

SUPPLEMENTAL MATERIALS

Developmental exposure to a mixture of 23 chemicals associated with unconventional oil and gas operations alters the immune system of C57Bl/6 mice

Lisbeth A. Boule, Timothy J. Chapman, Sara E. Hillman, Christopher D. Kassotis, Colleen O'Dell, Jacques Robert, Steve N. Georas, Susan C. Nagel, and B. Paige Lawrence

	Control	0.1 µg/mL	p-value^a	1 µg/mL	p-value^a
Time to Parturition	19.0 ± 0.00	18.9 ± 0.18	0.89	19.0 ± 0.00	1.0
Dam Weight GD0	18.3 ± 0.24	18.6 ± 0.48	0.82	18.4 ± 0.32	0.96
Dam Weight GD7	20.8 ± 0.35	21.5 ± 0.47	0.39	21.3 ± 0.32	0.68
Dam Weight GD14	27.6 ± 0.35	29.2 ± 0.69	0.14	29.2 ± 0.42	0.18
Dam Weight PND2	25.1 ± 0.42	26.0 ± 0.48	0.28	26.3 ± 0.29	0.17

Supplemental Materials, Table S1. Mean time to parturition and dam body weight before, during, and 2 days after giving birth. Time to parturition indicates the number of days between gestational day (GD) 0 and birth of pups. Dams were weighed on GD0, 7, 14, and 2 days after parturition (PND2). Data represent the means from 10 dams in each treatment group. ^aCompared to dams drinking control water.

Cell type	Female 0.1µg/mL vs. Control			Male 0.1µg/mL vs. Control			Female 1 µg/mL vs. Control		Male 1 µg/mL vs. Control		Female vs. Male Control	Female vs. Male 0.1 µg/mL	Female vs. Male 1µg/mL
	Control	0.1µg/mL	p-value ^a	Control	0.1µg/mL	p-value ^a	1 µg/mL	p-value ^a	1 µg/mL	p-value ^a	p-value ^b	p-value ^b	p-value ^b
MLN Cells													
Cell Number	7708333 ± 1097424	8505000 ± 1137502	0.87	5128500 ± 518984	4698750 ± 430391	0.85	7533150 ± 1040419	0.99	5384500 ± 972929	0.95	0.36	0.01*	0.15
CD4 ⁺ T cells	1450263 ± 168394	128895 ± 179973	0.78	637990 ± 90913	534007 ± 38120	0.64	1281035 ± 150390	0.76	667462 ± 107956	0.97	0.001*	0.001*	0.005*
Th2 cells	22181 ± 3585	27799 ± 3679	0.49	7975 ± 1008	9084 ± 469	0.76	20802 ± 2920	0.96	8137 ± 1669	0.99	0.003*	<0.0001*	0.002*
Th17 cells	25316 ± 3239	56982 ± 9006	0.008*	12343 ± 4758	11248 ± 2316	0.71	37912 ± 7257	0.39	15922 ± 4838	0.81	0.001*	0.01*	0.52
Tregs	117598 ± 12010	88245 ± 12673	0.26	60761 ± 11006	46341 ± 5405	0.51	97642 ± 13330	0.53	62711 ± 10343	0.99	0.01*	0.01*	0.06
BAL cells													
Macrophages	44808 ± 8681	179845 ± 32636	0.002*	92538 ± 18232	121104 ± 25160	0.61	126768 ± 23430	0.047*	112601 ± 18138	0.79	0.69	0.17	0.63
Eosinophils	174123 ± 63817	476702 ± 93240	0.02*	421327 ± 102220	662437 ± 150330	0.32	305157 ± 45367	0.41	538508 ± 72394	0.77	0.52	0.32	0.01*
Neutrophils	18141 ± 7747	83090 ± 34974	0.12	31046 ± 9986	35931 ± 9277	0.94	49694 ± 10276	0.59	40424 ± 13096	0.82	0.99	0.19	0.58
Lymphocytes	40426 ± 13250	95771 ± 17938	0.04*	48126 ± 12505	71727 ± 24996	0.64	54381 ± 11835	0.78	56912 ± 12799	0.94	0.99	0.45	0.88

Supplemental Materials, Table S2. The number of immune cell populations in male and female offspring after HDM sensitization and challenge.

At maturity (6-8 weeks of age), 9-10 female and 9-10 male offspring from each developmental exposure group were sensitized and challenged with house dust mite (HDM). Within each group, offspring of the same sex were from a different treated dam. Flow cytometry was used to identify CD4⁺ T cells in the MLN, based on the following parameters: as CD4⁺ T cells (CD3⁺CD4⁺); Th2 cells (GATA3⁺CD4⁺); Th17 cells (RORγt⁺CD4⁺); Tregs (Foxp3⁺CD25⁺CD4⁺). Differential cell counting was used to enumerate eosinophils, macrophages, lymphocytes, and neutrophils in the BAL. The mean number of cells ± SEM is shown. ^aMixture-exposed group compared to control group within the indicated sex. ^bFemale versus male offspring of dams given the indicated concentration of the mixture. *Demarcates $p \leq 0.05$.

Cell type	Female 0.1 µg/mL vs. Control			Male 0.1 µg/mL vs. Control			Female 1 µg/mL vs. Control		Male 1 µg/mL vs. Control		Female vs. Male Control	Female vs. Male 0.1 µg/mL	Female vs. Male 1µg/mL
	Control	0.1µg/mL	p-value ^a	Control	0.1µg/mL	p-value ^a	1 µg/mL	p-value ^a	1 µg/mL	p-value ^a	p-value ^b	p-value ^b	p-value ^b
MLN Cells													
CD4 ⁺ T cells	19.3 ± 1.04	15 ± 0.56	0.02*	12 ± 0.91	12 ± 0.62	0.80	18 ± 1.2	0.39	13 ± 0.61	0.92	<0.0001*	0.048*	0.004*
Th2 cells	1.5 ± 0.09	2.2 ± 0.15	0.0003*	1.4 ± 0.21	1.8 ± 0.20	0.20	1.6 ± 0.09	0.82	1.2 ± 0.09	0.77	0.99	0.37	0.45
Th17 cells	2.01 ± 0.234	3.7 ± 0.35	0.001*	1.7 ± 0.32	2.4 ± 0.66	0.75	2.99 ± 0.277	0.04*	2.65 ± 1.04	0.61	0.40	1.0	0.98
Tregs	8.3 ± 0.29	6.8 ± 0.19	0.005*	9.3 ± 0.44	8.5 ± 0.73	0.46	7.6 ± 0.37	0.24	9.3 ± 0.22	1.0	0.50	0.08	0.06
Treg:Th2	5.8 ± 0.43	3.2 ± 0.21	<0.0001*	8.8 ± 1.5	5.4 ± 0.75	0.09	4.9 ± 0.35	0.003*	8.4 ± 0.88	0.98	0.13	0.42	0.049*
Treg:Th17	5.6 ± 0.51	2.9 ± 0.25	0.0001*	6.7 ± 0.80	6.05 ± 1.22	0.89	3.5 ± 0.35	0.001*	6.19 ± 1.02	0.93	0.01*	0.13	0.01*
BAL cells													
Macrophages	0.24 ± 0.05	0.24 ± 0.04	0.99	0.26 ± 0.07	0.17 ± 0.03	0.63	0.23 ± 0.02	0.98	0.23 ± 0.07	0.94	1.00	0.22	0.93
Eosinophils	0.51 ± 0.06	0.55 ± 0.04	0.78	0.62 ± 0.07	0.696 ± 0.15	0.67	0.58 ± 0.02	0.43	0.66 ± 0.07	0.92	0.72	0.03*	0.35
Neutrophils	0.06 ± 0.01	0.08 ± 0.02	0.40	0.04 ± 0.01	0.06 ± 0.01	0.81	0.09 ± 0.01	0.30	0.05 ± 0.02	0.99	0.99	0.42	0.01*
Lymphocytes	0.13 ± 0.02	0.12 ± 0.02	0.95	0.08 ± 0.01	0.07 ± 0.01	0.90	0.09 ± 0.01	0.27	0.07 ± 0.01	0.77	0.09	0.01*	0.10

Supplemental Materials, Table S3. The percentage of immune cell populations in male and female offspring after HDM sensitization and challenge. At maturity (6-8 weeks of age), 9-10 female and 9-10 male offspring from each developmental exposure group were sensitized and challenged with house dust mite (HDM). Within each group, offspring of the same sex were from a different treated dam. Flow cytometry was used to identify CD4⁺ T cells in the MLN, based on the following parameters: as CD4⁺ T cells (CD3⁺CD4⁺); Th2 cells (GATA3⁺CD4⁺); Th17 cells (RORγt⁺CD4⁺); Tregs (Foxp3⁺CD25⁺CD4⁺). Differential cell counting was used to enumerate eosinophils, macrophages, lymphocytes, and neutrophils in the BAL. The mean percentage of the indicated cell type ± SEM is shown. ^aMixture-exposed group compared to control group within the indicated sex. ^bFemale versus male offspring of dams given the indicated concentration of the mixture. *Demarcates p ≤ 0.05.

Cell type	Female 0.1µg/mL vs. Control			Male 0.1µg/mL vs. Control			Female 1µg/mL vs. Control		Male 1µg/mL vs. Control		Female vs. Male Control	Female vs. Male 0.1 µg/mL	Female vs. Male 1µg/mL
	Control	0.1µg/mL	P-value ^a	Control	0.1µg/mL	P-value ^a	1µg/mL	P-value ^a	1µg/mL	P-value ^a	p-value ^b	p-value ^b	p-value ^b
Cell Number	4897563 ± 944506	4830563 ± 1421214	0.99	5818714 ± 1289135	5909625 ± 687895	0.99	3858214 ± 1020211	0.81	4029750 ± 525796	0.33	0.99	0.51	0.69
CD4 ⁺ T cells	749418 ± 134060	736576 ± 226789	0.99	712501 ± 150731	850933 ± 79669	0.64	559500 ± 118212	0.72	539570 ± 85843	0.50	1.00	0.64	0.88
Th1 cells	43093.3 ± 12678	34762 ± 10766	0.84	61928.9 ± 15790	69719 ± 11045	0.87	25216.2 ± 5612	0.47	41727 ± 3981	0.41	0.82	0.04*	0.03*
Th2 cells	335.9 ± 101.9	430.9 ± 120.9	0.77	3267.1 ± 899	4587 ± 519	0.31	183.7 ± 54.8	0.55	2915 ± 384	0.92	0.001*	<0.0001*	<0.0001*
Th17 cells	12406.2 ± 3226.3	14386.1 ± 4379.7	0.91	4813 ± 982	5059.4 ± 786	0.98	8871 ± 1786	0.75	4667 ± 1215	0.99	0.31	0.05	0.12
Tregs	79722.7 ± 15778	72622.8 ± 20524	0.96	99543 ± 20971	103334 ± 12872	0.98	73906.2 ± 17487	0.97	79909 ± 11096	0.64	0.96	0.23	0.60
Tfh cells	2994.6 ± 923.7	5248.8 ± 3062	0.73	28858 ± 8971	31237 ± 7782	0.97	3739.8 ± 1654.2	0.97	17829 ± 2785	0.51	0.01*	0.01*	0.001*
CD8 ⁺ T cells	181834 ± 29673	216851 ± 74308	0.88	572043 ± 118587	666815 ± 61383	0.71	156679 ± 35859	0.94	431571 ± 68438	0.48	0.004*	0.0004*	0.002*
CTL	7429.8 ± 1693.8	8468.3 ± 3161.1	0.98	76117 ± 22812	57961 ± 11293	0.64	12901.9 ± 5512.5	0.55	34918 ± 4610	0.13	0.0004*	0.001*	0.02*
NP specific CD8 ⁺ T cells	3057 ± 543	3329 ± 757	0.98	11143 ± 3012	9303 ± 1713	0.79	4341 ± 1574	0.65	6553 ± 960	0.19	0.01*	0.01*	0.20

Supplemental Materials, Table S4. The number of T cell sub-populations in male and female offspring after infection. At maturity (6-8 weeks of age), 9-10 female and 9-10 male offspring from each developmental exposure group were infected (i.n.) with influenza A virus (HKx31; H3N2). Within each group, offspring of the same sex were from a different treated dam. Flow cytometry was used to identify T cells in the MLN, based on the following parameters: CD8⁺ T cells (CD3⁺CD8⁺); CTL (CD44^{hi}CD62L^{lo}CD8⁺); NP-specific CD8⁺ T cells (D^bNP₃₆₆₋₃₇₄⁺CD8⁺); CD4⁺ T cells (CD3⁺CD4⁺); Th2 cells (GATA3⁺CD4⁺); Th17 cells (RORγt⁺CD4⁺); Tfh (CXCR5⁺PD-1⁺ CD4⁺); Tregs (Foxp3⁺CD25⁺CD4⁺). The mean number ± SEM are shown. ^aMixture-exposed group compared to control group within the indicated sex. ^bFemale versus male offspring from dams given the indicated concentration of the mixture. *Demarcates p ≤ 0.05.

Cell type	Female 0.1 µg/mL vs. Control			Male 0.1 µg/mL vs. Control			Female 1 µg/mL vs. Control		Male 1 µg/mL vs. Control		Female vs. Male Control	Female vs. Male 0.1 µg/mL	Female vs. Male 1µg/mL
	Control	0.1µg/mL	p-value ^a	Control	0.1µg/mL	p-value ^a	1µg/mL	p-value ^a	1µg/mL	p-value ^a	p-value ^b	p-value ^b	p-value ^b
CD4 ⁺ T cells	15.5 ± 1.04	14.8 ± 1.18	0.89	12.8 ± 0.850	15 ± 1.03	0.18	16 ± 1.1	0.93	13.1 ± 0.538	0.97	0.43	1.0	0.30
Th1 cells	0.82 ± 0.11	0.67 ± 0.14	0.66	1.04 ± 0.101	1.1 ± 0.11	0.80	0.66 ± 0.11	0.65	1.12 ± 0.133	0.89	0.81	0.07	0.12
Th2 cells	0.006 ± 0.001	0.008 ± 0.002	0.58	0.056 ± 0.005	0.079 ± 0.006	0.004*	0.005 ± 0.001	0.89	0.073 ± 0.003	0.04 *	<0.0001*	<0.0001*	<0.0001*
Th17 cells	0.303 ± 0.111	0.343 ± 0.123	0.96	0.11 ± 0.03	0.09 ± 0.02	0.88	0.31 ± 0.08	0.99	0.11 ± 0.02	0.99	0.53	0.22	0.48
Tregs	1.6 ± 0.18	1.5 ± 0.14	0.91	1.8 ± 0.11	1.8 ± 0.13	0.99	2.1 ± 0.19	0.19	2.05 ± 0.234	0.58	0.98	0.90	1.0
Tfh cells	0.06 ± 0.02	0.07 ± 0.02	0.77	0.44 ± 0.08	0.48 ± 0.08	0.93	0.07 ± 0.02	0.89	0.45 ± 0.06	0.99	<0.0001*	<0.0001*	0.0001*
CD8 ⁺ T cells	3.9 ± 0.41	4.1 ± 0.45	0.90	10.4 ± 0.835	11.7 ± 0.635	0.40	4.4 ± 0.26	0.67	10.5 ± 0.498	0.99	<0.0001*	<0.0001*	<0.0001*
CTL	0.15 ± 0.01	0.15 ± 0.02	0.99	1.2 ± 0.25	0.94 ± 0.13	0.56	0.26 ± 0.07	0.10	0.995 ± 0.212	0.69	<0.0001*	0.003*	0.01*
NP specific CD8 ⁺ T cells	0.065 ± 0.004	0.08 ± 0.01	0.44	0.22 ± 0.04	0.16 ± 0.02	0.29	0.11 ± 0.01	0.005*	0.16 ± 0.01	0.35	<0.0001*	0.08	0.53

Supplemental Materials, Table S5. The percentage of T cell sub-populations in male and female offspring after infection. At maturity (6-8 weeks of age), 9-10 female and 9-10 male offspring from each developmental exposure group were infected (i.n.) with influenza A virus (HKx31; H3N2). Within each group, offspring of the same sex were from a different treated dam. Flow cytometry was used to identify the indicated T cell types in the MLN, based on the following parameters: CD8⁺ T cells (CD3⁺CD8⁺); CTL (CD44^{hi}CD62L^{lo}CD8⁺); NP-specific CD8⁺ T cells (D^bNP₃₆₆₋₃₇₄⁺CD8⁺); CD4⁺ T cells (CD3⁺CD4⁺); Th2 cells (GATA3⁺CD4⁺); Th17 cells (RORγt⁺CD4⁺); Tfh (CXCR5⁺PD-1⁺CD4⁺); Tregs (Foxp3⁺CD25⁺CD4⁺). The mean percentage ± SEM is shown. ^aMixture-exposed group compared to control group within the indicated sex. ^bFemale versus male offspring from dams given the indicated concentration of the mixture. *Demarcates p ≤ 0.05.

Cell type	Female 0.1µg/mL vs. Control			Male 0.1µg/mL vs. Control			Female 1µg/mL vs. Control		Male 1µg/mL vs. Control		Female vs. Male Control	Female vs. Male 0.1 µg/mL	Female vs. Male 1µg/mL
	Control	0.1µg/mL	p-value ^a	Control	0.1µg/mL	p-value ^a	p-value ^b	p-value ^a	1µg/mL	p-value ^a	p-value ^b	p-value ^b	p-value ^b
DAY 7													
MLN cells	9978571 ± 1246035	13037500 ± 1031675	0.21	11778571 ± 1486431	13103125 ± 1663959	0.74	13396875 ± 1605984	0.15	11818750 ± 1030622	0.99	0.95	0.92	0.41
CD4 ⁺ T cells	1525836 ± 219598	1658047 ± 188466	0.91	1803307 ± 183874	2005097 ± 305007	0.84	1970091 ± 257145	0.37	1748992 ± 222254	0.99	0.97	0.90	0.98
Th1 cells	90810 ± 30735	162232 ± 34164	0.22	50130 ± 13281	37254 ± 5272	0.58	94160 ± 19381	0.99	41334 ± 7628	0.77	0.79	0.01*	0.52
Th2 cells	1673 ± 337	1313 ± 185	0.81	1770 ± 224	2815 ± 974	0.48	1997 ± 566	0.84	1279.9 ± 224.2	0.19	1.00	0.27	0.91
Th17 cells	69154 ± 5892	72420 ± 15035	0.99	74398 ± 6687	80427 ± 8591	0.88	116230 ± 22938	0.15	78659 ± 10310	0.94	0.99	1.0	0.36
Tregs	130587 ± 16380	159616 ± 19831	0.68	160451 ± 15644	213183 ± 31461	0.28	200500 ± 31538	0.13	163910 ± 17532	0.99	0.96	0.83	0.88
DAY 21													
MLN cells	13103571 ± 1545669	12406594 ± 1380055	0.96	11545625 ± 2873425	10905625 ± 2157050	0.98	14875313 ± 2150489	0.76	14059375 ± 2080860	0.74	0.86	0.99	0.99
CD4 ⁺ T cells	2135186 ± 280250	1729275 ± 192064	0.58	1688515 ± 353411	1567659 ± 287275	0.96	2364883 ± 1615598	0.84	2050241 ± 283712	0.69	0.48	0.99	0.81
Th1 cells	67862 ± 16381	94961 ± 24133	0.57	19248 ± 4454	20392 ± 5197	0.99	65723 ± 12496	0.99	28353 ± 4974	0.40	0.05	0.95	0.89
Th2 cells	2177 ± 349	1234 ± 153	0.09	415 ± 115	375 ± 94	0.97	1882 ± 365	0.77	611 ± 144	0.49	0.0003*	0.52	0.13
Th17 cells	123129 ± 21883	91393 ± 11430	0.51	55322 ± 17380	53921 ± 13712	0.99	141889 ± 24496	0.79	85498 ± 15768	0.38	0.02*	0.60	0.21
Tregs	233309 ± 36518	193893 ± 25318	0.73	231943 ± 53240	217953 ± 42181	0.97	270879 ± 44271	0.75	283672 ± 40044	0.71	0.74	1.0	0.97
DAY 42													
MLN cells	16756250 ± 3019859	15902778 ± 2171435	0.96	9770000 ± 1710798	23322222 ± 3489954	0.002*	18863889 ± 1647874	0.80	15780500 ± 2086808	0.21	0.34	0.27	0.94
CD4 ⁺ T cells	3006056 ± 546306	2827342 ± 410654	0.95	1710725 ± 277970	3362214 ± 484674	0.01*	3462128 ± 279403	0.73	2379627 ± 282119	0.37	0.19	0.92	0.34
Th1 cells	112823 ± 28704	92899 ± 16695	0.77	71941 ± 14380	186874 ± 34465	0.02*	108376 ± 14302	0.99	117446 ± 30449	0.46	0.85	0.10	0.99
Th2 cells	1918 ± 388	1198 ± 186	0.25	890 ± 185	1595 ± 276	0.05	2118 ± 326	0.89	1020 ± 133	0.89	0.07	0.88	0.03*
Th17 cells	172501 ± 26431	174585 ± 30157	0.99	37338 ± 9751	81499 ± 10623	0.01*	183473 ± 19926	0.95	64574 ± 10246	0.15	0.0001*	0.01*	0.001*
Tregs	368823 ± 59889	365668 ± 47395	0.99	228291 ± 37780	442805 ± 60917	0.01*	457275 ± 36033	0.41	329128 ± 40734	0.28	0.32	0.86	0.38

Supplemental Materials, Table S6. The number of CD4⁺ T cell subsets in developmentally exposed mice after MOG peptide administration.

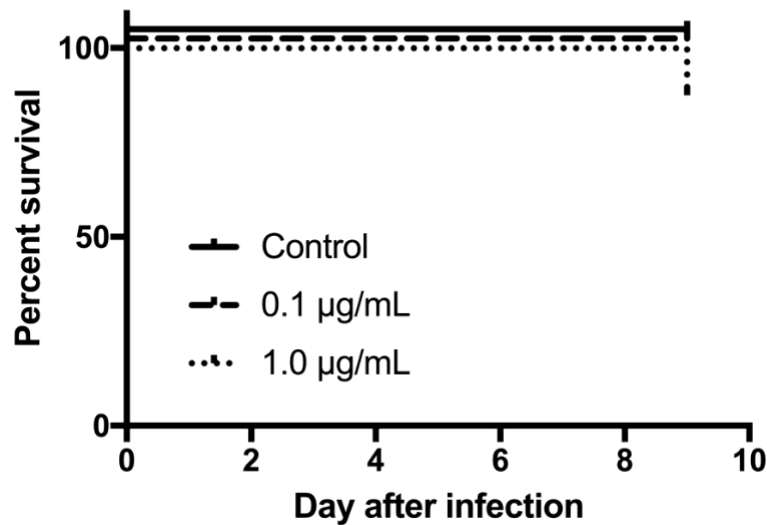
At maturity (6-8 weeks of age), EAE was induced in 8-10 female and 8-10 male offspring from each developmental exposure group. Within each group, offspring of the same sex were from a different treated dam. Mice were sacrificed 7, 21 or 42 days after MOG peptide administration, and flow cytometry was used to identify CD4⁺ T cells in the cervical lymph nodes, based on the following parameters: CD4⁺ T cells (CD3⁺CD4⁺); Th1 cells (Tbet⁺CD4⁺); Th2 cells (GATA3⁺CD4⁺); Th17 cells (RORγt⁺CD4⁺); Treg (Foxp3⁺CD25⁺CD4⁺). The mean percentage ± SEM is shown.

^aMixture-exposed group compared to control group within the indicated sex. ^bFemale versus male offspring from dams given the indicated concentration of the mixture. *Demarcates $p \leq 0.05$.

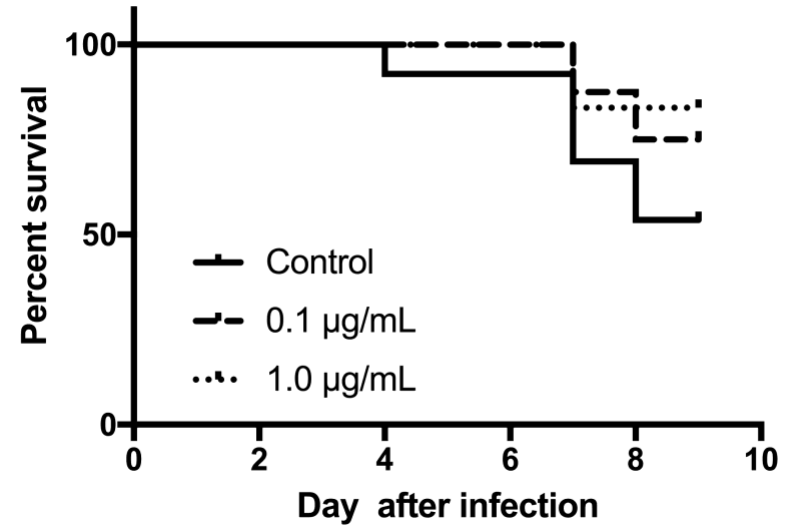
	Female 0.1µg/mL vs. Control			Male 0.1µg/mL vs. Control			Female 1µg/mL vs. Control		Male 1µg/mL vs. Control		Female vs. Male Control	Female vs. Male 0.1 µg/mL	Female vs. Male 1µg/mL
Cell type	Control	0.1µg/mL	P-value ^a	Control	0.1µg/mL	P-value ^a	1µg/mL	P-value ^a	1µg/mL	P-value ^a	p-value ^b	p-value ^b	p-value ^b
DAY 7													
CD4 ⁺ T cells	15 ± 0.67	13 ± 0.49	0.03*	16 ± 1.7	15 ± 0.69	0.87	15 ± 0.71	0.93	15 ± 1.2	0.76	0.98	0.57	1.0
Th1 cells	0.86 ± 0.25	1.3 ± 0.28	0.47	0.42 ± 0.09	0.29 ± 0.03	0.24	0.698 ± 0.142	0.87	0.33 ± 0.03	0.52	0.33	0.01*	0.68
Th2 cells	0.017 ± 0.002	0.010 ± 0.001	0.08	0.016 ± 0.002	0.018 ± 0.004	0.82	0.015 ± 0.003	0.77	0.011 ± 0.001	0.49	0.99	0.23	0.84
Th17 cells	0.75 ± 0.06	0.54 ± 0.09	0.21	0.67 ± 0.07	0.63 ± 0.04	0.89	0.86 ± 0.09	0.61	0.67 ± 0.06	0.99	0.99	0.99	0.47
Tregs	1.3 ± 0.04	1.2 ± 0.09	0.77	1.5 ± 0.17	1.6 ± 0.07	0.68	1.5 ± 0.11	0.34	1.4 ± 1.1	0.97	0.95	0.65	0.99
Treg:Th1	2.9 ± 0.78	1.2 ± 0.18	0.15	4.3 ± 0.79	6.1 ± 0.84	0.20	2.9 ± 0.67	0.99	4.5 ± 0.53	0.97	0.65	0.01*	0.72
Treg:Th2	84 ± 10	126 ± 12	0.34	95 ± 9.2	116 ± 19	0.66	133 ± 33	0.24	143 ± 18	0.13	0.99	0.97	0.99
Treg:Th17	1.8 ± 0.14	2.6 ± 0.32	0.05	2.2 ± 0.19	2.6 ± 0.19	0.41	1.8 ± 0.15	0.99	2.2 ± 0.29	0.98	0.94	0.99	0.70
DAY 21													
CD4 ⁺ T cells	16 ± 0.57	14 ± 0.65	0.15	16 ± 1.2	15 ± 0.69	0.56	16 ± 0.62	0.99	15 ± 0.71	0.62	0.94	0.82	0.26
Th1 cells	4.1 ± 1.1	5.8 ± 1.7	0.57	1.1 ± 0.14	1.2 ± 0.13	0.96	3.2 ± 0.73	0.88	1.5 ± 0.39	0.50	0.50	0.69	0.98
Th2 cells	0.11 ± 0.01	0.07 ± 0.01	0.04*	0.027 ± 0.005	0.022 ± 0.003	0.68	0.08 ± 0.01	0.24	0.029 ± 0.004	0.87	<0.0001*	0.03*	0.04*
Th17 cells	5.7 ± 0.38	5.3 ± 0.46	0.79	2.9 ± 0.41	3.4 ± 0.38	0.61	6.04 ± 0.378	0.82	4.1 ± 0.32	0.09	<0.0001*	<0.0001*	0.0001*
Tregs	10.5 ± 0.608	11 ± 0.56	0.76	13 ± 0.47	14 ± 0.39	0.67	11 ± 0.47	0.54	14 ± 0.64	0.75	0.84	0.99	0.93
Treg:Th1	5.2 ± 0.85	5.23 ± 1.2	1.0	13 ± 1.3	13 ± 1.5	0.98	6.04 ± 0.954	0.84	11 ± 1.4	0.73	0.07	0.41	0.78
Treg:Th2	122 ± 23	168 ± 16	0.40	739 ± 232	720 ± 102	0.99	194 ± 32	0.11	541 ± 75	0.64	0.01*	0.03*	0.47
Treg:Th17	1.9 ± 0.10	2.3 ± 0.73	0.13	5.2 ± 0.63	4.5 ± 0.51	0.59	1.96 ± 0.113	0.95	3.5 ± 0.28	0.07	<0.0001*	0.0004*	0.10
DAY 42													
CD4 ⁺ T cells	18 ± 0.57	17.8 ± 0.951	0.92	18 ± 1.3	15 ± 0.95	0.06	18 ± 0.32	0.95	16 ± 0.77	0.18	1.00	0.20	0.22
Th1 cells	3.4 ± 0.34	3.2 ± 0.32	0.83	4.2 ± 0.61	5.5 ± 0.54	0.41	3.1 ± 0.22	0.71	4.7 ± 0.87	0.87	0.92	0.35	0.82
Th2 cells	0.06 ± 0.01	0.04 ± 0.01	0.06	0.01 ± 0.01	0.047 ± 0.003	0.69	0.06 ± 0.01	0.79	0.044 ± 0.003	0.44	0.92	0.99	0.08
Th17 cells	6.01 ± 0.384	6.03 ± 0.364	0.99	2.03 ± 0.260	2.5 ± 0.28	0.47	5.3 ± 0.43	0.44	2.7 ± 0.32	0.28	<0.0001*	<0.0001*	<0.0001*
Tregs	13 ± 0.71	13 ± 0.37	0.79	13 ± 0.26	13 ± 0.34	0.99	13 ± 0.34	0.78	14 ± 0.43	0.64	0.95	0.34	0.51
Treg:Th1	4.01 ± 0.445	4.5 ± 0.46	0.69	3.8 ± 0.56	2.7 ± 0.35	0.24	4.4 ± 0.31	0.74	3.6 ± 0.45	0.94	0.99	0.07	0.74
Treg:Th2	217 ± 25	343 ± 49	0.09	324 ± 60	298 ± 25	0.89	256 ± 38	0.77	330 ± 24	0.89	0.44	0.97	0.78
Treg:Th17	2.1 ± 0.07	2.3 ± 0.21	0.82	7.8 ± 1.1	5.7 ± 0.47	0.22	2.6 ± 0.19	0.16	5.9 ± 0.73	0.27	<0.0001*	0.01*	0.01*

Supplemental Materials, Table S7. The percentage of CD4⁺ T cell subsets in developmentally exposed mice after MOG peptide administration. At maturity (6-8 weeks of age), EAE was induced in 8-10 female and 8-10 male offspring from each developmental exposure group. Within each group, offspring of the same sex were from a different treated dam. Mice were sacrificed 7, 21 or 42 days after MOG peptide administration, and flow cytometry was used to identify CD4⁺ T cells in the cervical lymph nodes, based on the following parameters: CD4⁺ T cells (CD3⁺CD4⁺); Th1 cells (Tbet⁺CD4⁺); Th2 cells (GATA3⁺CD4⁺); Th17 cells (RORγt⁺CD4⁺); Treg (Foxp3⁺CD25⁺CD4⁺). The mean percentage ± SEM is shown. ^aMixture-exposed group compared to control group within the indicated sex. ^bFemale versus male offspring from dams given the indicated concentration of the mixture. *Demarcates p ≤ 0.05.

A. Female offspring



B. Male offspring



Supplemental Materials, Figure S1. Effects of developmental exposure to chemicals associated with UOG on survival following primary influenza virus infection. At maturity (6-8 weeks of age), 9-10 female and 9-10 male offspring from each exposure group infected with influenza A virus (HKx31; H3N2). Within each group, offspring of the same sex were from a different treated dam. Post infection survival for (A) female and (B) male offspring was recorded daily for 9 days. There were no statistically significant differences in survival among same sex offspring.