HHS Public Access

Author manuscript

Food Chem Toxicol. Author manuscript; available in PMC 2020 December 22.

Published in final edited form as:

Food Chem Toxicol. 2020 February ; 136: 111114. doi:10.1016/j.fct.2020.111114.

Immunotoxicity and allergenic potential induced by topical application of perfluorooctanoic acid (PFOA) in a murine model

Hillary L. Shane[*], **Rachel Baur**, **Ewa Lukomska**, **Lisa Weatherly**, **Stacey E. Anderson**

Allergy and Clinical Immunology Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, WV, 26505, USA

Abstract

Perfluorooctanoic acid (PFOA) is a per- and polyfluoroalkyl substance (PFAS) once used as a surfactant in the polymerization of chemicals. Because of its ubiquitous nature and long half-life, PFOA is commonly detected in the environment, wildlife, and humans. While skin exposure to PFOA is of concern, studies evaluating the immunotoxicity of dermal exposure are lacking. These studies evaluated the immunotoxicity of PFOA (0.5–2% w/v, or 12.5–50 mg/kg/dose) following dermal exposure using a murine model. PFOA (0.5–2%) was not identified to be an irritant or sensitizer using the local lymph node assay. The IgM antibody response to sheep red blood cell. was significantly reduced in the spleen following 4-days of dermal exposure (2%). PFOA exposure produced a significant decrease in thymus (1 and 2%) and spleen (0.5–2%) weight along with an increase in liver weight (0.5–2%). Immune cell phenotyping identified a reduction in the frequency (1 and 2%) and number (0.5–2%) of splenic B-cells. To further define the mechanism of immunotoxicity, gene expression was also evaluated in the skin. The findings support a potential involvement of the nuclear receptor PPARα. These results demonstrate that dermal exposure to PFOA is immunotoxic and raise concern about potential adverse effects from dermal exposure.

Keywords

Allergy; Immune suppression; Immunotoxicity; Perfluorooctanoic acid

1. Introduction

Perfluorooctanoic acid (PFOA) is a per- and polyfluoroalkyl substance (PFAS) once widely used beginning in the 1950s primarily as a surfactant in the polymerization of chemicals including fluoroacrylic esters, fluoropolymers, and fluoroelastomers. Commercial

[*]Corresponding author. National Institute for Occupational Safety and Health (NIOSH), 1095 Willowdale Drive, Morgantown, WV, 26505, USA. hshane@cdc.gov (H.L. Shane).

CRediT authorship contribution statement

Hillary L. Shane: Conceptualization, Methodology, Investigation, Writing - review & editing. **Rachel Baur:** Investigation, Writing - review & editing. **Ewa Lukomska:** Investigation, Writing - review & editing. **Stacey E. Anderson:** Conceptualization, Methodology, Investigation, Writing - original draft, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2020.111114.

applications pertinent to these processes include manufacturing of flame retardants and extinguishers, surfactants, waxes and gloss finish enhancers, and water repellants in fabrics (Kudo et al., 2003; Starkov et al., 2002). Industrial production and emission of PFOA in North America and Europe was phased-out beginning in the early 2000s, with a complete cessation of PFOA production in the U.S. being achieved by 2015 (ITRC, 2017). Correspondingly, serum levels evaluated in human study populations have declined over time in these regions (Herrick et al., 2017). However, production of these chemicals or their precursors has increased in parts of Asia, and materials imported to the U.S. may still contain PFOA and/or related precursors. The hydrolytic half-life of PFOA in the environment is estimated to be greater than 97 years and the biological half-life in humans is reported to be 4.37 years (Kudo et al., 2003). Because of its ubiquitous nature, PFOA is commonly detected in the environment, wildlife, and humans (Giesy et al., 2001; Kannan et al., 2001, 2002a, 2002b, 2002c; Nakata et al., 2006). Additionally, PFOA has been identified in the blood of occupationally exposed individuals (Kudo et al., 2003; Ubel et al., 1980), as well as the general population (Emmett et al., 2006a, 2006b; Olsen et al., 1998, 2003). Occupational surveillance has demonstrated an increase in PFOA levels in the serum of individuals with and without workplace exposure; while several studies observed no discernable health effects (Olsen et al., 2003b; Olsen et al., 1998) the majority of evidence associates PFOA exposure with detrimental health outcomes including associations with numerous cancers (testicular and kidney), organ toxicity (hepatic, renal, etc.), endocrine, reproductive, developmental, and immunological effects (ATSDR, 2018; Gilliland et al., 1996; Kudo et al., 2003). Much of what we know about PFOA-mediated health effects was generated from epidemiological studies on occupationally exposed individuals with increased levels of PFOA present in their blood (Olsen et al., 2007) and individuals exposed to high environmental concentrations of PFOA (Barry et al., 2013; Frisbee et al., 2009).

The long clearance half-life of PFOA in humans has provoked intense interest in understanding the potential associated human health effects (Chang et al., 2016). Largely guided by evidence of immunotoxic effects of PFOA in *in vitro* and *in vivo* experimental systems, much epidemiologic research in recent years has focused on the possible effects of these chemicals on the immune system. Numerous reports have demonstrated PFOA-induced organ (liver, thymus, spleen) and systemic (body weight) toxicity following oral exposure (Betts, 2007; Kudo et al., 2003), in various animal models. 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., 2009b). PFOA is also immunosuppressive following oral exposure in murine models. A reduction in thymocyte and splenocyte populations, altered T lymphocyte populations, and an inhibition of T-cell-dependent IgM and IgG antibody responses have been documented (DeWitt et al., 2009a; Dewitt et al., 2008; DeWitt et al., 2009b; Yang et al., 2002; Yang et al., 2001). In addition to its immunosuppressive 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., 2007b). These data suggest that PFOA has the potential to augment IgE-mediated responses induced by other environmental and occupational allergens.

The potential for dermal exposure to PFOA is high, both during the manufacturing process, as well as in commercial products such as firefighting foams and carpet and fabric protectants. As one of the major uses of PFOA has been in carpet and fabric protectants, the potential exists for children to be exposed through dermal contact (as well as hand to mouth contact) and adults through both environmental and occupational exposures (Begley et al., 2005) (Kubwabo et al., 2005). Also, due it its persistance there is also concern for exposure in ground water which could occur during bathing or swimming, especially near sites where production once occured. (EPA, 2019). Despite the potential for dermal exposure, most research has focused on the immunotoxic effects of oral and inhalation PFOA exposure.

Previous data published in our laboratory suggest that PFOA is dermally absorbed and that under certain conditions the skin may be a significant route of exposure (Franko et al., 2012). Other than our previous findings, and a report of occupational dermal absorption in Chemolite workers (EPA, 2002), most investigations have ignored dermal PFOA exposure due to the assumption that it is not well absorbed by the skin (EPA, 2002). With the exception of a few older studies and unpublished reports that have conducted limited research looking at dermal irritancy and sensitization following PFOA exposure (Kennedy et al., 2004), there is an overall lack of data regarding the toxicity and/or immunogenicity of dermal exposure. Since the potential for PFOA dermal exposure still exists due to its environmental persistence, it is important to fully understand the potential for dermal penetration and the risk from exposure to PFOA through the dermal route.

In these studies, we show that dermal PFOA exposure induces immunotoxicity in a murine model, similar to that reported for oral and dietary exposure, and begin to define the associated mechanisms. These findings raise additional concerns about the immunotoxicity of PFOA and the dermal exposure route. In view of the ongoing environmental release and persistence of PFOA, widespread detection in humans, experimental and epidemiological evidence of immunotoxicity following oral exposure, and lack of data from the dermal route of exposure, these additional data enrich the database with respect to the immune hazard of PFOA.

2. Materials and methods

2.1. Test articles and chemicals

Acetone [CAS #67–64-1], perfluorooctanoic acid (PFOA; 96%) [CAS #335–67-1], α-hexylcinnamaldehyde (HCA) [CAS# 101–86-0], and cyclophosphamide [CP; CAS# 50–18-0] were purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI).

2.2. Species selection

Female BALB/c and $B_6C_3F_1$ mice were used in these studies. BALB/c mice have a T-helper (T_H)-2 bias and are commonly used to evaluate potential IgE-mediated sensitization, and were therefore used in the hypersensitivity studies (Klink et al., 2003; Woolhiser et al., 2000). $B_6C_3F_1$ mice are the strain of choice for immunotoxicity studies and were used to evaluate the IgM response to sheep red blood cells (SRBC) (Luster et al., 1992).

All mice were purchased from Taconic (Germantown, NY) at 6–8 weeks-of-age. Upon arrival, the animals were allowed to acclimate for a minimum of 5 days. Each shipment of animals was randomly assigned to treatment group, weighed, and individually identified via tail marking using a permanent marker. A preliminary analysis of variance on body weights was performed to insure a homogeneous distribution of animals across treatment groups. The animals were housed at a maximum of 5 mice/cage in ventilated plastic shoebox cages with hardwood chip bedding. 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 68 and 72°F and the relative humidity between 36 and 57%; a light/dark cycle was maintained at 12-h intervals. All animal experiments were performed in an AAALAC International 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.

2.3. PFOA exposures

For the sensitization study, BALB/c mice (5 mice/group) were topically treated on the dorsal surface of each ear with acetone vehicle, increasing concentrations of PFOA (0.5–2% w/v, or 12.5–50 mg/kg/dose) or positive control [30% HCA (v/v; sensitization positive control) once a day for three consecutive days. For the immune phenotyping and gene expression studies, BALB/c mice (5 mice/group) were topically exposed to acetone or increasing concentrations of PFOA (0.5–2% w/v, or 12.5–50 mg/kg/dose) on the dorsal surface of each ear once a day for 4 or 14 days. For analysis of the IgM response to SRBC, $B_6C_3F_1$ mice (N = 5) were topically exposed to acetone or increasing concentrations of PFOA (0.5–2%) on the dorsal surface of each ear once a day for 4 consecutive days. Cyclophosphamide (20 mg/kg in isotonic sterile saline) was included as the positive control for the analysis of the IgM response to SRBC and was injected intraperitoneally 4 days prior to sacrifice.

2.4. Combined local lymph node and irritancy assay

To determine the irritancy and sensitization potential of PFOA, a combined local lymph node assay (LLNA) was conducted according to the methods previously described (Anderson et al., 2013a). PFOA dosing concentrations (0.5–2% w/v, or 12.5–50 mg/kg/dose) and vehicle (acetone) were selected based on solubility and preliminary concentration range finding studies. HCA (30%) was included as the assay positive control.

2.5. Spleen in vivo response to the T-cell-dependent antigen SRBC

The primary IgM response to sheep red blood cells (SRBC) was enumerated using a modified hemolytic plaque assay of Jerne and Nordin (1963). Four days prior to euthanasia, the mice were immunized with 7.5×10^7 SRBC (in 200 μl volume) by intravenous injection. All SRBC for these studies were drawn from a single donor animal (Lampire Laboratories, Pipersville, PA). On the day of sacrifice, mice were euthanized by CO_2 asphyxiation, body and organ weights were recorded, and spleens were collected in 3 mL of Hank's balanced salt solution (HBSS). Single cell suspensions of the spleens from individual animals were prepared in HBSS by disrupting the spleen between the frosted ends of microscopic slides. To identify the total number of spleen cells, 20 μl of cells were added to 10 mL of Isoton II diluent (1:500; Beckman Coulter, Brea, CA) and two drops of Zap-o-globin (Beckman

Coulter, Brea, CA) were added to lyse red blood cells. Cells were then counted in the Coulter counter. Dilutions (1:30 and 1:120) of spleen cells were then prepared and 100 μl of each dilution were added to test tubes containing a 0.5 mL warm agar/dextran mixture (0.5% Bacto-Agar, DIFCO; and 0.05% DEAE dextran; Sigma, St. Louis, MO), 25 μl of 1:1 ratio of SRBC suspension, and 25 μl of 1:4 dilution (1 mL lyophilized) guinea pig complement (Cedarlane Labs, Burlington, Canada). Each sample was vortexed, poured into a petri dish, covered with a microscope coverslip, and incubated for 3 h at 37 °C. The plaques (representing antibody-forming B-cells) were then counted. Results were expressed in terms of both specific activity (IgM PFC per 10^6 spleen cells) and total activity (IgM PFC per spleen).

2.6. Immune phenotyping

Animals were euthanized by CO_2 inhalation 24 h after the final exposure, weighed, and examined for gross pathology. The liver, spleen, kidneys, and thymus were removed, cleaned of connective tissue and weighed. Left and right auricular draining lymph nodes (DLNs; drain the site of chemical application) and spleen were collected in 4 mL sterile phosphate-buffered saline (pH 7.4). Spleen and DLN cell suspensions (2 nodes/animal) were prepared by mechanical disruption of tissues between frosted microscope slides in phosphate buffered saline (PBS) and cells were counted after RBC lysis using a Z2 Coulter Particle Count and Size Analyzer (Beckman Coulter). One ear was collected in 2 mL of RPMI for immune phenotyping and one ear was collected in 0.5 mL of RNAlater for subsequent gene expression analysis (see below). Ear cell suspensions were prepared by splitting ear into ventral and dorsal halves, followed by an enzymatic digestion for 90 min at 37 °C with 0.25 mg/mL Liberase-TL Research grade (Roche) in RPMI with 100 μg/mL DNase I (Sigma-Aldrich). Digestion was stopped by the addition of 3 mL of RPMI +10% FBS, the ears + media were transferred to gentleMACS C Tubes (Miltenyi Biotec), then cells were mechanically disrupted on a gentleMACS™ Dissocciator (Miltenyi Biotec). Following disruption, cells were passed through a 70 μm cell strainer to make a single cell suspension, washed with RPMI 10% FBS, then live cells were counted on a Cellometer using AO/PI (Nexcelom) in order to quantify cells. For staining, single cell suspensions were resuspended in staining buffer containing a-mouse CD16/32 antibody (Fc Block) (BD Biosciences) then incubated with a cocktail of fluorochrome-conjugated antibodies specific for mouse cell surface antigens. For LN and Spleen cells: α-IgE-FITC (R35–72), α-B220-V500 (RA3–6B2), α-CD8a-PE (53–6.7) (BD Biosciences, San Jose, CA), α-CD4-BV-605 (GK1.4) (BioLegend, San Diego, CA), α-CD11b-PerCP-Cyanine5.5 (M1/70), α-CD11c-eF-450 (N418), α-CD25 PE-eF-601 (PC61.5), α-CD86-APC (GL1), α-MHC II-AF700 (M5/14.15.2), and α-CD44-eFluor 780 (IM7) (eBioscience). For ear cells: α-CD45-BV605 (30-F11), α-CD3-V500 (500A2), α-Siglec-F-PE (E50–2440), α-Ly6G-FITC (1A8) (BD Biosciences), Lineage-PerCP-Cyanine5.5 (α-Ter-119 [TER-119], α-CD19 [eBio1D3], α-CD11b [M1/70], and α-CD11c [N418]), α-CD90.2-Super Bright 780 (30-H12), α-CD117-eFlour 450 (2B8), α-FcεRI-APC (MAR-1) (eBioscience), and α-F4/80-APC-Fire750 (BM8) (Biolegend). Cells were then washed, fixed in Cytofix buffer (BD Biosciences), resuspended in staining buffer, and events were collected on an LSR II flow cytometer (BD Biosciences), compensation controls were prepared with eBioscience UltraComp eBeads.Analysis was performed using FlowJo v10 software (TreeStar Inc., Ashland, OR).

All cells were gated on single events prior to subsequent gating. The $IgE^{+}B220^{+}$ (IgE^{+} B-cells) population were analyzed as described by Manetz and Meade (1999). Gating for the spleen and LN phenotyping was performed as previously described (Shane et al., 2017). For the phenotyping of the ear, cellular populations were defined as follows: hematopoietic cells/leukocytes ($CD45^{+}$), T cells ($CD45^{+}$,SSC^{low},$CD45^{+}$ Lin^{-},$CD90^{+}$,$CD3^{+}$), mast cells ($CD45^{+}$,SSC^{hi},$CD45^{+}$,Lin^{-},$ckit^{+}$ $Fc\varepsilon RI^{+}$), neutrophils ($CD45^{+}$, $CD11b^{+}$ Ly-$6G^{+}$, $SiglecF^{-}$), eosinophils ($CD45^{+}$, $CD11b^{+}$ Ly-$6G^{int}$ $SiglecF^{+}$ SSC^{hi}), monocytes/macrophages ($CD45^{+}$, Lin^{+}, $F4/80^{+}$, SSC^{low}).

2.7. Gene expression analysis

Ears (1/mouse) were mechanically disrupted on a TissueLyser II in Buffer RLT (Qiagen). Total RNA was extracted using Qiagen's RNeasy mini spin column kits with DNase treatment on a QIAcube workstation. RNA concentrations and purity were analyzed on a NanoDrop spec-trophotomer (Thermo Fisher Scientific). The cDNA (1–2 μg) was prepared on an Eppendorf Mastercycler using Applied Biosystems' High Capacity Reverse Transcription kit. The cDNA was used as template for real-time PCR reactions containing TaqMan PCR Master Mix with gene-specific primers (Applied Biosystems) on a 7500 Real-Time PCR System. Relative fold gene expression changes ($2^{-\Delta\Delta CT}$) were determined compared to acetone controls and normalized for expression of housekeeping gene *beta-actin* (*Actb*). Genes that were evaluated include: *Tslp* (Mm01157588_m1), *Il1b* (Mm00434228_m1), *Il6* (Mm00446190_m1), *PPARα* (Mm00440930_m1), *Nfkb1* (Mm00476361_m1), *Flg2* (Mm02744902_ g1), *Lor* (Mm01962659_s1), and *Itgbl1* (Mm01200043_m1).

2.8. Statistical analyses

For analysis of the data generated from the described animal studies, the data were first tested for homogeneity using the Bartlett's Chi Square test. If homogeneous, a one-way analysis of variance (ANOVA) was conducted. If the ANOVA showed significance at $p < 0.05$ or less, the Dunnett's Multiple Range *t*-test was used to compare treatment groups with the control group. Linear trend analysis was performed to determine if PFOA had exposure concentration-related effects for the specified endpoints. Statistical analysis was performed using Graph Pad Prism version 5.0 (San Diego, CA). Statistical significance is designated by $*p \leq 0.05$ and $**p \leq 0.01$.

3. Results

3.1. In vivo studies did not identify PFOA to be an allergic sensitizer or dermal irritant

Dermal exposure to PFOA was found to be toxic (greater than 20% loss in body weigh) at concentrations greater than 2% (data not shown). For this reason, concentrations of PFOA up to 2% were tested in the subsequent studies. No ear swelling was observed in mice after dermal exposure to PFOA (data not shown). No increase in auricular DLN proliferation was identified after treatment with PFOA (Fig. 1). HCA (30%) was used as a positive control for these experiments and resulted in an average DPM/node of 4,704, indicating a 9.8 fold increase over vehicle (data not shown).

3.2. Dermal exposure to PFOA suppressed the splenic IgM response to SRBC

To evaluate if exposure to PFOA was immunosuppressive, the murine IgM response to SRBC was examined following a 4-day exposure to PFOA. Statistically significant reductions in the specific (PFC/10^6 cells) and PFC/spleen IgM antibody activity against SRBC were observed after exposure to PFOA (Fig. 2A and 2B). Exposure of mice to 2% PFOA resulted in a suppression of the values for PFC/10^6 cells and PFC/spleen (62 and 64%, respectively, vs. values for vehicle-treated mice); 1% PFOA resulted in suppressions of PFC/10^6 cells (20%) and PFC/spleen (27%); although it did not reach statistical significance (Fig. 2). However, there was a dose-responsive decrease in both PFC/10^6 cells and PFC/spleen (Linear Trend Test $p \leq 0.05$). Mice exposed to cyclophosphamide had a significantly reduced specific spleen IgM response (70% reduction) and total IgM response (67% reduction) compared to levels noted in vehicle-treated controls (data not shown).

3.3. Dermal exposure to PFOA for 4 and 14 days results in significant alterations in organ weights

A statistically significant decrease in thymus and spleen weights (% body weight) was observed following exposure to PFOA (Fig. 3A and B). Thymus weight was significantly decreased by 4-days of 2% PFOA exposure and for 1 and 2% for 14-days of exposure (Fig. 3A). Spleen weight was significantly decreased for all concentrations of PFOA at 4 and 14-days of exposures (Fig. 3B). Liver weight (% body weight) was significantly increased for all concentrations of PFOA at 4 and 14-days of exposures (Fig. 3C). Kidney weight (% body weight) was significantly decreased at 4-days but only following 2% exposure (Fig. 3D). In contrast, by 14-days of PFOA exposure, kidney weight was increased for both the 1% and 2% exposure groups. Organ weights not corrected for total body weight are reported in Supplemental Table 1, where significant decreases in mass of the thymus (14 days, 0.5%–2%) and spleen (4 and 14 days; 0.5%–2%) were observed with significant increases in liver mass (4 and 14 days; 0.5%–2%). Exposure to 2% PFOA was terminated after 10 days due to a greater than 20% loss in body weight. No changes in body weight were observed following 4 days of PFOA exposure however, 14 days of exposure resulted in dose responsive decreases in body weight (Supplemental Fig. 1).

3.4. Dermal exposure to PFOA for 4 and 14 days results in significant phenotypic changes in the spleen and DLN

Overall, the splenic cellularity was reduced, and reductions in cell numbers were observed after both 4 and 14 days of exposure to PFOA (0.5%, 1% and 2%) in all subsets of cells analyzed (Table 1), reflective of the reduction in total spleen mass (Fig. 3). However, certain subsets of cells were disproportionally reduced. Dermal exposure to PFOA for 4 days did not result in changes in the frequency of spleen CD4 T-cells, CD8 T-cells, B-cells, dendritic cells (DCs), or monocytes (Table 1). However, following 14 days of dermal PFOA exposure increases were observed in the frequency of CD4 T-cells (0.5%, 1%, and 2%) and CD8 T-cells (0.5%, 1%, and 2%) along with a significant decrease in the frequency of B-cells (1% and 2%) (Table 1). A dose dependent increase in monocytes (reaching statistical significance at the 2% PFOA concentration) was also observed. Phenotypic analysis of the DLN following 4 days of PFOA exposure resulted in a significant increase in the frequency of

DCs (2%) and a decrease in the frequency of monocytes (1%) (Table 2). The significant increase in DCs was also observed following 14 days of exposure to 2% PFOA. However, no changes in the frequency of monocytes was observed following 14-days of PFOA exposure. The extended PFOA exposure also resulted in a decrease in the frequency of CD4 T-cells in the LN (1% and 2%) and a decrease in the frequency of CD8 T-cells (1% and 2%) in the DLN. Exposure to 2% PFOA for 4 days produced statistically significant increases in the absolute number of total cells, B-cells, $CD4^+$ T-cells, $CD8^+$ T-cells, DCs, and monocytes (Table 2). However, for the 14-day exposure statistically significant decreases were observed for total cells (1% and 2%), B-cells (1%), and $CD4^+$ T-cells (1% and 2%).

3.5. Dermal PFOA exposure results in immunological changes in the skin

Dermal exposure to PFOA for 4 days did not result in changes in the frequency of CD45 cells, T-cells, mast cells, eosinophils, or monocytes in the ear tissue (Table 3). A statistically significant increase in the frequency of neutrophils was observed but only following 4 days of 2% PFOA exposure. Following 14 days of dermal PFOA exposure decreases were observed in the total number of cells (2%), frequency of CD45 cells (1% and 2%) and eosinophils (0.5%, 1%, 2%). Increases were observed in the frequency of T-cells (1%, and 2%) and mast cells (1%). A small but statistically significant decrease in cellularity was observed but only following 14 days of 2% PFOA ($1.21 \times 10^6 \pm 1.03 \times 10^5$) compared to the acetone control ($1.56 \times 10^6 \pm 8.5 \times 10^4$). Significant increases in absolute numbers of CD45, T-cells, neutrophils, and monocytes were observed but only following exposure to 2% PFOA for 4 days (Table 3). However, following 14 days of PFOA exposure decreases in CD45 cells (1% and 2%), eosinophils (0.5%, 1%, 2%) and monocytes (1% and 2%) were observed.

In an attempt to better define the mechanism of dermal immunotoxicity, select mRNA transcripts were evaluated in the skin following PFOA exposure. Increases in the Th2 skewing cytokine *Tslp* were observed at both PFOA exposure durations (Fig. 4A). A small but significant increase in the inflammatory cytokines *Il1b* and *Il6* were observed but only following the 4 day 2% PFOA exposure (Fig. 4B and C). Interestingly, decreases in peroxisome proliferator-activated receptor alpha (*PPARα*) and nuclear factor kappa B (*Nfkb1*) expression were observed at all concentrations but only for the 14-day exposure duration (Fig. 4D and E). Expression in genes involved in skin barrier integrity were also evaluated. Decreases in filaggrin (*Flg2*), integrin subunit beta like 1 (*Itgbl1*) and loricrin (*Lor*) were observed, but only for the extended PFOA exposure duration (Fig. 4F–H).

4. Discussion

A large number of workers in the United States are potentially exposed to chemicals that can be absorbed through the skin (Anderson et al., 2014). Since immune dysfunction can affect multiple organ systems, there is an increasing need to evaluate these chemicals and/or substances. A major role of the skin, as the largest organ in the body, is to serve as a barrier to protect from environmental and chemical insults. Disruption of barrier function may lead to inflammatory and immunological responses in the skin and other tissues (Hanel et al., 2013). In addition to providing protection from the outside environment, the skin is an extremely important player in immunological responses. The skin is an immunologically

active organ that must maintain a delicate balance between pro-inflammatory and anti-inflammatory immune responses in order to react against pathogens and yet mitigate unnecessary tissue damage. In order to achieve this balance, the skin is highly integrated with a diverse milieu of regulatory and inflammatory immune cells and mediators that are unique to this tissue. In an attempt to fill some of the data gaps associated with dermal PFOA exposure-related health effects, the immunotoxicity of PFOA was evaluated using a murine model in the studies described here. It is important to note that the overall purpose of this paper is for hazard identification, as such the concentrations of PFOA used in this manuscript were chosen based on preliminary data generated in our laboratory which showed immunotoxic effects following dermal exposure. Dermal exposure to 0.5%–2% PFOA resulted in detectable levels of PFOA in the serum, with 4 d of dermal exposure to 1% PFOA resulting in serum levels of 188 ± 16 μg/mL (Franko et al., 2012). These experimental concentrations are approximately 15- to 30-fold higher than those measured in the serum of the highest occupationally exposed human populations, (Kudo and Kawashima, 2003). It should be noted that comparisons between species can be difficult to make as human exposures are often chronic in nature and PFAS are excreted/eliminated at different rates in humans (Ubel et al., 1980) and in different experimental animal systems/sexes (Vanden Heuvel et al., 1991). However, the serum PFOA levels occurring after dermal exposure are for the purposes of hazard identification and are comparable to other experimental animal studies in the literature based on oral exposure to PFOA via gavage (Dewitt et al., 2008).

Similar to what has been described in the literature following oral exposure, suppression of the IgM response to SRBC was observed following dermal exposure to this chemical (Fig. 2). Phenotypic analysis of the spleen showed decreased cellularity in addition to decreases in absolute number and frequency of B-cells, further supporting the suppressive effect (Table 1). The T-cell dependent antibody response is one of the most sensitive indicators of immune integrity because it relies on an organized immune response that is dependent on the functional capacity and cooperation of numerous cell types including B-cells, T-cells, and macrophages (Anderson et al., 2006). Additional evidence for suppression and immunotoxicity was evidenced by decreases in spleen and thymus weights along with increases in liver weights as early as 4 days following dermal PFOA exposure (Fig. 3). In the DLN, by 14 days of exposure, total cell numbers and the frequency of $CD4^+$ subsets were significantly decreased (Table 2). While earlier studies identified immunotoxicity following a 4 day dermal PFOA exposure (Fairley et al., 2007a), recent findings in our laboratory suggest that exposure duration might also influence immunological response (Shane et al., 2017). Therefore, a 4 day and 14 day exposure duration were examined in the current study. While the exposure duration for the mice exposed to 2% PFOA had to be terminated due to the onset of overt toxicity (Supplemental Fig. 1), the majority of significant effects were also observed at lower concentrations that did not result in excessive loss in body weight.

In addition to other pathways, PFOA has been shown to trigger biological activity by activating the alpha isotype of peroxisome proliferator-activated receptors (PPARα), ligand-activated transcription factors that regulate gene expression (Li et al., 2017). Mechanistically, PPARα ligands block the NF-κB pathway and thereby modulate subsequent immune responses. Activation of PPARα modulates lipid and glucose

homeostasis, cell proliferation and differentiation, and inflammation (DeWitt et al., 2009b). However, PPARα independent PFOA induced immunological effects have also been demonstrated in PPARα knockout animal models and it has been suggested PFOA induced immune suppression is mediated via a PPARα independent pathway most likely due to B-cell disruption (DeWitt et al., 2016). PPARα is expressed in many cutaneous immune cells types including macrophages, keratinocytes, and T-lymphocytes where it regulates inflammatory responses (Dubrac et al., 2011). In an attempt to better define the dermal PFOA immunotoxicity, PPARα expression was examined in the skin. Interestingly, PFOA did not increase expression of *PPARα* in the skin following dermal exposure at the time points evaluated but instead resulted in significant decreases following the 14 days of exposure (Fig. 4). However, expression of *PPARα* at early time points (less than 4 days of exposure) was not evaluated. Consistent with PPARα activation, expression of *Nfkb1* was decreased by 14 days and no persistent signs of inflammation (evidenced by increases in *Il6 and Il1b*) were observed. However, a slight inflammatory response was observed after 4 days of exposure supported by increases in T-cell numbers in the DLN and skin, increases in eosinophil and neutrophil number and increases in inflammatory cytokines (*Il1b* and *Il6*) in the skin which completely resolved or decreased by 14 days (Tables 2 & 3 and Fig. 4).

While described as a PPARα agonist, no increases in *PPARα* were observed in the skin suggesting PFOA might not activate PPARα in the skin or that increases occurred at early time points which were not examined. Although this relationship has not been thoroughly investigated, differential expression and sensitivity of PFOA activation of PPARα has been reported (Abbott et al., 2012). The results from the present study demonstrate that PFOA is a non-irritating and non-sensitizing chemical as evidenced by the lack of increase in ear swelling and lymphocyte proliferation (Fig. 1). However, PFOA has also been shown to augment allergic disease in an animal model, but specific mechanisms have not been described (Fairley et al., 2007a). A lot of research has explored the therapeutic potential of PPARα agonists in inflammatory and allergic diseases and anti-inflammatory effects of PPARα activation have been reported in mouse models of irritant and allergic contact dermatitis (Furue et al., 2018). While PFOA might not induce direct activation of PPARα in the skin, there is involvement of this pathway as evidenced by significant reductions in expression. Research has demonstrated decreased expression of PPARα following permeability barrier abrogation and additional increases in Th2 cytokines in cultured normal human keratinocytes (Adachi et al., 2013). Due to the potential involvement of PPARα in skin barrier integrity, expression of the related mRNA transcripts *Flg2*, *Lor*, and *Itgbl1* were evaluated. Filaggrin is a protein involved in epidermal barrier function and research has shown that its expression is decreased in individuals with skin barrier disorder and atopic dermatitis (Cabanillas et al., 2016). Loricrin is a protein important for skin barrier formation and integrity and has been shown to be reduced in the skin of individuals with atopic dermatitis (Kim et al., 2008). Itgbl1 has been suggested to play a role in intracellular adhesions (Symington et al., 1993). Consistent with these studies, results from the current study demonstrate a significant decrease in the expression of *Flg2*, *Lor*, and *Itgbl1* following dermal PFOA exposure (Fig. 4). This suggests the potential involvement of PPARα in a cycle between barrier dysfunction and allergic inflammation. Although the findings support that PFOA might induce compromised skin integrity, no visual (irritation) or molecular

indicators (inflammation) were identified. In addition, previous work has shown that PFOA solid is corrosive, but not at the 1% applied dose when evaluated in a cultured epidermis *in vitro* model (Franko et al., 2012).

In addition to allergic responses in the skin, inverse relationships have been identified between PPARα expression and asthma (Kobayashi et al., 2005). While direct PPARα agonists have been shown to reduce airway inflammation, decreases in PPARα expression may cause elevations in this response (Trifilieff et al., 2003) and PPARα agonists have been shown to reduce TSLP expression (Jung et al., 2018). Consistent with the decrease in PPARα expression, expression of the Th2 skewing cytokine, *Tslp* was elevated in PFOA exposed skin by 4 days of exposure and persisted throughout exposure duration (Fig. 4). Elevations in *Tslp* following dermal chemical exposure have also previously been shown by our laboratory to contribute to immune ad-juvancy (Anderson et al., 2013b; Marshall et al., 2015). Additional studies have associated PFOA exposure with allergic disease including previous work conducted in our laboratory that demonstrated dermal exposure to PFOA enhances allergen-specific responses in a murine model (Fairley et al., 2007b). Consistent with this hypothesis, a significantly greater prevalence of self-reported cases of chronic bronchitis and asthma were 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). A recent study also associated PFOA exposure with earlier onset of atopic dermatitis further supporting the association of PFOA exposure with allergic disease (Wen et al., 2019).

These are the first studies to evaluate the allergenic potential and immunotoxicity induced by dermal exposure to PFOA using a murine model. The findings suggest the potential immunological pathway include direct and/or indirect PPARα effects which might be a result of compromised skin barrier integrity. As human PPARα expression is significantly less than that of rodents, potential PPARα independence indicates that future research should explore mechanisms of action of PFOA and similar compounds, including PPARα-dependent and independent pathways (Chang et al., 2016; DeWitt et al., 2009b). Although significant data gaps still exist for the complete toxicological evaluation of this chemical, these results suggest that PFOA is an immunotoxic chemical following dermal 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 can help reduce exposure, as well as provide insight into potential mechanisms of action.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding

This work was supported by internal funds from the Health Effects Laboratory Division of the National Institute for Occupational Safety and Health

Abbreviations:

ANOVA	analysis of variance
DC	dendritic cell
DLN	draining lymph nodes
HCA	α-hexylcinnamaldehyde
LLNA	local lymph node assay
NF- κB	nuclear factor kappa B
PFAS	per- and polyfluoroalkyl substance
PFC	plaque forming cells
PFOA	Perfluorooctanoic acid
PPARα	alpha isotype of peroxisome proliferator-activated receptors
SRBC	sheep red blood cell
TSLP	thymic stromal lymphopoietin

References

Abbott BD, Wood CR, Watkins AM, Tatum-Gibbs K, Das KP, Lau C, 2012 Effects of perfluorooctanoic acid (PFOA) on expression of peroxisome proliferator-activated receptors (PPAR) and nuclear receptor-regulated genes in fetal and postnatal CD-1 mouse tissues. Reprod. Toxicol. 33 (4), 491–505. 10.1016/j.reprotox.2011.11.005. [PubMed: 22154759]

Adachi Y, Hatano Y, Sakai T, Fujiwara S, 2013 Expressions of peroxisome proliferator-activated receptors (PPARs) are directly influenced by permeability barrier abrogation and inflammatory cytokines and depressed PPARalpha modulates expressions of chemokines and epidermal differentiation-related molecules in keratinocytes. Exp. Dermatol. 22 (9), 606–608. 10.1111/exd.12208. [PubMed: 23947677]

Anderson SE, Munson AE, Meade BJ, 2006 Analysis of Immunotoxicity by Enumeration of Antibody-Producing B cells.. Curr. Protoc. Toxicol. 29 (1). https://doi. org/10.1002/0471140856.tx1811s29.

Anderson-Mahoney P, Kotlerman J, Takhar H, Gray D, Dahlgren J, 2008 Self-reported health effects among community residents exposed to perfluorooctanoate. New Solut. 18 (2), 129–143. [PubMed: 18511391]

Anderson SE, Franko J, Anderson KL, Munson AE, Lukomska E, Meade BJ, 2013a Immunotoxicity and allergic potential induced by topical application of dimethyl carbonate (DMC) in a murine model. J. Immunotoxicol. 10 (1), 59–66. 10.3109/1547691x.2012.691124. [PubMed: 22953780]

Anderson SE, Franko J, Kashon ML, Anderson KL, Hubbs AF, Lukomska E, Meade BJ, 2013b Exposure to triclosan augments the allergic response to ovalbumin in a mouse model of asthma. Toxicol. Sci.: Off. J. Soc. Toxicol. 132 (1), 96–106. 10.1093/toxsci/kfs328.

Anderson SE, Meade BJ, 2014 Potential health effects associated with dermal exposure to occupational chemicals. Environ. Health Insights 8 (Suppl. 1), 51–62. 10.4137/ehi.s15258. [PubMed: 25574139]

ATSDR, 2018 DRAFT toxicological profile for perfluoroalkyls. Available at: https://www.atsdr.cdc.gov/toxprofiles/tp200.pdf Accessed.

Barry V, Winquist A, Steenland K, 2013 Perfluorooctanoic acid (PFOA) exposures and incident cancers among adults living near a chemical plant. Environ. Health Perspect. 121 (11–12), 1313–1318. 10.1289/ehp.1306615. [PubMed: 24007715]

Begley TH, White K, Honigfort P, Twaroski ML, Neches R, Walker RA, 2005 Perfluorochemicals: potential sources of and migration from food packaging. Food Addit. Contam. 22 (10), 1023–1031. [PubMed: 16227186]

Betts KS, 2007 Perfluoroalkyl acids: what is the evidence telling us? Environ. Health Perspect. 115 (5), A250–A256. [PubMed: 17520044]

Cabanillas B, Novak N, 2016 Atopic dermatitis and filaggrin. Curr. Opin. Immunol. 42, 1–8. 10.1016/j.coi.2016.05.002. [PubMed: 27206013]

Chang ET, Adami HO, Boffetta P, Wedner HJ, Mandel JS, 2016 A critical review of perfluorooctanoate and perfluorooctanesulfonate exposure and immunological health conditions in humans. Crit. Rev. Toxicol. 46 (4), 279–331. 10.3109/10408444.2015.1122573. [PubMed: 26761418]

DeWitt JC, Copeland CB, Luebke RW, 2009a Suppression of humoral immunity by perfluorooctanoic acid is independent of elevated serum corticosterone concentration in mice. Toxicol. Sci.: Off. J. Soc. Toxicol. 109 (1), 106–112. 10.1093/toxsci/kfp040. (Research Support, Non-U.S. Govt', Research Support, U.S. Govt', Non-P.H.S.).

Dewitt JC, Copeland CB, Strynar MJ, Luebke RW, 2008 Perfluorooctanoic acid-induced immunomodulation in adult C57BL/6J or C57BL/6N female mice. Environ. Health Perspect. 116 (5), 644–650. 10.1289/ehp.10896. [PubMed: 18470313]

DeWitt JC, Shnyra A, Badr MZ, Loveless SE, Hoban D, Frame SR, Cunard R, Anderson SE, Meade BJ, Peden-Adams MM, et al., 2009b Immunotoxicity of perfluorooctanoic acid and perfluorooctane sulfonate and the role of peroxisome proliferator-activated receptor alpha. Crit. Rev. Toxicol. 39 (1), 76–94. 10.1080/10408440802209804. [PubMed: 18802816]

DeWitt JC, Williams WC, Creech NJ, Luebke RW, 2016 Suppression of antigen-specific antibody responses in mice exposed to perfluorooctanoic acid: role of PPARalpha and T- and B-cell targeting. J. Immunotoxicol. 13 (1), 38–45. https://doi. org/10.3109/1547691x.2014.996682. [PubMed: 25594567]

Dubrac S, Schmuth M, 2011 PPAR-alpha in cutaneous inflammation. Derm. Endocrinol. 3 (1), 23–26. 10.4161/derm.3.1.14615.

Emmett EA, Shofer FS, Zhang H, Freeman D, Desai C, Shaw LM, 2006a Community exposure to perfluorooctanoate: relationships between serum concentrations and exposure sources. J. Occup. Environ. Med. 48 (8), 759–770. [PubMed: 16902368]

Emmett EA, Zhang H, Shofer FS, Freeman D, Rodway NV, Desai C, Shaw LM, 2006b Community exposure to perfluorooctanoate: relationships between serum levels and certain health parameters. J. Occup. Environ. Med. 48 (8), 771–779. [PubMed: 16902369]

EPA, U.S, 2002 Office of Pollution Prevention and Toxics, Risk Assessment Division, Revised Draft Hazard Assessment of Perfluorooctanoic Acid and its Salts.

EPA, U. S, 2019 USEPA Draft interim recommendations to address groundwater contaminated with Perfluorooctanoic Acid and Perfluorooctane Sulfonate.

Fairley KJ, Purdy R, Kearns S, Anderson SE, Meade B, 2007a Exposure to the immunosuppressant, perfluorooctanoic acid, enhances the murine IgE and airway hyperreactivity response to ovalbumin. Toxicol. Sci.: Off. J. Soc. Toxicol. 97 (2), 375–383. 10.1093/toxsci/kfm053.

Fairley KJ, Purdy R, Kearns S, Anderson SE, Meade BJ, 2007b Exposure to the immunosuppressant, perfluorooctanoic acid, enhances the murine IgE and airway hyperreactivity response to ovalbumin. Toxicol. Sci.: Off. J. Soc. Toxicol. 97 (2), 375–383. 10.1093/toxsci/kfm053.

Franko J, Meade BJ, Frasch HF, Barbero AM, Anderson SE, 2012 Dermal penetration potential of perfluorooctanoic acid (PFOA) in human and mouse skin. J. Toxicol. Environ. Health Part A 75 (1), 50–62. 10.1080/15287394.2011.615108.

Frisbee SJ, Brooks AP Jr., Maher A, Flensborg P, Arnold S, Fletcher T, Steenland K, Shankar A, Knox SS, Pollard C, et al., 2009 The C8 health project: design, methods, and participants. Environ. Health Perspect. 117 (12), 1873–1882. 10.1289/ehp.0800379. [PubMed: 20049206]

Furue K, Mitoma C, Tsuji G, Furue M, 2018 Protective role of peroxisome proliferator-activated receptor alpha agonists in skin barrier and inflammation. Immunobiology 223 (3), 327–330. 10.1016/j.imbio.2017.10.047. [PubMed: 29111315]

Giesy JP, Kannan K, 2001 Global distribution of perfluorooctane sulfonate in wildlife. Environ. Sci. Technol. 35 (7), 1339–1342. [PubMed: 11348064]

Gilliland FD, Mandel JS, 1996 Serum perfluorooctanoic acid and hepatic enzymes, lipoproteins, and cholesterol: a study of occupationally exposed men. Am. J. Ind. Med. 29 (5), 560–568. [PubMed: 8732932]

Hanel KH, Cornelissen C, Luscher B, Baron JM, 2013 Cytokines and the skin barrier. Int. J. Mol. Sci. 14 (4), 6720–6745. 10.3390/ijms14046720. [PubMed: 23531535]

Herrick RL, Buckholz J, Biro FM, Calafat AM, Ye X, Xie C, Pinney SM, 2017 Polyfluoroalkyl substance exposure in the mid-Ohio river valley, 1991–2012. Environ. Pollut. 228, 50–60. 10.1016/j.envpol.2017.04.092. [PubMed: 28505513]

ITRC ITRC, 2017 History and Use of Per- and Polyfluoroalkyl Substances (PFAS).

Jerne NK, Nordin AK, 1963 Plaque Formation in Agar by Single Antibody-Producing Cells. Science 140 (3565), 405 10.1126/science.140.3565.405.

Jung Y, Kim JC, Park NJ, Bong SK, Lee S, Jegal H, Jin LT, Kim SM, Kim YK, Kim SN, 2018 Eupatilin, an activator of PPARalpha, inhibits the development of oxazolone-induced atopic dermatitis symptoms in Balb/c mice. Biochem. Biophys. Res. Commun. 496 (2), 508–514. 10.1016/j.bbrc.2018.01.098. [PubMed: 29353040]

Kannan K, Choi JW, Iseki N, Senthilkumar K, Kim DH, Giesy JP, 2002a Concentrations of perfluorinated acids in livers of birds from Japan and Korea. Chemosphere 49 (3), 225–231. [PubMed: 12363300]

Kannan K, Corsolini S, Falandysz J, Oehme G, Focardi S, Giesy JP, 2002b Perfluorooctanesulfonate and related fluorinated hydrocarbons in marine mammals, fishes, and birds from coasts of the Baltic and the Mediterranean Seas. Environ. Sci. Technol. 36 (15), 3210–3216. [PubMed: 12188342]

Kannan K, Franson JC, Bowerman WW, Hansen KJ, Jones PD, Giesy JP, 2001 Perfluorooctane sulfonate in fish-eating water birds including bald eagles and albatrosses. Environ. Sci. Technol. 35 (15), 3065–3070. [PubMed: 11505980]

Kannan K, Newsted J, Halbrook RS, Giesy JP, 2002c Perfluorooctanesulfonate and related fluorinated hydrocarbons in mink and river otters from the United States. Environ. Sci. Technol. 36 (12), 2566–2571. [PubMed: 12099451]

Kennedy GL Jr., Butenhoff JL, Olsen GW, O'Connor JC, Seacat AM, Perkins RG, Biegel LB, Murphy SR, Farrar DG, 2004 The toxicology of perfluorooctanoate. Crit. Rev. Toxicol. 34 (4), 351–384. [PubMed: 15328768]

Kim BE, Leung DY, Boguniewicz M, Howell MD, 2008 Loricrin and involucrin expression is down-regulated by Th2 cytokines through STAT-6. Clin. Immunol. 126 (3), 332–337. 10.1016/j.clim.2007.11.006. [PubMed: 18166499]

Klink KJ, Meade BJ, 2003 Dermal exposure to 3-amino-5-mercapto-1,2,4-triazole (AMT) induces sensitization and airway hyperreactivity in BALB/c mice. Toxicol. Sci.: Off. J. Soc. Toxicol. 75 (1), 89–98. 10.1093/toxsci/kfg171.

Kobayashi M, Thomassen MJ, Rambasek T, Bonfield TL, Raychaudhuri B, Malur A, Winkler AR, Barna BP, Goldman SJ, Kavuru MS, 2005 An inverse relationship between peroxisome proliferator-activated receptor gamma and allergic airway inflammation in an allergen challenge model. Ann. Allergy Asthma Immunol.: Off. Publ. Am. Coll. Allergy Asthma Immunol. 95 (5), 468–473. 10.1016/s1081-1206(10)61173-8.

Kubwabo C, Stewart B, Zhu J, Marro L, 2005 Occurrence of perfluorosulfonates and other perfluorochemicals in dust from selected homes in the city of Ottawa, Canada. J. Environ. Monit. 7 (11), 1074–1078. [PubMed: 16252056]

Kudo N, Kawashima Y, 2003 Toxicity and toxicokinetics of perfluorooctanoic acid in humans and animals. J. Toxicol. Sci. 28 (2), 49–57. [PubMed: 12820537]

Li K, Gao P, Xiang P, Zhang X, Cui X, Ma LQ, 2017 Molecular mechanisms of PFOA-induced toxicity in animals and humans: implications for health risks. Environ. Int. 99, 43–54. 10.1016/j.envint.2016.11.014. [PubMed: 27871799]

Luster MI, Portier C, Pait DG, White KL Jr., Gennings C, Munson AE, Rosenthal GJ, 1992 Risk assessment in immunotoxicology. I. Sensitivity and predictability of immune tests. Fundam. Appl. Toxicol. 18 (2), 200–210. 10.1016/0272-0590(92)90047-l. [PubMed: 1534777]

Manetz TS, Meade BJ, 1999 Development of a flow cytometry assay for the identification and differentiation of chemicals with the potential to elicit irritation, IgE-mediated, or T cell-mediated hypersensitivity responses. Toxicological sciences: an official journal of the Society of Toxicology 48 (2), 206–217. [PubMed: 10353312]

Marshall NB, Lukomska E, Long CM, Kashon ML, Sharpnack DD, Nayak AP, Anderson KL, Jean Meade B, Anderson SE, 2015 Triclosan induces thymic stromal lymphopoietin in skin promoting Th2 allergic responses. Toxicol. Sci.: Off. J. Soc. Toxicol. 147 (1), 127–139. 10.1093/toxsci/kfv113.

Nakata H, Kannan K, Nasu T, Cho HS, Sinclair E, Takemurai A, 2006 Perfluorinated contaminants in sediments and aquatic organisms collected from shallow water and tidal flat areas of the Ariake Sea, Japan: environmental fate of perfluorooctane sulfonate in aquatic ecosystems. Environ. Sci. Technol. 40 (16), 4916–4921. [PubMed: 16955886]

Olsen GW, Burris JM, Burlew MM, Mandel JH, 2003 Epidemiologic assessment of worker serum perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) concentrations and medical surveillance examinations. J. Occup. Environ. Med. 45 (3), 260–270. [PubMed: 12661183]

Olsen GW, Burris JM, Ehresman DJ, Froehlich JW, Seacat AM, Butenhoff JL, Zobel LR, 2007 Half-life of serum elimination of perfluorooctanesulfonate,per-fluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. Environ. Health Perspect. 115 (9), 1298–1305. [PubMed: 17805419]

Olsen GW, Gilliland FD, Burlew MM, Burris JM, Mandel JS, Mandel JH, 1998 An epidemiologic investigation of reproductive hormones in men with occupational exposure to perfluorooctanoic acid. J. Occup. Environ. Med. 40 (7), 614–622. [PubMed: 9675720]

Shane HL, Lukomska E, Stefaniak AB, Anderson SE, 2017 Divergent hypersensitivity responses following topical application of the quaternary ammonium compound, didecyldimethylammonium bromide. J. Immunotoxicol. 14 (1), 204–214. 10.1080/1547691X.2017.1397826. [PubMed: 29124973]

Starkov AA, Wallace KB, 2002 Structural determinants of fluorochemical-induced mitochondrial dysfunction. Toxicol. Sci. 66 (2), 244–252. [PubMed: 11896291]

Symington BE, Takada Y, Carter WG, 1993 Interaction of integrins alpha 3 beta 1 and alpha 2 beta 1: potential role in keratinocyte intercellular adhesion. J. Cell Biol. 120 (2), 523–535. 10.1083/jcb.120.2.523. [PubMed: 8421064]

Trifilieff A, Bench A, Hanley M, Bayley D, Campbell E, Whittaker P, 2003 PPAR-alpha and -gamma but not -delta agonists inhibit airway inflammation in a murine model of asthma: in vitro evidence for an NF-kappaB-independent effect. Br. J. Pharmacol. 139 (1), 163–171. 10.1038/sj.bjp.0705232. [PubMed: 12746235]

Ubel FA, Sorenson SD, Roach DE, 1980 Health status of plant workers exposed to fluorochemicals–a preliminary report. Am. Ind. Hyg. Assoc. J. 41 (8), 584–589. 10.1080/15298668091425310. [PubMed: 7405826]

Vanden Heuvel JP, Kuslikis BI, Van Rafelghem MJ, Peterson RE, 1991 Tissue distribution, metabolism, and elimination of perfluorooctanoic acid in male and female rats. J. Biochem. Toxicol. 6 (2), 83–92. 10.1002/jbt.2570060202. [PubMed: 1941903]

Wen HJ, Wang SL, Chuang YC, Chen PC, Guo YL, 2019 Prenatal perfluorooctanoic acid exposure is associated with early onset atopic dermatitis in 5-year-old children. Chemosphere 231, 25–31. 10.1016/j.chemosphere.2019.05.100. [PubMed: 31128349]

Woolhiser MR, Munson AE, Meade BJ, 2000 Comparison of mouse strains using the local lymph node assay. Toxicology 146 (2–3), 221–227. [PubMed: 10814854]

Yang Q, Abedi-Valugerdi M, Xie Y, Zhao XY, Moller G, Nelson BD, DePierre JW, 2002 Potent suppression of the adaptive immune response in mice upon dietary exposure to the potent

peroxisome proliferator, perfluorooctanoic acid. Int. Immunopharmacol. 2 (2–3), 389–397. [PubMed: 11811941]

Yang Q, Xie Y, Eriksson AM, Nelson BD, DePierre JW, 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 (8), 1133–1140. [PubMed: 11597582]

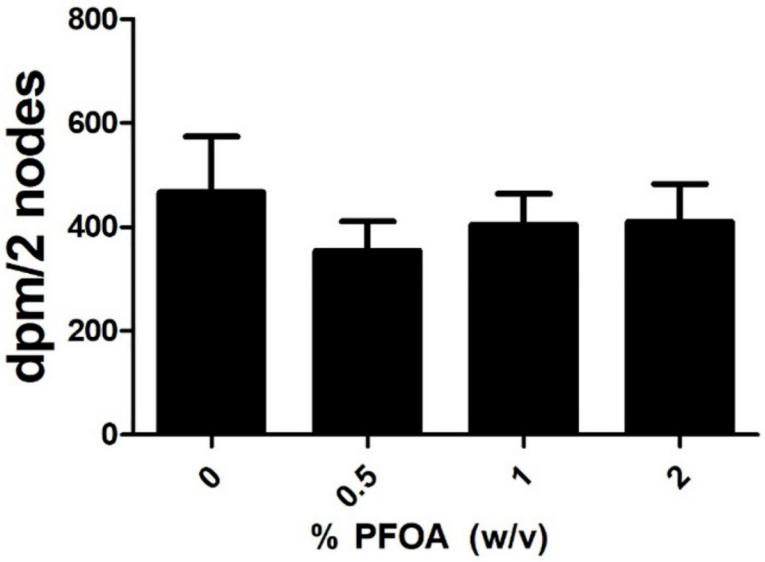

Fig. 1. Allergic sensitization potential after dermal exposure to PFOA.

Analysis of the allergic sensitization potential of PFOA using the LLNA. DPM represent [^{3}H]-thymidine incorporation into draining lymph node cells of BALB/c mice following exposure to vehicle (0%) or concentration of PFOA. Bars represent mean (± SE) of 5 mice per group.

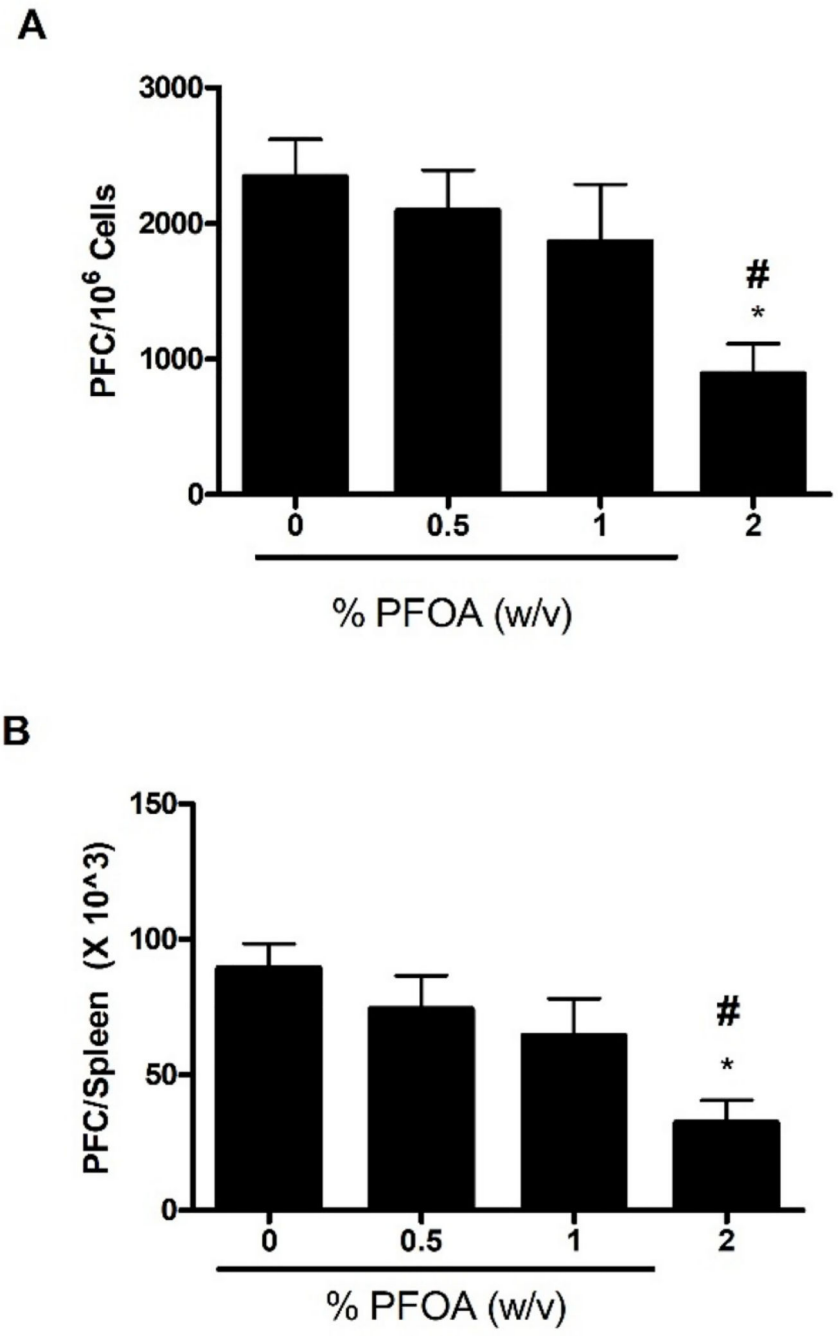

Fig. 2. Dermal PFOA exposure suppresses the spleen IgM response to SRBC.

Analysis of antibody producing spleen cells after a 4-day dermal exposure to PFOA suppressed the (A) specific and (B) total activity IgM response to SRBC. Bars represent mean fold-change (± SE) of 5 mice per group. Levels of statistical significance are denoted (*$p < 0.05$) as compared to acetone vehicle (0%). Linear Trend Test #$p \leq 0.05$.

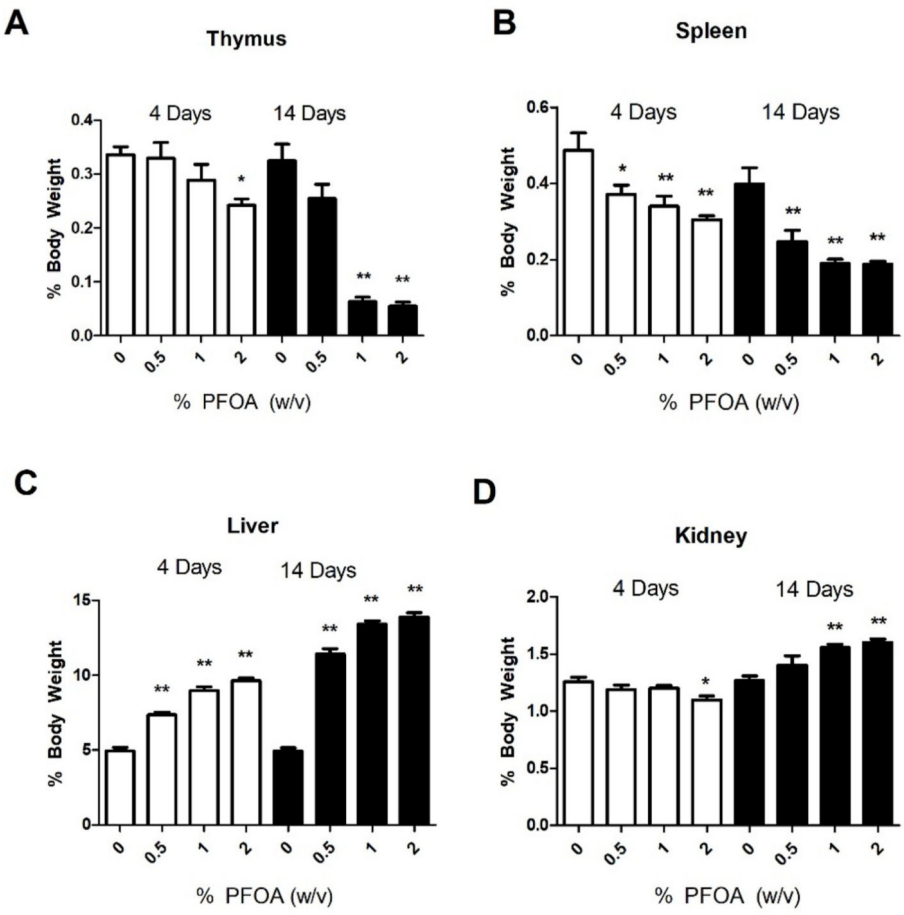

Fig. 3. Changes in organ weights after dermal exposure to PFOA.

Analysis of changes in thymus (A), spleen (B), liver (C), and kidney (D) weights following 4 and 14 days of PFOA exposure. Data is displayed as organ weight as % of body weight in order to normalize between different sizes of mice. Bars represent mean (± SE) of 5 mice per group. Levels of statistical significance are denoted (**$p < 0.01$ and * $p < 0.05$) as compared to acetone vehicle (0%).

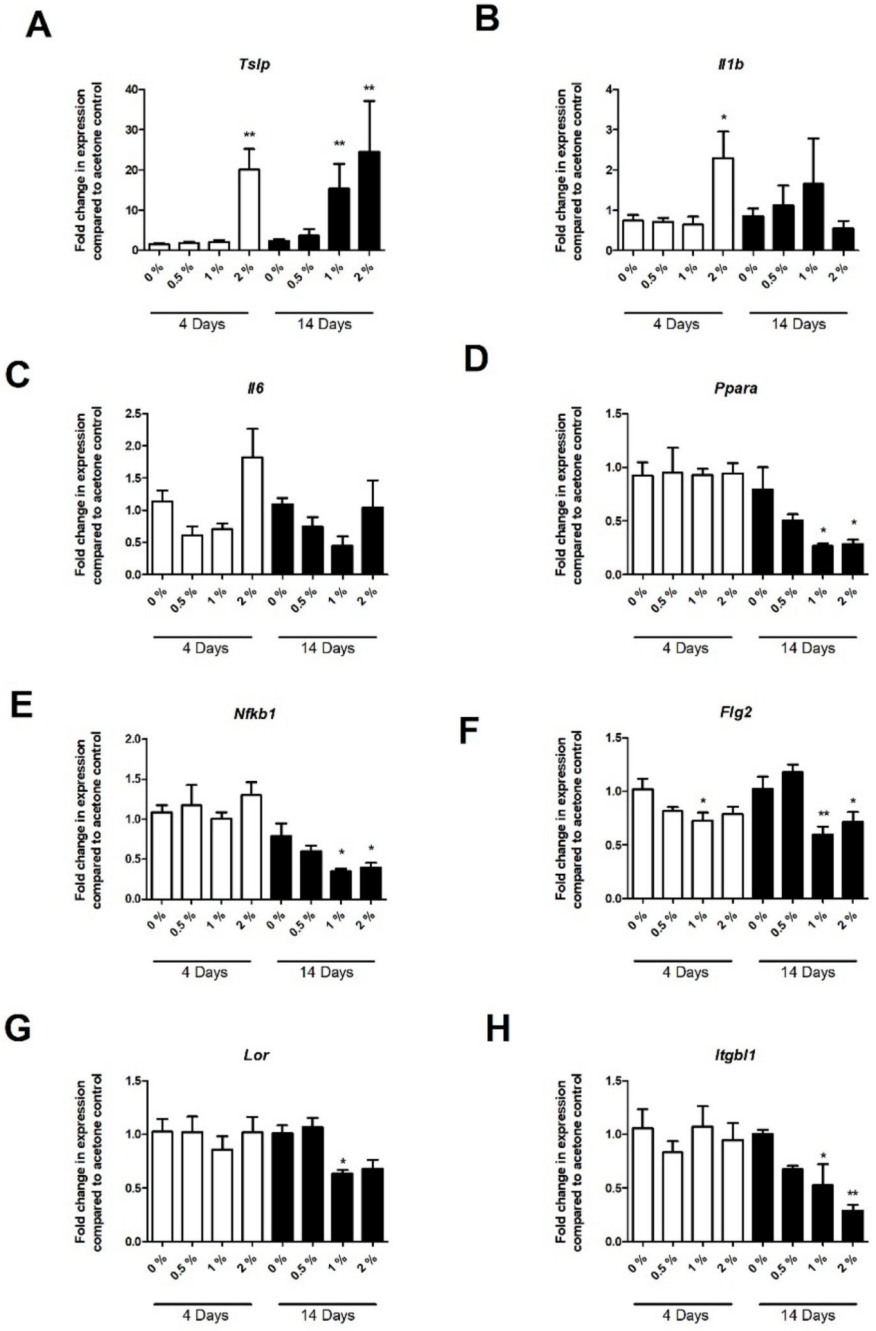

Fig. 4. Skin gene expression following dermal exposure to PFOA.

Gene expression in the skin following 4 and 14 days of PFOA exposure. Changes in *Tslp* (A), *Il-1beta* (B), *Il-6* (C), *PPARα* (D), *Nfkb1* (E), *Flg2* (F), *Lor* (G), and *Itgbl1* (H) were evaluated. Bars represent mean (± SE) of 5 mice per group. Levels of statistical significance are denoted (**$p < 0.01$ and * $p < 0.05$) as compared to acetone vehicle (0%).

Table 1

Spleen phenotyping following dermal exposure to PFOA.

Exposure Duration	**4 Days**				**14 Days**			
PFOA (w/v)	**0%**	**0.5%**	**1%**	**2%**	**0%**	**0.5%**	**1%**	**2%**
Cellularity (× 10^7)	9.48 ± 1.29	6.52 ± 0.40**	4.54 ± 0.38**	4.59 ± 0.47**	8.85 ± 0.75	3.78 ± 0.62**	0.86 ± 0.10**	0.82 ± 0.08**
$CD4^+$ (× 10^7)	2.24 ± 1.24	1.67 ± 1.38**	1.11 ± 0.85**	1.19 ± 0.13**	1.93 ± 0.17	1.14 ± 0.15**	0.30 ± 0.02**	0.27 ± 0.02**
$CD4^+$ (%)	23.70 ± 0.43	25.50 ± 0.53	24.72 ± 0.48	25.90 ± 1.22	21.82 ± 0.74	31.02 ± 1.69**	36.50 ± 1.95**	33.58 ± 1.00**
$CD8^+$ (× 10^6)	10.85 ± 0.57	7.62 ± 0.45**	5.07 ± 0.44**	5.32 ± 0.51**	9.19 ± 0.80	5.22 ± 0.78**	1.33 ± 0.11**	1.28 ± 0.12**
$CD8^+$ (%)	11.48 ± 0.19	11.70 ± 0.28	11.16 ± 0.32	11.64 ± 0.34	10.41 ± 0.39	14.00 ± 0.42**	15.84 ± 0.76**	15.62 ± 0.57**
B-cells (× 10^7)	3.50 ± 0.22	2.45 ± 0.14**	1.69 ± 0.16**	1.74 ± 0.19**	3.45 ± 0.33	1.32 ± 0.24**	0.27 ± 0.03**	0.21 ± 0.03**
B-cells (%)	36.94 ± 0.70	37.68 ± 0.73	37.14 ± 0.47	37.84 ± 0.51	38.82 ± 0.61	34.42 ± 1.22	25.72 ± 1.68**	25.20 ± 1.73**
Dendritic Cells (× 10^5)	9.98 ± 0.11	7.12 ± 0.15**	5.30 ± 0.54**	5.63 ± 0.54**	7.80 ± 0.68	4.32 ± 0.64**	0.76 ± 0.12**	0.88 ± 0.10**
Dendritic Cells (%)	1.06 ± 0.05	1.10 ± 0.05	1.16 ± 0.07	1.23 ± 0.04	0.88 ± 0.03	1.16 ± 0.06**	0.86 ± 0.07	1.06 ± 0.04
Monocytes (× 10^6)	3.73 ± 0.31	2.53 ± 0.78**	1.75 ± 0.17**	1.72 ± 0.18**	3.31 ± 0.35**	1.15 ± 0.18**	0.42 ± 0.07**	0.50 ± 0.03**
Monocytes (%)	3.92 ± 0.16	3.93 ± 0.20	3.84 ± 0.14	3.78 ± 0.23	3.71 ± 0.16	3.10 ± 0.18	4.97 ± 0.56	6.35 ± 0.62**

Spleen immune phenotyping following 4 and 14 days of PFOA exposure. Numbers represent mean (± SE) of 5 mice per group. Levels of statistical significance are denoted (**p < 0.01 and * p < 0.05) as compared to acetone vehicle (0%).

Table 2

Lymph node phenotyping following dermal exposure to PFOA.

Exposure Duration	**4 Days**				**14 Days**			
PFOA (w/v)	**0%**	**0.5%**	**1%**	**2%**	**0%**	**0.5%**	**1%**	**2%**
Cellularity (× 10^6)	4.50 ± 0.85	5.11 ± 0.66	4.94 ± 0.53	9.02 ± 1.31 *	4.69 ± 0.65	3.73 ± 0.29	2.34 ± 0.23 *	2.86 ± 0.42 **
$CD4^+$ (× 10^6)	2.34 ± 0.43	2.70 ± 0.35	2.60 ± 0.26	4.70 ± 0.68 *	2.57 ± 0.35	2.06 ± 0.19	1.09 ± 0.13 **	1.20 ± 0.19 **
$CD4^+$ (%)	52.12 ± 0.93	52.94 ± 0.50	52.96 ± 0.65	52.30 ± 1.06	54.82 ± 0.96	55.10 ± 0.78	46.18 ± 1.55 *	41.90 ± 1.73 **
$CD8^+$ (× 10^6)	1.09 ± 0.16	1.27 ± 0.16	1.18 ± 0.13	2.19 ± 0.31 *	1.11 ± 0.14	1.01 ± 0.61	0.73 ± 0.07	0.87 ± 0.01
$CD8^+$ (%)	24.90 ± 1.07	24.98 ± 0.28	23.84 ± 0.53	24.36 ± 0.23	23.78 ± 0.63	27.36 ± 0.91	31.53 ± 1.39 **	30.76 ± 0.30 **
B-cells (× 10^6)	0.72 ± 0.17	0.82 ± 0.11	0.85 ± 0.10	1.44 ± 0.23 *	0.87 ± 0.14	0.53 ± 0.04	0.39 ± 0.01 *	0.57 ± 0.10
B-cells (%)	15.42 ± 1.23	16.00 ± 0.78	17.18 ± 0.85	15.92 ± 0.96	18.42 ± 1.17	14.32 ± 0.58 *	17.28 ± 1.41	19.64 ± 1.17
Dendritic Cells (× 10^4)	3.02 ± 0.82	3.43 ± 0.44	3.39 ± 0.41	9.24 ± 0.13 **	2.36 ± 0.27	2.04 ± 0.19	1.63 ± 0.29	2.14 ± 0.23
Dendritic Cells (%)	0.63 ± 0.08	0.67 ± 0.04	0.69 ± 0.04	1.03 ± 0.08 **	0.51 ± 0.03	0.55 ± 0.05	0.68 ± 0.06	0.77 ± 0.07 **
Monocytes (× 10^5)	2.25 ± 0.44	1.82 ± 0.18	1.66 ± 0.19	4.06 ± 0.71 *	1.78 ± 0.28	1.36 ± 0.15	1.03 ± 0.16	1.41 ± 0.27
Monocytes (%)	5.20 ± 0.91	3.68 ± 0.29	3.35 ± 0.08 *	4.43 ± 0.29	3.80 ± 0.38	3.71 ± 0.46	4.34 ± 0.47	4.87 ± 0.54 **

Lymph node immune phenotyping following 4 and 14 days of PFOA exposure. Numbers represent mean (± SE) of 5 mice per group. Levels of statistical significance are denoted (**p < 0.01 and * p < 0.05) as compared to acetone vehicle (0%).

Table 3

Skin phenotyping following dermal exposure to PFOA.

Exposure Duration	**4 Days**				**14 Days**			
PFOA (w/v)	**0%**	**0.5%**	**1%**	**2%**	**0%**	**0.5%**	**1%**	**2%**
Cellularity (× 10^6)	2.82 ± 0.18	3.03 ± 0.15	2.86 ± 0.10	3.49 ± 0.35	1.55 ± 0.08	1.82 ± 0.09	1.41 ± 0.05	1.21 ± 0.01 *
$CD45^+$ (× 10^5)	2.38 ± 0.17	2.71 ± 0.07	2.11 ± 0.17	3.54 ± 0.36 **	1.05 ± 0.07	1.15 ± 0.07	0.65 ± 0.04 **	0.60 ± 0.05 **
$CD45^+$ (%)	8.48 ± 0.35	9.04 ± 0.55	7.34 ± 0.38	10.29 ± 1.00	6.81 ± 0.39	6.31 ± 0.25	4.62 ± 0.20 **	5.02 ± 0.43 **
T-cells (× 10^4)	0.91 ± 0.07	1.02 ± 0.14	0.85 ± 0.08	1.44 ± 0.79 **	0.48 ± 0.01	0.64 ± 0.06	0.41 ± 0.02	0.37 ± 0.06
T-cells (%)	3.84 ± 0.24	3.77 ± 0.50	4.04 ± 0.22	4.16 ± 0.25	4.60 ± 0.21	5.58 ± 0.44	6.36 ± 0.21	6.15 ± 0.54
Mast cells (× 10^4)	1.09 ± 0.98	1.32 ± 0.16	0.87 ± 0.05 *	1.62 ± 0.22	0.59 ± 0.79	0.54 ± 0.07	0.30 ± 0.01	0.38 ± 0.27
Mast cells (%)	4.57 ± 0.14	4.85 ± 0.51	4.20 ± 0.33	4.52 ± 0.27	5.52 ± 0.39	4.71 ± 0.38	4.61 ± 0.16 *	6.38 ± 0.24
Neutrophils (× 10^3)	1.41 ± 0.13	1.52 ± 0.19	1.22 ± 0.23	8.64 ± 1.97 **	0.69 ± 0.05	0.64 ± 0.05	0.81 ± 0.02	0.61 ± 0.01
Neutrophils (%)	0.59 ± 0.03	0.56 ± 0.06	0.56 ± 0.08	2.35 ± 0.47 **	0.65 ± 0.03	0.56 ± 0.03	1.19 ± 0.23	1.05 ± 0.22
Monocytes (× 10^5)	1.08 ± 0.03	1.25 ± 0.03	1.05 ± 0.07	1.47 ± 0.13 *	0.49 ± 0.03	0.56 ± 0.04	0.31 ± 0.02 **	0.30 ± 0.02 **
Monocytes (%)	45.94 ± 1.98	46.22 ± 1.13	49.86 ± 0.79	41.88 ± 1.18	47.02 ± 0.37	48.64 ± 1.12	48.22 ± 0.74	50.18 ± 0.92
Eosinophils (× 10^4)	1.16 ± 0.16	1.71 ± 0.12	0.86 ± 0.94	2.59 ± 0.62 *	0.67 ± 0.55	0.33 ± 0.56 **	0.05 ± 0.01 **	0.10 ± 0.03 **
Eosinophils (%)	4.82 ± 0.65	6.29 ± 0.36	4.18 ± 0.48	7.03 ± 1.25	6.33 ± 0.13	2.92 ± 0.47 **	0.81 ± 0.17 **	1.51 ± 0.46 **

Immune phenotyping of the skin following 4 and 14 days of PFOA exposure. Numbers represent mean (± SE) of 5 mice per group. Levels of statistical significance are denoted (**$p < 0.01$ and * $p < 0.05$) as compared to acetone vehicle (0%).