SUPPLEMENTARY MATERIAL

Perfluoroalkyl substances and immune cell counts in adults from the Mid-Ohio Valley (USA)

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Table S1. Absolute counts of WBCs and lymphocyte subsets and association between PFASs and absolute counts of these cells amongst the population in 2005–2006 (n=42,782) and in 2010 (n=526), Mid-Ohio Valley, USA

Outcome	Cell count Median (IQR)	PFHxS diff. (95%CI)	PFOA diff. (95%CI)	PFOS diff. (95%CI)	PFNA diff. (95%CI)
Total WBCs 2005–2006	7,100 (5,900, 8,600)	39 (17, 61)	-19 (-44, 6)	-39 (-60, -18)	-15 (-33, 4)
Total WBCs 2010	6,300 (5,300, 7,600)	57 (-93, 210)	53 (-139, 250)	35 (-85, 157)	-5 (-123, 115)
Neutrophils 2005–2006	4,400 (3,500, 5,500)	-2 (-19, 15)	-41 (-60, -21)	-69 (-85, -52)	-34 (-49, -19)
Neutrophils 2010	4,000 (3,200, 5,000)	-80 (-199, 43)	-57 (-205, 96)	-35 (-135, 69)	-46 (-147, 59)
Monocytes 2005–2006	400 (300, 500)	5 (3, 7)	2 (0, 5)	2 (0, 3)	1 (-1, 2)
Monocytes 2010	300 (300, 400)	6 (-8, 20)	13 (-3, 30)	4 (-7, 16)	2 (-8, 12)
Eosinophils 2005–2006	100 (100, 200)	-1 (-2, 0)	0 (-1, 1)	0 (-1, 0)	-1 (-1, 0)
Eosinophils 2010	100 (100, 200)	1 (-7, 10)	5 (-4, 14)	4 (-2, 11)	6 (1, 12)
Lymphocytes 2005–2006	2,000 (1,700, 2,500)	40 (33, 47)	22 (14, 31)	39 (31, 47)	29 (23, 36)
Lymphocytes 2010	1,800 (1,500, 2,200)	132 (74, 191)	99 (27, 173)	61 (13, 111)	23 (-19, 66)
CD3+ T cells	1,356 (1,085, 1,645)	101 (52, 152)	80 (20, 143)	42 (3, 83)	18 (-17, 53)
CD3+CD4+ T-helper cells	904 (714, 1105)	75 (39, 112)	67 (25, 111)	36 (5, 67)	18 (-8, 45)
CD3+CD8+ T-cytotoxic cells	401 (293, 534)	22 (2, 43)	16 (-10, 43)	6 (-9, 23)	-4 (-16, 9)
CD3+CD4+CD8+ DP T cells	16 (10, 25)	1 (0, 2)	2 (0, 4)	0 (-1, 1)	0 (-1, 1)
CD3−CD16+CD56+ NK cells	184 (129, 260)	16 (6, 26)	9 (-1, 20)	10 (2, 18)	1 (-6, 8)
CD3−CD19+ B cells	238 (170, 318)	17 (5, 30)	8 (-7, 24)	9 (-2, 21)	5 (-4, 15)

PFASs and counts of immune cells were log transformed (ln) for the analyses. Cell counts are expressed as cells/µL. Results are expressed as the difference in the outcomes (using the median as the reference point) associated with IQR increments in PFAS levels in 2005–2006. All models were adjusted for gender, age, smoking, month of sampling, alcohol intake, and educational level.

diff., Difference; CI, Confidence interval; DP, Double positive; IQR, Interquartile range; NK, Natural killer; PFASs, Perfluoroalkyl substances; PFHxS, Perfluorohexane sulfonate; PFNA, Perfluorononanoic acid; PFOA, Perfluorooctanoic acid; PFOS, Perfluorooctane sulfonate; WBCs, White blood cells.

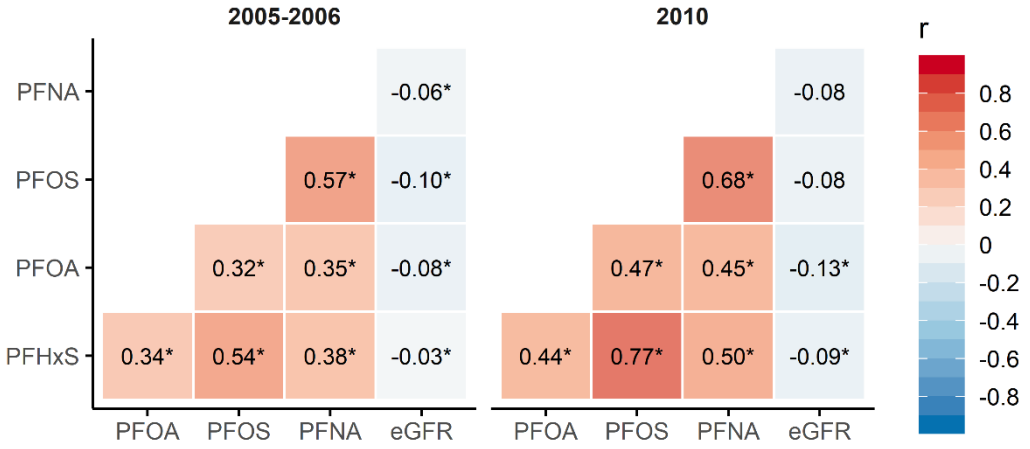

Figure S1. Correlation heatmap showing Pearson's correlations across all PFASs and eGFR amongst the population in 2005–2006 (n=42,782) and in 2010 (n=526), Mid-Ohio Valley, USA. PFASs were log-transformed (ln). Statistically significant correlations ($p<0.05$) are indicated by asterisks.

eGFR, Estimated glomerular filtration rate; PFASs, Perfluoroalkyl substances; PFHxS, Perfluorohexane sulfonate; PFNA, Perfluorononanoic acid; PFOA, Perfluorooctanoic acid; PFOS, Perfluorooctane sulfonate.

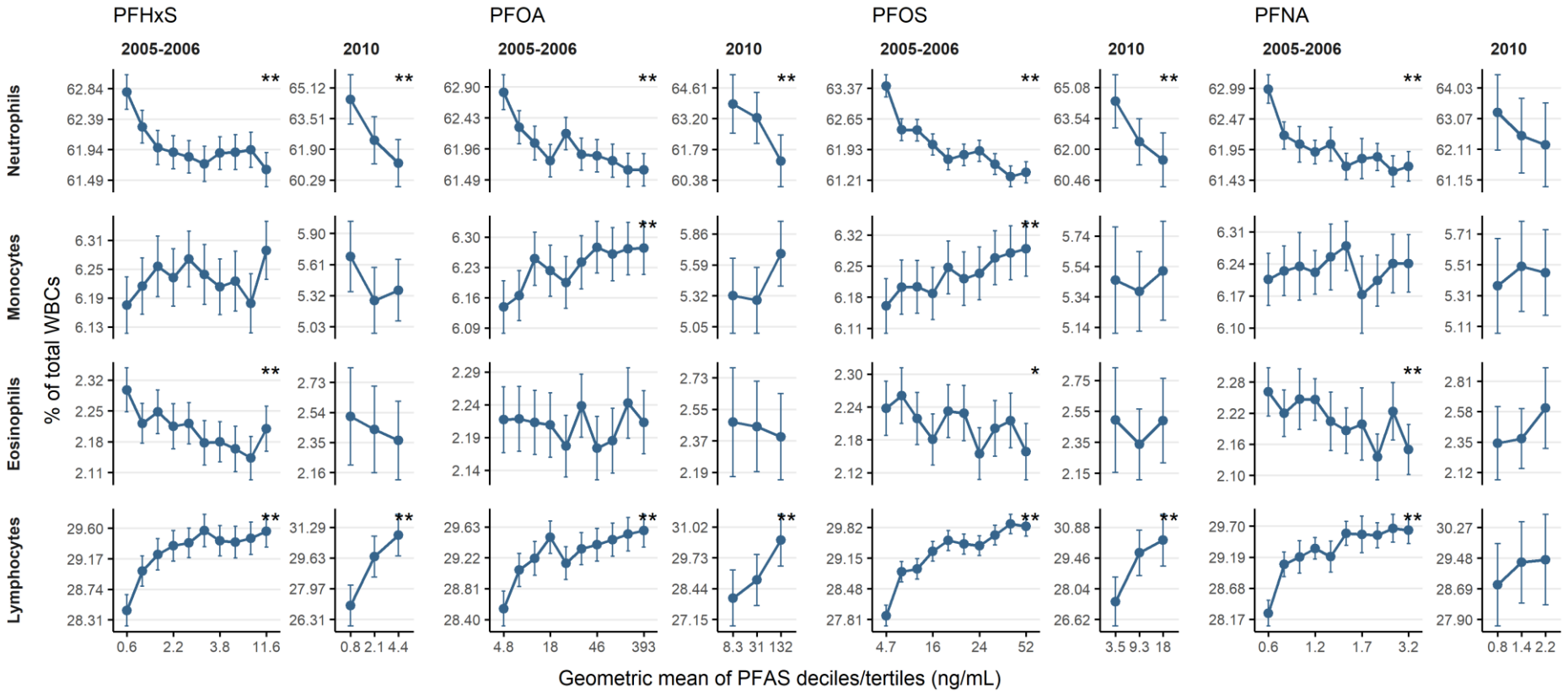

Figure S2. Adjusted WBC percentages (mean and 95% confidence interval) by PFAS deciles (2005–2006, n=42,782) or tertiles (2010, n=526), Mid-Ohio Valley, USA. Statistically significant trends at $p<0.05$ and $p<0.01$ are denoted by one and two asterisks, respectively. All models were adjusted for gender, age, smoking, month of sampling, alcohol intake, and educational level.

PFAS, Perfluoroalkyl substance; PFHxS, Perfluorohexane sulfonate; PFNA, Perfluorononanoic acid; PFOA, Perfluorooctanoic acid; PFOS, Perfluorooctane sulfonate; WBCs, White blood cells.

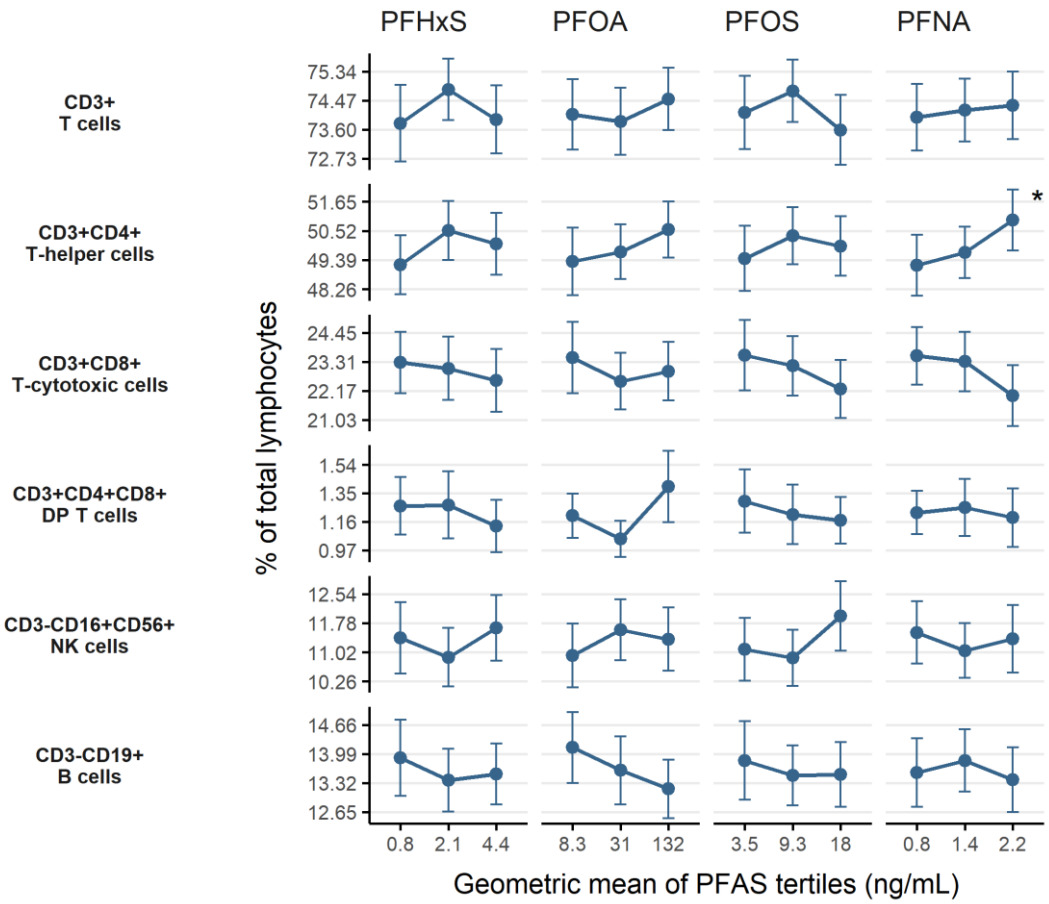

Figure S3. Adjusted lymphocyte subtype percentages (mean and 95% confidence interval) by PFAS tertiles (n=526), Mid-Ohio Valley, USA (2010). Statistically significant trends at $p<0.05$ and $p<0.01$ are denoted by one and two asterisks, respectively. All models were adjusted for gender, age, smoking, month of sampling, alcohol intake, and educational level.

DP, Double positive; NK, Natural killer; PFAS, Perfluoroalkyl substance; PFHxS, Perfluorohexane sulfonate; PFNA, Perfluorononanoic acid; PFOA, Perfluorooctanoic acid; PFOS, Perfluorooctane sulfonate.

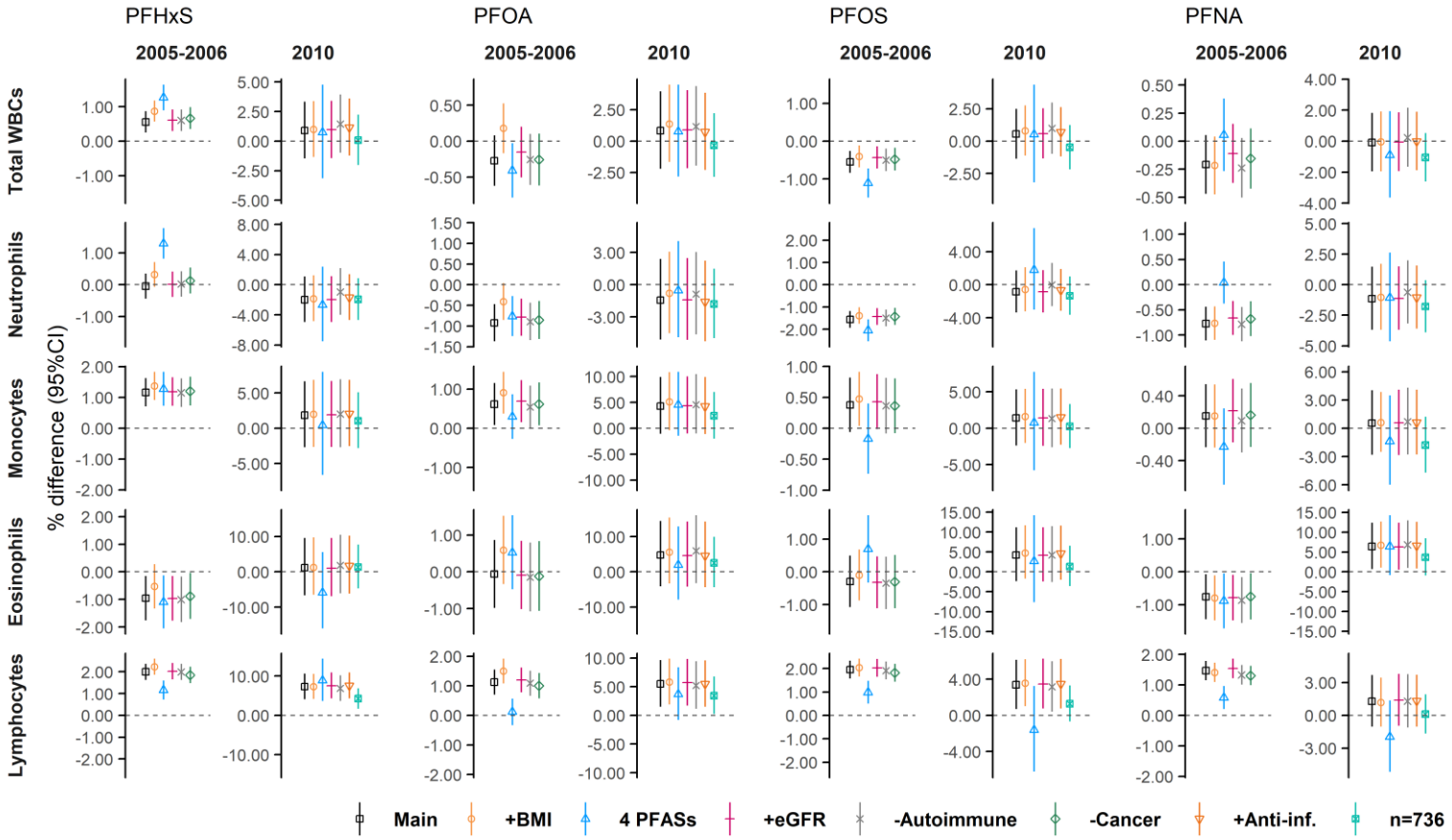

Figure S4. Association between PFASs and count (cells/µl) of types of WBCs amongst the population in 2005–2006 (n=42,782) and in 2010 (n=526), Mid-Ohio Valley, USA. Sensitivity analyses. PFASs and immune cell counts were log-transformed (ln). All models were adjusted for gender, age, smoking, month of sampling, alcohol intake, and educational level. Results are expressed as the percentage difference in the outcomes associated with IQR increments in PFAS levels in 2005–2006.

Anti-inf., Usage of anti-inflammatories; Autoimmune, People who reported autoimmune disease; BMI, Body mass index (only available for the 2005–2006 survey); Cancer, People who reported cancer; CI, Confidence interval; eGFR, Estimated glomerular filtration rate; n=736, Whole population in 2010 including people who reported infection or not; PFASs, Perfluoroalkyl substances; PFHxS, Perfluorohexane sulfonate; PFNA, Perfluorononanoic acid; PFOA, Perfluorooctanoic acid; PFOS, Perfluorooctane sulfonate; WBCs, White blood cells.

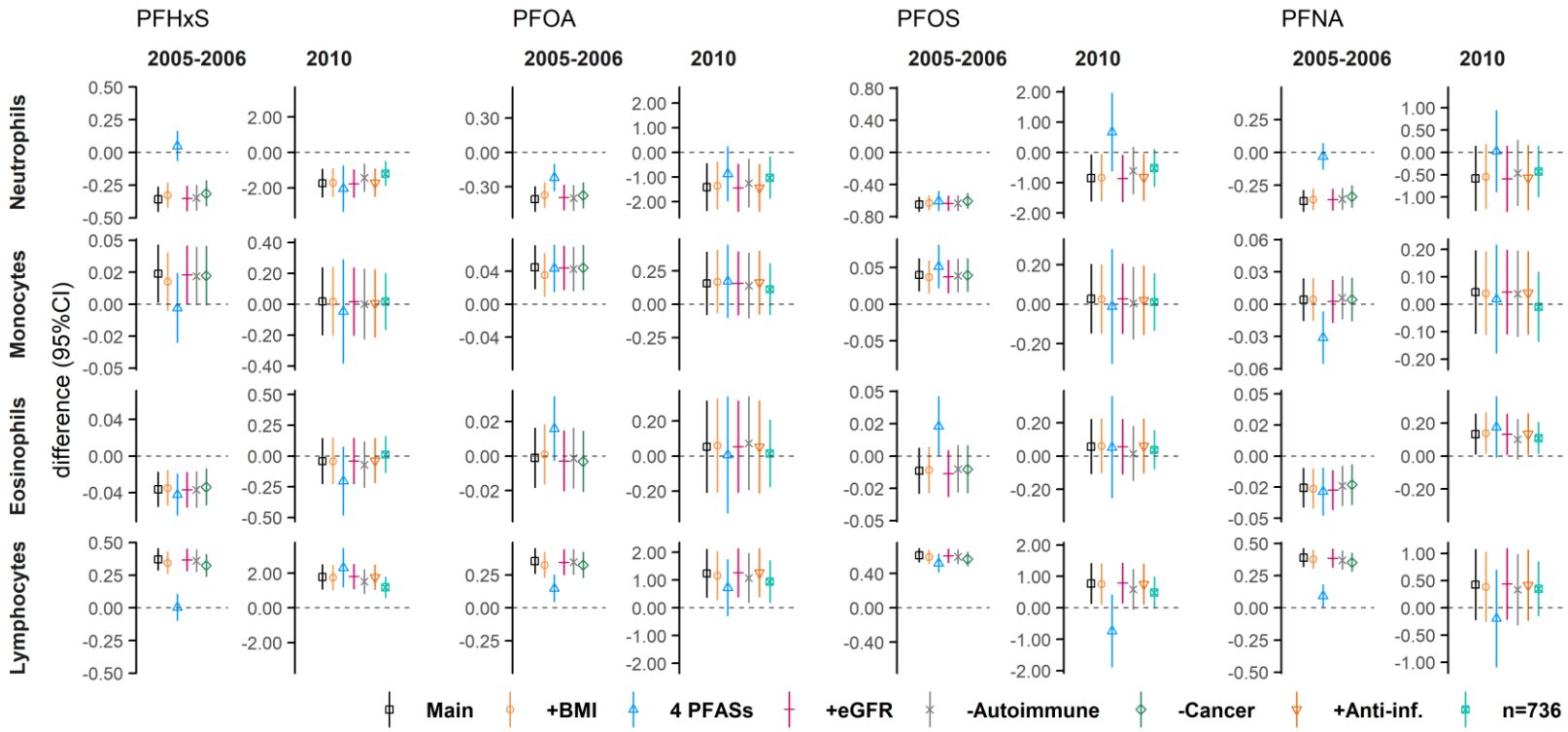

Figure S5. Association between PFASs and percentage of types of WBCs amongst the population in 2005–2006 (n=42,782) and in 2010 (n=526), Mid-Ohio Valley, USA. Sensitivity analyses. PFASs were log-transformed (ln). All models were adjusted for gender, age, smoking, month of sampling, alcohol intake, and educational level. Results are expressed as the difference in the outcomes associated with IQR increments in PFAS levels in 2005–2006.

Anti-inf., Usage of anti-inflammatories; Autoimmune, People who reported autoimmune disease; BMI, Body mass index (only available for the 2005–2006 survey); Cancer, People who reported cancer; CI, Confidence interval; eGFR, Estimated glomerular filtration rate; n=736, Whole population in 2010 including people who reported infection or not; PFASs, Perfluoroalkyl substances; PFHxS, Perfluorohexane sulfonate; PFNA, Perfluorononanoic acid; PFOA, Perfluorooctanoic acid; PFOS, Perfluorooctane sulfonate; WBCs, White blood cells.

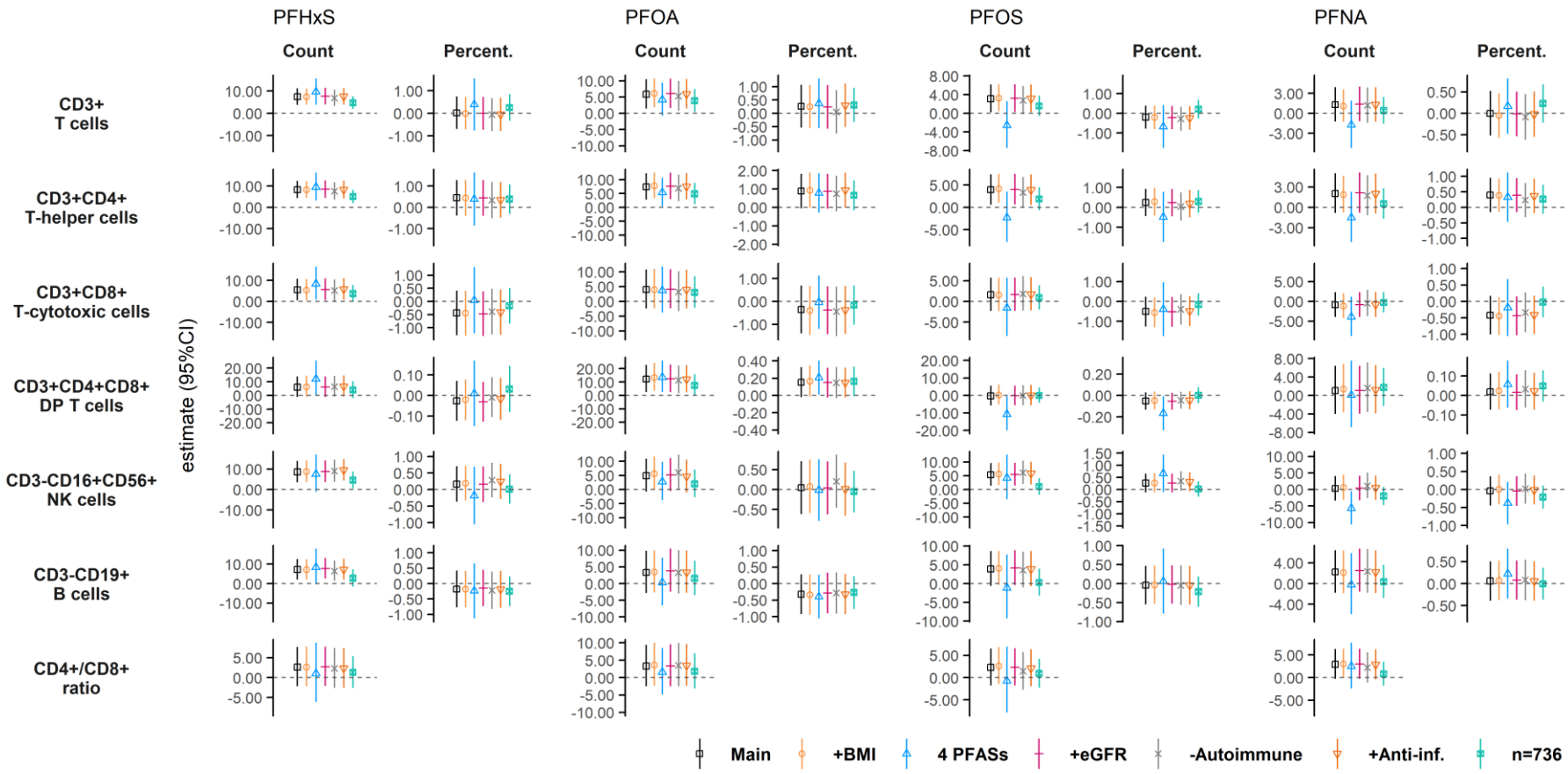

Figure S6. Association between PFASs and count (cells/µl) or percentage of lymphocyte subtypes amongst the population in 2010 (n=526), Mid-Ohio Valley, USA. Sensitivity analyses. PFAS and counts but not percentages of immune cells were log-transformed (ln). All models were adjusted for gender, age, smoking, month of sampling, alcohol intake, and educational level. Results are expressed as percentage difference (counts) or difference (percentages) in the outcomes associated with IQR increments in PFAS levels in 2005–2006.

Anti-inf., Usage of anti-inflammatories; Autoimmune, People who reported autoimmune disease; BMI, Body mass index (only available for the 2005–2006 survey); Cancer, People who reported cancer; CI, Confidence interval; DP, Double positive; eGFR, Estimated glomerular filtration rate; n=736, Whole population in 2010 including people who reported infection or not; NK, Natural killer; Percent., Percentage; PFASs, Perfluoroalkyl substances; PFHxS, Perfluorohexane sulfonate; PFNA, Perfluorononanoic acid; PFOA, Perfluorooctanoic acid; PFOS, Perfluorooctane sulfonate.

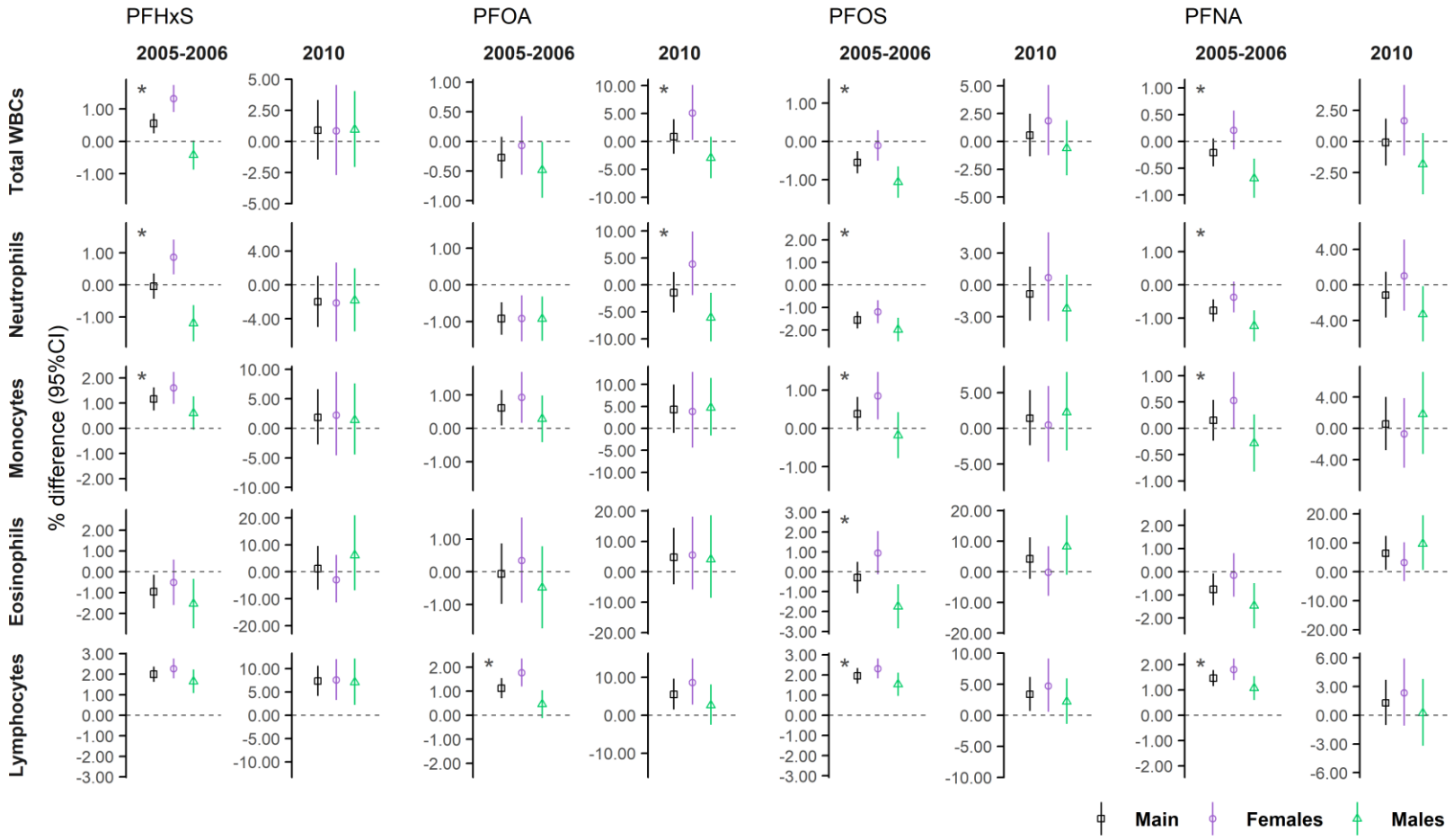

Figure S7. Association between PFASs and count (cells/µl) of types of WBCs amongst the population in 2005–2006 (n=42,782) and in 2010 (n=526), Mid-Ohio Valley, USA. Gender-interaction analyses. PFASs and counts of immune cells were log-transformed (ln). All models were adjusted for gender, age, smoking, month of sampling, alcohol intake, and educational level. Results are expressed as percentage difference in the outcomes associated with IQR increments in PFAS levels in 2005–2006. Statistically significant interactions ($p<0.05$) are indicated by asterisks.

CI, Confidence interval; PFASs, Perfluoroalkyl substances; PFHxS, Perfluorohexane sulfonate; PFNA, Perfluorononanoic acid; PFOA, Perfluorooctanoic acid; PFOS, Perfluorooctane sulfonate; WBCs, White blood cells.

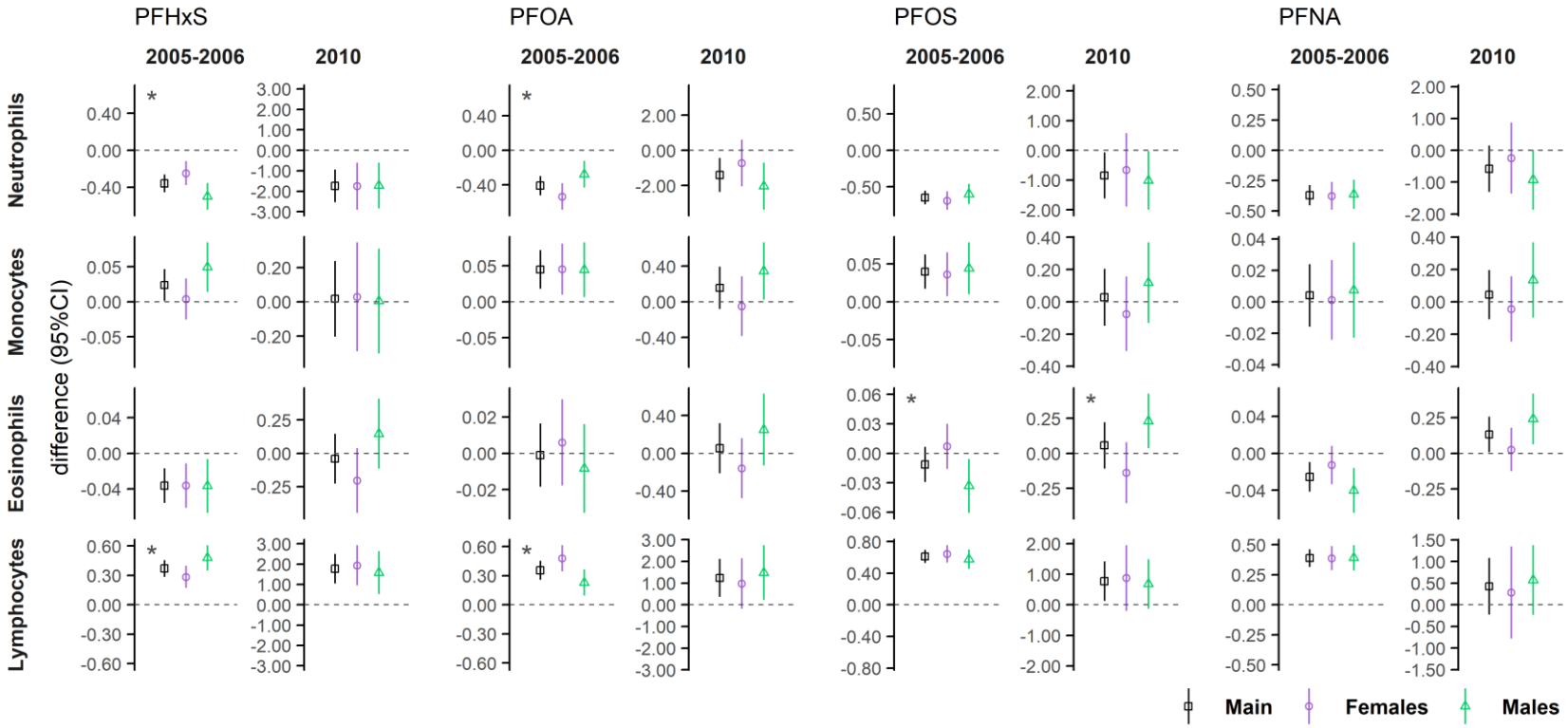

Figure S8. Association between PFASs and percentage of types of WBCs amongst the population in 2005–2006 (n=42,782) and in 2010 (n=526), Mid-Ohio Valley, USA. Gender-interaction analyses. PFASs were log-transformed (ln). All models were adjusted for gender, age, smoking, month of sampling, alcohol intake, and educational level. Results are expressed as the difference in the outcomes associated with IQR increments in PFAS levels in 2005–2006. Statistically significant interactions ($p<0.05$) are indicated by asterisks.

CI, Confidence interval; PFASs, Perfluoroalkyl substances; PFHxS, Perfluorohexane sulfonate; PFNA, Perfluorononanoic acid; PFOA, Perfluorooctanoic acid; PFOS, Perfluorooctane sulfonate; WBCs, White blood cells.

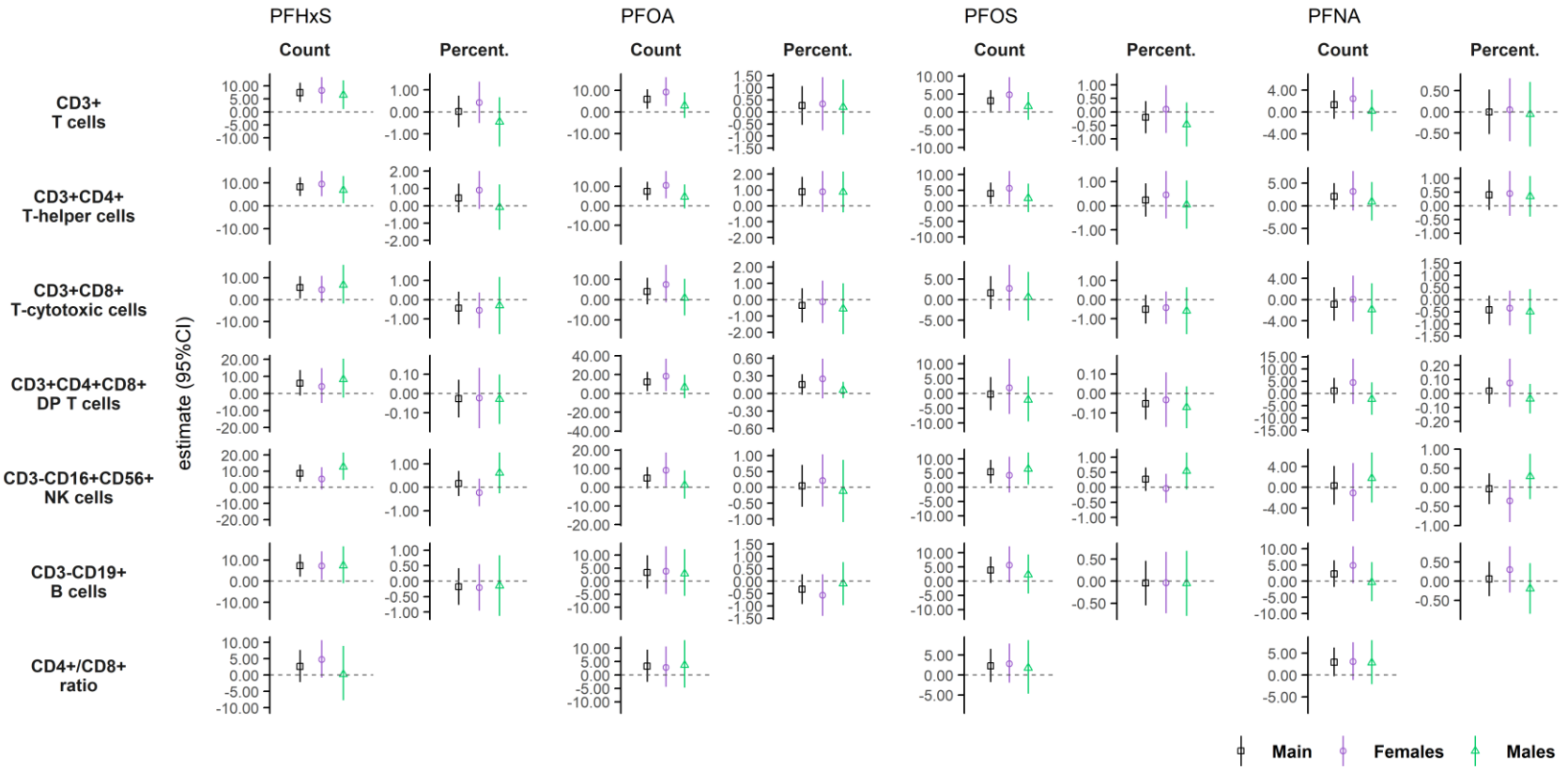

Figure S9. Association between PFASs and count (cells/µl) or percentage of lymphocyte subtypes amongst the population in 2010 (n=526), Mid-Ohio Valley, USA. Gender-interaction analyses. PFAS and counts but not percentages of immune cells were log-transformed (ln). All models were adjusted for gender, age, smoking, month of sampling, alcohol intake, and educational level. Results are expressed as percentage difference (counts) or difference (percentages) in the outcomes associated with IQR increments in PFAS levels in 2005–2006. Statistically significant interactions ($p<0.05$) are indicated by asterisks.

CI, Confidence interval; DP, Double positive; NK, Natural killer; Percent., Percentage; PFASs, Perfluoroalkyl substances; PFHxS, Perfluorohexane sulfonate; PFNA, Perfluorononanoic acid; PFOA, Perfluorooctanoic acid; PFOS, Perfluorooctane sulfonate.