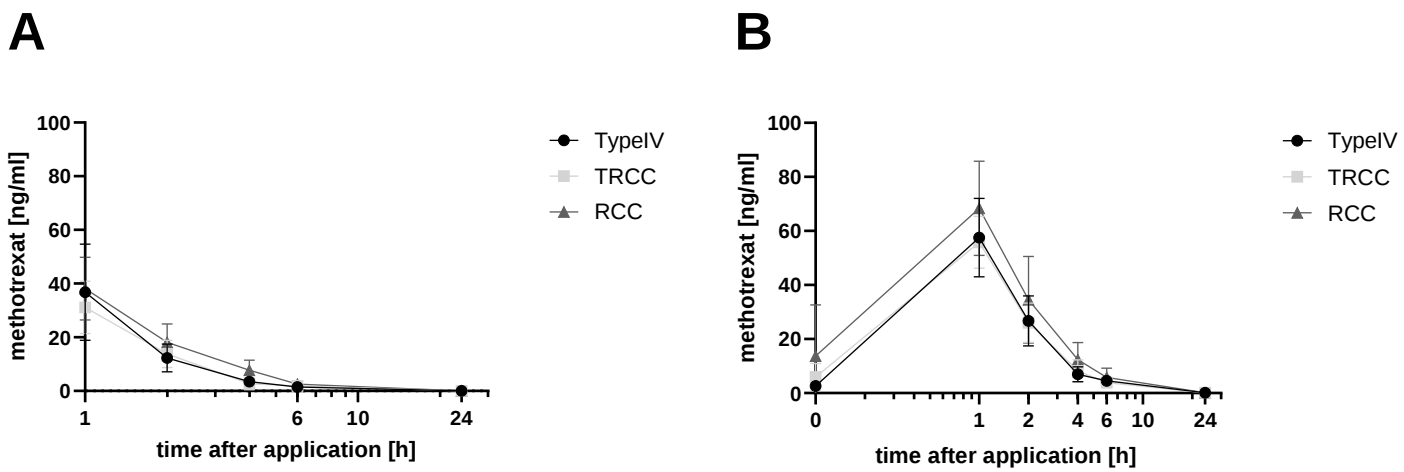
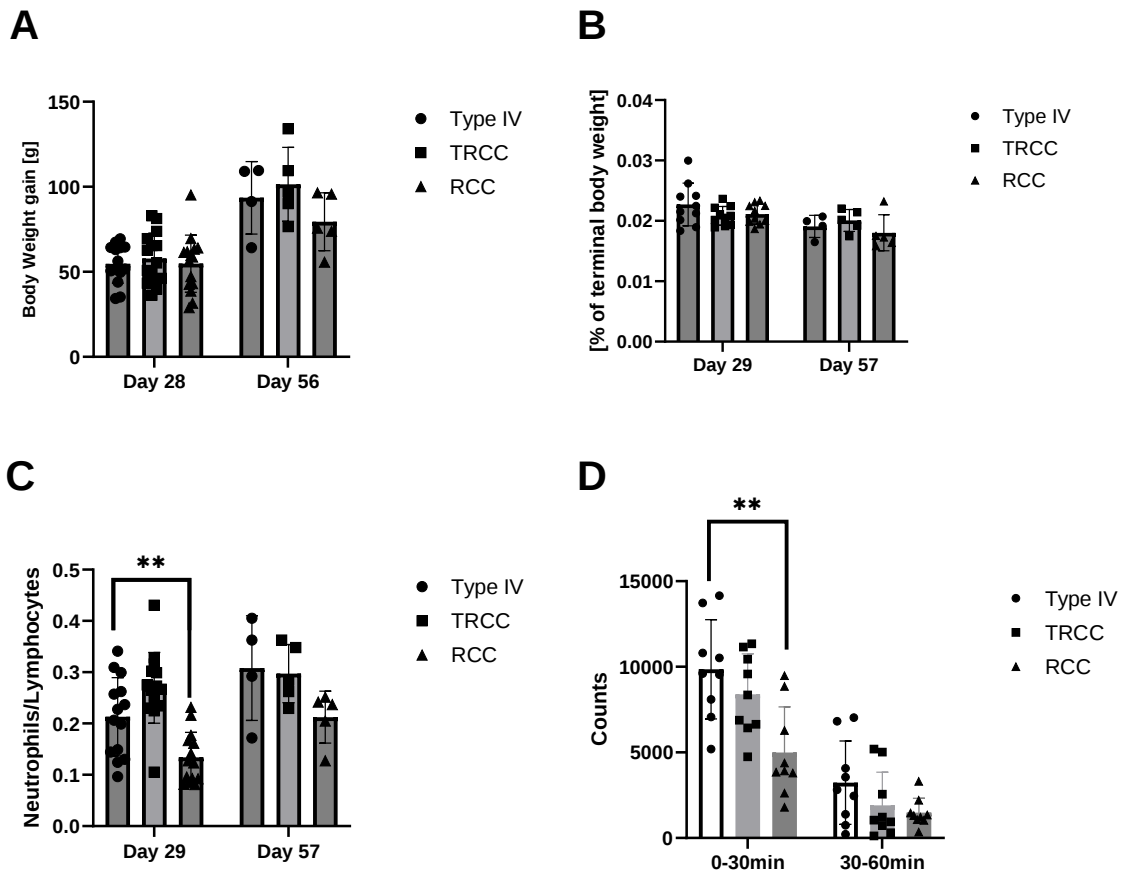
Additional file 2: Supplementary Plot 1



Supplementary Plot 1. Toxicokinetic evaluation on days 1 and 28.

Methotrexate serum concentration-time profiles on day 1 (A) and day 28 (B). Data are presented as mean ± SD (n = 10 per group).

Additional file 2: Supplementary Plot 2



Supplementary Plot 2. Animal welfare indicators across housing systems in methotrexate-treated animals. (A) Body-weight gain relative to study day 1 on study day 28 (Type IV: n = 14; TRCC and RCC: n = 15 per group) and study day 56 (Type IV: n = 4; TRCC and RCC: n = 5 per group). **(B)** Relative adrenal gland weight on study day 29 (n = 10 per group) and study day 57 (Type IV: n = 4; TRCC and RCC: n = 5 per group). **(C)** Ratio of absolute neutrophil and lymphocyte counts on study day 29 (Type IV: n = 14; TRCC and RCC: n = 15 per group) and study day 57 (Type IV: n = 4; TRCC and RCC: n = 5 per group). **(D)** Laser-beam counts indicating exploratory locomotor activity during the 0–30 min and 30–60 min intervals (n = 9 per group). Panels A–D show means ± SD and individual animal values. For each panel and time point, data were analyzed separately using one-way ANOVA followed by Dunnett's post hoc test, with the methotrexate-treated Type IV group as the reference. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.