**Supplemental Table 1** Body weight and body weight gain in female Harlan Sprague Dawley rats exposed to PFDA via oral gavage daily for 28 days

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Parameter | Vehicle | Perfluoro-N-Decanoic Acid (mg/kg/day) | | | | | Trend Analysis |
| 0.125 | 0.25 | 0.5 | 1.0 | 2.0 | p-value |
| *Body weight (g)* | |  |  |  |  |  |  |
| Day 1 | 171.1 ± 1.687 | 170.3 ± 2.180 | 171.3 ± 1.741 | 169.6 ± 2.019 | 170.7 ± 1.805 | 170.3 ± 1.771 | 0.632 |
| Day 8 | 186.3 ± 1.264 | 185.2 ± 2.197 | 185.2 ± 1.835 | 185.0 ± 1.841 | 186.8 ± 1.441 | 185.8 ± 1.441 | 0.664 |
| Day 15 | 198.4 ± 0.961 | 197.5 ± 1.888 | 199.1 ± 1.580 | 198.5 ± 1.761 | 198.7 ± 1.432 | **190.3 ± 1.683\*\*** | **<0.001** |
| Day 22 | 210.0 ± 0.809 | 209.7 ± 1.710 | 211.0 ± 1.672 | 209.9 ± 1.904 | 206.6 ± 1.481 | **183.4 ± 1.874\*\*** | **<0.001** |
| Day 29 | 217.3 ± 0.699 | 216.2 ± 1.425 | 219.3 ± 1.659 | 216.7 ± 1.546 | **207.2 ± 1.593\*\*** | **169.3 ± 2.421\*\*** | **<0.001** |
|  |  |  |  |  |  |  |  |
| *Body weight gain (g)* | |  |  |  |  |  |  |
| Day 8 - Day 1 | 15.20 ± 0.701 | 14.90 ± 0.736 | 13.93 ± 1.124 | 15.35 ± 0.649 | 16.12 ± 0.641 | 15.49± 0.794 | 0.143 |
| Day 15 - Day 1 | 27.29 ± 1.276 | 27.20 ± 1.271 | 27.86 ± 1.483 | 28.88 ± 1.325 | 28.08 ± 1.420 | **20.00 ± 1.798\*\*** | **<0.001** |
| Day 22 - Day 1 | 38.89 ± 1.606 | 39.41 ± 1.575 | 39.74 ± 1.994 | 40.29 ± 1.589 | 35.94 ± 1.576 | **13.13 ± 1.935\*\*** | **<0.001** |
| Day 29 - Day 1 | 46.21 ± 1.982 | 45.87 ± 1.427 | 48.01 ± 2.238 | 47.08 ± 1.890 | **36.52 ± 2.415\*\*** | **-1.20 ± 2.802\*\*** | **<0.001** |

Values represent the mean ± SE of all cohorts combined. Each cohort represented 8 animals per dose group. N = 11 cohorts for all dose groups.

\*p < 0.05; \*\*p < 0.01. Bolded entries indicate PFDA-exposed groups that differ significantly from the vehicle controls or significant trends across PFDA-exposed groups and vehicle controls.

PFDA, perfluoro-n-decanoic acid

**Supplemental Table 2** Body weight and body weight gain in female B6C3F1/N mice exposed to PFDA via oral gavage weekly for 4 weeks

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Parameter | Vehicle | Perfluoro-N-Decanoic Acid (mg/kg/day) | | | | | Trend Analysis |
| 0.3125 | 0.625 | 1.25 | 2.5 | 5.0 | p-value |
| *Body weight (g)* | |  |  |  |  |  |  |
| Day 1 | 22.33 ± 0.291 | 22.11 ± 0.344 | 22.06 ± 0.357 | 22.22 ± 0.280 | 22.36 ± 0.290 | 22.2 ± 0.228 | 0.445 |
| Day 8 | 22.96 ± 0.326 | 22.76 ± 0.375 | 22.53 ± 0.500 | 22.93 ± 0.383 | 23.08 ± 0.369 | 22.96 ± 0.242 | 0.926 |
| Day 15 | 23.56 ± 0.370 | 23.56 ± 0.442 | 23.26 ± 0.509 | 23.40 ± 0.384 | 23.71 ± 0.368 | 23.47 ± 0.221 | 0.613 |
| Day 22 | 23.96 ± 0.417 | 24.18 ± 0.602 | 23.80 ± 0.545 | 24.12 ± 0.448 | 24.04 ± 0.380 | 23.73 ± 0.250 | 0.102 |
| Day 29 | 24.93 ± 0.351 | 24.90 ± 0.497 | 24.53 ± 0.429 | 24.71 ± 0.403 | 24.75 ± 0.385 | **24.21 ± 0.190\*** | **0.001** |
|  |  |  |  |  |  |  |  |
| *Body weight gain (g)* | |  |  |  |  |  |  |
| Day 8 - Day 1 | 0.63 ± 0.116 | 0.65 ± 0.134 | 0.46 ± 0.154 | 0.71 ± 0.136 | 0.64 ± 0.185 | 0.77 ± 0.073 | 0.310 |
| Day 15 - Day 1 | 1.23 ± 0.148 | 1.45 ± 0.150 | 1.17 ± 0.177 | 1.30 ± 0.186 | 1.27 ± 0.168 | 1.27 ± 0.102 | 0.982 |
| Day 22 - Day 1 | 1.63 ± 0.242 | 2.07 ± 0.326 | 1.95 ± 0.212 | 1.88 ± 0.288 | 1.61 ± 0.162 | 1.53 ± 0.141 | 0.068 |
| Day 29 - Day 1 | 2.60 ± 0.176 | 2.79 ± 0.259 | 2.47 ± 0.137 | 2.48 ± 0.172 | 2.31 ± 0.184 | **2.02 ± 0.170\*\*** | **<0.001** |

Values represent the mean ± SE of all cohorts combined. Each cohort represented 8 animals per dose group. N = 11 cohorts for vehicle and 1.25-5.0 mg PFDA/kg/day. N = 8 cohorts for 0.3125 and 0.625 mg PFDA/kg/day, as these doses were not included in the host resistance study.

\*p < 0.05; \*\*p < 0.01. Bolded entries indicate PFDA-exposed groups that differ significantly from the vehicle controls or significant trends across PFDA-exposed groups and vehicle controls.

PFDA, perfluoro-n-decanoic acid

**Supplemental Table 3** Hematology and blood leukocyte differentials in female Harlan Sprague Dawley rats exposed to PFDA via oral gavage daily for 28 days

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Parameter | Vehicle | Perfluoro-N-Decanoic Acid (mg/kg/day) | | | CPS | Trend Analysis |
| 0.125 | 0.25 | 0.5 | 50 mg/kg | p-value |
| *Hematology* |  |  |  |  |  |  |
| Erythrocytes (106/mm3) | 6.58 ± 0.079 | 6.87 ± 0.135 | 7.09 ± 0.223 | 6.63 ± 0.134 | 6.52 ± 0.180 | 0.762 |
| Reticulocytes (%) | 3.18 ± 0.096 | 3.44 ± 0.224 | 2.91 ± 0.128 | 2.98 ± 0.096 | 0.05 ± 0.009\*\* | 0.103 |
| Hemoglobin (g/dl) | 14.21 ± 0.126 | 14.40 ± 0.254 | 14.14 ± 0.217 | 13.68 ± 0.233 | 13.60 ± 0.325 | 0.059 |
| Hematocrit (%) | 36.40 ± 0.461 | 37.90 ± 0.441 | 38.79 ± 1.177 | 36.64 ± 0.579 | 36.03 ± 1.030 | 0.711 |
| MCV (fl) | 55.33 ± 0.427 | 55.21 ± 0.655 | 54.73 ± 0.660 | 55.33 ± 0.392 | 55.24 ± 0.0577 | 0.920 |
| MCH (pg) | 21.64 ± 0.336 | 20.96 ± 0.283 | **20.01 ± 0.375\*\*** | 20.65 ± 0.204 | 20.89 ± 0.324 | **0.016** |
| MCHC (g/dl) | 39.10 ± 0.638 | 37.98 ± 0.307 | **36.58 ± 0.692\*** | **37.33 ± 0.381\*** | 37.79 ± 0.409 | **0.009** |
| Platelets (103/mm3) | 1232 ± 98.98 | 984 ± 131.9 | 1084 ± 99.48 | 1114 ± 77.9 | 458.6 ± 60.21\*\* | 0.814 |
| Leukocytes (103/mm3) | 11.40 ± 0.313 | 14.15 ± 1.416 | 12.11 ± 1.072 | 10.83 ± 1.300 | 2.08 ± 0.448\*\* | 0.933 |
|  |  |  |  |  |  |  |
| *Absolute leukocyte differentials (103/mm3)* |  |  |  |  |  |  |
| Lymphocytes | 6.90 ± 0.253 | 8.72 ± 0.745 | 6.99 ± 0.544 | 6.34 ± 0.761 | 0.93 ± 0.223\*\* | 0.687 |
| Neutrophils | 3.86 ± 0.075 | 4.53 ± 0.531 | 4.38 ± 0.468 | 3.80 ± 0.508 | 0.84 ± 0.146\*\* | 0.946 |
| Eosinophils | 0.16 ± 0.038 | 0.25 ± 0.064 | 0.20 ± 0.060 | 0.16 ± 0.044 | 0.11 ± 0.046 | 0.686 |
| Basophils | 0.03 ± 0.017 | 0.07 ± 0.042 | 0.05 ± 0.024 | 0.06 ± 0.023 | 0.05 ± 0.023 | 0.635 |
| Monocytes | 0.46 ± 0.52 | 0.58 ± 0.115 | 0.50 ± 0.063 | 0.47 ± 0.103 | 0.15 ± 0.032\*\* | 0.590 |
|  |  |  |  |  |  |  |
| *Percent leukocyte differentials* |  |  |  |  |  |  |
| % Lymphocytes | 60.45 ± 0.978 | 62.31 ± 1.739 | 58.30 ± 1.867 | 59.05 ± 2.696 | 44.08 ± 2.672\*\* | 0.168 |
| % Neutrophils | 33.94 ± 0.877 | 31.74 ± 1.427 | 35.76 ± 1.795 | 34.88 ± 2.134 | 42.20 ± 2.865\*\* | 0.214 |
| % Eosinophils | 1.39 ± 0.314 | 1.61 ± 0.266 | 1.47 ± 0.360 | 1.33 ± 0.256 | 3.90 ± 1.001\* | 0.650 |
| % Basophils | 0.23 ± 0.125 | 0.43 ± 0.194 | 0.36 ± 0.157 | 0.43 ± 0.157 | 1.54 ± 0.632\* | 0.814 |
| % Monocytes | 3.99 ± 0.405 | 3.91 ± 0.454 | 4.10 ± 0.342 | 4.30 ± 0.622 | 8.30 ± 1.385\* | 0.674 |

Values represent the mean (±SE) from 8 animals/group; \*p < 0.05; \*\*p < 0.01.

PFDA, perfluoro-n-decanoic acid; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; CPS, cyclophosphamide.

Bolded entries indicate PFDA-exposed groups that differ significantly from the vehicle controls or significant trends across PFDA-exposed groups and vehicle controls.

**Supplemental Table 4** Hematology and blood leukocyte differentials in female B6C3F1/N mice exposed to PFDA via oral gavage weekly for 4 weeks

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Parameter | Vehicle | Perfluoro-N-Decanoic Acid (mg/kg/week) | | | | | CPS | Trend Analysis |
| 0.3125 | 0.625 | 1.25 | 2.5 | 5.0 | 50 mg/kg | p-value |
| *Hematology* |  |  |  |  |  |  |  |  |
| Erythrocytes (106/mm3) | 9.28 ± 0.180 | 9.07 ± 0.295 | 8.92 ± 0.203 | 9.06 ± 0.163 | 9.07 ± 0.080 | 9.30 ± 0.052 | 8.12 ± 0.088\*\* | 0.563 |
| Reticulocytes (%) | 4.50 ± 0.183 | 3.95 ± 0.179 | 4.02 ± 0.237 | 4.06 ± 0.182 | 4.10 ± 0.174 | 4.23 ± 0.163 | 1.30 ± 0.155\*\* | 0.738 |
| Hemoglobin (g/dl) | 13.58 ± 0.255 | 13.16 ± 0.567 | 13.59 ± 0.299 | 13.13 ± 0.206 | 13.45 ± 0.247 | 13.55 ± 0.224 | 11.65 ± 0.130\*\* | 0.863 |
| Hematocrit (%) | 44.99 ± 1.016 | 44.68 ± 1.412 | 44.89 ± 1.028 | 45.15 ± 0.814 | 44.79 ± 0.409 | 45.40 ± 0.320 | 40.61 ± 0.510\*\* | 0.759 |
| MCV (fl) | 49.14 ± 0.551 | 49.31 ± 0.261 | 50.33 ± 0.212 | 49.83 ± 0.182 | 49.38 ± 0.267 | 48.84 ± 0.157 | 50.04 ± 0.187 | 0.066 |
| MCH (pg) | 14.83 ± 0.172 | 14.48 ± 0.195 | 15.25 ± 0.140 | 14.48 ± 0.184 | 14.83 ± 0.288 | 14.58 ± 0.230 | 14.35 ± 0.216 | 0.711 |
| MCHC (g/dl) | 30.23 ± 0.564 | 29.39 ± 0.466 | 30.30 ± 0.323 | 29.09 ± 0.342 | 30.06 ± 0.612 | 29.85 ± 0.502 | 28.70 ± 0.407 | 0.971 |
| Platelets (103/mm3) | 528.50 ± 79.46 | 586.25 ± 57.71 | 646.63 ± 53.71 | 783.25 ± 61.15 | 666.38 ± 66.05 | 463.63 ± 52.18 | 865.25 ± 80.52\* | 0.942 |
| Leukocytes (103/mm3) | 7.72 ± 1.065 | 7.47 ± 0.912 | 6.52 ± 0.848 | 6.21 ± 0.585 | 7.04 ± 0.593 | 7.08 ± 0.484 | 3.93 ± 0.201\* | 0.516 |
|  |  |  |  |  |  |  |  |  |
| *Absolute leukocyte differentials (103/mm3)* | |  |  |  |  |  |  |  |
| Lymphocytes | 5.30 ± 0.640 | 4.88 ± 0.508 | 4.12 ± 0.521 | 4.30 ± 0.408 | 4.41 ± 0.347 | 4.80 ± 0.246 | 2.40 ± 0.135\*\* | 0.396 |
| Neutrophils | 1.76 ± 0.331 | 1.86 ± 0.309 | 1.64 ± 0.226 | 1.26 ± 0.150 | 1.82 ± 0.188 | 1.80 ± 0.191 | 0.85 ± 0.108\* | 0.957 |
| Eosinophils | 0.22 ± 0.058 | 0.24 ± 0.064 | 0.29 ± 0.063 | 0.25 ± 0.055 | 0.34 ± 0.065 | 0.13 ± 0.030 | 0.29 ± 0.041 | 0.545 |
| Monocytes | 0.37 ± 0.064 | 0.40 ± 0.063 | 0.35 ± 0.051 | 0.30 ± 0.040 | 0.34 ± 0.030 | 0.31 ± 0.042 | 0.25 ± 0.027 | 0.329 |
| Basophils | 0.08 ± 0.022 | 0.09 ± 0.028 | 0.12 ± 0.027 | 0.11 ± 0.031 | 0.13 ± 0.025 | 0.05 ± 0.011 | 0.13 ± 0.023 | 0.581 |
|  |  |  |  |  |  |  |  |  |
| *Percent leukocyte differentials* | |  |  |  |  |  |  |  |
| % Lymphocytes | 70.19 ± 2.159 | 66.90 ± 2.565 | 63.53 ± 1.256 | 69.88 ± 2.825 | 63.06 ± 1.433 | 68.34 ± 1.753 | 61.77 ± 3.267\* | 0.396 |
| % Neutrophils | 21.76 ± 2.059 | 23.93 ± 1.735 | 25.22 ± 1.002 | 20.14 ± 1.238 | 25.62 ± 0.790 | 25.01 ± 1.081 | 21.22 ± 1.994 | 0.170 |
| % Eosinophils | 2.46 ± 0.574 | 2.75 ± 0.605 | 4.06 ± 0.592 | 3.43 ± 0.898 | 4.56 ± 0.633 | 1.68 ± 0.310 | 7.33 ± 0.970\*\* | 0.843 |
| % Monocytes | 4.68 ± 0.289 | 5.33 ± 0.334 | 5.52 ± 0.501 | 4.91 ± 0.519 | 4.99 ± 0.422 | 4.31 ± 0.424 | 6.33 ± 0.585\* | 0.371 |
| % Basophils | 0.91 ± 0.221 | 1.08 ± 0.251 | 1.66 ± 0.271 | 1.65 ± 0.449 | 1.77 ± 0.252 | 0.65 ± 0.128 | 3.36 ± 0.532\*\* | 0.971 |

Values represent the mean (±SE) from 8 animals/group; \*p < 0.05; \*\*p < 0.01.

PFDA, perfluoro-n-decanoic acid; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; CPS, cyclophosphamide.

**Supplemental Table 5** Humoral mediated immunity in female Harlan Sprague Dawley rats exposed to PFDA via oral gavage daily for 28 days

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Parameter | Vehicle | Perfluoro-N-Decanoic Acid (mg/kg/day) | | | CPSa | Trend Analysis |
| 0.125 | 0.25 | 0.5 |  | p-value |
| *Serum IgM* |  |  |  |  |  |  |
| Serum anti-KLH IgM (μg/ml) | 14.9 ± 4.004 | 23.52 ± 12.73 | 21.01 ± 5.957 | 14.17 ± 2.713 | 0.12 ± 0.027\*\* | 0.502 |
| Serum anti-SRBC IgM (log 2) | 7.60 ± 0.601 | 7.31 ± 0.206 | 7.34 ± 0.291 | 7.94 ± 0.524 | 1.33 ± 0.329 | 0.650 |
|  |  |  |  |  |  |  |
| *T-Dependent antibody response to SRBC*  *Study 1* |  |  |  |  |  |  |
| IgM AFC/106 spleen cells | 470.2 ± 98.36 | 241.3 ± 38.12 | 315.1 ± 61.98 | 507.5 ± 104.8 | 1.99 ± 0.350\*\* | 0.545 |
| IgM AFC/spleen X 103 | 446.6 ± 113.6 | 207.8 ± 43.02 | 275.6 ± 64.69 | 403.9 ± 80.23 | 1.00 ± 0.000\*\* | 0.749 |
| Cells/spleen X 107 | 89.81 ± 5.945 | 82.53 ± 6.927 | 84.93 ± 4.778 | 80.42 ± 3.378 | 14.06 ± 0.836\*\* | 0.449 |
| Spleen weight (mg) | 627.1 ± 38.36 | 603.6 ± 41.15 | 571.6 ± 18.19 | 557.4 ± 23.02 | 290.5 ± 29.30\*\* | 0.139 |
|  |  |  |  |  |  |  |
| *T-Dependent antibody response to SRBC*  *Study 2* |  |  |  |  |  |  |
| IgM AFC/106 spleen cells | 627.6 ± 155.0 | 619.2 ± 155.9 | 578.0 ± 88.3 | 502.6 ± 133.2 | 2.65 ± 0.477\*\* | 0.347 |
| IgM AFC/spleen X 103 | 546.4 ± 124.8 | 512.6 ± 126.8 | 487.9 ± 81.61 | 417.8 ± 112.4 | 1.00 ± 0.000\*\* | 0.246 |
| Cells/spleen X 107 | 89.45 ± 2.312 | 80.47 ± 3.399 | 82.22 ± 4.090 | 83.47 ± 2.741 | 13.01 ± 0.772\*\* | 0.290 |
| Spleen weight (mg) | 622.0 ± 12.21 | 604.0 ± 32.77 | 563.1 ± 14.85 | 572.0 ± 20.33 | 272.9 ± 8.539\*\* | **0.022** |
|  |  |  |  |  |  |  |
| *T-Dependent antibody response to SRBC*  *Study 3* |  |  |  |  |  |  |
| IgM AFC/106 spleen cells | 582.6 ± 88.04 | 566.5 ± 75.02 | 688.4 ± 101.3 | 520.8 ± 90.87 | 2.95 ± 0.773\*\* | 0.893 |
| IgM AFC/spleen X 103 | 608.6 ± 97.56 | 517.5 ± 60.63 | 670.9 ± 106.7 | 452.6 ± 68.53 | 1.00 ± 0.000\*\* | 0.626 |
| Cells/spleen X 107 | 105.3 ± 5.603 | 92.85 ± 3.583 | 97.10 ± 3.210 | **89.97 ± 4.199\*** | 14.99 ± 1.465\*\* | **0.039** |
| Spleen weight (mg) | 638.8 ± 26.42 | 580.0 ± 14.02 | 610.0 ± 11.34 | 588.8 ± 24.45 | 255.0 ± 16.58\*\* | 0.200 |

aCPS, cyclophosphamide, 25 mg/kg for serum IgM studies, 50 mg/kg for T-dependent antibody response studies

Values represent the mean (±SE) from 8 animals/group; \*p < 0.05; \*\*p < 0.01.

PFDA, perfluoro-n-decanoic acid; IgM, immunoglobulin M; KLH, keyhole limpet hemocyanin; SRBC, sheep red blood cells; AFC, antibody forming cells;

Bolded entries indicate PFDA-exposed groups that differ significantly from the vehicle controls or significant trends across PFDA-exposed groups and vehicle controls.

**Supplemental Table 6** Humoral mediated immunity in female B6C3F1/N mice exposed to PFDA via oral gavage weekly for 4 weeks

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Parameter | Vehicle | Perfluoro-N-Decanoic Acid (mg/kg/week) | | | | | CPS | Trend Analysis |
| 0.3125 | 0.625 | 1.25 | 2.5 | 5.0 | 50 mg/kg | p-value |
| *T-Dependent antibody response to SRBC* | |  |  |  |  |  |  |  |
| IgM AFC/106 spleen cells | 1492.7 ± 133.0 | 1340.5 ± 144 | 1591.5 ± 210.1 | 1638.6 ± 110 | 1671.0 ± 97.65 | 1323.3 ± 128 | 1.20 ± 0.27\*\* | 0.986 |
| IgM AFC/spleen X 103 | 308.6 ± 20.66 | 259.5 ± 40.29 | 307.9 ± 44.69 | 305.3 ± 26.10 | 339.5 ± 17.10 | 228.6 ± 20.91 | 0.09 ± 0.02\*\* | 0.607 |
| Cells/spleen X 107 | 20.99 ± 0.886 | 18.79 ± 1.365 | 20.45 ± 1.693 | 18.54 ± 0.605 | 20.48 ± 0.777 | 17.43 ± 0.659 | 7.49 ± 0.40\*\* | 0.191 |
| Spleen weight (mg) | 81.25 ± 3.797 | 76.25 ± 5.706 | 100.38 ± 15.91 | 87.38 ± 2.927 | 82.25 ± 2.48 | 70.75 ± 2.889 | 42.00 ± 2.60\*\* | 0.458 |

Values represent the mean (±SE) from 8 animals/group; \*p < 0.05; \*\*p < 0.01.

PFDA, perfluoro-n-decanoic acid; IgM, immunoglobulin M; SRBC, sheep red blood cells; AFC, antibody forming cells; CPS, cyclophosphamide

**Supplemental Table 7** Cell mediated immunity in female Harlan Sprague Dawley rats exposed to PFDA via oral gavage daily for 28 days

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Parameter | Vehicle | Perfluoro-N-Decanoic Acid (mg/kg/day) | | | CPS | Challenge only | Trend Analysis |
| 0.125 | 0.25 | 0.5 | 50 mg/kg |  | p-value |
| *Delayed type hypersensitivity* |  |  |  |  |  |  |  |
| Foot pad swelling (mm X 100) | 117.1 ± 13.21 | 77.38 ± 17.20 | 86.50 ± 14.52 | 86.19 ± 14.16 | 27.94 ± 4.03\*\* | 5.81 ± 4.19\*\* | 0.144 |
|  |  |  |  |  |  |  |  |
| *Anti-CD3 proliferation* |  |  |  |  |  |  |  |
| Total cells (X107) per spleen | 82.49 ± 3.795 | 82.92 ± 5.100 | **67.68 ± 2.985\*** | **63.90 ± 2.232\*\*** | 10.73 ± 0.820\*\* |  | **≤0.001** |
| Spleen cells stimulated with anti-CD3 (cpm) | 151125 ± 20246 | 159189 ± 18947 | 176059 ± 31930 | 121894 ± 32032 | 60562 ± 25597\* |  | 0.662 |
| Unstimulated spleen cells (cpm) | 7011 ± 881.4 | 8721 ± 1533 | 10194 ± 2306 | 9483 ± 2759 | 3624 ± 1329 |  | 0.460 |

Values represent the mean (±SE) from 8 animals/group; \*p < 0.05; \*\*p < 0.01.

PFDA, perfluoro-n-decanoic acid; CPS, cyclophosphamide,

Bolded entries indicate PFDA-exposed groups that differ significantly from the vehicle controls or significant trends across PFDA-exposed groups and vehicle controls.

**Supplemental Table 8** Cell mediated immunity in female B6C3F1/N mice exposed to PFDA via oral gavage weekly for 4 weeks

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Parameter | Vehicle | Perfluoro-N-Decanoic Acid (mg/kg/week) | | | | | CPS | Challenge only | Trend Analysis |
| 0.3125 | 0.625 | 1.25 | 2.5 | 5.0 | 50 mg/kg |  | p-value |
| *Delayed type hypersensitivity* | |  |  |  |  |  |  |  |  |
| Foot pad swelling (mm X 100) | 83.50 ± 9.359 | 85.44 ± 5.567 | 79.19 ± 5.524 | 88.50 ± 10.53 | 64.13 ± 7.170 | 74.56 ± 10.02 | 9.81 ± 2.842\*\* | 7.75 ± 1.79\*\* | 0.164 |
|  |  |  |  |  |  |  |  |  |  |
| *Mixed Leukocyte Response* | |  |  |  |  |  |  |  |  |
| Responder (B6C3F1/N) cells only | 2023.8 ± 435.5 | 2041.6 ± 272.0 | 2400.3 ± 715.3 | 2573.5 ± 455.2 | 1607.4 ± 161.2 | 1275.5 ± 169.5 | 892.0 ± 154.3\*\* |  | 0.120 |
| Responders + Stimulator (DBA/2) cells | 55388 ± 16174 | 57927 ± 14399 | 58349 ± 13890 | 65015 ± 18621 | 74835 ± 19490 | 67852 ± 18497 | 41771 ± 13997 |  | 0.516 |
|  |  |  |  |  |  |  |  |  |  |
| *Anti CD3 proliferation* |  |  |  |  |  |  |  |  |  |
| Total cells (X107) per spleen | 14.94 ± 0.654 | 14.71 ± 0.742 | 13.81 ± 0.685 | 12.94 ± 0.504 | 13.75 ± 1.056 | **11.39 ± 0.524\*\*** | 4.74 ± 0.449\*\* |  | **<0.001** |
| Spleen cells stimulated with anti-CD3 (cpm) | 249603 ± 11663 | 275463 ± 14183 | 272030 ± 11389 | 280357 ± 10507 | 260946 ± 16032 | 237083 ± 11164 | 72652 ± 3711\*\* |  | 0.504 |
| Unstimulated spleen cells (cpm) | 2928.8 ± 307.4 | 3426.0 ± 174.8 | 3688.0 ± 480.2 | 4433.0 ± 959.7 | 4322.0 ± 487.1 | 3132.6 ± 413.0 | 799.25 ± 139.2\*\* |  | 0.279 |

Values represent the mean (±SE) from 8 animals/group; \*p < 0.05; \*\*p < 0.01.

PFDA, perfluoro-n-decanoic acid; CPS, cyclophosphamide,

Bolded entries indicate PFDA-exposed groups that differ significantly from the vehicle controls or significant trends across PFDA-exposed groups and vehicle controls.

**Supplemental Table 9** Natural killer cell activity in female Harlan Sprague Dawley rats exposed to PFDA via oral gavage daily for 28 days

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Parameter | Vehicle | Perfluoro-N-Decanoic Acid (mg/kg/day) | | | Positive Controla | Trend Analysis |
| 0.125 | 0.25 | 0.5 |  | p-value |
| Spleen Weight (mg) | 586.0 ± 22.89 | 568.6 ± 26.60 | 575.5 ± 36.61 | 553.5 ± 13.28 | 518.1 ± 12.73\* | 0.355 |
|  |  |  |  |  |  |  |
| *Percent Cytotoxicity* |  |  |  |  |  |  |
| 200:1 E:T ratio | 43.46 ± 1.766 | 49.66 ± 3.177 | 46.24 ± 2.734 | 47.00 ± 3.185 | 4.74 ± 0.729\*\* | 0.364 |
| 100:1 E:T ratio | 32.72 ± 1.420 | 37.80 ± 2.193 | 34.74 ± 2.180 | 35.69 ± 2.967 | 4.05 ± 0.565\*\* | 0.321 |
| 50:1 E:T ratio | 23.50 ± 1.075 | 28.58 ± 1.160 | 25.14 ± 1.214 | 25.82 ± 2.154 | 3.92 ± 0.398\*\* | 0.512 |
| 25:1 E:T ratio | 16.72 ± 0.864 | 21.02 ± 1.524 | 18.28 ± 1.334 | 19.45 ± 1.755 | 3.56 ± 0.438\*\* | 0.401 |
| 12.5:1 E:T ratio | 12.48 ± 0.702 | **15.67 ± 0.767\*** | 13.07 ± 1.087 | 14.14 ± 1.161 | 3.70 ± 0.478\*\* | 0.233 |
| 6.25:1 E:T ratio | 9.09 ± 0.689 | 10.87 ± 0.660 | 9.06 ± 0.688 | 9.95 ± 0.793 | 3.70 ± 0.499\*\* | 0.827 |

aPositive control CPS, cyclophosphamide, 50 mg/kg for cells/spleen; AAGM1, 10% (v/v) solution of rabbit anti-asialo GM1 antibody for spleen weight and cytotoxicity;

Values represent the mean (±SE) from 8 animals/group; \*p < 0.05; \*\*p < 0.01.

PFDA, perfluoro-n-decanoic acid;

Bolded entries indicate PFDA-exposed groups that differ significantly from the vehicle controls or significant trends across PFDA-exposed groups and vehicle controls.

**Supplemental Table 10** Natural killer cell activity in female B6C3F1/N mice exposed to PFDA via oral gavage weekly for 4 weeks

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Parameter | Vehicle | Perfluoro-N-Decanoic Acid (mg/kg/week) | | | | | Positive Controla | Trend Analysis |
| 0.3125 | 0.625 | 1.25 | 2.5 | 5.0 |  | p-value |
| Spleen Weight (mg) | 66.25 ± 3.115 | 66.50 ± 4.062 | 65.00 ± 2.079 | 68.63 ± 2.220 | 68.50 ± 4.119 | 59.75 ± 2.776 | 83.86 ± 3.07\*\* | 0.411 |
| Cells/spleen X 107 | 14.94 ± 0.654 | 14.71 ± 0.742 | 13.81 ± 0.685 | 12.94 ± 0.504 | 13.75 ± 1.056 | **11.39 ± 0.052\*\*** | 4.74 ± 0.45\*\* | **<0.001** |
|  |  |  |  |  |  |  |  |  |
| *Percent Cytotoxicity* |  |  |  |  |  |  |  |  |
| 200:1 E:T ratio | 16.49 ± 4.000 | 15.85 ± 3.312 | 12.95 ± 3.030 | 17.05 ± 4.502 | 18.05 ± 5.101 | 21.29 ± 4.896 | 0.21 ± 1.74\*\* | 0.481 |
| 100:1 E:T ratio | 11.69 ± 3.056 | 11.44 ± 2.749 | 8.80 ± 2.668 | 13.96 ± 3.683 | 12.60 ± 3.488 | 17.53 ± 3.127 | 0.64 ± 1.44\*\* | 0.223 |
| 50:1 E:T ratio | 8.50 ± 2.166 | 8.82 ± 2.021 | 6.39 ± 1.754 | 8.57 ± 3.186 | 9.42 ± 2.773 | 11.35 ± 2.628 | 0.46 ± 1.401\* | 0.652 |
| 25:1 E:T ratio | 4.23 ± 1.631 | 4.78 ± 1.787 | 4.04 ± 1.708 | 4.39 ± 2.246 | 4.92 ± 2.434 | 7.33 ± 2.366 | -0.82 ± 1.387 | 0.576 |
| 12.5:1 E:T ratio | 1.50 ± 1.504 | 1.50 ± 1.569 | 1.45 ± 1.353 | 1.52 ± 1.638 | 1.55 ± 1.678 | 4.98 ± 2.146 | -0.36 ± 1.824 | 0.241 |
| 6.25:1 E:T ratio | 4.16 ± 2.307 | 0.39 ± 1.217 | 0.25 ± 0.947 | -0.24 ± 1.497 | 0.20 ± 1.417 | 2.19 ± 1.540 | 2.02 ± 3.251 | 0.570 |

aPositive control CPS, cyclophosphamide, 50 mg/kg for cells/spleen; AAGM1, 10% (v/v) solution of rabbit anti-asialo GM1 antibody for spleen weight and cytotoxicity;

Values represent the mean (±SE) from 8 animals/group; \*p < 0.05; \*\*p < 0.01.

PFDA, perfluoro-n-decanoic acid;

Bolded entries indicate PFDA-exposed groups that differ significantly from the vehicle controls or significant trends across PFDA-exposed groups and vehicle controls.

**Supplemental Table 11** Bone marrow DNA synthesis and colony forming units in female Harlan Sprague Dawley rats exposed to PFDA via oral gavage daily for 28 days

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Parameter | Vehicle | Perfluoro-N-Decanoic Acid (mg/kg/day) | | | CPS | Trend Analysis |
| 0.125 | 0.25 | 0.5 | 50 mg/kg | p-value |
| *DNA Synthesis Study 1* |  |  |  |  |  |  |
| Total cells/femur X 106 | 62.41 ± 5.246 | 69.21 ± 5.181 | 62.85 ± 4.887 | 62.91 ± 3.988 | 8.88 ± 1.358\*\* | 0.762 |
| DNA Synthesis (cpm) | 77119 ± 5629 | 80170 ± 10099 | 86032 ± 5353 | 80572 ± 6324 | 5189 ± 1293\*\* | 0.401 |
|  |  |  |  |  |  |  |
| *DNA Synthesis Study 2* |  |  |  |  |  |  |
| Total cells/femur X 106 | 64.46 ± 6.140 | 59.52 ± 2.604 | 56.36 ± 6.410 | 60.06 ± 3.893 | 10.32 ± 1.043\*\* | 0.712 |
| DNA Synthesis (cpm) | 41784 ± 5122 | 50905 ± 9202 | 55818 ± 4634 | 53523 ± 4466 | 4122 ± 344.2\*\* | 0.122 |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
| *Bone Marrow Colony Forming Units* |  |  |  |  |  |  |
| Total cells/femur X 106 (CFU-GM/CFU-M) | 62.41 ± 5.246 | 69.21 ± 5.181 | 62.85 ± 4.887 | 62.91 ± 3.988 | 8.88 ± 1.358\*\* | 0.762 |
| Total cells/femur X 106 (CFU-E/BFU-E) | 64.46 ± 6.140 | 59.52 ± 2.604 | 56.36 ± 6.410 | 60.06 ± 3.893 | 10.32 ± 1.043\*\* | 0.712 |
|  |  |  |  |  |  |  |
| *CFU (BFU)/105 cells* |  |  |  |  |  |  |
| CFU-GM/105 cells | 117.4 ± 5.677 | 105.3 ± 5.431 | 113.4 ± 5.976 | 109.6 ± 2.070 | 8.75 ± 2.717\*\* | 0.685 |
| CFU-M/105 cells | 178.1 ± 4.026 | 172.3 ± 9.029 | 165.1 ± 5.680 | 168.1 ± 5.680 | 7.50 ± 2.360\*\* | 0.198 |
| CFU-E/105 cells | 180.6 ± 14.49 | 186.5 ± 16.14 | 184.3 ± 7.365 | 158.1 ± 8.987 | 5.63 ± 2.367\*\* | 0.264 |
| BFU-E/2X105 cells | 20.50 ± 2.841 | 17.00 ± 2.315 | 19.25 ± 3.016 | 17.00 ± 1.773 | 1.00 ± 0.500\*\* | 0.352 |
|  |  |  |  |  |  |  |
| *CFU (BFU)/femur* |  |  |  |  |  |  |
| CFU-GM/femur X 104 | 7.20 ± 0.725 | 7.29 ± 0.669 | 7.21 ± 0.814 | 6.92 ± 0.544 | 0.07 ± 0.023\*\* | 0.725 |
| CFU-M/femur X 104 | 10.98 ± 1.001 | 11.73 ± 0.762 | 10.37 ± 0.936 | 10.42 ± 0.404 | 0.07 ± 0.029\*\* | 0.307 |
| CFU-E/femur X 104 | 5.78 ± 0.649 | 5.55 ± 0.471 | 5.24 ± 0.700 | 4.82 ± 0.501 | 0.03 ± 0.013\*\* | 0.179 |
| BFU-E/femur X 104 | 0.65 ± 0.096 | 0.51 ± 0.074 | 0.49 ± 0.044 | 0.45 ± 0.080 | 0.01 ± 0.003\*\* | 0.114 |

Values represent the mean (±SE) from 7-8 animals/group; \*p < 0.05; \*\*p < 0.01.

PFDA, perfluoro-n-decanoic acid; CPS, cyclophosphamide; CFU, colony forming units; BFU, burst forming units; GM, granulocyte-macrophage; M, macrophage; E, erythropoietin

**Supplemental Table 12** Bone marrow cell immunophenotyping in female Harlan Sprague Dawley rats exposed to PFDA via oral gavage daily for 28 days

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Treatment | Cells/femur | CD45RA+ | CD3+ | CD11b/c+ | CD71+ |
| *Absolute Values (x106)* | |  |  |  |  |
| Vehicle | 64.46 ± 6.140 | 12.44 ± 1.646 | 1.25 ± 0.237 | 26.43 ± 2.171 | 18.40 ± 1.967 |
| PFDA |  |  |  |  |  |
| 0.125 mg/kg | 59.52 ± 2.604 | 11.67 ± 0.598 | 1.11 ± 0.122 | 22.96 ± 1.487 | 17.51 ± 0.942 |
| 0.250 mg/kg | 56.36 ± 6.410 | 11.86 ± 1.547 | 1.14 ± 0.194 | 20.25 ± 2.279 | 17.73 ± 2.187 |
| 0.500 mg/kg | 60.06 ± 3.893 | 11.65 ± 1.152 | 1.13 ± 0.070 | 23.55 ± 1.755 | 18.82 ± 1.343 |
| CPS (50mg/kg) | 10.32 ± 1.043\*\* | 0.01 ± 0.013\*\* | 0.01 ± 0.013\*\* | 0.15 ± 0.027\*\* | 0.08 ± 0.037 |
| Trend Analysis | 0.712 | 0.880 | 0.893 | 0.260 | 0.853 |
|  |  |  |  |  |  |
| *Percent Values* | | | |  |  |
| Vehicle |  | 19.01 ± 0.999 | 1.93 ± 0.224 | 41.65 ± 1.461 | 28.38 ± 1.127 |
| PFDA |  |  |  |  |  |
| 0.125 mg/kg |  | 19.64 ± 0.571 | 1.86 ± 0.143 | 38.39 ± 1.249 | 29.46 ± 1.016 |
| 0.250 mg/kg |  | 20.83 ± 1.226 | 1.91 ± 0.184 | **36.15 ± 1.163\*** | 31.24 ± 0.853 |
| 0.500 mg/kg |  | 19.20 ± 1.003 | 1.91 ± 0.143 | 39.16 ± 1.129 | 31.26 ± 0.612 |
| CPS (50mg/kg) |  | 0.30 ± 0.065\*\* | 0.33 ± 0.080\*\* | 1.23 ± 0.140\*\* | 0.76 ± 0.224\*\* |
| Trend Analysis |  | 0.699 | 0.636 | 0.226 | 0.014 |

Values represent the mean (± SE) from 8 animals per group; \*p ≤ 0.05; \*\*p ≤ 0.01

CD45RA+ = B-lymphocyte lineage; CD3+ = T-lymphocyte lineage; CD11b/c+ = Granulocytes and monocytes; CD71+ = Erythroid lineage;

PFDA, perfluoro-n-decanoic acid; CPS, cyclophosphamide;

Bolded entries indicate PFDA-exposed groups that differ significantly from the vehicle controls or significant trends across PFDA-exposed groups and vehicle controls.

**Supplemental Table 13** Bone marrow DNA synthesis in female B6C3F1/N mice exposed to PFDA via oral gavage weekly for 4 weeks

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Parameter | Vehicle | Perfluoro-N-Decanoic Acid (mg/kg/week) | | | | | CPS | Trend Analysis |
| 0.3125 | 0.625 | 1.25 | 2.5 | 5.0 | 50 mg/kg | p-value |
| *DNA Synthesis Study 1* | |  |  |  |  |  |  |  |
| Total cells/femur X 106 | 9.70 ± 0.651 | 9.36 ± 0.344 | 8.18 ± 0.705 | 9.35 ± 0.558 | 9.97 ± 0.860 | 10.04 ± 1.088 | 5.17 ± 0.461\*\* | 0.899 |
| DNA Synthesis (cpm) | 84457 ± 2935 | 76796 ± 3895 | 83150 ± 7304 | 71971 ± 4811 | 82535 ± 4793 | 79777 ± 2072 | 76151 ± 6449 | 0.732 |
|  |  |  |  |  |  |  |  |  |
| *DNA Synthesis Study 2* | |  |  |  |  |  |  |  |
| Total cells/femur X 106 | 1.29 ± 0.068 | 0.95 ± 0.071 | **0.79 ± 0.057\*\*** | 1.04 ± 0.083 | 1.14 ± 0.143 | 1.02 ± 0.079 | 0.59 ± 0.051\*\* | 0.386 |
| DNA Synthesis (cpm) | 109712 ± 7669 | 129404 ± 10416 | 115094 ± 5331 | 114859 ± 8294 | 106487 ± 9711 | 107040 ± 9008 | 137237 ± 7016 | 0.406 |
|  |  |  |  |  |  |  |  |  |
| *DNA Synthesis Study 3* | |  |  |  |  |  |  |  |
| Total cells/femur X 106 | 10.20 ± 0.661 | 10.46 ± 0.968 | 9.80 ± 0.494 | 10.19 ± 0.701 | 9.15 ± 0.751 | 9.57 ± 0.827 | 3.89 ± 0.567\*\* | 0.213 |
| DNA Synthesis (cpm) | 86662 ± 6631 | 82745 ± 4290 | 76829 ± 4723 | 90240 ± 7558 | 84182 ± 1461 | 73034 ± 3879 | 63541 ± 4918\* | 0.427 |

Values represent the mean (± SE) from 8 animals per group; \*p ≤ 0.05; \*\*p ≤ 0.01

PFDA, perfluoro-n-decanoic acid; CPS, cyclophosphamide;

Bolded entries indicate PFDA-exposed groups that differ significantly from the vehicle controls or significant trends across PFDA-exposed groups and vehicle controls.

**Supplemental Table 14** Resistance to *Influenza* virus in female B6C3F1/N mice exposed to PFDA via oral gavage weekly for 4 weeks

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Parameter | Vehicle | Perfluoro-N-Decanoic Acid (mg/kg/week) | | | AZA | Trend Analysis |
| 1.25 | 2.5 | 5.0 | 200 mg/kg | p-value |
| *Challenge A,* 1:2420 dilution | |  |  |  |  |  |
| # Surviving to End of Study | 12 | 12 | 12 | 12 | 10 | 1.000 |
| # Early Deaths | 0 | 0 | 0 | 0 | 2 |  |
| % Moribundity | 0 | 0 | 0 | 0 | 17 |  |
|  |  |  |  |  |  |  |
| *Challenge B,* 1:440 dilution | |  |  |  |  |  |
| # Surviving to End of Study | 9 | 8 | 7 | 10 | 2\* | 0.933 |
| # Early Deaths | 3 | 4 | 4 | 2 | 10 |  |
| % Moribundity | 25 | 33 | 36 | 17 | 83 |  |
|  |  |  |  |  |  |  |
| *Challenge C,* 1:80 dilution | |  |  |  |  |  |
| # Surviving to End of Study | 7 | 6 | 7 | 5 | 0\*\* | 0.596 |
| # Early Deaths | 5 | 6 | 5 | 7 | 12 |  |
| % Moribundity | 42 | 50 | 42 | 58 | 100 |  |

Values represent the mean (± SE) from 12 animals per group; \*p ≤ 0.05; \*\*p ≤ 0.01

PFDA, perfluoro-n-decanoic acid; AZA, azathioprine;