

## M06: Spleen Immunophenotyping

Study Number: I20263

Test Compound: Tris(chloropropyl) phosphate

Date: 29 May 2024

DTTID: 104-016-002-000-4

CAS Number: 13674-84-5

Time: 9:39:18 AM

Study Type: Immune screening with perinatal exposure

DTXSID: DTXSID201016652

Species/Strain: Rat/Harlan Sprague Dawley

### F1 Males: Immunophenotyping

	Treatment Groups (ppm)				
	0	2500	5000	10000	15 (mg/kg CPS)
Spleen Cells ( $\times 10^6$ )	206.44 ± 19.10[12]	211.80 ± 12.72[12]	220.43 ± 20.13[12]	225.41 ± 14.72[11]	57.78 ± 11.65[8]**
<b>Absolute counts</b>					
Total Lymphocytes	204.529 ± 19.051[12]	210.477 ± 12.629[12]	218.297 ± 20.007[12]	223.862 ± 14.640[11]	56.256 ± 11.477[8]**
Total T Cells	55.703 ± 7.095[12]	58.494 ± 3.852[12]	57.848 ± 5.324[12]	61.860 ± 3.832[11]	27.142 ± 4.362[8]**
CD4 <sup>+</sup> T Cells <sup>a</sup>	30.618 ± 3.405[12]	32.401 ± 2.227[12]	32.326 ± 2.754[12]	35.047 ± 3.460[11]	14.060 ± 2.129[8]**
CD8 <sup>+</sup> T Cells <sup>b</sup>	22.562 ± 3.551[12]	23.700 ± 1.814[12]	23.206 ± 2.625[12]	24.304 ± 1.568[11]	12.167 ± 2.099[8]**
B Cells	102.460 ± 8.873[12]	104.165 ± 6.826[12]	112.310 ± 11.655[12]	107.027 ± 7.324[11]	15.497 ± 4.205[8]**
NK Cells	6.558 ± 0.524[12]	7.521 ± 0.542[12]	6.833 ± 0.537[12]	6.970 ± 0.779[11]	2.807 ± 0.611[8]**
Mono/Mac Cells <sup>c</sup>	3.671 ± 0.280[12]	4.502 ± 0.310[12]	3.721 ± 0.458[12]	3.798 ± 0.344[11]	0.516 ± 0.121[8]**
Neutrophils	2.435 ± 0.237[12]**	2.068 ± 0.192[12]	1.858 ± 0.192[12]	1.728 ± 0.219[11]*	0.374 ± 0.068[8]**
Eosinophils	1.729 ± 0.113[12]	1.817 ± 0.099[12]	1.759 ± 0.193[12]	2.032 ± 0.197[11]	0.851 ± 0.198[8]**
Total T Cells: B Cells Ratio	0.535 ± 0.026[12]	0.566 ± 0.026[12]	0.528 ± 0.022[12]	0.584 ± 0.020[11]	2.077 ± 0.239[8]**
CD4 <sup>+</sup> T Cells: CD8 <sup>+</sup> T Cells Ratio	1.448 ± 0.096[12]	1.401 ± 0.078[12]	1.457 ± 0.105[12]	1.505 ± 0.196[11]	1.194 ± 0.085[8]*
<b>Relative counts</b>					
Percent Total Lymphocytes (%)	99.050 ± 0.258[12]	99.376 ± 0.047[12]	99.988 ± 0.229[12]	99.306 ± 0.047[11]	97.766 ± 1.847[8]
Percent Total T Cells (%)	26.668 ± 0.925[12]	27.752 ± 0.720[12]	26.686 ± 0.764[12]	27.788 ± 0.805[11]	50.509 ± 2.419[8]**
Percent CD4 <sup>+</sup> T Cells <sup>a</sup> (%)	55.823 ± 1.455[12]	55.508 ± 1.315[12]	56.218 ± 1.476[12]	55.820 ± 2.416[11]	52.229 ± 1.483[8]
Percent CD8 <sup>+</sup> T Cells <sup>b</sup> (%)	39.483 ± 1.324[12]	40.396 ± 1.265[12]	39.768 ± 1.518[12]	40.052 ± 2.344[11]	44.528 ± 1.577[8]*
Percent B Cells (%)	50.244 ± 0.875[12]	49.481 ± 1.020[12]	50.871 ± 0.894[12]	47.730 ± 0.620[11]	25.925 ± 2.065[8]**
Percent NK Cells (%)	3.306 ± 0.198[12]	3.555 ± 0.122[12]	3.212 ± 0.149[12]	3.051 ± 0.168[11]	4.946 ± 0.194[8]**
Percent Mono/Mac Cells <sup>c</sup> (%)	1.843 ± 0.130[12]	2.136 ± 0.111[12]	1.674 ± 0.125[12]	1.721 ± 0.151[11]	0.873 ± 0.047[8]**
Percent Neutrophils (%)	1.179 ± 0.031[12]**	0.969 ± 0.062[12]**	0.834 ± 0.039[12]**	0.754 ± 0.061[11]**	0.690 ± 0.077[8]**
Percent Eosinophils (%)	0.919 ± 0.099[12]	0.868 ± 0.038[12]	0.817 ± 0.061[12]	0.915 ± 0.080[11]	1.479 ± 0.163[8]*

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### F1 Females: Immunophenotyping

	Treatment Groups (ppm)				
	0	2500	5000	10000	15 (mg/kg CPS)
Spleen Cells (x10 <sup>6</sup> )	71.74 ± 3.33[12]	72.47 ± 6.23[12]	72.04 ± 3.89[12]	72.79 ± 5.53[12]	22.47 ± 2.23[8]**
<b>Absolute counts</b>					
Total Lymphocytes	69.817 ± 3.294[12]	70.919 ± 6.116[12]	66.819 ± 4.049[12]	64.791 ± 5.832[12]	17.859 ± 2.616[8]**
Total T Cells	22.003 ± 1.023[12]	21.412 ± 1.946[12]	19.958 ± 1.195[12]	19.240 ± 1.713[12]	9.559 ± 1.307[8]**
CD4 <sup>+</sup> T Cells <sup>a</sup>	11.965 ± 0.681[12]	11.628 ± 0.935[12]	10.603 ± 0.608[12]	10.645 ± 1.164[12]	4.736 ± 0.661[8]**
CD8 <sup>+</sup> T Cells <sup>b</sup>	9.279 ± 0.477[12]	9.057 ± 0.976[12]	8.688 ± 0.648[12]	7.899 ± 0.685[12]	4.510 ± 0.666[8]**
B Cells	33.909 ± 1.845[12]	34.531 ± 2.944[12]	32.541 ± 2.183[12]	30.068 ± 3.094[12]	3.968 ± 0.850[8]**
NK Cells	2.075 ± 0.141[12]*	2.117 ± 0.206[12]	1.916 ± 0.143[12]	1.656 ± 0.142[12]	0.796 ± 0.084[8]**
Mono/Mac Cells <sup>c</sup>	0.986 ± 0.065[12]*	0.979 ± 0.108[12]	0.814 ± 0.085[12]	0.706 ± 0.062[12]	0.152 ± 0.024[8]**
Neutrophils	0.805 ± 0.045[12]*	0.713 ± 0.069[12]	0.685 ± 0.044[12]	0.672 ± 0.040[12]	0.202 ± 0.031[8]**
Eosinophils	0.533 ± 0.029[12]**	0.602 ± 0.065[12]	0.443 ± 0.043[12]	0.372 ± 0.033[12]**	0.290 ± 0.087[8]**
Total T Cells: B Cells Ratio	0.656 ± 0.021[12]	0.619 ± 0.014[12]	0.623 ± 0.027[12]	0.649 ± 0.024[12]	2.752 ± 0.284[8]**
CD4 <sup>+</sup> T Cells: CD8 <sup>+</sup> T Cells Ratio	1.308 ± 0.075[12]	1.391 ± 0.103[12]	1.259 ± 0.078[12]	1.378 ± 0.123[12]	1.090 ± 0.092[8]*
<b>Relative counts</b>					
Percent Total Lymphocytes (%)	97.283 ± 0.407[12]**	97.794 ± 0.197[12]	92.515 ± 1.548[12]	88.133 ± 1.370[12]**	76.859 ± 4.929[8]**
Percent Total T Cells (%)	31.607 ± 0.627[12]	30.097 ± 0.475[12]	29.987 ± 0.727[12]	29.698 ± 0.681[12]	54.103 ± 1.271[8]**
Percent CD4 <sup>+</sup> T Cells <sup>a</sup> (%)	54.268 ± 1.231[12]	55.506 ± 1.541[12]	53.354 ± 1.284[12]	54.791 ± 1.935[12]	49.848 ± 1.809[8]
Percent CD8 <sup>+</sup> T Cells <sup>b</sup> (%)	42.278 ± 1.243[12]	41.133 ± 1.529[12]	43.267 ± 1.331[12]	41.498 ± 1.805[12]	46.879 ± 1.969[8]
Percent B Cells (%)	48.402 ± 0.680[12]	48.699 ± 0.505[12]	48.548 ± 0.943[12]	46.048 ± 0.797[12]	20.950 ± 1.827[8]**
Percent NK Cells (%)	2.979 ± 0.168[12]	3.033 ± 0.189[12]	2.888 ± 0.178[12]	2.598 ± 0.142[12]	4.795 ± 0.429[8]**
Percent Mono/Mac Cells <sup>c</sup> (%)	1.379 ± 0.068[12]**	1.392 ± 0.113[12]	1.119 ± 0.094[12]	0.973 ± 0.059[12]**	0.660 ± 0.061[8]**
Percent Neutrophils (%)	1.122 ± 0.038[12]**	0.973 ± 0.031[12]*	0.949 ± 0.028[12]**	0.945 ± 0.048[12]**	0.874 ± 0.056[8]**
Percent Eosinophils (%)	0.749 ± 0.037[12]**	0.851 ± 0.062[12]	0.608 ± 0.040[12]	0.525 ± 0.043[12]**	1.158 ± 0.233[8]

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### LEGEND

Data are displayed as mean  $\pm$  SEM (N) unless otherwise noted.

All units are  $\times 10^6$ /spleen unless otherwise noted.

Statistical analysis performed by Jonckheere (trend) and Shirley or Dunn (pairwise) tests. The positive control group (15 mg/kg CPS) was excluded from trend test.

Statistical analysis for the positive control group (15 mg/kg CPS) compared to the vehicle control group was performed using the Wilcoxon rank sum test.

Statistical significance for the control group indicates a significant trend test.

Statistical significance for a treatment group indicates a significant pairwise test compared to the vehicle control group.

\* Statistically significant at  $P \leq 0.05$

\*\* Statistically significant at  $P \leq 0.01$

<sup>a</sup>CD4 $^{+}$  T Cells = Helper/DTH - T Cell

<sup>b</sup>CD8 $^{+}$  T Cells = Cytotoxic T Cell

<sup>c</sup>Mono/Mac Cells = Monocytes/Macrophage

SD = Study Day

SEM = Standard Error of the Mean

CPS = Cyclophosphamide

Males from the vehicle control and treatment groups were removed on SD 97; females were removed on SD 89.

Animals from the positive control group (15 mg/kg CPS) were purchased from a commercial source, age matched to the F1 animals, and removed on the same day as the F1 animals.

\*\* END OF REPORT \*\*