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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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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).

Table S1. Number of total observations and litters represented in mixed effect models (observations, litters)

Embryonic day	Dose group	Embryo Weight	Embryo length	Placenta weight	Embryo:Placental weight ratio
11.5	Vehicle Control	68, 13	24, 8	68, 13	68, 13
11.5	1 mg/kg bw/day PFOA	64, 11	18, 6	62, 11	62, 11
11.5	5 mg/kg bw/day PFOA	66, 11	18, 6	65, 11	65, 11
11.5	2 mg/kg bw/day GenX (HFPO-DA)	71, 12	21, 7	70, 12	70, 12
11.5	10 mg/kg bw/day GenX (HFPO-DA)	62, 12	21, 7	62, 12	62, 12
17.5	Vehicle Control	80, 13	24, 8	80, 13	80, 13
17.5	1 mg/kg bw/day PFOA	66, 12	21, 7	66, 12	66, 12
17.5	5 mg/kg bw/day PFOA	73, 12	18, 6	73, 12	73, 12
17.5	2 mg/kg bw/day GenX (HFPO-DA)	68, 12	21, 7	68, 12	68, 12
17.5	10 mg/kg bw/day GenX (HFPO-DA)	66, 13	24, 8	66, 13	66, 13

Table S2. Internal dosimetry of tissues at embryonic day 11.5 including maternal serum, maternal liver, amniotic fluid, and whole embryo (Mean ± SD, N = 6-8)

Biological matrix	1 mg/kg bw/day PFOA	5 mg/kg bw/day PFOA	2 mg/kg bw/day GenX (HFPO-DA)	10 mg/kg bw/day GenX (HFPO-DA)
Maternal serum (µg/mL)	25.4 ± 3.7‡	117.3 ± 20.6	33.5 ± 15.7**	118.1 ± 10.4
Amniotic fluid (µg/mL)	4.6 ± 2.8*,‡	8.8 ± 2.7	3.6 ± 2.2**,§	9.3 ± 2.0
Maternal liver (µg/g)	48.3 ± 12.5*,†,‡	151.5 ± 18.5	5.45 ± 3.43§	19.9 ± 4.2††
Whole embryo (µg/g)	0.80 ± 0.10*,‡	2.34 ± 0.27	0.91 ± 0.22**,§	3.21 ± 0.51††

ANOVA with *post hoc* multiple comparison correction using Tukey contrasts:
* $P < 0.05$ 1 mg/kg/day PFOA vs 5 mg/kg/day PFOA
† $P < 0.05$ 1 mg/kg/day PFOA vs 2 mg/kg/day GenX
‡ $P < 0.05$ 1 mg/kg/day PFOA vs 10 mg/kg/day GenX
§ $P < 0.05$ 2 mg/kg/day GenX vs 5 mg/kg/day PFOA
** $P < 0.05$ 2 mg/kg/day GenX vs 10 mg/kg/day GenX
†† $P < 0.05$ 10 mg/kg/day GenX vs 5 mg/kg/day PFOA
Note: Vehicle control (VC) samples were quantified for PFOA and GenX (HFPO-DA); all VC means were below the limit of detection (LOD) of 10 ng/mL for both PFOA and GenX (HFPO-DA)

Table S3. Internal dosimetry of tissues at embryonic day 17.5 including maternal serum, maternal liver, male whole embryo and female whole embryo (Mean ± SD, N = 6-8)

Biological matrix	1 mg/kg bw/day PFOA	5 mg/kg bw/day PFOA	2 mg/kg bw/day GenX (HFPO-DA)	10 mg/kg bw/day GenX (HFPO-DA)
Maternal serum (μg/mL)	18.7 ± 3.2*,‡	95.1 ± 14.1	22.9 ± 17.1§,**	58.5 ± 34.5
Maternal liver (μg/g)	181.1 ± 46.0†,‡	159.2 ± 21.7	4.56 ± 2.80§	14.2 ± 7.6††
Whole embryo (μg/g)	5.78 ± 0.71*,†	16.4 ± 1.75	3.23 ± 1.28§,**	7.69 ± 2.92††
Male embryo (μg/g)	5.78 ± 0.82*	16.9 ± 1.88	3.04 ± 1.27§,**	7.55 ± 3.35††
Female embryo (μg/g)	5.78 ± 0.81*	16.1 ± 2.75	3.39 ± 1.44§,**	6.89 ± 2.72††

ANOVA with *post hoc* multiple comparison correction using Tukey contrasts:
* $P < 0.05$ 1 mg/kg/day PFOA vs 5 mg/kg/day PFOA
† $P < 0.05$ 1 mg/kg/day PFOA vs 2 mg/kg/day GenX
‡ $P < 0.05$ 1 mg/kg/day PFOA vs 10 mg/kg/day GenX
§ $P < 0.05$ 2 mg/kg/day GenX vs 5 mg/kg/day PFOA
** $P < 0.05$ 2 mg/kg/day GenX vs 10 mg/kg/day GenX
†† $P < 0.05$ 10 mg/kg/day GenX vs 5 mg/kg/day PFOA
Note: N = 3 for PFOA 5 mg/kg male embryo; Vehicle control (VC) samples were quantified for PFOA and GenX (HFPO-DA); all VC means were below the LOD of 10 ng/mL for both PFOA and GenX (HFPO-DA) except for maternal serum (0.211 ± 0.55 μg/mL)

Table S4. Litter parameters in mice gestationally exposed to PFOA or GenX from embryonic day 0.5 to 11.5 or 17.5 (Mean ± SD, N = 11-13)

Embryonic day	Litter parameter	Vehicle Control	1 mg/kg bw/day PFOA	5 mg/kg bw/day PFOA	2 mg/kg bw/day GenX (HFPO-DA)	10 mg/kg bw/day GenX (HFPO-DA)
11.5	No. implantation sites	13.7 ± 2.8	15.5 ± 2.7	14.5 ± 2.3	14.7 ± 2.7	14.8 ± 3.7
11.5	No. resorptions	0.6 ± 1.1	0.6 ± 0.5	0.5 ± 0.9	0.7 ± 0.8	0.9 ± 1.3
11.5	No. dead embryos	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.3	0.0 ± 0.0	0.1 ± 0.3
11.5	No. viable embryos	13.1 ± 3.0	14.8 ± 2.5	13.9 ± 2.2	14.0 ± 2.9	13.8 ± 3.7
11.5	% resorbed[†]	4.6 ± 8.6	4.0 ± 3.3	3.6 ± 5.9	5.0 ± 6.2	5.6 ± 7.7
11.5	% nonviable[†]	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 1.9	0.0 ± 0.0	2.1 ± 7.2
11.5	% viable[†]	95.4 ± 8.6	96.0 ± 3.3	95.9 ± 5.9	95.0 ± 6.2	92.4 ± 9.3
17.5	No. implantation sites	14.2 ± 2.9	13.5 ± 2.0	13.7 ± 2.1	13.8 ± 2.0	12.5 ± 3.1
17.5	No. resorptions	0.3 ± 0.5	0.3 ± 0.5	0.3 ± 0.5	0.2 ± 0.6	0.5 ± 0.7
17.5	No. dead embryos	0.1 ± 0.3	0.1 ± 0.3	0.0 ± 0.0	0.1 ± 0.3	0.0 ± 0.0
17.5	No. viable embryos	13.8 ± 3.2	13.1 ± 2.1	13.3 ± 1.9	13.5 ± 2.1	12.0 ± 3.0
17.5	% resorbed[†]	2.7 ± 4.6	2.6 ± 3.9	2.3 ± 3.4	1.2 ± 4.1	3.5 ± 5.3
17.5	% nonviable[†]	0.6 ± 2.3	0.6 ± 2.1	0.0 ± 0.0	0.7 ± 2.4	0.0 ± 0.0
17.5	% viable[†]	96.7 ± 4.8	96.8 ± 4.1	97.7 ± 3.4	98.1 ± 4.6	96.5 ± 5.3

[†]Calculated as percent of implantation sites

Note: $P > 0.05$ relative to vehicle control for all values (ANOVA with *post hoc* multiple comparison correction using Tukey contrasts)

Table S5. Relative gestational weight gain (% gain from embryonic day 0.5) at necropsy adjusting for litter size (adjusted model estimate and 95% confidence intervals; N = 11-13)

Dose group	E11.5 stimate (95% CI)	E11.5 Estimate (95% CI) relative to VC	E17.5 Estimate (95% CI)	E11.5 Estimate (95% CI) relative to VC
Vehicle control	17.3 (7.2, 27.4)	0 (0,0)	36.2 (10.8, 61.5)	0 (0,0)
1 mg/kg bw/day PFOA	16.2 (-0.2, 32.5)	-1.1 (-7.4, 5.1)	44.9 (6.5, 83.3)	8.7 (-4.3, 21.8)
5 mg/kg bw/day PFOA	21.6 (5.4, 37.9)	4.3 (-1.8, 10.5)	50.7 (12.2, 89.0)*	14.5 (1.4, 27.5)*
2 mg/kg bw/day GenX (HFPO-DA)	20.8 (4.7, 36.9)	3.5 (-2.5, 9.5)	48.7 (10.3, 87.1)	12.5 (-0.5, 25.6)
10 mg/kg bw/day GenX (HFPO-DA)	24.4 (8.1, 40.6)*	7.1 (0.9, 13.2)*	55.3 (16.7, 93.7)*	19.1 (5.9, 32.2)*

Abbr: E = embryonic day; VC = vehicle control

*$P < 0.05$; Beta estimate 95% confidence intervals relative to VC do not overlap zero (Generalized linear regression adjusted for litter size, with vehicle control as reference group)

Table S6. Incidence of liver histopathology in maternal livers at embryonic day 11.5

Lesion type and severity	Vehicle Control N (%)	1 mg/kg/day PFOA N (%)	5 mg/kg/day PFOA N (%)	2 mg/kg/day GenX (HFPO-DA) N (%)	10 mg/kg/day GenX (HFPO-DA) N (%)
CA*, any severity	0 (0)	5 (100)	5 (100)	5 (100)	5 (100)
CA, minimal to mild	0 (0)	5 (100)	0 (0)	5 (100)	0 (0)
CA, moderate	0 (0)	0 (0)	0 (0)	0 (0)	2 (40)
CA, marked	0 (0)	0 (0)	5 (100)	0 (0)	3 (60)
Increase in mitotic figures	0 (0)	2 (40)	5 (100)	3 (60)	4 (80)
Increase in cell death†	0 (0)	1 (20)	3 (60)	4 (80)	3 (60)
Focal regions of classic necrosis	1 (20)	0 (0)	2 (40)	0 (0)	1 (20)
Vacuolation‡, any severity	0 (0)	0 (0)	5 (100)	0 (0)	5 (100)
Vacuolation, minimal	0 (0)	0 (0)	0 (0)	0 (0)	5 (100)
Vacuolation, mild	0 (0)	0 (0)	5 (100)	0 (0)	0 (0)
Vacuolation, moderate	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Abbr: CA = cytoplasmic alteration

*CA was characterized as hepatocellular hypertrophy with decreased glycogen and intensely eosinophilic granular cytoplasm, with extension from periportal to midzonal regions with increasing severity

†Cell death included both apoptosis and single cell necrosis of individual hepatocytes

‡Vacuolation was graded by severity based on occurrence of clear, small, and round vacuoles in the cytoplasm of centrilobular hepatocytes in areas of cytoplasmic alteration as: minimal—rarely or occasionally seen as small clusters; mild—frequently seen as small clusters; moderate—seen in all centrilobular hepatocytes

Note: Histopathology was evaluated by a pathology working group and observations of increased mitotic figures and cell death were made relative to the vehicle control group; increase in mitotic figures and cell death was scored as minimal

Table S7. Incidence of liver histopathology in maternal livers at embryonic day 17.5

Lesion type and severity	Vehicle Control N (%)	1 mg/kg/day PFOA N (%)	5 mg/kg/day PFOA N (%)	2 mg/kg/day GenX (HFPO-DA) N (%)	10 mg/kg/day GenX (HFPO-DA) N (%)
CA*, any severity	0 (0)	5 (100)	6 (100)	5 (100)	5 (100)
CA, minimal to mild	0 (0)	0 (0)	0 (0)	2 (40)	0 (0)
CA, moderate	0 (0)	5 (100)	0 (0)	3 (60)	0 (0)
CA, marked	0 (0)	0 (0)	6 (100)	0 (0)	5 (100)
Increase in mitotic figures	5 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Increase in cell death[†]	0 (0)	5 (100)	6 (100)	0 (0)	5 (100)
Focal regions of classic necrosis	0 (0)	0 (0)	0 (0)	1 (20)	1 (20)
Vacuolation[‡], any severity	0 (0)	0 (0)	6 (100)	0 (0)	5 (100)
Vacuolation, minimal	0 (0)	0 (0)	0 (0)	0 (0)	2 (40)
Vacuolation, mild	0 (0)	0 (0)	0 (0)	0 (0)	3 (60)
Vacuolation, moderate	0 (0)	0 (0)	6 (100)	0 (0)	0 (0)

Abbr: CA = cytoplasmic alteration

*CA was characterized as hepatocellular hypertrophy with decreased glycogen and intensely eosinophilic granular cytoplasm, with extension from periportal to midzonal regions with increasing severity

[†]Cell death included both apoptosis and single cell necrosis of individual hepatocytes

[‡]Vacuolation was graded by severity based on occurrence of clear, small, and round vacuoles in the cytoplasm of centrilobular hepatocytes in areas of cytoplasmic alteration as: minimal—rarely or occasionally seen as small clusters; mild—frequently seen as small clusters; moderate—seen in all centrilobular hepatocytes

Note: Histopathology was evaluated by a pathology working group and observations of increased mitotic figures and cell death were made relative to the vehicle control group; increase in mitotic figures and cell death was scored as minimal

Table S8. Embryo and placental mixed effect model adjusted estimates and 95% confidence intervals (N = 11-13 dams with 62-80 observations per group)

Embryonic day	Dose group	Embryo weight (mg)	Embryo length (mm)	Placental weight (mg)	Embryo:Placental Weight Ratio
11.5	Vehicle Control	49.8 (29.2, 70.5)	18.4 (16.4, 20.3)	40.1 (19.0, 61.1)	1.08 (0.73, 1.43)
11.5	1 mg/kg bw/day PFOA	44.7 (11.1, 78.2)	17.5 (14.5, 20.4)	30.5 (-3.8, 64.7)	1.19 (0.62, 1.76)
11.5	5 mg/kg bw/day PFOA	45.3 (11.8, 78.7)	18.5 (15.6, 21.4)	31.2 (-2.9, 65.3)	1.21 (0.64, 1.77)
11.5	2 mg/kg bw/day GenX (HFPO-DA)	49.9 (16.7, 83.1)	17.6 (14.7, 20.5)	34.0 (0.1, 67.8)	1.22 (0.66, 1.78)
11.5	10 mg/kg bw/day GenX (HFPO-DA)	45.2 (12.0, 78.3)	17.4 (14.5, 20.3)	38.4 (4.5, 72.2)	1.08 (0.52, 1.64)
17.5	Vehicle Control	1378.6 (1206.3, 1550.8)	33.6 (31.1, 36.1)	130.8 (109.8, 151.8)	11.2 (9.2, 13.3)
17.5	1 mg/kg bw/day PFOA	1350.7 (1091.9, 1609.4)	33.6 (29.6, 37.6)	129.7 (98.2, 161.2)	11.1 (8.0, 14.3)
17.5	5 mg/kg bw/day PFOA	1249.5 (991.0, 1508.0)*	31.2 (27.2 35.3)*	151.9 (120.5, 183.4)*	8.5 (5.4, 11.6)*
17.5	2 mg/kg bw/day GenX (HFPO-DA)	1369.8 (1111.3, 1628.4)	32.7 (28.7, 36.6)	137.1 (105.6, 168.6)	10.6 (7.5, 13.7)
17.5	10 mg/kg bw/day GenX (HFPO-DA)	1337.0 (1077.5, 1596.4)	33.8 (29.8, 37.7)	146.3 (114.7, 177.9)*	9.5 (6.4, 12.7)*

Note: N = 6-8 dams with 18-24 observations per group for embryo length

*$P < 0.05$; 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 S9. Placental lesion index at embryonic day 11.5

Placental outcome or sample size	Vehicle control	1 mg/kg bw/day PFOA	5 mg/kg bw/day PFOA	2 mg/kg bw/day GenX (HFPO-DA)	10 mg/kg bw/day GenX (HFPO-DA)
Total no. placenta evaluated	37	33	36	43	30
No. WNL (%)	35 (94.6)	32 (97.0)	32 (88.9)	41 (95.3)	30 (100)
No. abnormal (%)	2 (5.4)	4 (3.0)	4 (11.1)	2 (4.7)	0 (0.0)
No. labyrinth atrophy (%)	0 (0)	0 (0)	3 (8.3)	0 (0)	0 (0)
No. labyrinth congestion (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
No. labyrinth necrosis (%)	0 (0)	0 (0)	1 (2.7)	0 (0)	0 (0)
No. early fibrin clot (%)	0 (0)	0 (0)	0 (0)	1 (2.8)	0 (0)
No. placental nodule (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
No. other (%)[†]	2 (5.4)	1 (3.0)	0 (0)	1 (2.3)	0 (0)
No. litters evaluated	5	5	5	5	5
Mean no. placenta evaluated per litter (min, max)	7.4 (5, 10)	6.6 (4, 10)	7.4 (5, 10)	8.6 (4, 11)	6.2 (3, 11)
Mean WNL ± SD (%) per litter	6.4 ± 1.9 (95.0)	6.4 ± 1.9 (98.0)	6.4 ± 1.7 (88.3)	8.2 ± 2.8 (95.6)	6.0 ± 3.1 (97.1)
Mean abnormal ± SD (%) per litter	0.4 ± 0.9 (5.0)	0.2 ± 0.4 (2.0)	1.0 ± 1.4 (11.7)	0.4 ± 0.9 (4.4)	0 ± 0.0 (0)

Abbr: WNL = within normal limits

*$P < 0.05$ relative to vehicle control (general linear model using a Poisson distribution with *post hoc* multiple comparison correction using Tukey contrasts)

[†]Other lesions were considered spontaneous and not treatment related and included: Increased thickness of trilaminar trophoblast layers (vehicle control), decreased labyrinth area (vehicle control), absent labyrinth (PFOA 1 mg/kg), large focal labyrinth hemorrhage and thrombus (GenX 2 mg/kg)

Table S10. Placental lesion incidence at embryonic day 17.5

Placental outcome or sample size	Vehicle control	1 mg/kg bw/day PFOA	5 mg/kg bw/day PFOA	2 mg/kg bw/day GenX (HFPO-DA)	10 mg/kg bw/day GenX (HFPO-DA)
Total no. placenta evaluated	41	32	40	31	35
No. WNL (%)	40 (97.6)	29 (90.6)	13 (32.5)*	13 (41.9)	6 (17.1)*
No. abnormal (%)	1 (2.4)	3 (9.4)	27 (67.5)*	18 (58.1)	29 (82.9)*
No. labyrinth atrophy (%)	0 (0)	0 (0)	3 (7.5)	15 (48.4)	16 (45.7)
No. labyrinth congestion (%)	0 (0)	0 (0)	23 (57.5)	1 (3.2)	8 (22.9)
No. labyrinth necrosis (%)	0 (0)	1 (3.1)	0 (0)	0 (0)	1 (2.9)
No. early fibrin clot (%)	0 (0)	0 (0)	1 (2.5)	1 (3.2)	4 (11.4)
No. placental nodule (%)	0 (0)	2 (6.3)	0 (0)	1 (3.2)	0 (0)
No. other (%)[†]	1 (2.4)	0 (0)	0 (0)	0 (0)	0 (0)
No. litters evaluated	5	5	6	5	5
Mean no. placenta evaluated per litter (min, max)	8.2 (7, 10)	6.4 (5, 8)	6.7 (5, 9)	6.2 (2, 8)	7.0 (3, 10)
Mean WNL ± SD (%) per litter	8.0 ± 0.7 (98.0)	5.8 ± 1.3 (91.4)	2.2 ± 2.7 (38.1)*	2.6 ± 2.8 (51.7)*	1.2 ± 2.7 (12.0)*
Mean abnormal ± SD (%) per litter	0.2 ± 0.4 (2.0)	0.6 ± 0.9 (8.6)	4.5 ± 3.8 (61.9)*	3.6 ± 3.6 (48.3)*	5.8 ± 3.0 (88.0)*

Abbr: WNL = within normal limits

*$P < 0.05$ relative to vehicle control (general linear regression using a Poisson distribution with *post hoc* multiple comparison correction using Tukey contrasts)

[†]Other lesions were considered spontaneous and not treatment related and included: Cortical necrosis with inflammatory cells (vehicle control)

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

Hormone	Sex	Vehicle control	1 mg/kg/day PFOA	5 mg/kg/day PFOA	2 mg/kg/day GenX (HFPD-DA)	10 mg/kg/day GenX (HFPO-DA)
rT3 (ng/g)	Female	0.9 ± 0.8[†]	0.8 ± 0.4	1.6 ± 1.0	2.3 ± 0.6	1.9 ± 0.1
rT3 (ng/g)	Male	1.4 ± 0.6	0.5 ± 0.4	1.2 ± 0.1	1.0 ± 0.2	1.3 ± 0.3
T3 (ng/g)	Female	0.4 ± 0.3	0.2 ± 0	0.2 ± 0	0.3 ± 0.2	0.2 ± 0
T3 (ng/g)	Male	0.2 ± 0	0.2 ± 0	0.2 ± NA[†]	0.2 ± 0	0.2 ± 0
T4 (ng/g)	Female	3.6 ± 0.8	2.0 ± 0.4	3.5 ± 1.7	5.9 ± 2.3	6.5 ± 1.4
T4 (ng/g)	Male	4.0 ± 0.4	3.1 ± 1.2	2.2 ± 0.7	4.7 ± 1.1	5.8 ± 0.7
T3:T4 ratio	Female	0.095 ± 0.06	0.104 ± 0.02	0.065 ± 0.02	0.053 ± 0.02	0.032 ± 0.01
T3:T4 ratio	Male	0.05 ± 0.005	0.071 ± 0.02	0.083 ± NA[†]	0.044 ± 0.01	0.035 ± 0.004
rT3:T4 ratio	Female	0.291 ± 0.30	0.401 ± 0.22	0.441 ± 0.05	0.409 ± 0.08	0.302 ± 0.09
rT3:T4 ratio	Male	0.355 ± 0.17	0.196 ± 0.17	0.454 ± 0.07	0.225 ± 0.08	0.231 ± 0.04

Abbr: rT3 = reverse triiodothyronine, T3 = triiodothyronine, T4 = thyroxine, MDL = method detection limit
Note: $P > 0.05$ relative to vehicle control for all values including sex*treatment as an interaction term in the model (ANOVA with *post hoc* multiple comparison correction using Tukey contrasts); Sample sizes ranged from N = 1-3; Non-quantifiable samples below the MDL were imputed using the calculation MDL*0.5. MDL values were: T4: 0.84 ng/g, T3: 0.42 ng/g, rT3: 0.67 ng/g.

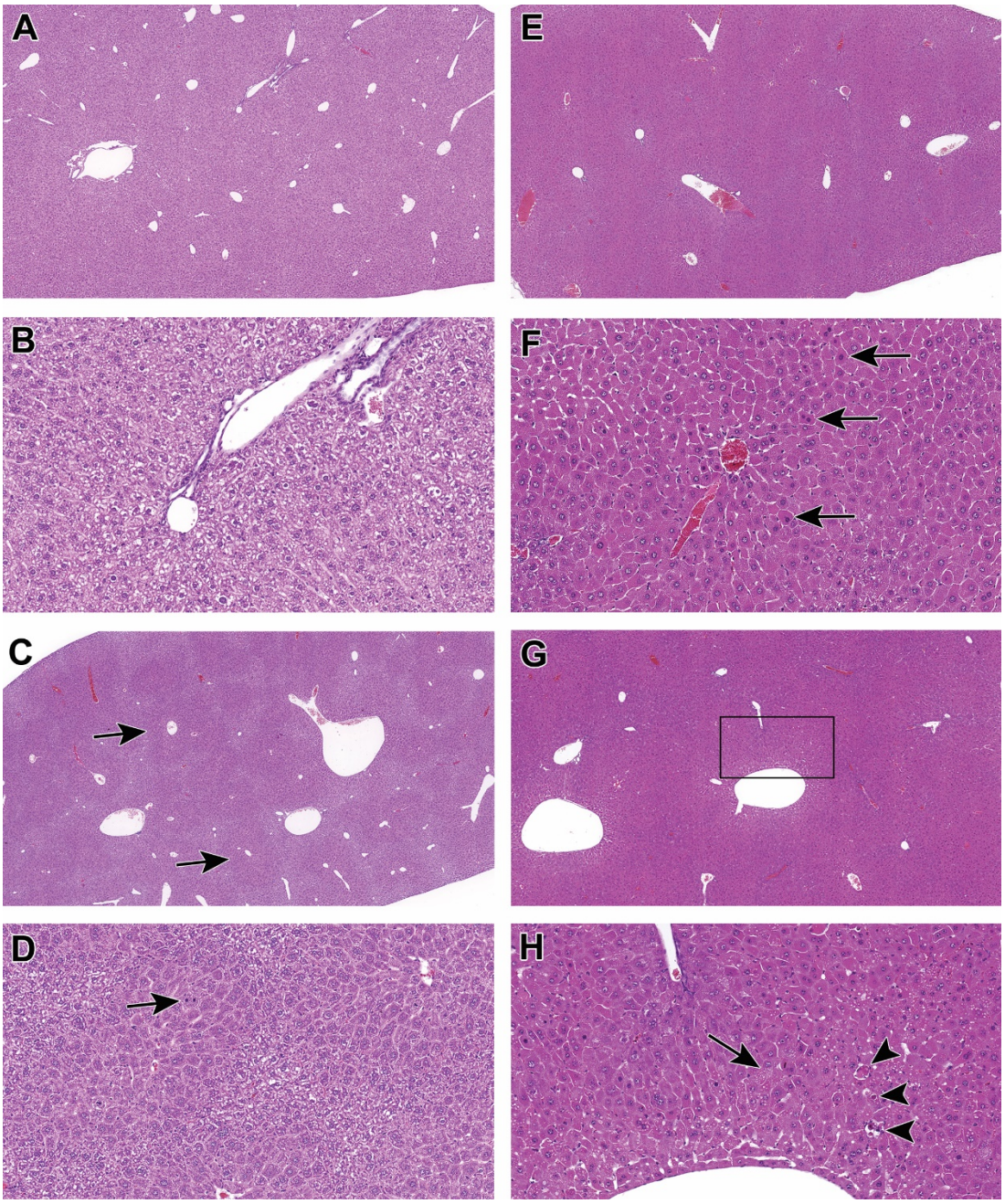

Figure S1. Representative examples of liver histology in pregnant dams at gestation days 11.5 and 17.5 exposed to either vehicle control (A, B, C, D) or treated with GenX (also HFPO-DA; E, F) or PFOA (G, H). (A) Liver from a pregnant dam at 11.5 days of gestation, exposed to vehicle control (4X). (B) 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).

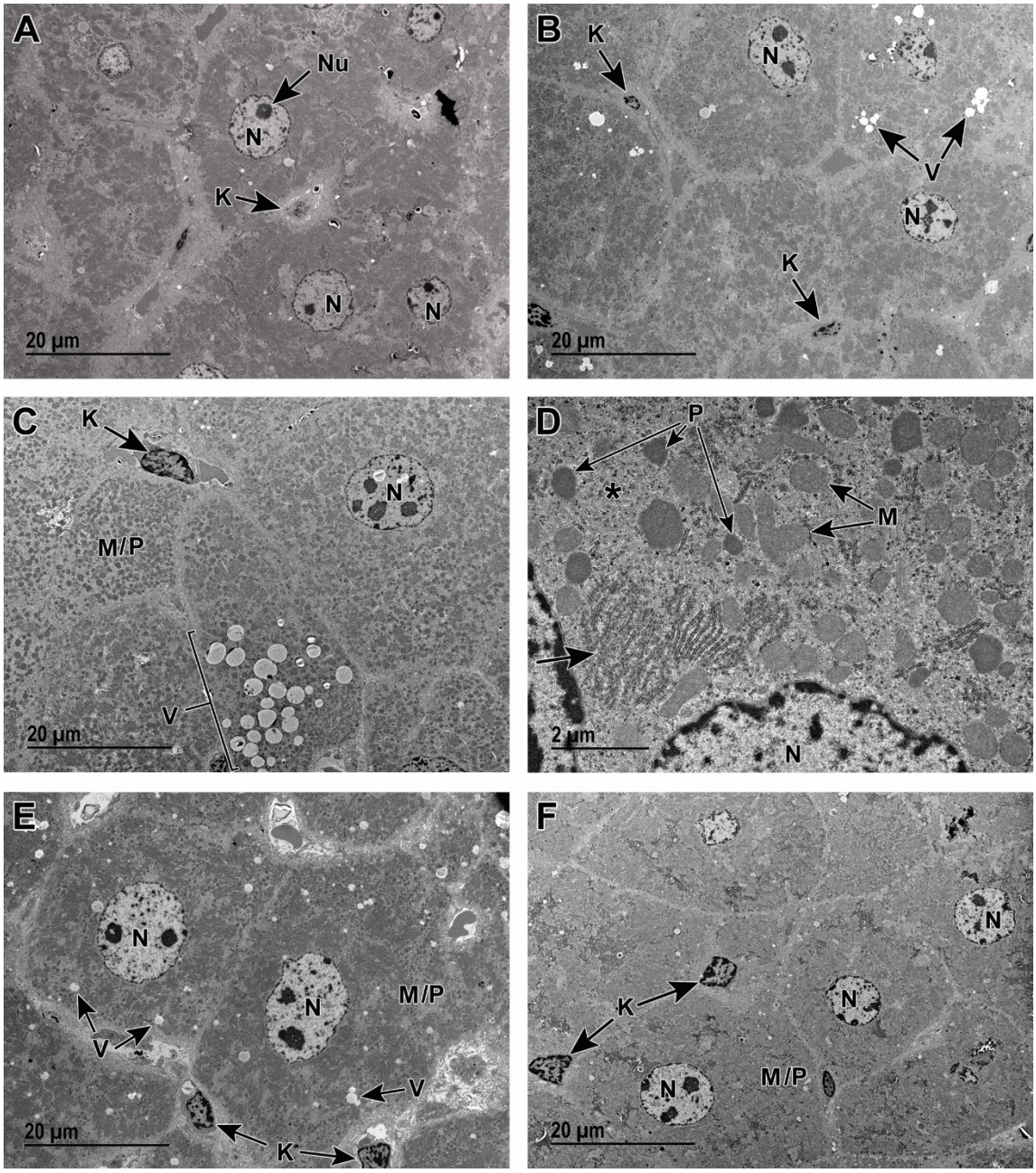

Figure S2. Transmission electron microscopy (TEM) of normal liver and livers exposed to PFOA or GenX (also HFPO-DA). (A) TEM of normal liver from a vehicle control pregnant dam at gestation day 17.5 showing prominent rough endoplasmic reticulum with abundant ribosomes and evenly dispersed, abundant glycogen (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.

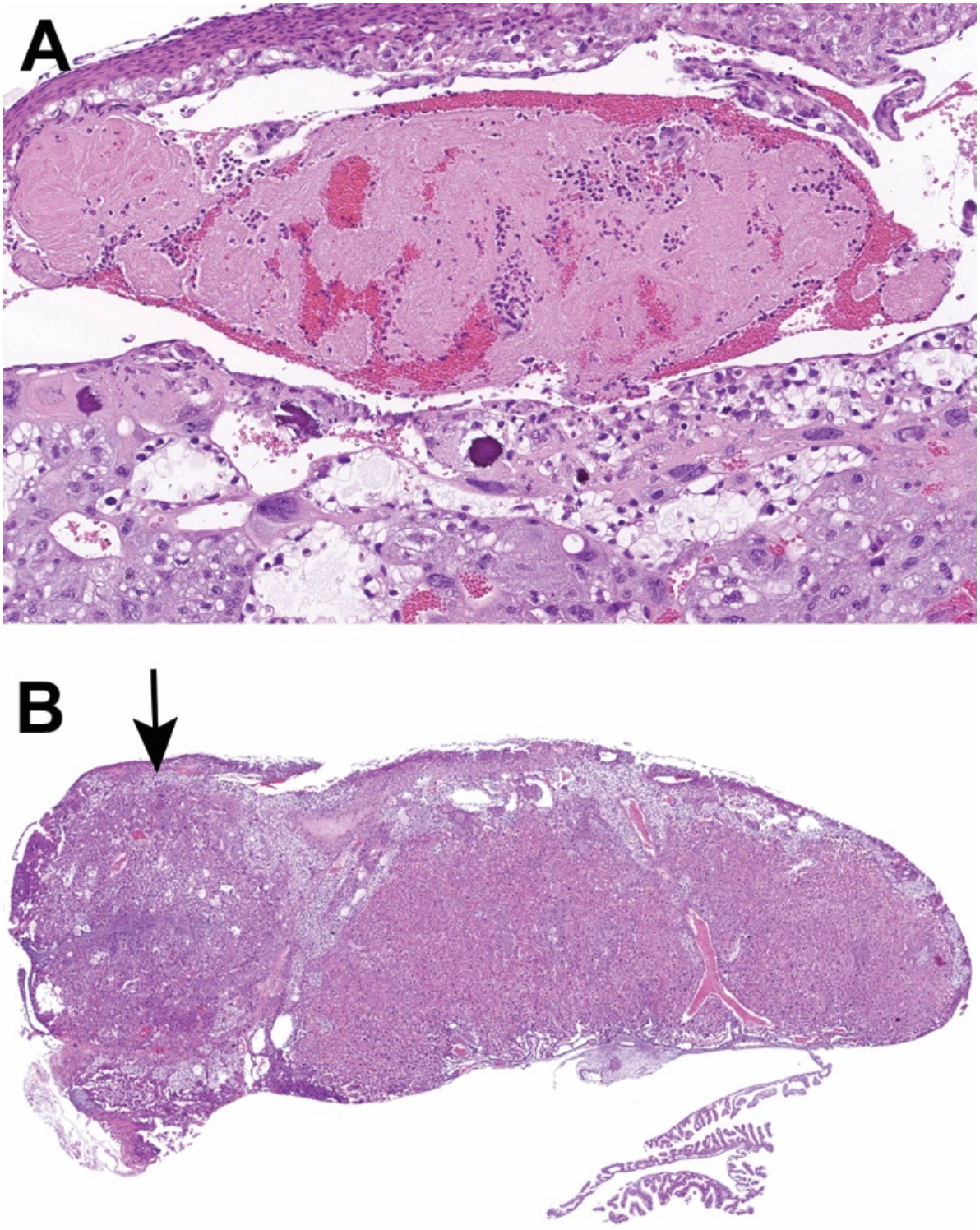

Figure S3. Representative examples of occasional histopathological placenta findings observed in dams at gestation day 17.5. (A) Early clot formation in a maternal artery in the decidua region of the placenta (20X). This dam was at gestation day 17.5 and treated with 10 mg/kg/day GenX (also HFPO-DA). 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).

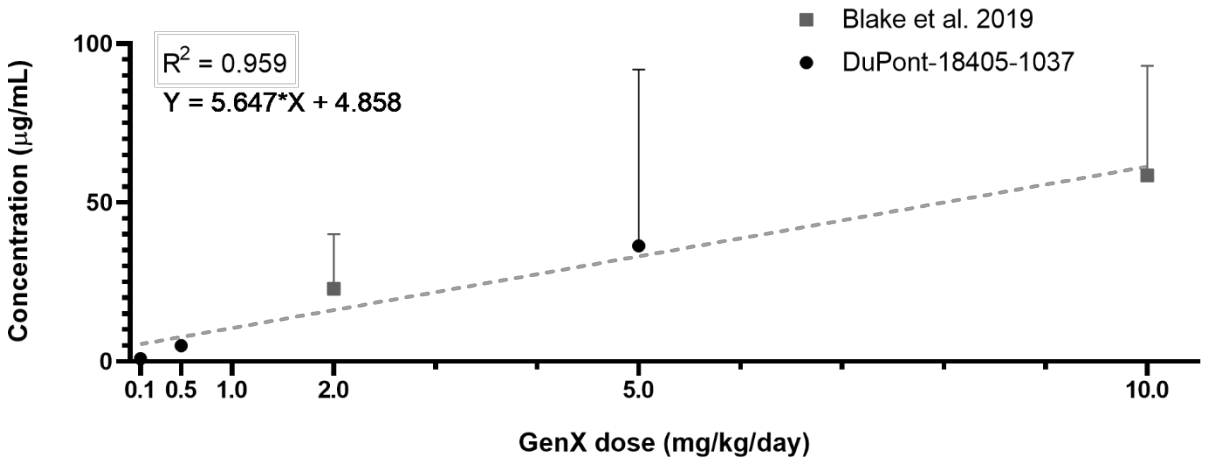

Figure S4. Comparison of maternal serum or plasma levels (mean ± SD) in CD-1 mice gestationally exposed to GenX (also HFPO-DA) at varying dose levels in a study conducted by DuPont-18405-1037 (Edwards 2010b; plasma measured on lactation day 21) and the present study (Blake et al. 2019; serum measured 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).