickness mouse skin, and 48% to 69% in human skin (epidermis and full thickness, respectively). From the data described in Table 2, only 69–87% of the applied dose is accounted for as penetration, surface, and skin amounts for mouse and human skin. In considering the vapor pressure of solid PFOA (Table 1), these suboptimal recoveries (missing up to 30%) may be explained by the potential for sublimation of surface PFOA (Kaiser et al. 2010). Despite low recoveries, the results nevertheless demonstrate significant dermal absorption of PFOA from these finite-dose applications.

While significant PFOA penetration was observed in both mouse and human skin samples, previous studies performed by Fasano et al. (2005) reported that only a small amount of APFO, the aluminum salt of PFOA, penetrates human epidermal membranes in vitro. To try and explain this discrepancy, the effect of PFOA ionization state on dermal penetration was explored. The pH-partition hypothesis, originally proposed more than 50 years ago, holds that passive transport of charged chemical species across biological membranes is small, owing to their poor solubility in lipids (Shore et al. 1957). The acid dissociation constant (pK_a) of PFOA is a matter of controversy, with reported values in the approximate range of 0.5–3 (Rayne and Forest 2009). The fraction of non-ionized PFOA in an aqueous solution can be predicted from the Henderson–Hasselbalch equation. Assuming the larger pK_a value, it is clear that PFOA is almost fully ionized at the pH of stratum cornuem (approximately 5.5). Therefore, PFOA dermal permeation, first as the substantially un-ionized free acid in water (measured pH 2.25), and at its near fully ionized state at normal skin surface pH, was examined. The median permeability coefficient of the free acid (5.5×10^{-2} cm/h) was found to be more than 1000-fold greater than that of the charged species (4.4×10^{-5} cm/h) from a buffered (pH 5.5) solution. These differences are supported by considerations of lipid solubility. Based on the log K_{ow} and log D values listed in Table 1, the lipophilicity of the un-ionized species is 870 times greater than that of the ionized. If penetration can be attributed primarily to the un-ionized species (Vecchia and Bunge 2003), then a permeability coefficient ratio of 1000-fold, as measured here, can be explained by the ionization status of PFOA at the experimental pH. This premise is supported by results from predictive models of skin permeability. The estimated

permeability coefficients listed in Table 3, using appropriate values of log K_{ow} as inputs, compare favorably with the measured k_p values. The human epidermal permeability coefficient of APFO measured by Fasano et al. (2005), approximately 10^{-6} cm/h, is comparable to our ionized PFOA permeability of 5.8×10^{-5} cm/h. These results suggest that the ionization state of PFOA is a major consideration in the dermal absorption of this compound. At normal stratum corneum pH, PFOA is largely ionized and very little penetration occurs. If, however, the compound is present in a nonionized state, then a substantial penetration is expected. It should be noted that most real-world PFOA exposures, particularly very low-level environmental exposures, would be to the ionized state. While the permeability under these conditions is low, the dermal exposure route should nevertheless be considered in evaluating the total exposure to this chemical.

As a result of its acidic nature, these studies also evaluated corrosivity as a potential mechanism mediating PFOA dermal absorption. EpiDerm and other corrosivity tests using human skin models have been validated by both the European Center for the Validation of Alternative Methods (ECVAM, 2000) and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM, 2002). The corrosive potential of PFOA was determined by assessing cytotoxicity, measured as a reduction in mitochondrial dehydrogenase activity, following dermal application of the test substance. Following a 1-h exposure, 50 mg undiluted PFOA solid was classified as corrosive (14% viability), while a 1% solution PFOA in acetone was classified as noncorrosive (18% viability). Both values are near the cutoff point of 15% required for the classification of "corrosive." The classification scheme set forth by the manufacturer is supported independently by correlation of EpiDerm results with a large data set of skin corrosivity measurements (ICCVAM 2002). However, given the small differences in values the likelihood for a true biological difference between these two test articles is questionable.

Independent of the specific classification, exposure to the 1% PFOA solution is detrimental to underlying epidermal cellular function, as cell viability was only 18% following a 1-h exposure. Since PFOA is corrosive to epidermis, it might be suggested that the observed increase in serum PFOA levels, following dermal application in mice, may simply be the result of deterioration of the skin barrier. However, no visual signs of irritation were detected on mouse ears following topical application of PFOA at concentrations up to 2% (data not shown).

Although a 1% PFOA solution was categorized as noncorrosive in EpiDerm studies, the high level of cell death <15% compared to control (Table 4) associated with the exposure raised the question of whether dermal exposure induced inflammation that may contribute to injury and/or modulation of immunologic responses. Various environmental stimuli have previously been shown to induce epidermal keratinocytes to release inflammatory cytokines (IL-1, TNF-α), chemotactic factors (IL-8, Ccl2), and growth-promoting cytokines (IL-6, GM-CSF, TGF-β) (Corsini and Galli 1998). In addition, certain inflammatory cytokines produced by keratinocytes, such as IL-18 and IL-33, have the potential to engage the adaptive immune response and can regulate the development of humoral versus cellular immune responses (Moussion et al. 2008; Wittmann et al. 2009). Since dermal exposure to PFOA was shown to suppress T-cell-dependent IgM and IgG antibodies response and enhance IgE-mediated responses to protein and chemical allergens, evaluation of these cytokines was included in these studies (Anderson et al. 2009; Dewitt et al. 2008; 2009a; 2009b; Fairley et al. 2007; Yang et al. 2001; 2002).

The present data suggest that despite its corrosive potential, PFOA did not induce the expression of cytokines that may be involved in the modulation of immunologic responses and/or inflammation. Although a time course was evaluated, these studies only examined the effect of a single PFOA exposure. Repeated-exposure experiments will be required to fully

investigate the effect of dermal PFOA application on skin inflammatory cytokine production.

In summary, PFOA has been detected in the blood of virtually all Americans tested during the last decade (Calafat et al. 2007). Although it was long believed that PFOA was biologically inactive, more recent experiments, using controlled animal studies, showed an association between PFOA exposure and hepatic toxicity, carcinogenicity, reproductive toxicity, hormone disruption, and immunotoxicity (DeWitt et al. 2009b). While the majority of animal studies investigated the health effects associated with oral and inhalation routes of PFOA exposure, little is known about the significance of dermal exposure in the human population. The current studies indicate that the potential for dermal absorption is significant in both mouse and human skin and emphasize that the extent of PFOA dermal absorption is dependent on its ionization state. These results raise concern regarding the possibility for dermal exposure in both occupationally exposed individuals and the general population.

REFERENCES

Anderson-Mahoney, P., Kotlerman, J., Takhar, H., Gray, D., and Dahlgren, J. 2008. Self-reported health effects among community residents exposed to perfluorooctanoate. *N. Solut*. 18: 129–43.

Anderson, S. E., Butterworth, L., Jackson, L. G., Frasch, H. F., Franko, J., and Meade, B. J. 2009. Adjuvancy and immunosuppression: Mechanisms of immunomodulation following dermal exposure to PFOA in mice. *Toxicologist* 108: 467.

Arp, H. P., Niederer, C., and Goss, K. U. 2006 Predicting the partitioning behavior of various highly fluorinated compounds. *Environ. Sci. Technol*. 40: 7298–304.

Barton, C. A., Botelho, M. A., and Kaiser, M. A. 2007 Solid vapor pressure and enthalpy of sublimation for perfluorooctanoic acid. *J. Chem. Eng. Data* 53: 7298–303.

Betts, K. S. 2007. Perfluoroalkyl acids: What is the evidence telling us? *Environ. Health Perspect*. 115: A250–56.

Burns, D. C., Ellis, D. A., Li, H., McMurdo, C. J., and Webster, E. 2008. Experimental pKa determination for perfluorooctanoic acid (PFOA) and the potential impact of pKa concentration dependence on laboratory-measured partitioning phenomena and environmental modeling. *Environ. Sci. Technol*. 42: 9283–88.

Calafat, A. M., Needham, L. L., Kuklenyik, Z., Reidy, J. A., Tully, J. S., Aguilar-Villalobos, M., and Naeher, L. P. 2006. Perfluorinated chemicals in selected residents of the American continent. *Chemosphere* 63: 490–96.

Calafat, A. M., Wong, L. Y., Kuklenyik, Z., Reidy, J. A., and Needham, L. L. 2007. Polyfluoroalkyl chemicals in the U.S. population: Data from the National Health and Nutrition Examination Survey (NHANES) 2003–2004 and comparisons with NHANES 1999–2000. *Environ. Health Perspect*. 115: 1596–602.

Corsini, E., and Galli, C. L. 1998. Cytokines and irritant contact dermatitis. *Toxicol. Lett*. 102–103: 277–82.

DeWitt, J. C., Copeland, C. B., and Luebke, R. W. 2009b. Suppression of humoral immunity by perfluorooctanoic acid is independent of elevated serum corticosterone concentration in mice. *Toxicol. Sci*. 109: 106–12.

Dewitt, J. C., Copeland, C. B., Strynar, M. J. and Luebke, R. W. 2008. Perfluorooctanoic acid-induced immunomodulation in adult C57BL/6J or C57BL/6N female mice. *Environ. Health Perspect*. 116: 644–50.

DeWitt, J. C., Shnyra, A., Badr, M. Z., Lovelss, S. E., Hoban, D., Frame, S. R., Cunard, R., Anderson, S. E., Meade, B. J., Peden-Adams, M. M., Luebke, R. W., and Luster, M. I. 2009a. Immunotoxicity of perfluorooctanoic acid and perfluorooctane sulfonate and the role of peroxisome proliferator-activated receptor alpha. *Crit. Rev. Toxicol*. 39: 76–94.

European Centre for the Validation of Alternative Methods. 2000 *ESAC statement on the application of the EpiDerm human skin model for skin corrosivity testing*. European Centre for the Validation

of Alternative Methods. Available at: http://ecvam.jrc.ec.europa.eu/publication/EpiDerm_statement.pdf (accessed March 14, 2011).

Fairley, K. J., Purdy, R., Kearns, S., Anderson, S. E., and Meade, B. J. 2007. Exposure to the immunosuppressant, perfluorooctanoic acid, enhances the murine IgE and airway hyperreactivity response to ovalbumin. *Toxicol. Sci.* 97: 375–83.

Fasano, W. J., Kennedy, G. L., Szostek, B., Farrar, D. G., Ward, R. J., Haroun, L., and Hinderliter, P. M. 2005. Penetration of ammonium perfluorooctanoate through rat and human skin in vitro. *Drug Chem. Toxicol.* 28: 79–90.

Frasch, H. F., and Barbero, A. M. 2005. Application of solid-phase microextraction to in vitro skin permeation experiments: example using diethyl phthalate. *Toxicol. In Vitro* 19: 253–59.

ICCVAM. 2002. *ICCVAM Evaluation of EPISKIN™, EpiDerm™ (EPI-200), and the rat skin transcutaneous electrical resistance (TER) assay: In vitro test methods for assessing the dermal corrosivity potential of chemicals*. The Interagency Coordinating Committee on the Validation of Alternative Methods and the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicicological Methods. U.S. Department of Health and Human Services, National Institutes of Health, National Institute of Environmental Health Sciences, NIH publication number 02-4502. Available at: http://iccvam.niehs.nih.gov/methods/dermal/epiderm.htm (accessed March 14, 2011).

Kaiser, M. A., Dawson, B. J., Barton, C. A., and Botelho, M. A. 2010. Understanding potential exposure sources of perfluorinated carboxylic acids in the workplace. *Ann. Occup. Hyg.* 54: 915–22.

Kennedy, G. L., Butenhoff, J. L., Olsen, G. W., O'Connor, J.C., Seacat, A. M., Perkins, R. G., Biegel, L. B., Murphy, S. R., and Farrar, D. G. 2004. The toxicology of perfluorooctanoate. *Crit. Rev. Toxicol.* 34: 351–84.

Kudo, N., and Kawashima, Y. 2003. Toxicity and toxicokinetics of perfluorooctanoic acid in humans and animals. *J. Toxicol. Sci.* 28: 49–57.

Kuklenyik, Z., Needham, L. L., and Calafat, A. M. 2005. Measurement of 18 perfluorinated organic acids and amides in human serum using on-line solid-phase extraction. *Anal. Chem.* 77: 6085–91.

Liu, X., Testa, B., and Fahr, A. 2010. Lipophilicity and its relationship with passive drug permeation. *Pharm. Res.* 28: 962–77.

Moussion, C., Ortega, N., and Girard, J. P. 2008. The IL-1-like cytokine IL-33 is constitutively expressed in the nucleus of endothelial cells and epithelial cells in vivo: A novel 'alarmin'? *PLoS One* 3: e3331.

Olsen, G. W., Burris, J. M., Ehresman, D. J., Froehlich, J. W., Seacat, A. M., Butenhoff, J. L., and Zobel, L. R. 2007. Half-life of serum elimination of perfluorooctanesulfonate, perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. *Environ. Health Perspect.* 115: 1298–305.

Ping, J., Rodgers, P. J., and Ameniya, S. 2009 High lipophilicity of perfluoroalkyl carboxylate and solfonate: Implications for their membrane permeability. *J. Am. Chem. Soc.* 131: 2290–96.

Rayne, S., and Forest, K. 2009. Perfluoroalkyl sulfonic and carboxylic acids: A critical review of physicochemical properties, levels and patterns in waters and wastewaters, and treatment methods. *J. Environ. Sci. Health A Tox. Hazard. Subst. Environ. Eng.* 44: 1145–99.

Sabourin, C. L., Petrali, J. P., and Casillas, R. P. 2000. Alterations in inflammatory cytokine gene expression in sulfur mustard-exposed mouse skin. *J. Biochem. Mol. Toxicol.* 14: 291–302.

Shinoda, K., Hato, M., and Hayashi, T. 1972. Physicochemical properties of aqueous solutions of fluorinated surfactants. *J. Phys. Chem.* 76: 909–14.

Shore, P. A., Brodie, B. B., and Hogben, C. A. 1957. The gastric secretion of drugs: A pH

partition hypothesis. *J. Pharmacol. Exp. Ther.* 119: 361–69.

U.S. Environmental Protection Agency. 2006. *Revised draft hazard assessment of perfluorooctanoic acid and its salts*. Office of Pollution Prevention and Toxics, Risk Assessment Division. http://www.epa.gov/sab/pdf/sab_06_006.pdf

Vecchia, B. E., and Bunge, A. L. 2003. Skin absorption databases and predictive equations. In *Transdermal drug delivery*, 2nd ed., R. H. Guy and J. Hadgraft, eds., 59–63. New York: Marcel Dekker.

Wittmann, M., Macdonald, A., and Renne, J. 2009. IL-18 and skin inflammation. *Autoimmun. Rev.* 9: 45–48.

Yang, Q., Abedi-Valugerdi, M., Xie, Y., Zhao, X. Y., Möller, G., Nelson, B. D., and DePierre, J. W. 2002. Potent suppression of the adaptive immune response in mice upon dietary exposure to the potent peroxisome proliferator, perfluorooctanoic acid. *Int. Immunopharmacol.* 2: 389–97.

Yang, Q., Xie, Y., Eriksson, A. M., Nelson, B. D., and DePierre, J. W. 2001. Further evidence for the involvement of inhibition of cell proliferation and development in thymic and splenic atrophy induced by the peroxisome proliferator perfluoroctanoic acid in mice. *Biochem. Pharmacol.* 62: 1133–40.