# Supplementary Materials: Orally Administered 6:2 Chlorinated Polyfluorinated Ether Sulfonate (F-53B) Causes Thyroid Dysfunction in Rats

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**Table S1.** Hematology data for male rats in the 28-day gavage study of F-53B.

| Parameter | Groups (mg/kg/day) | | | |
|---|---|---|---|---|
| | Vehicle Control | 5 | 20 | 100 |
| RBCs (× $10^6$ cells/μL) | 8.65±0.491 | 8.99±0.322 | 8.92±0.264 | 8.74±0.245 |
| HGB (g/dL) | 16.4±0.67 | 16.7±0.63 | 17.0±0.28 | 16.9±0.40 |
| HCT (%) | 46.9±2.72 | 48.8±1.86 | 49.1±1.47 | 48.5±1.07 |
| MCV (fL) | 54.2±0.84 | 54.3±1.53 | 55.0±1.14 | 55.4±0.50 |
| MCH (pg) | 19.0±0.91 | 18.5±0.87 | 19.0±0.41 | 19.3±0.63 |
| MCHC (g/dL) | 35.0±1.52 | 34.2±1.26 | 34.6±0.82 | 34.8±1.05 |
| PLTs (× $10^3$ cells/μL) | 986±110.1 | 980±116.5 | 984±72.7 | 991±72.9 |
| WBCs (× $10^3$ cells/μL) | 8.89±2.146 | 8.94±2.353 | 8.46±2.191 | 7.56±1.199 |
| NEU (% of WBCs) | 15.3±3.56 | 15.8±5.23 | 13.6±2.98 | 16.7±9.74 |
| LYM (% of WBCs) | 78.8±4.29 | 78.3±6.14 | 80.3±2.78 | 78.0±9.33 |
| MONO (% of WBCs) | 3.3±1.28 | 3.8±1.46 | 3.5±0.49 | 3.2±0.94 |
| EOS (% of WBCs) | 1.4±1.08 | 0.7±0.25 | 1.2±0.30 | 1.2±0.35 |
| BASO (% of WBCs) | 0.2±0.07 | 0.2±0.08 | 0.3±0.08 | 0.2±0.07 |
| LUC (% of WBCs) | 0.9±0.55 | 1.2±0.42 | 1.1±0.41 | 0.9±0.41 |
| RET (%) | 2.0±0.198 | 1.86±0.324 | 2.19±0.289 | 1.94±0.180 |

RBC, red blood cell; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelet; WBC, white blood cell; NEU, neutrophil; LYM, lymphocyte; MONO, monocyte; EOS, eosinophil; BASO, basophil; LUC, large unstained cell; RET, reticulocyte. Data are expressed as mean ± SD values ($n$ = 7/group). *Significantly different from the vehicle control group ($p < 0.05$) Statistics: One-way analysis of variance (ANOVA) followed by the Dunnett's test.

**Table S2.** Hematology data for female rats in the 28-day gavage study of F-53B s.

| Parameter | Groups (mg/kg/day) | | | |
|---|---|---|---|---|
| | **Vehicle Control** | **5** | **20** | **100** |
| RBCs (× $10^6$ cells/μL) | 7.93±0.319 | 7.97±0.329 | 7.9±0.22 | 8.08±0.344 |
| HGB (g/dL) | 14.9±0.37 | 15.1±0.32 | 15.1±0.36 | 15.8±0.51 |
| HCT (%) | 43.6±0.79 | 43.3±1.46 | 43.4±1.09 | 44.7±1.95 |
| MCV (fL) | 43.6±2.94 | 54.4±0.92* | 55.1±1.05* | 55.4±1.34* |
| MCH (pg) | 18.8±0.42 | 19.0±0.68 | 19.2±0.41 | 19.5±0.63 |
| MCHC (g/dL) | 34.3±0.73 | 35.0±0.96 | 34.8±0.75 | 35.3±0.71 |
| PLTs (× $10^3$ cells/μL) | 1103±78.1 | 1111±65.9 | 1124±56.1 | 1175±81.1 |
| WBCs (× $10^3$ cells/μL) | 5.78±1.247 | 5.81±1.652 | 5.34±0.545 | 5.23±1.658 |
| NEU (% of WBCs) | 15.3±5.26 | 15.8±9.99 | 16.6±5.71 | 13.5±3.78 |
| LYM (% of WBCs) | 79.1±5.67 | 78.9±10.39 | 77.7±5.85 | 81.0±3.81 |
| MONO (% of WBCs) | 3.3±0.72 | 2.9±0.74 | 3.0±0.62 | 2.7±0.92 |
| EOS (% of WBCs) | 0.9±0.29 | 1.1±0.48 | 1.5±0.46 | 1.2±0.49 |
| BASO (% of WBCs) | 0.2±0.08 | 0.2±0.04 | 0.2±0.10 | 0.1±0.10 |
| LUC (% of WBCs) | 1.2±0.17 | 1.1±0.46 | 1.0±0.33 | 1.0±0.53 |
| RET (%) | 2.6±0.69 | 2.4±0.46 | 2.5±0.31 | 2.9±0.61 |

RBC, red blood cell; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelet; WBC, white blood cell; NEU, neutrophil; LYM, lymphocyte; MONO, monocyte; EOS, eosinophil; BASO, basophil; LUC, large unstained cell; RET, reticulocyte. Data are expressed as mean ± SD values ($n$ = 7/group). *Significantly different from the vehicle control group ($p < 0.05$) Statistics: One-way analysis of variance (ANOVA) followed by the Dunnett's test.

**Table S3.** Clinical biochemistry data for male rats in the 28-day gavage study of F-53B.

| Parameter | Groups (mg/kg/day) | | | |
|---|---|---|---|---|
| | **Vehicle Control** | **5** | **20** | **100** |
| ALT (IU/L) | 43.19±7.730 | 45.06±9.208 | 54.60±29.974 | 39.56±6.988 |
| AST (IU/L) | 125.23±23.005 | 125.63±20.611 | 131.27±29.366 | 119.03±25.106 |
| ALP (IU/L) | 340.47±80.153 | 339.63±60.051 | 335.96±86.256 | 305.66±40.462 |
| GGT (IU/L) | 0.51±0.240 | 0.31±0.108 | 0.77±0.629 | 0.35±0.140 |
| BUN (mg/dL) | 21.44±2.946 | 21.77±2.578 | 21.23±1.746 | 20.29±1.079 |
| CREA (mg/dL) | 0.64±0.072 | 0.69±0.116 | 0.67±0.073 | 0.65±0.052 |
| TP (g/dL) | 5.96±0.175 | 6.00±0.234 | 6.03±0.066 | 5.97±0.110 |
| T-BIL (mg/dL) | 0.18±0.036 | 0.19±0.039 | 0.20±0.016 | 0.19±0.016 |
| GLU (mg/dL) | 126.89±17.108 | 129.79±13.362 | 128.21±19.569 | 136.64±24.992 |
| TG (mg/dL) | 33.90±8.909 | 28.20±6.383 | 30.81±7.936 | 38.26±9.567 |
| ALB (g/dL) | 3.88±0.037 | 3.88±0.140 | 3.91±0.057 | 3.93±0.034 |
| A/G ratio | 1.87±0.114 | 1.83±0.098 | 1.84±0.056 | 1.93±0.056 |
| Ca (mg/dL) | 9.86±0.294 | 9.96±0.450 | 9.82±0.309 | 9.80±0.350 |
| Na (mmol/L) | 141.57±1.134 | 142.57±2.299 | 142.71±0.951 | 143.29±1.113 |
| Cl (mmol/L) | 101.71±0.488 | 102.86±0.690* | 103.29±1.254* | 104.14±0.690* |
| K (mmol/L) | 4.87±0.233 | 4.93±0.337 | 4.86±0.264 | 4.91±0.191 |
| IP (mg/dL) | 8.72±0.458 | 8.90±0.978 | 8.39±1.123 | 8.29±0.763 |

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase; BUN, blood urea nitrogen; CREA, creatinine; TP, total protein; T-BIL, total bilirubin; T-CHOL, cholesterol; GLU, glucose; TG, triglyceride; ALB, albumin; A/G ratio, albumin/globulin ratio; Ca, calcium; Na, sodium; Cl, chloride; K, potassium; IP, phosphorus; HDL, high-density lipoprotein; LDL, low-density lipoprotein. Data are expressed as mean ± SD values ($n$ = 7/group). *Significantly different from the vehicle control group ($p < 0.05$). Statistics: One-way analysis of variance (ANOVA) followed by the Dunnett's test.

**Table S4.** Clinical biochemistry data for male rats in the 28-day gavage study of F-53B.

| Parameter | Groups (mg/kg/day) | | | |
|---|---|---|---|---|
| | **Vehicle Control** | **5** | **20** | **100** |
| ALT (IU/L) | 35.20±3.097 | 34.83±6.698 | 37.99±10.808 | 36.70±3.462 |
| AST (IU/L) | 110.50±27.220 | 104.29±21.954 | 111.00±20.353 | 112.10±17.523 |
| ALP (IU/L) | 249.77±31.380 | 250.94±41.536 | 242.17±41.349 | 237.51±37.243 |
| GGT (IU/L) | 0.84±0.234 | 0.68±0.324 | 0.84±0.253 | 0.90±0.283 |
| BUN (mg/dL) | 19.21±1.642 | 22.53±4.673 | 19.34±4.451 | 21.86±4.275 |
| CREA (mg/dL) | 0.68±0.073 | 0.74±0.062 | 0.72±0.114 | 0.76±0.083 |
| TP (g/dL) | 6.25±0.221 | 6.24±0.226 | 6.30±0.341 | 6.61±0.242* |
| T-BIL (mg/dL) | 0.24±0.053 | 0.24±0.027 | 0.22±0.019 | 0.25±0.046 |
| GLU (mg/dL) | 111.77±15.741 | 109.34±16.450 | 116.90±3.804 | 113.71±19.619 |
| TG (mg/dL) | 21.44±6.702 | 24.73±7.372 | 22.93±2.453 | 21.64±10.168 |
| ALB (g/dL) | 4.06±0.109 | 4.13±0.156 | 4.14±0.184 | 4.33±0.165 |
| A/G ratio | 1.86±0.069 | 1.96±0.079 | 1.92±0.148 | 1.90±0.076 |
| Ca (mg/dL) | 10.05±0.228 | 10.06±0.245 | 9.97±0.113 | 10.14±0.228 |
| Na (mmol/L) | 142.71±0.756 | 143.57±0.535 | 144.00±1.414 | 145.00±1.633* |
| Cl (mmol/L) | 105.29±1.380 | 106.14±1.215 | 107.29±0.756* | 107.29±2.138* |
| K (mmol/L) | 4.42±0.138 | 4.42±0.289 | 4.60±0.319 | 4.76±0.486 |
| IP (mg/dL) | 8.47±0.795 | 8.37±0.748 | 7.85±0.871 | 8.16±1.420 |

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase; BUN, blood urea nitrogen; CREA, creatinine; TP, total protein; T-BIL, total bilirubin; T-CHOL, cholesterol; GLU, glucose; TG, triglyceride; ALB, albumin; A/G ratio, albumin/globulin ratio; Ca, calcium; Na, sodium; Cl, chloride; K, potassium; IP, phosphorus; HDL, high-density lipoprotein; LDL, low-density lipoprotein. Data are expressed as mean ± SD values ($n$ = 7/group). *Significantly different from the vehicle control group ($p < 0.05$). Statistics: One-way analysis of variance (ANOVA) followed by the Dunnett's test.

**Table S5.** Relative organ weight data for male rats in the 28-day gavage study of F-53B.

| Parameter | Groups (mg/kg/day) | | | |
|---|---|---|---|---|
| | Vehicle Control | 5 | 20 | 100 |
| Body weight (g) | 388.13±21.907 | 363.58±16.671 | 370.66±17.582 | 373.15±21.014 |
| Liver (%) | 2.554±0.0717 | 2.500±0.0943 | 2.500±0.0930 | 2.581±0.0682 |
| Kidneys (%) | 0.647±0.0199 | 0.653±0.0285 | 0.664±0.0209 | 0.647±0.0309 |
| Adrenal glands (%) | 0.014±0.0018 | 1.015±0.0017 | 0.014±0.0015 | 0.014±0.0008 |
| Heart (%) | 0.344±0.0211 | 0.353±0.0213 | 0.343±0.0219 | 0.346±0.0205 |
| Brain (%) | 0.517±0.0282 | 0.534±0.0215 | 0.535±0.0323 | 0.530±0.0253 |
| Pituitary gland (%) | 0.003±0.0002 | 0.003±0.0005 | 0.003±0.0007 | 0.003±0.0009 |
| Spleen (%) | 0.214±0.0155 | 0.206±0.0174 | 0.214±0.0255 | 0.206±0.0155 |
| Thymus (%) | 0.120±0.0140 | 0.099±0.0187* | 0.112±0.0144 | 0.096±0.0106* |
| Testes (%) | 1.004±0.0734 | 1.143±0.0790* | 1.072±0.0315 | 1.073±0.0535 |
| Epididymis (%) | 0.338±0.0290 | 0.362±0.0309 | 0.366±0.0221 | 0.359±0.0246 |
| Prostate, seminal vesicle and coagulating gland (%) | 0.689±0.0504 | 0.698±0.0750 | 0.680±0.0884 | 0.695±0.0646 |

Data are expressed as mean ± SD values ($n$ = 7/group). *Significantly different from the vehicle control group ($p < 0.05$) Statistics: One-way analysis of variance (ANOVA) followed by the Dunnett's test.

**Table S6.** Relative organ weight data for male rats in the 28-day gavage study of F-53B.

| Parameter | Groups (mg/kg/day) | | | |
|---|---|---|---|---|
| | Vehicle Control | 5 | 20 | 100 |
| Body weight (g) | 219.79±6.615 | 223.45±15.666 | 214.96±6.592 | 209.93±6.639 |
| Liver (%) | 2.525±0.0558 | 2.471±0.0822 | 2.552±0.1260 | 2.493±0.1338 |
| Kidneys (%) | 0.683±0.0361 | 0.650±0.0283 | 0.643±0.0310 | 0.654±0.0255 |
| Adrenal glands (%) | 0.030±0.0017 | 0.031±0.0022 | 0.032±0.0020 | 0.032±0.0035 |
| Heart (%) | 0.379±0.0156 | 0.375±0.0246 | 0.394±0.0179 | 0.380±0.0138 |
| Brain (%) | 0.816±0.0379 | 0.798±0.0406 | 0.827±0.0268 | 0.850±0.0319 |
| Pituitary gland (%) | 0.006±0.0006 | 0.005±0.0014 | 0.006±0.0006 | 0.006±0.0003 |
| Spleen (%) | 0.270±0.0290 | 0.266±0.0225 | 0.266±0.0469 | 0.272±0.0226 |
| Thymus (%) | 0.144±0.0159 | 0.146±0.0117 | 0.154±0.0239 | 0.272±0.0226 |
| Ovaries (%) | 0.040±0.0030 | 0.039±0.0046 | 0.040±0.0062 | 0.039±0.0071 |
| Uterus (%) | 0.227±0.0380 | 0.250±0.1359 | 0.294±0.1291 | 0.299±0.1188 |

Data are expressed as mean ± SD values ($n$ = 7/group). *Significantly different from the vehicle control group ($p < 0.05$) Statistics: One-way analysis of variance (ANOVA) followed by the Dunnett's test.