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Supplemental Material

Evaluation of Maternal, Embryo, and Placental Effects in CD-1 Mice following Gestational Exposure to Perfluorooctanoic Acid (PFOA) or Hexafluoropropylene Oxide Dimer Acid (HFPO-DA or GenX)

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Table S10. Placental lesion incidence at embryonic day 17.5.

Table S11. Sex stratified placental thyroid hormone measurements at embryonic day 17.5 (Mean ± SD, N = 1-3).

Figure S1. Representative examples of liver histology in pregnant dams at gestation days 11.5 and 17.5 exposed to either vehicle control (A, B, C, D) or treated with GenX (also HFPO-DA; E, F) or PFOA (G, H). (A) Liver from a pregnant dam at 11.5 days of gestation, exposed to vehicle control (4X). (B) Higher magnification of (A) illustrating the normal uniform hepatocellular size and cytoplasmic glycogen accumulation (20X). (C) Example of a liver from a pregnant dam at 17.5 days of gestation, exposed to vehicle control. The features of centrilobular hepatocellular hypertrophy (arrows), karyomegaly, increased mitotic figures, decreased glycogen, and increased basophilic granular cytoplasm are normal features for dam livers at this stage of pregnancy (4X). (D) Higher magnification of (C) illustrating the increased mitotic figures (arrow) decreased glycogen, and increased basophilic granular cytoplasm in the areas of centrilobular hepatocellular hypertrophy (20X). (E) Example of a liver from a pregnant dam at 11.5 days of gestation, exposed to 10 mg/kg/day of GenX. There is diffuse moderate cytoplasmic alteration in this liver affecting the centrilobular, midzonal and periportal regions (4X). (F) Higher magnification of (E) illustrating the hepatocellular hypertrophy with decreased glycogen and eosinophilic granular cytoplasm. The arrows show examples of early hepatocellular apoptosis with condensed cytoplasm and condensed dark basophilic nuclear chromatin (20X). (G) Example of a liver from a pregnant dam at gestation day 17.5, exposed to 5 mg/kg/day PFOA with diffuse cytoplasmic alteration (4X). (H) Higher magnification of the boxed region in (G) showing cytoplasmic alteration with apoptosis (arrowheads) as well as accumulation of hepatocellular cytoplasmic small vacuoles with distinct borders (arrow; 20X).

Figure S2. Transmission electron microscopy (TEM) of normal liver and livers exposed to PFOA or GenX (also HFPO-DA). (A) TEM of normal liver from a vehicle control pregnant dam at gestation day 17.5 showing prominent rough endoplasmic reticulum with abundant ribosomes and evenly dispersed, abundant glycogen (see Figures 4A or 5A H&E and 4B or 5B TEM). (B) TEM of liver from a pregnant dam at gestation day 17.5 and treated with 1 mg/kg/day PFOA. Although at 40X magnification light microscopy this liver appeared to be within normal limits (see Figures 4C H&E and D TEM), TEM reveals increased vacuolation (V), evenly dispersed glycogen, as well as abundant mitochondria and peroxisomes. (C and D) TEM of liver from a pregnant dam at gestation day 17.5 and treated with 5 mg/kg/day PFOA (see figures 4E H&E and 4F TEM). Note the abundant cytoplasmic organelles consistent with mitochondria (M) and peroxisomes (P), extensive vacuoles (V), less prominent rough endoplasmic reticulum (arrow) with fewer ribosomes and less abundant glycogen (asterisk). (E and F) Transmission electron microscopy of liver from a pregnant dam at gestation day 17.5 treated with 2 mg/kg/day GenX (E; see Figures 5C H&E and 5D TEM) or 10 mg/kg/day GenX (F; see Figures 5E H&E and 5F TEM). Note the abundance of cytoplasmic organelles consistent with mitochondria (M) and peroxisomes (P). K = Kupffer cell, N = nucleus, NU = nucleolus.

Figure S3. Representative examples of occasional histopathological placenta findings observed in dams at gestation day 17.5. (A) Early clot formation in a maternal artery in the decidua region of the placenta (20X). This dam was at gestation day 17.5 and treated with 10 mg/kg/day GenX (also HFPO-DA). Note the fibrin formation with trapped cells. (B) Nodule (arrow) of tissue from the junction zone of the placenta from a dam at gestational day 17.5 that was treated with 2 mg/kg/day GenX (2X).

Figure S4. Comparison of maternal serum or plasma levels (mean ± SD) in CD-1 mice gestationally exposed to GenX (also HFPO-DA) at varying dose levels in a study conducted by DuPont-18405-1037 (Edwards 2010b; plasma measured on lactation day 21) and the present study (Blake et al. 2019; serum measured on E17.5). Maternal serum or plasma was collected less than 6 hours after oral gavage in both studies. Administered GenX dose and maternal serum or plasma concentration was linearly correlated across data from both studies ($R^2 = 0.959$, $P < 0.05$ for non-zero slope).