**Supplemental Data**

**Latent, sex-specific metabolic health effects in CD-1 mouse offspring exposed to PFOA or HFPO-DA (GenX) during gestation**

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|  |  |  |  |
| --- | --- | --- | --- |
| **Table S1.** Nutritional composition of study diets | | | |
|  | Low Fat Diet (D12450B) | High Fat Diet (D12492) | NIH31 Diet |
| % Kcal Protein | 20 | 20 | 24 |
| % Kcal Fat | 10 | 60 | 14 |
| % Kcal Carbohydrate | 70 | 20 | 62 |
| Energy Density (Kcal/g) | 3.82 | 5.21 | 3.00 |
| *Note: Nutrient breakdown by percent Kcal made up of protein, fat, and carbohydrate. Experimental diets include high fat diet (HFD, 60% kcal% fat diet; Product #D12492, Research Diets) or control low fat diet (LFD, 10% kcal% diet; Product# D12450B, Research Diets), compared to NIH-31 diet (Zeigler) that was eaten by dams and litters prior to weaning.* | | | |

**Table S2.** Primer list for RT-qPCR of white adipose tissue

|  |  |  |  |
| --- | --- | --- | --- |
| Gene of Interest | Gene Name | Molecular Function | IDT DNA Catalog # |
| *Acaca* | Acetyl-CoA Carboxylase Alpha | Multifunctional enzyme system for fatty acid synthesis | Mm.PT.58.12492865 |
| *Acacb* | Acetyl-CoA Carboxylase Beta | Multifunctional enzyme system for fatty acid synthesis | Mm.PT.58.32720646 |
| *Adipoq* | Adiponectin | Adipose specific protein hormone & adipokine | Mm.PT.58.29300341 |
| *Akt1* | Akt Serine/Threonine Kinase 1 | Protein kinase | Mm.PT.58.8333433 |
| *Esr1* | Estrogen Receptor Alpha | Nuclear receptor ligand-activated transcription factor | Mm.PT.58.8025728 |
| *Esr2* | Estrogen Receptor Beta | Nuclear receptor ligand-activated transcription factor | Mm.PT.58.42880809 |
| *Esrrg* | Estrogen Related Receptor Gamma | Nuclear hormone receptor | Mm.PT.58.29781526 |
| *Fabp4* | Fatty Acid Binding Protein 4 | Adipose specific fatty acid binding protein | Mm.PT.58.43866459 |
| *Fasn* | Fatty Acid Synthase | Multifunctional enzyme system for fatty acid synthesis | Mm.PT.58.14276063 |
| *Slc2a4 (Glut4)* | Glucose Transporter 4 | Solute carrier family 2 insulin regulated glucose transporter | Mm.PT.58.9683859 |
| *Nr3c1 (GR)* | Glucocorticoid Receptor | Nuclear receptor transcription factor | Mm.PT.58.42952901 |
| *Insr* | Insulin Receptor | Receptor tyrosine kinase | Mm.PT.58.7145585 |
| *Irs1* | Insulin Receptor Substrate 1 | Signaling adapter protein | Mm.PT.58.43919344 |
| *Lep* | Leptin | Adipose tissue hormone | Mm.PT.58.13515402 |
| *Lpl* | Lipoprotein Lipase | Lipase enzyme | Mm.PT.58.45869933 |
| *Mapk3 (Erk1)* | Mitogen-activated Protein Kinase 3 | Extracellular signal-regulated kinase | Mm.PT.58.16043493 |
| *Pparg* | Peroxisome Proliferator-activated Receptor Gamma | Nuclear receptor ligand-activated transcription factor | Mm.PT.58.10379967 |
| *Rpl19* | 60S Ribosomal protein L19 | Ribosomal protein | Mm.PT.58.12385796 |
| *Rxra* | Retinoid X Receptor Alpha | Nuclear receptor ligand-activated transcription factor | Mm.PT.58.42702972 |
| *Srebf1* | Sterol Regulatory Binding Factor 1 | Transcription factor | Mm.PT.58.42313188 |
| *Note: Gene specific primers were purchased as predesigned PrimeTime qPCR primer assays by Integrated DNA technologies (IDTDNA.com)* | | | |

**Table S3.** Quantification of PFOA and GenX in offspring urine and serum at PND 5.5

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Treatment** | **Serum (µg/mL)** | **Serum (ug/mL)/Dose** | **Urine (µg/mL)** | **Urine (ug/mL)/Dose** |
| Vehicle | < LOD | N/A | < LOD | N/A |
| 0.2 mg/kg GenX | 0.42 ± 0.12 | 2.10 | 0.51 ± 0.16 | 2.55 |
| 1.0 mg/kg GenX | 1.32 ± 0.23 | 1.32 | 1.81 ± 0.74 | 1.81 |
| 2.0 mg/kg GenX | 2.15 ± 0.53 | 1.08 | 3.4 ± 1.49 | 1.70 |
| 0.1 mg/kg PFOA | 2.27 ± 0.84 | 22.70 | < LOD | < LOD |
| 1.0 mg/kg PFOA | 13.02 ± 2.58 | 13.02 | 0.42 ± 0.15 | 0.42 |

*Note: Vehicle control samples were quantified for PFOA and GenX. Vehicle control samples were run against a low concentration calibration curve with a limit of detection (LOD) of 5 ng/mL. GenX and PFOA experimental samples were run against higher calibrations curve with a LOD of 100 ng/mL. All vehicle control samples were below the LOD of 5 ng/mL for both PFOA and GenX and all 0.1 mg/kg PFOA urine samples were below the LOD of 100 ng/mL for PFOA. Sufficient serum and urine sample quantities were achieved by pooling across pups within the same litter. Data are shown as mean ± SD (N = 5 litters).*

**Table S4.** P-values associated with measured PFOA or GenX in offspring urine and serum at PND 5.5

|  |  |  |
| --- | --- | --- |
| **Comparison** | **Serum** | **Urine** |
| Vehicle control vs 0.1 mg/kg PFOA | 0.005 | 1.00 |
| Vehicle control vs 1.0 mg/kg PFOA | < 0.001 | 0.83 |
| Vehicle control vs 0.2 mg/kg GenX | 0.98 | 0.67 |
| Vehicle control vs 1.0 mg/kg GenX | 0.21 | < 0.001 |
| Vehicle control vs 2.0 mg/kg GenX | 0.007 | < 0.001 |
| 0.1 mg/kg PFOA vs 1.0 mg/kg PFOA | < 0.001 | 0.97 |
| 0.1 mg/kg PFOA vs 0.2 mg/kg GenX | 0.08 | 0.90 |
| 0.1 mg/kg PFOA vs 1.0 mg/kg GenX | 0.69 | 0.002 |
| 0.1 mg/kg PFOA vs 2.0 mg/kg GenX | 1.00 | < 0.001 |
| 1.0 mg/kg PFOA vs 0.2 mg/kg GenX | < 0.001 | 1.00 |
| 1.0 mg/kg PFOA vs 1.0 mg/kg GenX | < 0.001 | 0.02 |
| 1.0 mg/kg PFOA vs 2.0 mg/kg GenX | < 0.001 | <0.001 |
| 0.2 mg/kg GenX vs 1.0 mg/kg GenX | 0.73 | 0.03 |
| 0.2 mg/kg GenX vs 2.0 mg/kg GenX | 0.11 | < 0.001 |
| 1.0 mg/kg GenX vs 2.0 mg/kg GenX | 0.79 | 0.004 |

*Note: P-values were determined by ANOVA with post hoc multiple comparison correction using Tukey contrasts*

**Table S5.** Quantification of PFOA and GenX in male and female offspring serum at PND 22

|  |  |  |
| --- | --- | --- |
| **Treatment** | **Females (mg/mL)** | **Males (mg/mL)** |
| Vehicle | < LOD | < LOD |
| 0.2 mg/kg GenX | < LOD | < LOD |
| 1.0 mg/kg GenX | < LOD | < LOD |
| 2.0 mg/kg GenX | < LOD | < LOD |
| 0.1 mg/kg PFOA | 0.34 ± 0.04 | 0.27 ± 0.04 |
| 1.0 mg/kg PFOA | 3.59 ± 1.08 | 4.26 ± 4.55 |

*Note: Vehicle control samples were quantified for PFOA and GenX. Vehicle control samples were run against a low concentration calibration curve with a limit of detection of 5 ng/mL. GenX and PFOA experimental samples were run against higher calibrations curve with a LOD of 100 ng/mL. All vehicle control samples were below the LOD of 5 ng/mL for both PFOA and GenX and all GenX samples were below the LOD of 100 ng/mL for GenX. Data shown are mean ± SD (N = 3).*

**Table S6.** Pup number and litter and pup weights at PND 0.5 and PND 5.5

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Number of pups per litter PND 0.5** | **Average pup weight PND 0.5 (g)** | **Litter weight PND 0.5 (g)** | **Number of pups per litter**  **PND 5.5** | **Pup weight PND 5.5 (g)** | **Litter weight PND 5.5 (g)** | **Absolute mean pup weight gain PND 0.5 to 5.5 (g)** | **Relative mean pup weight gain**  **PND 0.5 to 5.5 (% change)** | **Adjusted mixed effect model estimate†**  **PND 5.5 weight (g) (95% CI)** |
| Control | 13.1 ± 1.4 | 1.64 ± 0.05 | 21.5 ± 1.9 | 12.7 ± 1.2 | 3.7 ± 0.2 | 46.9 ± 3.3 | 2.07 ± 0.19 | 126.0 ± 11.4 | 5.24 (4.8, 5.7) |
| GenX 0.2 mg/kg | 12.7 ± 1.5 | 1.60 ± 0.09 | 20.3 ± 2.2 | 12.7 ± 1.2 | 3.7 ± 0.2 | 47.1 ± 4.5 | 2.12 ± 0.20 | 132.5 ± 12.1 | 5.22 (4.5, 5.9) |
| GenX 1.0 mg/kg | 12.2 ± 3.3 | 1.58 ± 0.12 | 18.9 ± 4.6 | 12.2 ± 3.3 | 3.5 ± 0.5 | 41.1 ± 9.3 | 1.90 ± 0.44 | 119.8 ± 21.8 | 4.91 (4.2, 5.6)\* |
| GenX 2.0 mg/kg | 11.9 ± 2.8 | 1.66 ± 0.1 | 19.5 ± 3.8 | 11.3 ± 2.6 | 3.5 ± 0.6 | 38.6 ± 6.7 | 1.84 ± 0.50 | 110.3 ± 25.5 | 4.89 (4.2, 5.6)\* |
| PFOA 0.1 mg/kg | 12.2 ± 2.5 | 1.63 ± 0.1 | 19.5 ± 4.7 | 12.1 ± 2.4 | 3.8 ± 0.4 | 45.0 ± 5.8 | 2.15 ± 0.33 | 131.8 ± 18.7 | 5.21 (4.5, 5.9) |
| PFOA 1.0 mg/kg | 13.0 ± 2.7 | 1.58 ± 0.13 | 20.4 ± 3.4 | 12.9 ± 2.8 | 3.5 ± 0.3 | 45.0 ± 7.5 | 1.95 ± 0.20 | 123.3 ± 11.6 | 5.06 (4.4, 5.8) |

*Note: Pup weights at PND 0.5 were determined by dividing the newborn litter weight by the number of pups. Pups were individually weighed at PND 5.5, and the average pup weight per litter was used to calculate treatment group means shown here. Except for pup weight at PND 5.5, all data were analyzed by ANOVA with post hoc multiple comparison correction using Tukey contrasts (P > 0.05 relative to vehicle control for all values, mean ± SD and N = 9-10 litters with N=4-19 pups/litter).†P < 0.05 for PND 5.5 pup weight; Beta estimate 95% confidence intervals do not overlap zero (Mixed effect model adjusting a priori for litter size as a fixed effect and the dam as a random effect, vehicle control as reference group)*

**Table S7**. Female offspring body and liver weight at PND 22

|  |  |  |  |
| --- | --- | --- | --- |
| **Treatment** | **Body weight (g)** | **Liver weight (g)** | **Relative liver weight (%)** |
| Vehicle control | 14.3 ± 1.4 | 0.78 ± 0.09 | 5.6 ± 0.3 |
| GenX 0.2 mg/kg | 13.8 ± 0.9 | 0.74 ± 0.09 | 5.3 ± 0.4 |
| GenX 1.0 mg/kg | 13.8 ± 1.6 | 0.76 ± 0.12 | 5.5 ± 0.4 |
| GenX 2.0 mg/kg | 13.9 ± 1.7 | 0.76 ± 0.19 | 5.5 ± 0.5 |
| PFOA 0.1 mg/kg | 14.7 ± 0.9 | 0.77 ± 0.07 | 5.3 ± 0.3 |
| PFOA 1.0 mg/kg | 13.6 ± 1.4 | 0.80 ± 0.09 | 5.9 ± 0.3 |

*Note: P > 0.05 for all values, one-way ANOVA with Dunnett’s post hoc test*

*Data shown are mean ± SD (N = 9-10)*

**Table S8.** Male offspring body and liver weight at PND 22

|  |  |  |  |
| --- | --- | --- | --- |
| **Treatment** | **Body weight (g)** | **Liver weight (g)** | **Relative liver weight (%)** |
| Vehicle control | 14.2 ± 1.0 | 0.77 ± 0.06 | 5.4 ± 0.3 |
| GenX 0.2 mg/kg | 14.9 ± 0.6 | 0.82 ± 0.09 | 5.5 ± 0.5 |
| GenX 1.0 mg/kg | 14.2 ± 1.4 | 0.81 ± 0.15 | 5.6 ± 0.6 |
| GenX 2.0 mg/kg | 13.8 ± 1.4 | 0.76 ± 0.13 | 5.5 ± 0.4 |
| PFOA 0.1 mg/kg | 14.8 ± 1.6 | 0.82 ± 0.13 | 5.5 ± 0.5 |
| PFOA 1.0 mg/kg | 14.0 ± 1.1 | 0.84 ± 0.08 | **6.0 ± 0.4 \*** |

*Note: One-way ANOVA with Dunnett’s post hoc test, \*P < 0.05 relative to vehicle control. Data shown are mean ± SD (N = 8-10)*

**Table S9**. Female offspring body and liver weights at Week 6 (PND 42 ± 2)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Treatment** | | **Body weight (g)** | **Liver weight (g)** | **Relative liver weight (%)** |
| Low Fat Diet | Vehicle control | 25.5 ± 1.3 | 1.4 ± 0.1 | 5.5 ± 0.4 |
| GenX 0.2 mg/kg | 25.9 ± 1.8 | 1.4 ± 0.2 | 5.5 ± 0.5 |
| GenX 1.0 mg/kg | 27.2 ± 1.6 | 1.6 ± 0.3 | 5.8 ± 0.7 |
| GenX 2.0 mg/kg | 25.6 ± 2.1 | 1.4 ± 0.2 | 5.5 ± 0.5 |
| PFOA 0.1 mg/kg | 27.2 ± 3.3 | 1.5 ± 0.3 | 5.6 ± 0.6 |
| PFOA 1.0 mg/kg | 24.9 ± 2.6 | 1.4 ± 0.2 | 5.5 ± 0.4 |
| High Fat Diet | Vehicle control | 28.0 ± 2.0 | 1.4 ± 0.2 | 5.0 ± 0.6 |
| GenX 0.2 mg/kg | 30.6 ± 2.2 | 1.5 ± 0.2 | 4.9 ± 0.8 |
| GenX 1.0 mg/kg | 30.7 ± 2.9 | 1.5 ± 0.2 | 4.9 ± 0.4 |
| GenX 2.0 mg/kg | 27.7 ± 2.6 | 1.4 ± 0.1 | 5.0 ± 0.4 |
| PFOA 0.1 mg/kg | 28.3 ± 2.7 | 1.5 ± 0.2 | 5.4 ± 0.8 |
| PFOA 1.0 mg/kg | 30.4 ± 2.3 | 1.5 ± 0.2 | 4.9 ± 0.5 |

*Note: One-way ANOVA with Dunnett’s post hoc test, P < 0.05. Data shown are mean ± SD (N = 8-10)*

**Table S10**. Male offspring body and liver weights at Week 6

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Treatment** | | **Body weight (g)** | **Liver weight (g)** | **Relative liver weight (%)** |
| Low Fat Diet | Vehicle control | 34.2 ± 2.2 | 1.9 ± 0.3 | 5.5 ± 0.8 |
| GenX 0.2 mg/kg | 34.8 ± 2.4 | 2.1 ± 0.3 | 5.9 ± 0.6 |
| GenX 1.0 mg/kg | 35.7 ± 1.5 | **2.3 ± 0.2 \*** | **6.4 ± 0.6 \*** |
| GenX 2.0 mg/kg | **37.3 ± 3.6\*** | **2.2 ± 0.3 \*** | 6.0 ± 0.4 |
| PFOA 0.1 mg/kg | 35.5 ± 1.8 | 2.1 ± 0.2 | 5.8 ± 0.3 |
| PFOA 1.0 mg/kg | 34.3 ± 3.1 | 2.2 ± 0.2 | **6.2 ± 0.4 \*** |
| High Fat Diet | Vehicle control | 38.3 ± 2.2 | 2.0 ± 0.1 | 5.3 ± 0.3 |
| GenX 0.2 mg/kg | 37.7 ± 3.0 | 2.0 ± 0.2 | 5.2 ± 0.4 |
| GenX 1.0 mg/kg | 37.7 ± 4.4 | 2.1 ± 0.4 | 5.4 ± 0.5 |
| GenX 2.0 mg/kg | 37.6 ± 5.7 | 2.0 ± 0.4 | 5.3 ± 0.5 |
| PFOA 0.1 mg/kg | 39.4 ± 2.8 | 2.1 ± 0.2 | 5.3 ± 0.3 |
| PFOA 1.0 mg/kg | 41.2 ± 3.2 | 2.3 ± 0.5 | 5.6 ± 0.8 |

*Note: One-way ANOVA with Dunnett’s post hoc test, \*P < 0.05 relative to diet matched vehicle control. Data shown are mean ± SD (N = 7-12)*

**Table S11**. Female offspring body and liver weights at Week 18 (PND 126 ± 2)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Treatment** | | **Body weight (g)** | **Liver weight (g)** | **Relative liver weight (%)** |
| Low Fat Diet | Vehicle control | 35.5 ± 6.5 | 1.6 ± 0.3 | 4.5 ± 0.3 |
| GenX 0.2 mg/kg | 35.2 ± 5.4 | 1.6 ± 0.5 | 4.5 ± 0.8 |
| GenX 1.0 mg/kg | 36.8 ± 4.5 | 1.8 ± 0.3 | 4.9 ± 0.6 |
| GenX 2.0 mg/kg | 36.3 ± 6.2 | 1.6 ± 0.5 | 4.4 ± 0.8 |
| PFOA 0.1 mg/kg | 37.1 ± 4.7 | 1.6 ± 0.3 | 4.4 ± 0.5 |
| PFOA 1.0 mg/kg | 36.1 ± 6.2 | 1.7 ± 0.5 | 4.7 ± 0.8 |
| High Fat Diet | Vehicle control | 54.2 ± 11.1 | 1.8 ± 0.5 | 3.3 ± 0.6 |
| GenX 0.2 mg/kg | 52.4 ± 6.8 | 1.7 ± 0.3 | 3.2 ± 0.5 |
| GenX 1.0 mg/kg | 56.1 ± 11.6 | 2.0 ± 0.7 | 3.5 ± 0.8 |
| GenX 2.0 mg/kg | 49.7 ± 7.4 | 1.5 ± 0.2 | 3.1 ± 0.3 |
| PFOA 0.1 mg/kg | 53.0 ± 9.8 | 1.8 ± 0.7 | 3.3 ± 0.8 |
| PFOA 1.0 mg/kg | 55.6 ± 11.6 | 2.5 ± 1.1 | **4.4 ± 1.2 \*** |

*Note: One-way ANOVA with Dunnett’s post hoc test, \*P < 0.05 relative to diet matched vehicle control. Data shown are mean ± SD (N = 7-11)*

**Table S12**. Male offspring body and liver weights at Week 18

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Treatment** | | **Body weight (g)** | **Liver weight (g)** | **Relative liver weight (%)** |
| Low Fat Diet | Vehicle control | 45.1 ± 6.6 | 1.9 ± 0.3 | 4.2 ± 0.2 |
| GenX 0.2 mg/kg | 47.1 ± 4.8 | 2.1 ± 0.2 | 4.4 ± 0.3 |
| GenX 1.0 mg/kg | 49.6 ± 4.4 | 2.1 ± 0.2 | 4.4 ± 0.3 |
| GenX 2.0 mg/kg | **53.3 ± 6.5 \*** | **2.3 ± 0.3 \*** | 4.4 ± 0.6 |
| PFOA 0.1 mg/kg | **53.0 ± 6.7 \*** | 2.3 ± 0.4 | 4.3 ± 0.4 |
| PFOA 1.0 mg/kg | 52.3 ± 8.5 | **2.4 ± 0.5 \*** | 4.6 ± 0.9 |
| High Fat Diet | Vehicle control | 61.8 ± 5.7 | 3.1 ± 0.7 | 4.9 ± 0.8 |
| GenX 0.2 mg/kg | 59.7 ± 8.9 | 2.9 ± 1.3 | 4.7 ± 1.4 |
| GenX 1.0 mg/kg | 66.9 ± 7.9 | 3.7 ± 1.3 | 5.4 ± 1.5 |
| GenX 2.0 mg/kg | 60.6 ± 8.7 | 2.9 ± 0.8 | 4.7 ± 0.7 |
| PFOA 0.1 mg/kg | 61.2 ± 8.2 | 2.8 ± 1.0 | 4.5 ± 0.9 |
| PFOA 1.0 mg/kg | 62.7 ± 3.2 | 2.9 ± 0.7 | 4.7 ± 1.1 |

*Note: One-way ANOVA with Dunnett’s post hoc test, \*P < 0.05 relative to diet matched vehicle control. Data shown are mean ± SD (N = 8-11)*

**Table S13**. Postnatal weight gain (g) from weaning until 18 weeks of age shown as repeated measures mixed effect model

estimates (95% CI)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Treatment** | **Female LFD** | **Female HFD** | **Male LFD** | **Male HFD** |
| Vehicle Control | 15.46 (13.37, 17.54) | 13.56 (10.07, 17.04) | 20.08 (17.49, 22.66) | 20.07 (16.97, 23.17) |
| 0.2 mg/kg GenX | 15.40 (10.46, 20.33) | 13.46 (4.9, 22.01) | 20.87 (14.75, 27.00) | 19.03 (11.64, 26.42) |
| 1.0 mg/kg GenX | 16.19 (11.16, 21.21) | 14.29 (5.55, 23.01) | 21.20 (15.08, 27.32) | 23.91 (16.64, 31.17) |
| 2.0 mg/kg GenX | 16.42 (11.39, 21.43) | 11.42 (2.86, 19.97) | **24.57 (18.63, 30.52)\*** | 18.57 (11.41, 25.73) |
| 0.1 mg/kg PFOA | 17.16 (12.22, 22.09) | 14.06 (5.49, 22.61) | 22.59 (16.47, 28.72) | 21.15 (13.88, 28.41) |
| 1.0 mg/kg PFOA | 15.68 (10.81, 20.54) | 16.34 (7.78, 24.9) | 23.41 (17.29, 29.53) | 19.43 (12.16, 26.69) |

*Note: Point estimates are derived from a repeated measure mixed effects model accounting for repeated weight measurements of individuals*

*and time (age) as random and fixed effects, respectively.*

**Table S14**. Male offspring serum lipid levels at Week 6

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Treatment** | | **Cholesterol (mg/dL)** | **HDL (mg/dL)** | **LDL (mg/dL)** | **HDL:LDL** | **Triglycerides (mg/dL)** |
| Low Fat Diet | Vehicle control | 149.6 ± 39.7 | 90.2 ± 26.7 | 27.4 ± 9.3 | 3.3 ± 0.6 | 189.1 ± 71.9 |
| GenX 0.2 mg/kg | **203.7 ± 44.9 \*** | 106.8 ± 11.3 | 36.2 ± 13.8 | 3.2 ± 0.8 | 160.2 ± 46.2 |
| GenX 1.0 mg/kg | **216.0 ± 31.1 \*** | 109.3 ± 13.2 | 38.8 ± 8.0 | 2.9 ± 0.4 | 151.8 ± 37.4 |
| GenX 2.0 mg/kg | **193.0 ± 20.8 \*** | 106.2 ± 10.7 | 33.1 ± 6.1 | 3.3 ± 0.5 | 185.8 ± 37.2 |
| PFOA 0.1 mg/kg | 172.0 ± 37.2 | 103.1 ± 20.0 | 36.1 ± 12.8 | 3.0 ± 0.5 | 156.0 ± 27.6 |
| PFOA 1.0 mg/kg | 188.1 ± 23.9 | 99.8 ± 11.9 | 37.8 ± 5.6 | 2.7 ± 0.3 | 201.5 ± 47.5 |
| High Fat Diet | Vehicle control | 175.8 ± 31.2 | 116.4 ± 14.0 | 26.8 ± 6.1 | 4.5 ± 0.6 | 168 ± 41.0 |
| GenX 0.2 mg/kg | 177.8 ± 35.7 | 108.2 ± 19.9 | 27.2 ± 8.0 | 4.1 ± 0.5 | 142.7 ± 52.3 |
| GenX 1.0 mg/kg | 166.6 ± 31.4 | 102 ± 12.0 | 23.4 ± 5.3 | 4.5 ± 0.6 | 123.9 ± 29.9 |
| GenX 2.0 mg/kg | 188.3 ± 27.3 | 113.4 ± 10.6 | 27.5 ± 5.9 | 4.2 ± 0.6 | 141.6 ± 75.2 |
| PFOA 0.1 mg/kg | 193.9 ± 33.6 | 126.2 ± 20.1 | 31.6 ± 6.9 | 4.1 ± 0.4 | 171.7 ± 67.1 |
| PFOA 1.0 mg/kg | 201.3 ± 26.1 | 116.9 ± 19.7 | 33.5 ± 8.1 | **3.6 ± 0.7 \*** | 162.9 ± 52.9 |

*Note: One-way ANOVA with Dunnett’s post hoc test, \*P < 0.05 relative to diet matched vehicle control. Data shown are mean ± SD (N = 8-12)*

**Table S15.** Female offspring serum lipid levels at Week 6

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Treatment** | | **Cholesterol (mg/dL)** | **HDL (mg/dL)** | **LDL (mg/dL)** | **HDL:LDL** | **Triglycerides (mg/dL)** |
| Low Fat Diet | Vehicle control | 121.2 ± 19.0 | 66.0 ± 7.8 | 31.1 ± 8.4 | 2.3 ± 0.6 | 213.4 ± 66.4 |
| GenX 0.2 mg/kg | 138.1 ± 23.9 | 70.4 ± 11.7 | 25.6 ± 4.0 | **2.8 ± 0.3 \*** | 159.4 ± 35.5 |
| GenX 1.0 mg/kg | 144.0 ± 21.1 | 65.4 ± 6.6 | 27.4 ± 5.1 | 2.4 ± 0.3 | **123.5 ± 27.8 \*** |
| GenX 2.0 mg/kg | 146.0 ± 14.4 | 67.6 ± 6.2 | 29.1 ± 3.4 | 2.3 ± 0.3 | 168.4 ± 68.1 |
| PFOA 0.1 mg/kg | 133.0 ± 26.5 | 72.4 ± 12.5 | 26.6 ± 5.8 | **2.8 ± 0.3 \*** | **148.6 ± 53.1 \*** |
| PFOA 1.0 mg/kg | 130.9 ± 15.5 | 60.6 ± 5.1 | 27.3 ± 3.6 | 2.3 ± 0.4 | **140.7 ± 30.9 \*** |
| High Fat Diet | Vehicle control | 158.6 ± 20.7 | 92.0 ± 8.9 | 27.7 ± 4.0 | 3.4 ± 0.4 | 113.2 ± 25.5 |
| GenX 0.2 mg/kg | 151.8 ± 15.6 | 80.7 ± 7.3 | 25.3 ± 3.0 | 3.2 ± 0.3 | 125.0 ± 29.2 |
| GenX 1.0 mg/kg | 157.9 ± 31.4 | 85.6 ± 12.5 | 25.0 ± 6.5 | 3.5 ± 0.5 | 149.0 ± 50.5 |
| GenX 2.0 mg/kg | 160.7 ± 22.0 | 89.3 ± 9.6 | 27.2 ± 5.2 | 3.3 ± 0.4 | 135.3 ± 57.0 |
| PFOA 0.1 mg/kg | 146.5 ± 24.5 | 90.8 ± 10.7 | 25.0 ± 5.0 | 3.7 ± 0.5 | 105.6 ± 25.7 |
| PFOA 1.0 mg/kg | 162.7 ± 29.7 | 93.9 ± 18.4 | 27.2 ± 7.6 | 3.6 ± 0.7 | 123.8 ± 39.9 |

*Note: One-way ANOVA with Dunnett’s post hoc test, \*P < 0.05 relative to diet matched vehicle control. Data shown are mean ± SD (N = 8-10)*

**Table S16**. Male offspring serum lipid levels at Week 18

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Treatment** | | **Cholesterol (mg/dL)** | **HDL (mg/dL)** | **LDL (mg/dL)** | **HDL: LDL** | **Triglycerides (mg/dL)** |
| Low Fat Diet | Vehicle control | 166.0 ± 39.5 | 97.8 ± 14.7 | 24.0 ± 7.4 | 4.3 ± 0.9 | 123.9 ± 47.7 |
| GenX 0.2 mg/kg | 195.2 ± 31.2 | 114.4 ± 14.5 | 27.8 ± 6.3 | 4.2 ± 0.7 | 147.4 ± 21.5 |
| GenX 1.0 mg/kg | **216.3 ± 52.8 \*** | **118.1 ± 14.5 \*** | 34.2 ± 12.9 | 3.7 ± 0.9 | 121.7 ± 25.8 |
| GenX 2.0 mg/kg | 204.9 ± 46.6 | 112.3 ± 19.1 | 32.3 ± 11.3 | 3.8 ± 1.1 | 134.4 ± 67.0 |
| PFOA 0.1 mg/kg | 186.7 ± 35.2 | 105.9 ± 15.1 | 27.7 ± 6.6 | 3.9 ± 0.6 | 137.7 ± 52.5 |
| PFOA 1.0 mg/kg | 192.8 ± 29.6 | 107.6 ± 16.2 | 30.3 ± 5.1 | 3.6 ± 0.6 | 147.6 ± 60.0 |
| High Fat Diet | Vehicle control | 237.1 ± 51 | 132.0 ± 18.7 | 35.3 ± 14.4 | 4.2 ± 1.2 | 91.6 ± 23.7 |
| GenX 0.2 mg/kg | 246.1 ± 61.3 | 140.6 ± 26.5 | 41.1 ± 17.2 | 3.8 ± 1.0 | 114.6 ± 29.2 |
| GenX 1.0 mg/kg | 254.1 ± 66.9 | 129.9 ± 13.2 | 42.9 ± 16.9 | 3.4 ± 1.3 | 100.8 ± 26.3 |
| GenX 2.0 mg/kg | 197.0 ± 31.8 | 121.3 ± 15.5 | 28.1 ± 6.6 | 4.4 ± 0.6 | 118.1 ± 41.6 |
| PFOA 0.1 mg/kg | 220.1 ± 57.8 | 121.0 ± 17.5 | 37.8 ± 14.0 | 3.5 ± 0.9 | 100.3 ± 24.9 |
| PFOA 1.0 mg/kg | 221.3 ± 38.0 | 124.3 ± 10.2 | 35.1 ± 11.3 | 3.8 ± 0.8 | 84.8 ± 15.2 |

*Note: One-way ANOVA with Dunnett’s post hoc test, \*P < 0.05 relative to diet matched vehicle control. Data shown are mean ± SD (N = 8-11)*

**Table S17**. Female offspring serum lipid levels at Week 18

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Treatment** | | **Cholesterol (mg/dL)** | **HDL (mg/dL)** | **LDL (mg/dL)** | **HDL: LDL** | **Triglycerides (mg/dL)** |
| Low Fat Diet | Vehicle control | 150.1 ± 40.1 | 70.8 ± 17.5 | 24.2 ± 7.1 | 3.0 ± 0.6 | 83.2 ± 20.6 |
| GenX 0.2 mg/kg | 134.6 ± 25.2 | 66.1 ± 9.9 | 21.9 ± 5.7 | 3.1 ± 0.5 | 71.1 ± 12.4 |
| GenX 1.0 mg/kg | 143.4 ± 51.6 | 63.4 ± 17.7 | 24.4 ± 10.2 | 2.7 ± 0.5 | 68.6 ± 24.9 |
| GenX 2.0 mg/kg | 150.6 ± 34.4 | 75.5 ± 23.5 | 23.4 ± 4.9 | 3.3 ± 0.9 | 82.3 ± 15.5 |
| PFOA 0.1 mg/kg | 126.7 ± 34.4 | 60.3 ± 13.6 | 21.9 ± 6.1 | 2.8 ± 0.3 | 76.8 ± 15.5 |
| PFOA 1.0 mg/kg | 117.1 ± 22.7 | 57.9 ± 11.1 | 20.3 ± 4.1 | 2.9 ± 0.5 | 76.1 ± 21.7 |
| High Fat Diet | Vehicle control | 185.5 ± 33.6 | 101.7 ± 16 | 24.7 ± 5.7 | 4.2 ± 0.5 | 86.2 ± 22.2 |
| GenX 0.2 mg/kg | 176.3 ± 17.6 | 97.2 ± 9.7 | 24.6 ± 3.8 | 4.0 ± 0.4 | 82.8 ± 15.3 |
| GenX 1.0 mg/kg | 184.3 ± 29.1 | 97.0 ± 13.6 | 26.4 ± 6.6 | 3.8 ± 0.7 | 83.4 ± 20.3 |
| GenX 2.0 mg/kg | 184.0 ± 46.3 | 100.7 ± 22.6 | 28.7 ± 12.0 | 3.7 ± 0.5 | 79.4 ± 11.6 |
| PFOA 0.1 mg/kg | 175.8 ± 38.9 | 90.4 ± 16.2 | 26.3 ± 8.6 | 3.7 ± 0.8 | 71.6 ± 16.6 |
| PFOA 1.0 mg/kg | 227.2 ± 69.9 | 95.6 ± 11.9 | **41.0 ± 18.9 \*** | **2.8 ± 1.2 \*** | 84.1 ± 25.8 |

*Note: One-way ANOVA with Dunnett’s post hoc test, \*P < 0.05 relative to diet matched vehicle control. Data shown are mean ± SD (N = 7-11)*

**Table S18**. Glucose tolerance test area under the curve in female offspring

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Treatment** | **Week 9 AUC** | **Week 14 AUC** |
| Low Fat Diet | Vehicle | 3531 ± 1760 | 4746 ± 3649 |
| GenX 0.2 mg/kg | 2813 ± 1606 | 4698 ± 4066 |
| GenX 1.0 mg/kg | 1331 ± 1804 | 3305 ± 2061 |
| GenX 2.0 mg/kg | 3201 ± 1715 | 1968 ± 2525 |
| PFOA 0.1 mg/kg | 2392 ± 773 | 2626 ± 1768 |
| PFOA 1.0 mg/kg | 2131 ± 1992 | 2950 ± 2260 |
| High Fat Diet | Vehicle | 9462 ± 4866 | 12070 ± 4576 |
| GenX 0.2 mg/kg | 9606 ± 3008 | 14064 ± 4684 |
| GenX 1.0 mg/kg | 9535 ± 4488 | 12536 ± 5684 |
| GenX 2.0 mg/kg | 10257 ± 3316 | 8960 ± 4420 |
| PFOA 0.1 mg/kg | 8408 ± 3297 | 13903 ± 5212 |
| PFOA 1.0 mg/kg | 7246 ± 5602 | 12952 ± 9192 |

*Note: One-way ANOVA with Dunnett’s post hoc test, \*P < 0.05. Data shown are mean ± SD (N = 7-11)*

**Table S19**. Glucose tolerance test area under the curve in male offspring

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Treatment** | **Week 9 AUC** | **Week 14 AUC** |
| Low Fat Diet | Vehicle | 10066 ± 6929 | 9876 ± 6091 |
| GenX 0.2 mg/kg | 10499 ± 5489 | 14333 ± 9693 |
| GenX 1.0 mg/kg | 12816 ± 6972 | 16003 ± 14905 |
| GenX 2.0 mg/kg | 12654 ± 6486 | 16874 ± 6067 |
| PFOA 0.1 mg/kg | 13118 ± 8702 | 20364 ± 13937 |
| PFOA 1.0 mg/kg | 8200 ± 3788 | 19801 ± 11984 |
| High Fat Diet | Vehicle | 20373 ± 9791 | 30982 ± 11081 |
| GenX 0.2 mg/kg | 23265 ± 10527 | 26080 ± 10761 |
| GenX 1.0 mg/kg | 29326 ± 13856 | 29702 ± 9713 |
| GenX 2.0 mg/kg | 21055 ± 11048 | 31841 ± 13537 |
| PFOA 0.1 mg/kg | 22495 ± 4582 | 31954 ± 13589 |
| PFOA 1.0 mg/kg | 18584 ± 10282 | 22251 ± 12055 |

*Note: One-way ANOVA with Dunnett’s post hoc test, \*P < 0.05. Data shown are mean ± SD (N = 8-11)*

**Table S20.** Body mass composition at Week 12 in female offspring

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Treatment** | **Body weight (g)** | **Fat mass (g)** | **Lean mass (g)** | **Fluid mass (g)** | **Fat: Lean** | **% Fat** | **% Fluid** | **% Lean** |
| Low Fat Diet | Vehicle Control | 32.0 ± 4.3 | 5.7 ± 2.3 | 19.9 ± 1.9 | 1.9 ± 0.4 | 0.28 ± 0.09 | 17.3 ± 4.6 | 5.9 ± 0.6 | 62.4 ± 3.3 |
| GenX 0.2 mg/kg | 31.4 ± 3.8 | 5.8 ± 1.9 | 19.1 ± 1.8 | 1.8 ± 0.4 | 0.30 ± 0.08 | 18.3 ± 4.0 | 5.5 ± 0.6 | 60.9 ± 2.9 |
| GenX 1.0 mg/kg | 31.8 ± 3.2 | 5.7 ± 2.9 | 19.4 ± 0.6 | 1.8 ± 0.3 | 0.29 ± 0.15 | 17.2 ± 7.9 | 5.7 ± 0.4 | 61.5 ± 5.6 |
| GenX 2.0 mg/kg | 33.1 ± 4.4 | 5.8 ± 2.5 | 20.3 ± 2.8 | 1.8 ± 0.4 | 0.29 ± 0.14 | 17.2 ± 6.4 | 5.3 ± 0.7 | 61.4 ± 4.6 |
| PFOA 0.1 mg/kg | 33.7 ± 3.7 | 6.7 ± 3.3 | 20.2 ± 0.9 | 2.0 ± 0.5 | 0.30 ± 0.20 | 19.3 ± 7.5 | 5.9 ± 0.8 | 60.5 ± 5.5 |
| PFOA 1.0 mg/kg | 31.8 ± 4.4 | 6.2 ± 2.4 | 19.1 ± 2.0 | 1.8 ± 0.3 | 0.32 ± 0.11 | 18.9 ± 5.5 | 5.6 ± 0.4 | 60.3 ± 4.0 |
| High Fat Diet | Vehicle Control | 43.7 ± 10.7 | 15.3 ± 8.2 | 21.6 ± 2.6 | 3.5 ± 1.3 | 0.68 ± 0.32 | 32.6 ± 11.4 | 7.8 ± 1.2 | 51.3 ± 8.2 |
| GenX 0.2 mg/kg | 44.0 ± 5.8 | 16.2 ± 5.3 | 21.0 ± 1.5 | 3.5 ± 0.9 | 0.77 ± 0.25 | 36 ± 7.2 | 7.8 ± 1.0 | 48.3 ± 5.3 |
| GenX 1.0 mg/kg | 44.5 ± 8.6 | 16.6 ± 6.8 | 20.9 ± 1.7 | 3.7 ± 1.1 | 0.78 ± 0.28 | 35.9 ± 8.9 | 8.1 ± 1.1 | 48.1 ± 6.7 |
| GenX 2.0 mg/kg | 40.0 ± 4.7 | 13.2 ± 3.7 | 20.2 ± 1.4 | 3.0 ± 0.6 | 0.65 ± 0.17 | 32.6 ± 5.5 | 7.4 ± 0.6 | 50.8 ± 3.8 |
| PFOA 0.1 mg/kg | 44.2 ± 4.4 | 17.4 ± 3.5 | 20.4 ± 1.6 | 3.6 ± 0.5 | 0.90 ± 0.2 | 39.0 ± 5.0 | 8.1 ± 0.7 | 46.4 ± 3.7 |
| PFOA 1.0 mg/kg | 47.4 ± 9.9 | 19.9 ± 8.8 | 21.0 ± 1.4 | 4.1 ± 1.4 | 0.93 ± 0.39 | 40.0 ± 11.3 | 8.3 ± 1.3 | 45.8 ± 8.2 |

*Note: One-way ANOVA with Dunnett’s post hoc test, \*P < 0.05. Data shown are mean ± SD (N = 7-11)*

**Table S21**. Body mass composition at Week 12 in male offspring

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Treatment** | **Body weight (g)** | **Fat mass (g)** | **Lean mass (g)** | **Fluid mass (g)** | **Fat: Lean** | **% Fat** | **% Fluid** | **% Lean** |
| Low Fat Diet | Vehicle Control | 43.5 ± 5.1 | 8.2 ± 3.9 | 26.4 ± 1.6 | 2.2 ± 0.6 | 0.31 ± 0.15 | 18.1 ± 7.6 | 5.0 ± 0.8 | 61.2 ± 5.8 |
| GenX 0.2 mg/kg | 44.4 ± 5.3 | 6.8 ± 4.0 | 27.3 ± 2.1 | 2.1 ± 0.6 | 0.25 ± 0.14 | 16.0 ± 6.9 | 4.9 ± 0.9 | 62.5 ± 4.9 |
| GenX 1.0 mg/kg | 44.4 ± 4.6 | 10.2 ± 2.6 | 25.4 ± 2.6 | 2.2 ± 0.5 | 0.41 ± 0.11 | 22.9 ± 5.1 | 5.0 ± 0.8 | 57.4 ± 3.8 |
| GenX 2.0 mg/kg | 48.6 ± 6.0 | 11.0 ± 5.7 | 27.9 ± 3.0 | 2.5 ± 0.7 | 0.40 ± 0.21 | 21.9 ± 9.5 | 5.2 ± 1.0 | 58.0 ± 7.6 |
| PFOA 0.1 mg/kg | 46.1 ± 7.4 | 9.7 ± 4.8 | 27.0 ± 3.2 | 2.7 ± 0.8 | 0.4 ± 0.2 | 20.4 ± 7.4 | 5.7 ± 1.0 | 59.1 ± 5.2 |
| PFOA 1.0 mg/kg | 47.5 ± 6.7 | 12.3 ± 4.4 | 26.5 ± 2.3 | 2.8 ± 0.6 | 0.46 ± 0.14 | 25.2 ± 6.0 | 5.8 ± 0.5 | 56.2 ± 4.2 |
| High Fat Diet | Vehicle Control | 55.1 ± 4.6 | 18.7 ± 4.0 | 27.4 ± 1.1 | 3.8 ± 0.6 | 0.68 ± 0.15 | 33.6 ± 4.6 | 6.8 ± 0.6 | 49.9 ± 3.4 |
| GenX 0.2 mg/kg | 52.7 ± 7.0 | 17.1 ± 5.1 | 26.8 ± 2.1 | 3.5 ± 0.7 | 0.63 ± 0.16 | 31.8 ± 5.1 | 6.6 ± 0.6 | 51.1 ± 3.6 |
| GenX 1.0 mg/kg | 58.0 ± 6.2 | 20.2 ± 4.9 | 28.0 ± 2.0 | 4.2 ± 0.9 | 0.72 ± 0.17 | 34.5 ± 5.5 | 7.3 ± 0.9 | 48.6 ± 3.6 |
| GenX 2.0 mg/kg | 54.7 ± 5.5 | 17.6 ± 5.3 | 27.9 ± 1.6 | 3.6 ± 0.7 | 0.63 ± 0.19 | 31.5 ± 8.1 | 6.6 ± 1.0 | 51.5 ± 6.4 |
| PFOA 0.1 mg/kg | 55.5 ± 4.9 | 18.5 ± 3.6 | 27.6 ± 1.2 | 3.7 ± 0.6 | 0.7 ± 0.1 | 33.1 ± 3.6 | 6.5 ± 0.6 | 50.0 ± 2.7 |
| PFOA 1.0 mg/kg | 53.8 ± 5.3 | 18.9 ± 3.8 | 26.0 ± 2.0 | 3.9 ± 0.7 | 0.73 ± 0.14 | 34.8 ± 4.3 | 7.2 ± 0.6 | 48.5 ± 3.2 |

*Note: One-way ANOVA with Dunnett’s post hoc test, \*P < 0.05. Data shown are mean ± SD (N = 8-11)*

**Table S22**. Body mass composition at Week 17 in female offspring

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Treatment** | **Body weight (g)** | **Fat mass (g)** | **Lean mass (g)** | **Fluid mass (g)** | **Fat: Lean** | **% Fat** | **% Fluid** | **% Lean** |
| Low Fat Diet | Vehicle Control | 33.9 ± 5.2 | 6.4 ± 3.2 | 20.4 ± 1.8 | 1.8 ± 0.5 | 0.31 ± 0.13 | 18.0 ± 6.7 | 5.3 ± 0.8 | 60.9 ± 4.9 |
| GenX 0.2 mg/kg | 34.5 ± 5.1 | 6.9 ± 3.3 | 20.5 ± 1.9 | 1.9 ± 0.4 | 0.33 ± 0.14 | 19.4 ± 6.5 | 5.6 ± 0.3 | 60.0 ± 5.1 |
| GenX 1.0 mg/kg | 36.1 ± 2.6 | 7.7 ± 3.2 | 21.1 ± 0.9 | 2.1 ± 0.4 | 0.37 ± 0.16 | 20.9 ± 7.6 | 5.7 ± 0.7 | 58.8 ± 5.7 |
| GenX 2.0 mg/kg | 36.6 ± 5.8 | 8.3 ± 4.4 | 21.0 ± 2.7 | 2.1 ± 0.7 | 0.4 ± 0.23 | 21.9 ± 9.1 | 5.7 ± 1.1 | 57.8 ± 6.4 |
| PFOA 0.1 mg/kg | 36.4 ± 4.4 | 8.2 ± 4.5 | 21.2 ± 0.8 | 2.2 ± 0.6 | 0.39 ± 0.21 | 21.6 ± 9.5 | 6.0 ± 1.0 | 58.9 ± 6.9 |
| PFOA 1.0 mg/kg | 34.8 ± 5.8 | 7.9 ± 4.3 | 20.0 ± 1.8 | 2 ± 0.6 | 0.39 ± 0.20 | 21.6 ± 8.6 | 5.8 ± 0.8 | 58.2 ± 6.1 |
| High Fat Diet | Vehicle Control | 54.0 ± 12.3 | 24.8 ± 10.1 | 22.4 ± 2.3 | 4.8 ± 1.6 | 1.08 ± 0.37 | 44.0 ± 9.8 | 8.7 ± 1.2 | 42.8 ± 6.7 |
| GenX 0.2 mg/kg | 53.2 ± 7.1 | 24.7 ± 6.9 | 21.7 ± 1.1 | 4.8 ± 1 | 1.14 ± 0.31 | 45.7 ± 7.3 | 8.9 ± 0.7 | 41.3 ± 4.9 |
| GenX 1.0 mg/kg | 55.6 ± 10.4 | 26.1 ± 7.5 | 22.5 ± 2.8 | 5.1 ± 1.3 | 1.15 ± 0.23 | 46.2 ± 5.6 | 9.0 ± 0.7 | 40.9 ± 4.0 |
| GenX 2.0 mg/kg | 49.4 ± 7.2 | 20.9 ± 6.1 | 21.9 ± 1.4 | 4.2 ± 0.8 | 0.95 ± 0.24 | 41.5 ± 6.8 | 8.4 ± 0.6 | 44.8 ± 4.8 |
| PFOA 0.1 mg/kg | 52.4 ± 9.4 | 25.6 ± 8.1 | 20.7 ± 1.9 | 4.7 ± 1.3 | 1.23 ± 0.34 | 47.8 ± 8.2 | 8.8 ± 1.1 | 40.3 ± 5.9 |
| PFOA 1.0 mg/kg | 56.6 ± 12.1 | 26.6 ± 9.4 | 23.2 ± 2.5 | 5.1 ± 1.5 | 1.12 ± 0.30 | 45.6 ± 7.7 | 8.8 ± 0.9 | 42.0 ± 5.4 |

*Note: One-way ANOVA with Dunnett’s post hoc test, \*P < 0.05. Data shown are mean ± SD (N = 7-11)*

**Table S23**. Body mass composition at Week 17 in male offspring

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Treatment** | **Body weight (g)** | **Fat mass (g)** | **Lean mass (g)** | **Fluid mass (g)** | **Fat: Lean** | **% Fat** | **% Fluid** | **% Lean** |
| Low Fat Diet | Vehicle Control | 44.0 ± 5.9 | 7.0 ± 3.6 | 27.4 ± 2.3 | 2.0 ± 0.5 | 0.25 ± 0.12 | 15.2 ± 6.4 | 4.5 ± 0.7 | 62.7 ± 4.7 |
| GenX 0.2 mg/kg | 45.8 ± 4.7 | 6.3 ± 2.3 | 29.6 ± 3.4 | 2.0 ± 0.4 | 0.22 ± 0.09 | 13.7 ± 4.6 | 4.3 ± 0.7 | 64.5 ± 3.6 |
| GenX 1.0 mg/kg | 48.4 ± 4.4 | 11.0 ± 3.1 | 27.6 ± 3.0 | 2.4 ± 0.4 | 0.40 ± 0.13 | 22.6 ± 5.9 | 5.0 ± 0.7 | 57.1 ± 4.8 |
| GenX 2.0 mg/kg | **52.1 ± 6.8\*** | **13.6 ± 5.6\*** | 28.3 ± 2.0 | 2.7 ± 0.8 | **0.48 ± 0.20\*** | **25.3 ± 8.0\*** | 5.2 ± 1.0 | **55.0 ± 6.5\*** |
| PFOA 0.1 mg/kg | 51.1 ± 7.3 | 10.7 ± 6.4 | 30.3 ± 3.1 | **2.8 ± 0.8\*** | 0.36 ± 0.22 | 19.9 ± 10.2 | 5.4 ± 1.0 | 59.9 ± 7.5 |
| PFOA 1.0 mg/kg | **51.9 ± 8.1\*** | **12.9 ± 4.8\*** | 29.1 ± 3.1 | **3.0 ± 0.8\*** | 0.44 ± 0.15 | 24.2 ± 7.2 | **5.7 ± 0.8\*** | 56.7 ± 5.5 |
| High Fat Diet | Vehicle Control | 61.3 ± 5.8 | 22.5 ± 5.0 | 29.4 ± 1.4 | 4.5 ± 0.9 | 0.76 ± 0.16 | 36.3 ± 4.8 | 7.3 ± 0.8 | 48.3 ± 3.5 |
| GenX 0.2 mg/kg | 59.2 ± 8.5 | 20.7 ± 6.0 | 29.1 ± 2.5 | 4.2 ± 1.1 | 0.71 ± 0.16 | 34.4 ± 4.7 | 7.0 ± 0.9 | 49.6 ± 3.7 |
| GenX 1.0 mg/kg | 65.8 ± 7.3 | 25.3 ± 6.0 | 30.7 ± 2.6 | 5.0 ± 1 | 0.82 ± 0.19 | 38.1 ± 5.6 | 7.6 ± 0.7 | 47.0 ± 3.9 |
| GenX 2.0 mg/kg | 59.6 ± 8.2 | 20.1 ± 7.0 | 30.0 ± 1.8 | 4.2 ± 1.1 | 0.67 ± 0.22 | 32.7 ± 8.7 | 7.0 ± 1.1 | 51.0 ± 6.9 |
| PFOA 0.1 mg/kg | 60.2 ± 7.2 | 19.7 ± 7.1 | 30.6 ± 1.3 | 4 ± 0.9 | 0.64 ± 0.23 | 32.0 ± 7.9 | 6.6 ± 0.7 | 51.4 ± 5.7 |
| PFOA 1.0 mg/kg | 62.8 ± 4.0 | 24.4 ± 3.5 | 29.0 ± 1.9 | 4.9 ± 0.5 | 0.84 ± 0.14 | 38.7 ± 3.9 | 7.8 ± 0.5 | 46.3 ± 3.1 |

*Note: One-way ANOVA with Dunnett’s post hoc test, \*P < 0.05. Data shown are mean ± SD (N = 8-11)*

**Table S24.** Liver Pathology at Week 18 in Low Fat Diet Offspring

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Control** | | **0.2 mg/kg GenX** | | **1.0 mg/kg GenX** | | **2.0 mg/kg GenX** | | **0.1 mg/kg PFOA** | | **1.0 mg/kg PFOA** | |
|  | **M** | **F** | **M** | **F** | **M** | **F** | **M** | **F** | **M** | **F** | **M** | **F** |
| **N** | **8** | **9** | **9** | **9** | **8** | **8** | **11** | **8** | **9** | **9** | **9** | **10** |
| **Hepatocyte single cell necrosis** | **2** | **1** | **4** | **3** | **4** | **3** | **7** | **2** | **5** | **3** | **5** | **4** |
| Mild | 1 |  | 4 | 3 | 4 | 3 | 6 | 2 | 4 | 3 | 3 | 3 |
| Moderate | 1 | 1 |  |  |  |  | 1 |  | 1 |  | 1 | 1 |
| Severe |  |  |  |  |  |  |  |  |  |  | 1 |  |
| **Mixed Cell Infiltrate** | **3** | **5** | **3** | **4** | **7** | **3** | **8** | **3** | **5** | **4** | **5** | **6** |
| Mild | 3 | 3 | 3 | 3 | 7 | 1 | 7 | 3 | 5 | 4 | 5 | 5 |
| Moderate |  | 1 |  | 1 |  |  | 1 |  |  |  |  | 1 |
| Severe |  | 1 |  |  |  | 2 |  |  |  |  |  |  |
| **Fatty change, microvesicular** | **0** | **0** | **1** | **4** | **2** | **3** | **7\*** | **1** | **4** | **2** | **3** | **2** |
| Mild |  |  | 1 | 2 | 2 | 2 | 5 |  | 3 | 1 | 3 | 1 |
| Moderate |  |  |  | 2 |  | 1 | 2 |  |  | 1 |  | 1 |
| Severe |  |  |  |  |  |  |  | 1 | 1 |  |  |  |
| **Fatty change, macrovesicular** | **0** | **2** | **0** | **1** | **0** | **1** | **3** | **0** | **2** | **5** | **2** | **2** |
| Mild |  | 1 |  |  |  |  | 2 |  | 1 | 4 | 1 | 2 |
| Moderate |  | 1 |  | 1 |  | 1 | 0 |  |  |  | 1 |  |
| Severe |  |  |  |  |  |  | 1 |  |  | 1 |  |  |

*Note: Fisher’s exact test with Holm’s post hoc test, \*P < 0.05, †P < 0.10 relative to vehicle control. Data shown are mean ± SD (N = 8-11 per sex)*

**Table S25**. Liver Pathology at Week 18 in High Fat Diet-fed Offspring

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Control** | | **0.2 mg/kg GenX** | | **1.0 mg/kg GenX** | | **2.0 mg/kg GenX** | | **0.1 mg/kg PFOA** | | **1.0 mg/kg PFOA** | |
|  | **M** | **F** | **M** | **F** | **M** | **F** | **M** | **F** | **M** | **F** | **M** | **F** |
| **N** | **9** | **8** | **8** | **9** | **9** | **7** | **10** | **9** | **8** | **8** | **8** | **9** |
| **Hepatocyte single cell necrosis** | **7** | **1** | **6** | **0** | **9** | **5**† | **8** | **3** | **5** | **2** | **8** | **8\*** |
| Mild | 3 | 1 | 2 |  | 1 | 5 | 3 | 2 | 2 | 1 | 2 | 6 |
| Moderate |  |  | 1 |  | 3 |  | 4 | 1 | 1 |  | 3 |  |
| Severe | 4 |  | 3 |  | 5 |  | 1 |  | 2 | 1 | 3 | 2 |
| **Mixed Cell Infiltrate** | **5** | **5** | **6** | **7** | **6** | **5** | **8** | **4** | **5** | **6** | **6** | **6** |
| Mild | 5 | 3 | 5 | 5 | 3 | 4 | 5 | 4 | 4 | 5 | 6 | 5 |
| Moderate |  | 2 | 1 | 2 | 2 | 1 | 3 |  | 1 |  |  | 1 |
| Severe |  |  |  |  | 1 |  |  |  |  | 1 |  |  |
| **Fatty change, microvesicular** | **9** | **5** | **6** | **7** | **9** | **7** | **8** | **7** | **6** | **4** | **8** | **7** |
| Mild | 1 | 2 | 1 | 3 | 2 | 2 |  | 6 | 2 | 2 |  | 2 |
| Moderate | 2 | 2 | 2 | 1 |  | 1 | 1 |  | 1 |  | 5 |  |
| Severe | 6 | 1 | 3 | 3 | 7 | 4 | 7 | 1 | 3 | 2 | 3 | 5 |
| **Fatty change, macrovesicular** | **6** | **5** | **4** | **3** | **6** | **1** | **2** | **5** | **6** | **3** | **6** | **8** |
| Mild | 4 | 5 | 3 | 1 | 4 |  | 1 | 5 | 4 | 2 | 3 | 5 |
| Moderate |  |  | 1 | 2 | 1 | 1 | 1 |  | 1 |  | 1 | 3 |
| Severe | 2 |  |  |  | 1 |  | 0 |  | 1 | 1 | 2 |  |

*Note: Fisher’s exact test with Holm’s post hoc test, \*P < 0.05, †P < 0.10 relative to vehicle control. Data shown are mean ± SD (N = 7-10 per sex)*

**Table S26**. Liver enzymes at week 18 in female offspring

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Treatment** | **AST** | **ALT** | **ALP** |
| Low Fat Diet | Vehicle Control | 238.7 ± 62.0 | 55.8 ± 22.0 | 84.6 ± 22.5 |
| GenX 0.2 mg/kg | 216.3 ± 67.6 | 48.7 ± 23.9 | 71.9 ± 17.8 |
| GenX 1.0 mg/kg | 212.3 ± 39.2 | 44.8 ± 19.7 | 84.5 ± 18.7 |
| GenX 2.0 mg/kg | 254.6 ± 56.1 | 77.1 ± 89.3 | 75.6 ± 17.5 |
| PFOA 0.1 mg/kg | 197.3 ± 37.1 | 39.0 ± 12.1 | 76.1 ± 26.4 |
| PFOA 1.0 mg/kg | 243.1 ± 55.0 | 50.5 ± 17.7 | 83.9 ± 22.2 |
| High Fat Diet | Vehicle Control | 199.5 ± 46.4 | 54.4 ± 19.4 | 41.6 ± 11.7 |
| GenX 0.2 mg/kg | 190.0 ± 37.6 | 57.0 ± 48.7 | 40.0 ± 9.0 |
| GenX 1.0 mg/kg | 250.4 ± 55.6 | 77.0 ± 36.4 | 53.7 ± 11.9 |
| GenX 2.0 mg/kg | 248.6 ± 112.1 | **132.0 ± 100.8\*** | **57.9 ± 17.7\*** |
| PFOA 0.1 mg/kg | 208.1 ± 51.2 | 53.6 ± 26.7 | 44.6 ± 11.5 |
| PFOA 1.0 mg/kg | **245.0 ± 81.8\*** | **125.0 ± 73.9\*** | **59.3 ± 14.2\*** |

*Note: One-way ANOVA with Dunnett’s post hoc test, \*P < 0.05. Data shown are mean ± SD (N = 7-11)*

**Table S27**. Liver enzymes at week 18 in male offspring

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Treatment** | **AST** | **ALT** | **ALP** |
| Low Fat Diet | Vehicle Control | 173.1 ± 25.9 | 40.3 ± 14.7 | 44.1 ± 11.3 |
| GenX 0.2 mg/kg | 177.2 ± 36.0 | 34.8 ± 6.9 | **29.8 ± 6.7\*** |
| GenX 1.0 mg/kg | 226.2 ± 89.3 | 65.9 ± 66.1 | 42.8 ± 6.6 |
| GenX 2.0 mg/kg | 167.9 ± 43.6 | 44.1 ± 17.8 | 42.1 ± 9.3 |
| PFOA 0.1 mg/kg | 173.9 ± 33.1 | 60.2 ± 32.8 | 36.9 ± 12.5 |
| PFOA 1.0 mg/kg | 208.6 ± 61.8 | 87.1 ± 78.8 | 47.2 ± 15.0 |
| High Fat Diet | Vehicle Control | 185.7 ± 35.8 | 117.4 ± 76.0 | 43.6 ± 14.1 |
| GenX 0.2 mg/kg | 187.0 ± 84.1 | 143.5 ± 140.3 | 40.5 ± 16.6 |
| GenX 1.0 mg/kg | 196.9 ± 62.6 | 110.9 ± 58.1 | 53.8 ± 20.5 |
| GenX 2.0 mg/kg | 197.1 ± 48.2 | 75.9 ± 33.6 | 34.9 ± 7.0 |
| PFOA 0.1 mg/kg | 193.9 ± 40.1 | 107.1 ± 78.0 | 37.4 ± 11.2 |
| PFOA 1.0 mg/kg | 198.1 ± 55.3 | 172.1 ± 77.4 | 48.9 ± 15.1 |

*Note: One-way ANOVA with Dunnett’s post hoc test, \*P < 0.05. Data shown are mean ± SD (N = 8-11)*