Supplementary Table 1

GC/MS parameters for the isomer specific detection of PBDE.

GC Type	Agilent 6890
Column	Rtx-1614, 15 m, 0.25 mm ID, 0.1 µm film thickness (Restek)
Temperature program	75 °C, 1.5 min, 18 °C/min, 210 °C, 8 °C/min, 310 °C, 5 min
Carrier gas	helium
Flow	constant flow 1.6 mL/min
Injector	cooled injection system CIS 4 (Gerstel)
Temperature line	320 °C
Injection volutransferme	1 µL splitless
MS Type	MAT 95XL (Thermo)
Ionization mode	EI+, 47 eV, 260°C
Resolution	>9000

Supplementary Table 2. Limits of quantification for PND 30 samples. **(Related to Figure 1)**

	Mean	Standard deviation
	µg/kg wet weight	µg/kg wet weight
BDE-7	0.008	0.006
BDE-10	0.006	0.005
BDE-15	0.15	0.04
BDE-17	0.008	0.002
BDE-28	0.15	0.04
BDE-30	0.01	0.01
BDE-47	1.5	0.37
BDE-49	0.02	0.006
BDE-66	0.06	0.05
BDE-71	0.02	0.004
BDE-77	0.05	0.04
BDE-85	0.05	0.04
BDE-99	0.95	0.23
BDE-100	0.43	0.10
BDE-119	0.05	0.01
BDE-126	0.05	0.04
BDE-138	0.07	0.06
BDE-139	0.06	0.05
BDE-140	0.06	0.05
BDE-153	0.75	0.18
BDE-154	0.05	0.04
BDE-156	0.07	0.06
BDE-171	0.12	0.05
BDE-180	0.12	0.05
BDE-183	0.09	0.04
BDE-184	0.09	0.04
BDE-191	0.09	0.04
BDE-196	0.02	0.02
BDE-197	0.02	0.02
BDE-201	0.02	0.02
BDE-203	0.02	0.02
BDE-204	0.02	0.02
BDE-205	0.03	0.04
BDE-206	0.31	0.07
BDE-207	0.36	0.09
BDE-208	0.21	0.05
BDE-209	5.0	1.2

Supplementary Table 3. Mass spectrometric analysis (HRGC/HRMS) of PBDE congeners in PND 30 F1 male offspring brain on a wet-weight basis after transplacental and lactational low dose exposure to DE-71 through the dam. **(Related to Figure 1)**

Compound/Substituents	IUPAC Number	Male Offspring Brain PND 30 (ng/g ww)		
Treatment		**VEH/CON**	**0.1 mg/kg DE-71**	**0.4 mg/kg DE-71**
n		3	3	3
2,4-Dibromdiphenylether	BDE-7	<LOQ	<LOQ	NR
2,6-Dibromdiphenylether	BDE-10	<LOQ	<LOQ	<LOQ
4,4'-Dibromdiphenylether	BDE-15	<LOQ	<LOQ	<LOQ
2,2',4-Tribromdiphenylether	BDE-17	<LOQ	<LOQ	0.015±0.003
2,4,4'-Tribromdiphenylether	BDE-28	<LOQ	NR	<LOQ
2,4,6-Tribromdiphenylether	BDE-30	<LOQ	<LOQ	<LOQ
2,2',4,4'-Tetrabromdiphenylether	BDE-47	<LOQ	5.625±4.014	8.344±2.125*
2,2',4,5'-Tetrabromdiphenylether	BDE-49	<LOQ	<LOQ	<LOQ
2,3',4,4'-Tetrabromdiphenylether	BDE-66	NR	<LOQ	<LOQ
2,3',4',6-Tetrabromdiphenylether	BDE-71	<LOQ	<LOQ	<LOQ
3,3',4,4'-Tetrabromdiphenylether	BDE-77	<LOQ	<LOQ	<LOQ
2,2',3,4,4'-Pentabromdiphenylether	BDE-85	0.010±0.0003	1.014±0.644	1.238±0.087**
2,2',4,4',5-Pentabromdiphenylether	BDE-99	<LOQ	16.400±9.083	46.843.5±7.706*
2,2',4,4',6-Pentabromdiphenylether	BDE-100	<LOQ	6.007±3.292	20.065±3.30*
2,3',4,4',6-Pentabromdiphenylether	BDE-119	<LOQ	<LOQ	<LOQ
3,3',4,4',5-Pentabromdiphenylether	BDE-126	<LOQ	NR	<LOQ
2,2',3,4,4',5'-Hexabromdiphenylether	BDE-138	<LOQ	0.358±0.235	0.612±0.117*
2,2',3,4,4',6-Hexabromdiphenylether	BDE-139	NR	1.706±0.806 (*P*=.09)	4.704±0.877*
2,2',3,4,4',6'-Hexabromdiphenylether	BDE-140	<LOQ	0.391±0.327	0.541±0.132*
2,2',4,4',5,5'-Hexabromdiphenylether	BDE-153	<LOQ	15.032±4.805*	60.736±11.3*
2,2',4,4',5,6'-Hexabromdiphenylether	BDE-154	0.018±0.001	1.214±0.666	2.801±0.493*
2,3,3',4,4',5-Hexabromdiphenylether	BDE-156	<LOQ	<LOQ	<LOQ
2,2',3,3',4,4',6-Heptabromdiphenylether	BDE-171	<LOQ	<LOQ	<LOQ
2,2',3,4,4',5,5'-Heptabromdiphenylether	BDE-180	<LOQ	<LOQ	<LOQ
2,2',3,4,4',5',6-Heptabromdiphenylether	BDE-183	<LOQ	NR	0.214±0.008****
2,2',3,4,4',6,6'-Heptabromdiphenylether	BDE-184	<LOQ	0.119±0.069	0.353±0.09*
2,3,3',4,4',5',6-Heptabromdiphenylether	BDE-191	<LOQ	<LOQ	<LOQ
2,2',3,3',4,4',5,6'-Octabromdiphenylether	BDE-196	NR	0.026±0.022	<LOQ
2,2',3,3',4,4',6,6'-Octabromdiphenylether	BDE-197	NR	0.038±0.034	0.034±0.014
2,2',3,3',4,5',6,6'-Octabromdiphenylether	BDE-201	<LOQ	NR	<LOQ
2,2',3,4,4',5',5',6-Octabromdiphenylether	BDE-203	NR	0.032±0.027	<LOQ
2,2',3,4,4',5',6,6'-Octabromdiphenylether	BDE-204	<LOQ	NR	<LOQ
2,3,3',4,4',5,5',6-Octabromdiphenylether	BDE-205	<LOQ	<LOQ	<LOQ
2,2',3,3',4,4',5,5',6-Nonabromdiphenylether	BDE-206	<LOQ	<LOQ	<LOQ
2,2',3,3',4,4',5,6,6'-Nonabromdiphenylether	BDE-207	<LOQ	<LOQ	<LOQ
2,2',3,3',4,5,5',6,6'-Nonabromdiphenylether	BDE-208	<LOQ	<LOQ	<LOQ
2,2',3,3',4,4',5,5',6,6'-Decabromdiphenylether	BDE-209	<LOQ	<LOQ	<LOQ
ΣPBDEs		0.038±0.009	45.4±21.0*	147±26.1**,^

Content was measured as ng/g wet weight
Values expressed as mean ± s.e.m.

Values for all biological replicates are listed; all values were identical when <LOQ is listed
**P*<.05, ***P*<.01 compared to VEH/CON; ^*P*<.05 compared to L-DE-71 (Student's t-test with or without Welch's Correction or Mann Whitney test)
'n' indicates the number of biological replicates per group.
BDE, brominated diphenyl ether; IUPAC, International Union of Pure and Applied Chemistry; LOQ, limit of quantification; NR, not repeatable, only 1 sample yielded detectable values

Supplementary Table 4. Mass spectrometric analysis (GC/ECNI/MS) of PBDE congeners in PND 110 F1 male offspring brain on a lipid-weight basis after transplacental and lactational low dose exposure to DE-71 through the dam. **(Related to Figure 1)**

Compound/Substituents	IUPAC Number	Male Offspring Brain PND 110 (ng/g lw)		
Treatment		**VEH/CON**	**0.1 mg/kg DE-71**	**0.4 mg/kg DE-71**
n		4	4	4
2,2′,4-tri BDE	BDE 17	<MDL	<MDL	<MDL
2,3′,4-tri BDE	BDE 25	<MDL	<MDL	<MDL
2,4,4′-tri BDE, 2′,3,4-tri BDE	BDE 28, 33	<MDL	13.27, 8.66, 10.14, 18.02 (12.5 ± 2.01)**	<MDL
2,4,6,-tri BDE	BDE 30	<MDL		
2,2′,4,4′-tetra BDE	BDE 47	<MDL	29.41, 23.29, 15.04, 40.78 (27.1 ± 5.42)**	42.07, 106.31, 57.02, 47.97 (63.3 ± 14.6)*
2,2′,4,5′-tetra BDE	BDE 49	<MDL	<MDL	<MDL
2,3′,4,4′-tetra BDE	BDE 66	<MDL	<MDL	<MDL
2,3′,4′,6-tetra BDE	BDE 71	<MDL	<MDL	<MDL
2,4,4′,6-tetra BDE	BDE 75	<MDL	<MDL	<MDL
2,2′,3,4,4′-penta BDE, 2,2′,4,4′,6,6′-hexa BDE	BDE 85, 155	<MDL	<MDL	<MDL
2,2′,4,4′,5-penta BDE	BDE 99	<MDL	#55.59, <MDL, <MDL, <MDL	<MDL
2,2′,4,4′,6-penta BDE	BDE 100	<MDL	14.70, 5.96. 5.56, 67.33 (23.4 ± 14.8)	10.76, 27.20, 10.61, 13.71 (15.6 ± 3.94)*
2,3,4,5,6-penta BDE	BDE 116	<MDL	<MDL	<MDL
2,3′,4,4′,6 –penta BDE	BDE 119	<MDL	<MDL	<MDL
2,2′,3,4,4′,5′-hexa BDE	BDE 138	<MDL	<MDL	<MDL
2,2′,4,4′,5,5′-hexa BDE (mean ± S.E.M.)	BDE 153	<MDL	10.76, 11.37, 5.89, 34.14 (15.5 ± 6.32)*	129.14, 222.51, 135.25, 117.49 (151 ± 24.1)**
2,2′,4,4′,5,6′-hexa BDE	BDE 154	<MDL	<MDL	<MDL
2, 3,3′,4,4′,5-hexa BDE	BDE 156	<MDL	<MDL	<MDL
2,2′, 3,4,4′,5,6-hepta BDE	BDE 181	<MDL	<MDL	<MDL
2,2′, 3, 4,4′,5′,6-hepta BDE	BDE 183	<MDL	<MDL	<MDL
2,3,3′,4,4′,5,6-hepta BDE	BDE 190	<MDL	<MDL	<MDL
2, 3,3′,4,4′,5′,6-hepta BDE	BDE 191	<MDL	<MDL	<MDL
2,2′,3,3′,4,5,6,6′-octa BDE, 2,2′,3,4,4′,5,5′,6-octa BDE	BDE 200, 203	<MDL	<MDL	<MDL
2, 3,3′,4,4′,5,5′,6-octa BDE	BDE 205	<MDL	<MDL	<MDL
2,2′,3,3′,4,4′,5,5′,6-nona BDE	BDE 206	<MDL	<MDL	<MDL
2,2′,3,3′,4,4′,5,5′,6,6′-deca BDE	BDE 209	<MDL	<MDL	<MDL
ΣPBDEs		<MDL	78.6 ± 28.0*	230 ± 42.3**,^

Content was measured as ng/g lipid weight
Values expressed as mean ± S.E.M.
Values for all biological replicates are listed; all values were identical when <MDL is listed
*compared to VEH/CON, *P<.05, **P<.01 (Student's t-test with Welch's Correction)
^compared to L-DE-71, ^P<.05 (Student's t-test with Welch's Correction)
Group comparisons for ∑PBDEs were done using Brown-Forsythe ANOVA
'n' indicates the number of biological replicates per group. BDE-99 was detected in only one 0.1 mg/kg DE-71 biological replicate.
BDE, brominated diphenyl ether; IUPAC, International Union of Pure and Applied Chemistry; MDL, method detection limit

Supplementary Table 5. Mass spectrometric analysis (GC/ECNI/MS) of PBDE congeners in PND 110 F1 male offspring brain on a wet-weight basis after transplacental and lactational low dose exposure to DE-71 through the dam. **(Related to Figure 1)**

Compound/Substituents	IUPAC Number	Male Offspring Brain PND 110 (ng/g ww)		
Treatment		**VEH/CON**	**0.1 mg/kg DE-71**	**0.4 mg/kg DE-71**
n		4	4	4
2,2′,4-tri BDE	BDE 17	<MDL	<MDL	<MDL
2,3′,4-tri BDE	BDE 25	<MDL	<MDL	<MDL
2,4,4′-tri BDE, 2′,3,4-tri BDE	BDE 28, 33	<MDL	0.30, 0.13, 0.25, 0.17 (0.213 ± 0.038)**	<MDL
2,4,6,-tri BDE	BDE 30	<MDL	<MDL	<MDL
2,2′,4,4′-tetra BDE	BDE 47	<MDL	0.67, 0.35, 0.37, 0.38 (0.443 ± 0.076)**	0.57, 0.56, 0.56, 0.55 (0.560 ± 0.004)****
2,2′,4,5′-tetra BDE	BDE 49	<MDL	<MDL	<MDL
2,3′,4,4′-tetra BDE	BDE 66	<MDL	<MDL	<MDL
2,3′,4′,6-tetra BDE	BDE 71	<MDL	<MDL	<MDL
2,4,4′,6-tetra BDE	BDE 75	<MDL	<MDL	<MDL
2,2′,3,4,4′-penta BDE, 2,2′,4,4′,6,6′-hexa BDE	BDE 85, 155	<MDL	<MDL	<MDL
2,2′,4,4′,5-penta BDE	BDE 99	<MDL	#1.27, <MDL, <MDL, <MDL	<MDL
2,2′,4,4′,6-penta BDE	BDE 100	<MDL	0.34, 0.09, 0.14, 0.62 (0.298 ± 0.12) *P*=.06	0.15, 0.14, 0.10, 0.16 (0.138 ± 0.013)**
2,3,4,5,6-penta BDE	BDE 116	<MDL	<MDL	<MDL
2,3′,4,4′,6 –penta BDE	BDE 119	<MDL	<MDL	<MDL
2,2′,3,4,4′,5′-hexa BDE	BDE 138	<MDL	<MDL	<MDL
2,2′,4,4′,5,5′-hexa BDE (mean ± S.E.M.)	BDE 153	<MDL	0.25, 0.17, 0.14, 0.31 (0.218 ± 0.039)**	1.76, 1.16, 1.32, 1.36 (1.40 ± 0.128)***
2,2′,4,4′,5,6′-hexa BDE	BDE 154	<MDL	<MDL	<MDL
2, 3,3′,4,4′,5-hexa BDE	BDE 156	<MDL	<MDL	<MDL
2,2′, 3,4,4′,5,6-hepta BDE	BDE 181	<MDL	<MDL	<MDL
2,2′, 3, 4,4′,5′,6-hepta BDE	BDE 183	<MDL	<MDL	<MDL
2,3,3′,4,4′,5,6-hepta BDE	BDE 190	<MDL	<MDL	<MDL
2, 3,3′,4,4′,5′,6-hepta BDE	BDE 191	<MDL	<MDL	<MDL
2,2′,3,3′,4,5,6,6′-octa BDE, 2,2′,3,4,4′,5,5′,6-octa BDE	BDE 200, 203	<MDL	<MDL	<MDL
2, 3,3′,4,4′,5,5′,6-octa BDE	BDE 205	<MDL	<MDL	<MDL
2,2′,3,3′,4,4′,5,5′,6-nona BDE	BDE 206	<MDL	<MDL	<MDL
2,2′,3,3′,4,4′,5,5′,6,6′-deca BDE	BDE 209	<MDL	<MDL	<MDL
ΣPBDEs		<MDL	1.17 ± 0.205*	2.10 ± 0.134**,^

Content was measured as ng/g wet weight

Values expressed as mean ± s.e.m.

Values for all biological replicates are listed; all values were identical when <MDL is listed

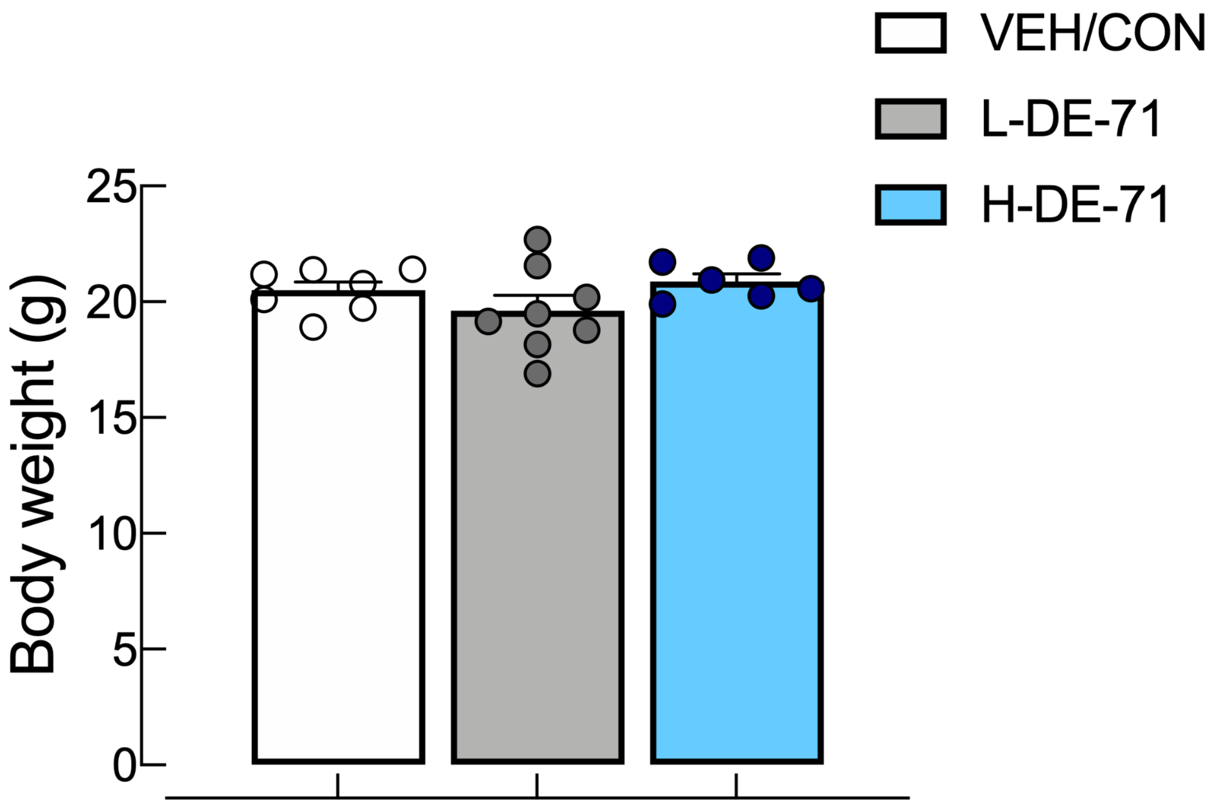
*compared to VEH/CON, *P<.05, **P<.01, ***P<.001, ****P<.0001 (Student's t-test with or without Welch's Correction or Brown-Forsythe ANOVA)

^compared to L-DE-71, ^P<.05 (Student's t-test with or without Welch's Correction)

'n' indicates the number of biological replicates per group. BDE-99 was detected in only one 0.1 mg/kg DE-71 replicate.

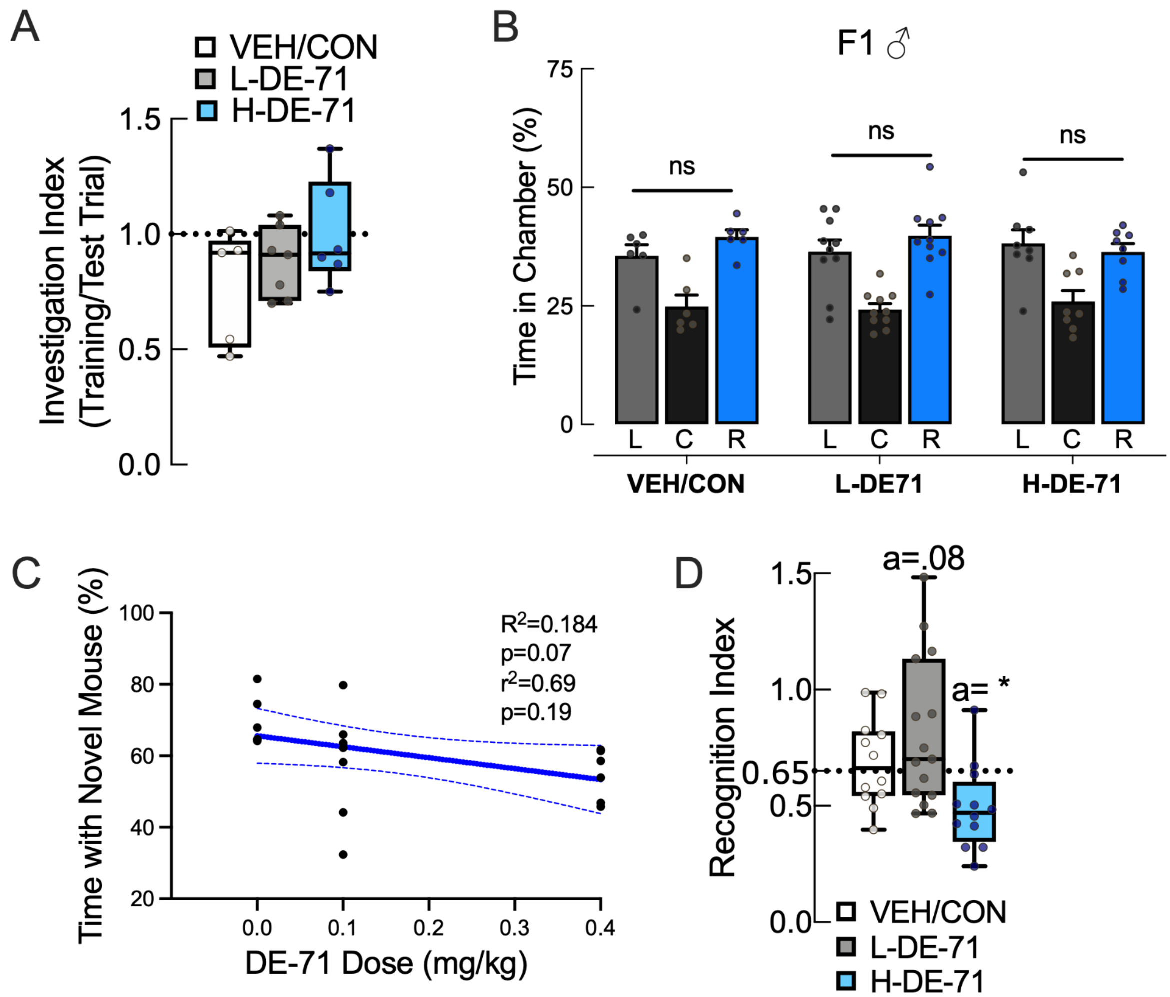
BDE, brominated diphenyl ether; IUPAC, International Union of Pure and Applied Chemistry; MDL, method detection limit

Supplementary Figure 1



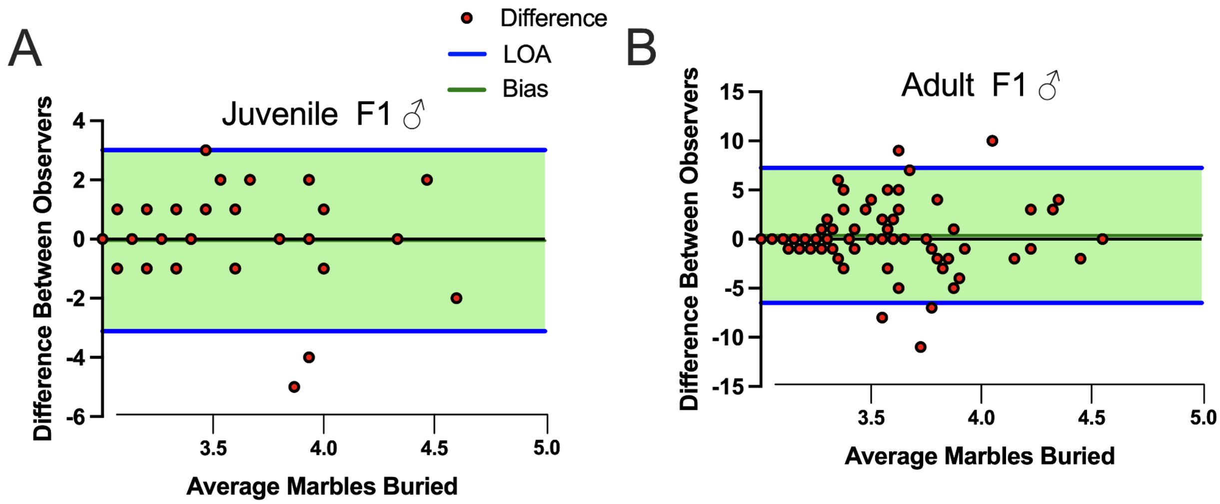
Supplementary Figure 1. Average body weights (mean +/- s.e.m) of male offspring at PND 44. *n*, 6-8 litters/group

Supplementary Figure 2



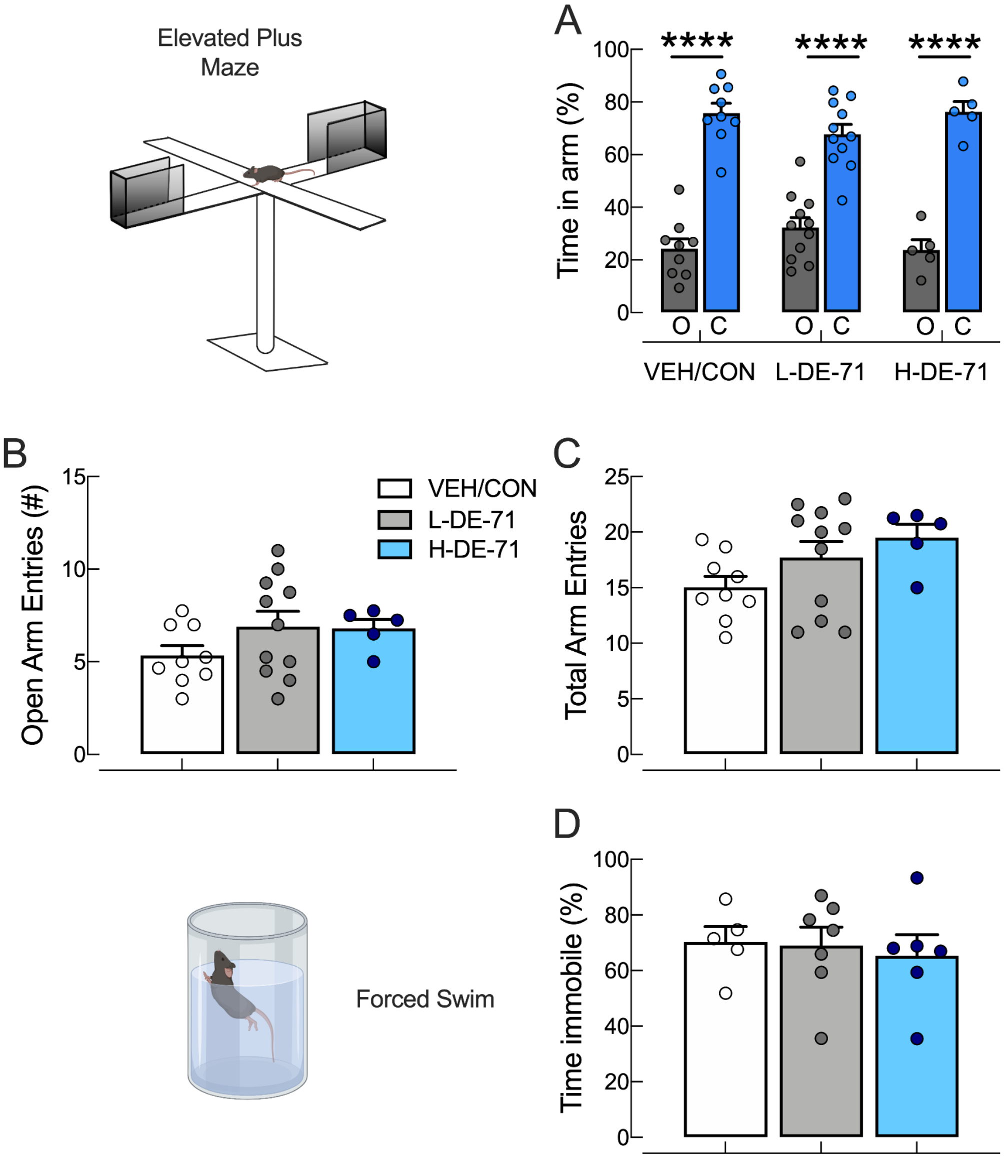
Supplementary Figure 2. Supplemental information for Figures 2 and 3. A. Investigation Index scores on SNP (when exploring both social stimuli) indicate the ratio of time spent investigating the first social stimulus on a training trial to the total investigation time during test trial. A score of near 1 shown here indicates equal investigation time on training and test trials indicating that SNP deficit in DE-71 mice is not due to lack of social engagement. **B**. Adult male mice were tested for sociability using a three-chamber apparatus. In the first habituation phase, mice were allowed to explore the middle chamber only. During the second habituation phase test mice were allowed to explore all three chambers of an empty apparatus and time spent in left, right and center chambers was recorded over 10 min. Males in all exposure groups showed similar times spent in both side chambers during the second habituation phase indicating no inherent side preference. **C**. Dose-response relationship for investigation time of novel mouse on SNP indicates an apparent dose responsivity of investigation. **D**. Mean RI scores obtained for SRM, using individual mice as the statistical unit, indicated an apparent effect of L-DE-71. [a]statistical difference vs 0.65, one-sample t-test, *$P<.05$; *n*, 5-7 litters/group (a), 5-10 litters/group (b). C, Center; L, Left; R, Right

Supplementary Figure 3



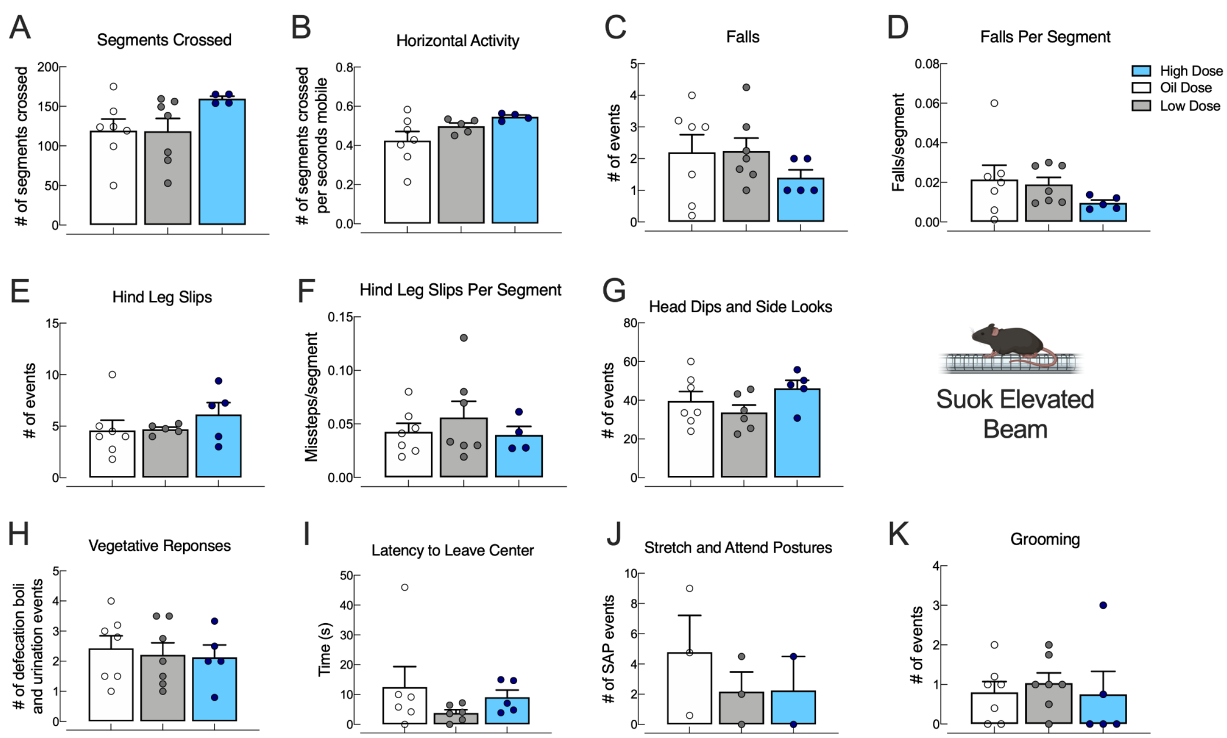
Supplementary Figure 3. Scores for marble burying and nestle shredding tests (Related to Figure 2). **a** Bland-Altman bias plot (mean+s.d.) was used to test the validity and reproducibility of marble burying scores between two independent judges blind to exposure group. Analysis revealed a very small mean of the differences between judge scores (Bias, -0.05±1.56) and a precision measured as limits of agreement (LOA), average difference ± 1.96 standard deviation of the difference, of -3.1-3.0, indicating negligible skewing by either judge. **b** Bland-Altman plot of adult marble burying (Bias, 0.37±3.51) LOA range from -6.5-7.2.

Supplementary Figure 4



Supplementary Figure 4. DE-71 exposure does not affect anxiety nor produce depressive-like behavior in exposed adult F1 male. a Time spent in open and closed arms on an elevated plus maze. All groups, regardless of exposure, spent significantly greater time in closed arm. **b, c** Open and total arm entries were similar across all groups. **d** Time spent immobile on the Forced Swim test showed no differences across groups indicating no effects of DE-71 on depressive-like behavior. *vs % time spent in closed arm, ****$P<.0001$. n, 5-11 litters/group (a, b, c); n, 5-7 litters/group (d).

Supplementary Figure 5



Supplementary Figure 5 Suok Test Parameters. Male adults were tested on SUOK platform for different sensorimotor abilities: **a, b** locomotion; **c-f** sensorimotor coordination; **g** exploratory activity; **h-j** anxiety behaviors; and **k** autogrooming. *n* 6-8 litters/group (a-i, k); 2-3 litters/group (j)

Supplementary Statistical Results

Figure 1. Maternal dosing paradigm for DE-71 produces persistent BDE congener penetration in male F1 offspring brain. (**b**) Σ_{12}PBDEs in PND 30 F1 brain. One-way ANOVA, Exposure: $F_{(2,6)}=15.0$, $P<.001$. Sidak's post hoc, VEH/CON vs H-DE-71, $P=.01$, L-DE-71 vs H-DE-71, $P=.05$. (**c**) ∑PBDE in PND 110 F1 brain. Brown-Forsythe ANOVA, Exposure: $F_{(2,5.26)}=53.2$, $P<.001$. Dunnet's T3 post-hoc, VEH/CON vs L-DE-71, $P<.01$; VEH/CON vs H-DE-71, $P<.01$; L- vs H-DE-71, $P<05$. (**e**) BDE-47. One-sided Welch's t-test, VEH/CON vs H-DE-71, $t_{(2)}=3.9$, $P<.05$; BDE-28. One-sided Welch's t-test, VEH/CON vs H-DE-71, $t_{(2)}=14.1$, $P<.01$. BDE99. One-sided Welch's t-test, VEH/CON vs H-DE-71, $t_{(2)}=6.1$, $P<.05$. BDE100. One-sided Welch's t-test, VEH/CON vs H-DE-71, $t_{(2)}=6.1$, $P<.05$. BDE138. One-sided Welch's t-test, VEH/CON vs H-DE-71, $t_{(2)}=5.1$, $P<.05$. BDE139. One-sided Welch's t-test, VEH/CON vs L-DE-71, $t_{(2)}=2.1$, $P=.09$; VEH/CON vs H-DE-71, $t_{(2)}=5.3$, $P<.05$. BDE140. One-sided Welch's t-test, VEH/CON vs H-DE-71, $t_{(2)}=4.0$, $P<.05$. BDE153. One-sided Welch's t-test, VEH/CON vs L-DE-71, $t_{(2)}=3.1$, $P<.05$; VEH/CON vs H-DE-71, $t_{(2)}=3.1$, $P<.05$. BDE153. One-sided Welch's t-test, VEH/CON vs H-DE-71, $t_{(2)}=5.3$, $P<.05$. BDE154. One-sided Welch's t-test, VEH/CON vs H-DE-71, $t_{(2)}=5.7$, $P<.05$. BDE183. One-sided t-test, VEH/CON vs H-DE-71, $t_{(4)}=18.7$, $P<.0001$. BDE184. One-sided Welch's t-test, VEH/CON vs H-DE-71, $t_{(2)}=3.8$, $P<.05$. (**f**) BDE28. One-sided t-test, VEH/CON vs L-DE-71, $t_{(6)}=4.2$, $P<.01$. BDE47. One-sided Welch's t-test, VEH/CON vs L-DE-71, $t_{(3.4)}=5.2$, $P<.01$. VEH/CON vs H-DE-71, $t_{(3.3)}=27.1$, $P<.0001$. BDE100. One-sided Welch's t-test, VEH/CON vs L-DE-71, $t_{(3.1)}=2.2$, $P=.06$. One-sided t-test, VEH/CON vs H-DE-71, $t_{(6)}=4.5$, $P<.01$. BDE153. One-sided t-test, VEH/CON vs L-DE-71, $t_{(6)}=4.3$, $P<.01$. One-sided Welch's t-test, VEH/CON vs H-DE-71, $t_{(3.1)}=10.6$, $P<.001$.

Figure 2. **Early-life exposure to DE-71 induces deficits relevant to core symptoms of ASD in juvenile and adult F1 males.** (**a**) F1 social novelty preference, Effect of stimulus. Paired t-test, VEH/CON: $t_{(5)}=6.8$, $P<.01$; L-DE-71: $t_{(6)}=1.4$, ns. H-DE-71: $t_{(5)}=1.6$, ns. (**b**) F1 Recognition Index. One-way ANOVA: $F_{(2,16)}=3.0$, $P=.07$. Holm-Sidak's post-hoc test, VEH/CON vs L-DE-71, $P=.08$. VEH/CON vs H-DE-71, $P<.05$; One-sample t-test vs hypothetical value of 0 VEH/CON, $t_{(5)}=6.8$, $P<.01$ (**c**) F1 three-chamber apparatus, Effect of stimulus (sniffing time). Paired t-test, VEH/CON: $t_{(4)}=8.2$, $P<.01$, L-DE-71: $t_{(6)}=3.1$, $P<.05$. H-DE-71: $t_{(5)}=5.8$, $P<.01$. (**d**) F1 three-chamber apparatus, Effect of stimulus (chamber time). Paired t-test, VEH/CON: $t_{(5)}=2.6$, $P<.05$, L-DE-71: $t_{(9)}=2.9$, $P<.05$. H-DE-71: $t_{(7)}=2.5$, $P<.05$. (e) Marble Burying test (juvenile). One-way ANOVA, $F_{(2,18)}=10.5$, $P<.001$. Tukey's post hoc test, VEH/CON vs L-DE-71, $P<.05$; L-DE-71 vs H-DE-71, $P<.001$. (**f**) Marble Burying test (adult). One-way ANOVA, $F_{(2,27)}=3.9$, $P<.05$. Tukey's post hoc test , VEH/CON vs L-DE-71, $P<.05$.

Figure 3. Exposure to L-DE-71 compromises long-term social recognition memory (SRM) in adult F1 male mice. (**a**) F1 Investigation Time Familiar Stimulus (Day 2 Familiar), Day 1 vs Day

2: Paired t-test: VEH/CON $t_{(4)}$=11.2, P<.001; L-DE-71 $t_{(4)}$=2.2, ns; H-DE-71 $t_{(3)}$=5.3, P<.05. (**b**) Recognition Index: Across-groups: One-way ANOVA, $F_{(2,11)}$=4.8, P<.05. Sidak's post-hoc test, VEH/CON vs H-DE-71, P=.08. One-Sample t-test vs hypothetical value of 0.65: VEH/CON, $t_{(4)}$=0.6, ns., L-DE-71, $t_{(4)}$=2.3, ns, H-DE-71, $t_{(3)}$=2.6, ns; (**c**) F1 Investigation Time Familiar Stimulus (Day 2 Novel), Day 1 vs Day 2: Paired t-test: VEH/CON $t_{(5)}$=1.1, ns; L-DE-71 $t_{(7)}$=1.8, P<.05; H-DE-71 $t_{(4)}$=1.1, ns. (**d**) Recognition Index: Across-groups: One-way ANOVA, $F_{(2,16)}$=0.14, ns. One-Sample t-test vs hypothetical value of 1.0: VEH/CON, $t_{(5)}$=1.0, ns, L-DE-71, $t_{(7)}$=1.4, ns, H-DE-71, $t_{(4)}$=1.1, ns.

Figure 4. Perinatal exposure to L-DE-71 and H-DE-71 compromised short- and long-term novel object recognition memory in adult F1 male mice. (a) F1 Investigation Time Familiar vs Novel (30 min retention): paired t-test: VEH/CON $t_{(3)}$=3.5, P<.05; L-DE-71 $t_{(4)}$=0.5, ns; H-DE-71 $t_{(4)}$=1.6, ns. (**b**) Discrimination Index: One-way ANOVA, Exposure: $F_{(2,11)}$=3.4, P=0.14, Holm-Sidak's post hoc, VEH/CON vs L-DE71, P=.07; L-DE-71 vs DE-71, ns. One-Sample t- test vs a hypothetical value of 0: VEH/CON, $t_{(3)}$=3.5, P<.05, L-DE-71, $t_{(4)}$=0.25, ns; H-DE-71, $t_{(4)}$=1.6, ns. (**c**) Distance traveled: One-way ANOVA, Exposure: $F_{(2,10)}$=2.7, P=0.11, Tukey's post hoc test VEH/CON vs L-DE-71, ns; VEH/CON vs H-DE-71, ns. (**d**) Velocity: One-way ANOVA, Exposure: $F_{(2,10)}$=2.8, P=0.11, VEH/CON vs L-DE-71, ns; VEH/CON vs H-DE-71, ns. (**e**) F1 Investigation Time Familiar vs Novel (24 h retention): paired t-test: VEH/CON $t_{(3)}$=3.6, P<.05; L-DE-71 $t_{(4)}$=2.3, P<.08; H-DE-71 $t_{(4)}$=1.1, ns. (**f**) Discrimination Index: One-way ANOVA, Exposure: $F_{(2,11)}$=2.3, P=0.15. Holm-Sidak's post hoc, VEH/CON vs L-DE-71, P=.09; VEH/CON vs H-DE-71, ns. One-Sample t-test vs a hypothetical value of 0: VEH/CON, $t_{(3)}$=3.6, P<.05, L-DE-71, $t_{(4)}$=2.3, ns, H-DE-71, $t_{(4)}$=1.1, ns. (**g**) Distance traveled: One-way ANOVA, Exposure: $F_{(2,11)}$=0.27, P=0.77. (**h**) Velocity: One-way ANOVA, Exposure: $F_{(2,11)}$=0.3, P=0.74.

Figure 5. Perinatal exposure to DE-71 does not alter general olfaction but disrupts olfactory discrimination of social odors in adult F1 male offspring. (**a**) F1 Olfactory Preference Test: RM Mixed Effects Two-way ANOVA: Exposure $F_{(2,30)}$=0.06, P=0.94; Odor treatment $F_{(2.14,64.2)}$=33.2, P<.0001; Interaction $F_{(4.3,64.2)}$=0.8, ns; Holm-Sidak's post hoc test, odor treatment: peanut butter vs water: VEH/CON P<.001, L-DE-71, P<.01, H-DE-71, P<.01; peanut butter vs butyric acid: VEH/CON P<.01, L-DE-71, P<.05, H-DE-71, P<.01; peanut butter vs vanilla: VEH/CON P<.001, L-DE-71, P<.01, H-DE-71, P<.01; Holm-Sidak's post hoc test, odor treatment: ns. (**b**) F1 Olfactory Habituation/Dishabituation Test: RM Mixed Effects Two-way ANOVA: Exposure $F_{(2,47)}$=2.1, P=0.1, Odor-trial treatment $F_{(14,646)}$=58.7, P<.0001; Interaction $F_{(28,646)}$=3.0, P<.0001; (see Table 1). Between group comparisons, Sidak's post hoc test (see Table 1).

Figure 6. Effects of early-life PBDE exposure on the open field test. (**a**) F1 Distance Traveled Arena (PND 30): RM Mixed Effects Two-way ANOVA: Exposure $F_{(2,16)}$=1.2, P=0.3; Time $F_{(5.8,92.2)}$=43.6, P<.0001; Interaction $F_{(11.5,92.2)}$=1.6, P=0.11; Holm-Sidak's post hoc test, baseline vs time bin 9, 10, 11, 12: VEH/CON, P<.01; L-DE-71, P<.05, ns, ns, P<.05; H-DE-71, P<.0001, P<01, P<.0001, P<.01. Holm-Sidak's post hoc test (exposure), VEH/CON vs L-DE-71, P<.05; L-

DE-71 vs H-DE-71, $P<.05$. (**b**) Cumulative Distance Traveled, Center vs Periphery (PND 30), Two-Way ANOVA: Zone $F_{(1,32)}=236$, $P<.0001$; Exposure, $F_{(2,32)}=1.2$, $P=0.32$; Interaction, $F_{(2,32)}=0.16$, $P=0.9$; Holm-Sidak's post hoc test (zone), VEH/CON $P<.0001$; L-DE-71, $P<.0001$; H-DE-71, $P<.0001$. (**c**) F1 Cumulative defecation (PND 30): RM Two-way ANOVA: Exposure $F_{(2,15)}=0.94$, $P=0.4$; Time $F_{(1.0,15.5)}=52.6$, $P<.0001$; Interaction $F_{(2.1,15.5)}=0.02$, $P=1.0$. Holm-Sidak's post hoc test (time): 5 vs 10 min: VEH/CON, ns, L-DE-71, ns, H-DE-71, ns; 5 vs 60 min: VEH/CON, $P<.01$; L-DE-71, $P<.01$; H-DE-71, $P<.05$; 10 vs 60 min: VEH/CON, $P<.01$; L-DE-71, $P<.01$; H-DE-71, $P<.05$. Holm-Sidak's post hoc test (exposure at 5 min): L-DE-71 vs VEH/CON, $P<.05$; H-DE-71 vs VEH/CON, ns; L-DE-71 vs H-DE-71, $P<.05$; exposure at 10 and 60 min: L-DE-71 vs VEH/CON, ns; H-DE-71 vs VEH/CON, ns. (**d**) F1 Distance Traveled Arena (PND 100): RM Mixed Effects Two-way ANOVA: Exposure $F_{(2,13)}=0.4$, $P=0.7$; Time $F_{(11,142)}=22.6$, $P<.0001$; Interaction $F_{(2,142)}=1.1$, $P=0.4$; Holm-Sidak's post hoc test (time), baseline vs time bin 9, 10, 11, 12: VEH/CON, $P=0.35$, $P=.06$, $P<.05$, $P<.05$; L-DE-71, $P<.0001$, $P<.0001$, $P<.0001$, $P<.0001$; H-DE-71, $P<.0001$, $P<0001$, $P<.0001$, $P<.0001$. Holm-Sidak's post hoc test (exposure), L-DE-71 vs VEH/CON, ns; H-DE-71 vs VEH/CON, $P<.05$ (bin 1). (**e**) Cumulative Distance Traveled, Center vs Periphery (PND 100), Two-Way ANOVA: Zone $F_{(1,13)}=1431$, $P<.0001$; Exposure, $F_{(2,13)}=0.7$, $P=0.52$; Interaction, $F_{(2,13)}=0.78$, $P=0.5$; Holm-Sidak's post hoc test (zone), VEH/CON, $P<.0001$; L-DE-71, $P<.0001$; H-DE-71, $P<.0001$. (**f**) F1 Cumulative defecation (PND 100): RM Two-way ANOVA: Exposure $F_{(2,13)}=0.83$, $P=0.46$; Time $F_{(1.1,14.0)}=23.3$, $P<.001$; Interaction $F_{(2.1,14.0)}=0.67$, $P=0.54$. Holm-Sidak's post hoc test (time): 5 vs 10 min: VEH/CON, $P<.01$; L-DE-71, ns; H-DE-71, ns; 5 vs 60 min: VEH/CON, $P<.05$; L-DE-71, ns; H-DE-71, ns; 10 vs 60 min: VEH/CON, $P<.05$; L-DE-71, ns; H-DE-71, ns. Holm-Sidak's post hoc test (exposure at 5 min): L-DE-71 vs VEH/CON, ns; H-DE-71 vs VEH/CON, ns; Exposure at 10 min: L-DE-71 vs VEH/CON, ns; H-DE-71 vs VEH/CON, $P<.05$. Exposure at 60 min: L-DE-71 vs VEH/CON, ns; H-DE-71 vs VEH/CON, ns.

Figure 7. DE-71 exposure alters mRNA expression of social neuropeptide markers in select regions of the social neural network in adult F1 male.

PVN: *Oxt*, Brown-Forsythe ANOVA, Exposure: $F_{(2,9.9)}=11.1$, $P<.01$, Dunnet's T3 post hoc test, L-DE-71 vs VEH/CON, $P<.01$; H-DE-71 vs VEH/CON, $P<.05$. *Oxtr*, Brown-Forsythe ANOVA, Exposure: $F_{(2,22)}=0.62$, ns. *Avp*, One-way ANOVA, Exposure: $F_{(2,17)}=9.6$, $P<.01$. Tukey's post hoc test: L-DE-71 vs VEH/CON, $P<.01$: H-DE-71 vs L-DE-71, $P<.01$, *Avp1ar*, One-way ANOVA, Exposure: $F_{(2,24)}=1.4$, $P=0.26$. *Adcyap1*, One-way ANOVA, Exposure: $F_{(2,16)}=0.5$. *Adcyap1r1*, One-way ANOVA, Exposure: $F_{(2,22)}=7.6$, $P<.01$, n=14-17/group. Tukey's post hoc test: H-DE-71 vs VEH/CON, $P<.01$. **SON:** *Oxt*, One-way ANOVA, Exposure: $F_{(2,18)}=0.94$, $P=0.4$. *Oxtr*, Brown-Forsythe ANOVA, Exposure: $F_{(2,18)}=0.2$, $P=0.82$. *Avp*, One-way ANOVA, Exposure: $F_{(2,30)}=0.29$, $P=0.75$. *Avp1ar*, One-way ANOVA, Exposure: $F_{(2,28)}=1.5$, $P=.23$. *Adcyap1*, One-way ANOVA, Exposure: $F_{(2,30)}=0.36$, $P=0.70$. *Adcyap1r1*, One-way ANOVA, Exposure: $F_{(2,32)}=2.8$, $P=0.07$, Sidak's post hoc test, H-DE-71 vs VEH/CON, $P<.05$. **BNST:** *Oxt*, Brown-Forsythe ANOVA, Exposure: $F_{(2,12.2)}=0.17$, $P=.84$. *Oxtr*, One-way ANOVA, Exposure: $F_{(2,22)}=0.45$, $P=.64$. *Avp*, Brown-Forsythe One-way ANOVA, Exposure: $F_{(2,13.5)}=3.8$, $P<.05$. Dunnet's T3 post hoc test, VEH/CON vs H-DE-71, $P<.05$. *Avp1ar*, One-way ANOVA, Exposure: $F_{(2,34)}=4.1$, $P<.05$. Dunnet's post hoc test, VEH/CON vs H-DE-71, $P<.05$. *Adcyap1*, Brown-Forsythe ANOVA, Exposure: $F_{(2,15.4)}=4.7$, $P<.05$, Dunnet's T3 post hoc test, VEH/CON vs H-DE-71, $P<.05$. *Adcyap1r1*, One-way ANOVA, Exposure: $F_{(2,32)}=4.8$, $P<.05$. Dunnet's post hoc test, VEH/CON vs H-DE-71, *P*<.05. **AMG:** *Oxtr*, One-way ANOVA: Exposure: $F_{(2,27)}$=1.4, *P*=.26. *Avp*, One-way ANOVA, Exposure: $F_{(2,18)}$=1.4, *P*=.27. *Avp1ar*, One-way ANOVA, Exposure: $F_{(2,20)}$=5.3, *P*<.05. Sidak's post hoc test H-DE-71 vs VEH/CON, *P*<.01. *Adcyap1*, One-way ANOVA, Exposure: $F_{(2,24)}$=1.0, *P*=.38. *Adcyap1r1*, One-way ANOVA, Exposure: $F_{(2,27)}$=.72, *P*=.5. **LS:** *Oxtr*, One-way ANOVA, Exposure: $F_{(2,30)}$=.28, p=.76. *Avp*, Brown-Forsythe One-way ANOVA, Exposure: $F_{(2,14.1)}$=1.6, *P*=.24, Dunnet's T3 post hoc test, L-DE-71 vs VEH/CON, *P*<.05; H-DE-71 vs VEH/CON, *P*=.9. *Avp1ar*, One-way ANOVA, Exposure: $F_{(2,27)}$=0.5, *P*=.61. *Adcyap1*, One-way ANOVA: Exposure: $F_{(2,27)}$=.68, *P*=.51. *Adcyap1r1*, One-way ANOVA, Exposure: $F_{(2,33)}$=1.2, *P*=.32.

Figure 8. Perinatal exposure to DE-71 produced dose-specific responses in plasma OXT levels of adult F1 male. (**a**) Plasma Arg8-vasopressin F1 male: One-way ANOVA, Exposure: $F_{(2,24)}$=.63, *P*<.54. (**b**) Plasma Oxytocin F1 male: Brown-Forsythe ANOVA, Exposure: $F_{(2,68.6)}$=12, *P*<.0001, Dunnet's T3 post hoc test: L-DE-71 vs VEH/CON, *P*<.05; H-DE-71 vs VEH/CON *P*<.01; L-DE-71 vs H-DE-71, *P*<.0001.

Supplementary Figure 1. F1 male body weight: One-way ANOVA, Exposure: $F_{(2,18)}$=1.7, *P*=.22.

Supplementary Figure 2. Social novelty preference test (SNP) and time in chamber on the three-chamber sociability test (SOC) in F1 male. (**a**) F1 SNP Investigation Index. One-way ANOVA: $F_{(2,15)}$=1.6, *P*=.23. (**b**) F1 Phase 2 Chamber Preference on SOC, Paired t-test: VEH/CON $t_{(5)}$=1.3, *P*=0.13; L-DE-71 $t_{(9)}$=0.74, *P*=.24; H-DE-71 $t_{(7)}$=0.41, *P*=0.35.

Supplementary Figure 3. Scores for marble burying and nestle shredding tests (Related to Figure 2). Analysis revealed a very small mean of the differences between judge scores (Bias, -0.05±1.56) and a precision measured as limits of agreement (LOA), average difference ± 1.96 standard deviation of the difference, of -3.1-3.0, indicating negligible skewing by either judge. **b** Bland-Altman plot of adult marble burying (Bias, 0.37±3.51) LOA range from -6.5-7.2.

Supplementary Figure 4. DE-71 exposure does not affect anxiety nor produce depressive-like behavior in exposed adult F1 male. (a) Time spent in open and closed arms. Two-way ANOVA, Exposure: $F_{(2,44)}=2 \times 10^{-21}$, *P*>.99; Stimulus: $F_{(1,44)}$=183.5, *P*<.0001; Interaction: $F_{(2,44)}$=3.2, *P<.05*. Holm-Sidak's post hoc test (Open vs Closed): VEH/CON, P <.0001, L-DE-71, *P*<.0001 ; H-DE-71, *P*<.0001. (**b**) Open arm entries on EPM: Brown-Forsythe ANOVA Exposure: $F_{(2,20)}$=2.1, *P*=.15. (**c**) total arm entries on EPM: ANOVA Exposure: $F_{(2,22)}$=2.4, *P*=.11. **d** Time spent immobile on Forced Swim test, One-way ANOVA Exposure: $F_{(2,15)}$=.14, *P*=.87.

Supplementary Figure 5. Suok Test Parameters. (**a**) F1 Segments Crossed: One-way ANOVA, Exposure: $F_{(2,15)}$=2.0, *P*=.18; (b) F1 Horizontal Activity: Brown-Forsythe ANOVA, Exposure: $F_{(2,7.6)}$=4.2, *P*=.06; (**c**) F1 Falls: One-way ANOVA, Exposure: $F_{(2,16)}$=.94, *P*=.41. (**d**) F1 Falls per Segments Crossed: Brown-Forsythe ANOVA, Exposure: $F_{(2,9.2)}$=1.5, *P*=.23. (**e**) F1 Hind Leg

Slips: One-way ANOVA, Exposure $F_{(2,14)}$=.81, *P*=.47. (**f**) F1 Hind Leg slips per segments crossed: One-way ANOVA, Exposure $F_{(2,15)}$=.54, *P*=.6. (**g**) F1 Head Dips and Side Looks: One-way ANOVA, Exposure $F_{(2,15)}$=1.8, *P*=.20. (**h**) F1 Vegetative Responses: One-way ANOVA, Exposure: $F_{(2,16)}$=0.14, *P*=.87. (**i**) F1 Latency to Leave Center: Brown-Forsythe ANOVA, Exposure $F_{(2,6.4)}$=1.1, *P*=0.38. (**j**) F1 Stretch and Attend Postures (risk assessment): One-way ANOVA, Exposure: $F_{(2,5)}$=.56, *P*=.6. (**k**) F1 Autogrooming: One-way ANOVA, Exposure: $F_{(2,16)}$=.19, *P*=.83.