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EFFECTS OF POLYSTYRENE MICROPLASTIC EXPOSURE ON GROWTH AND PHYSIOLOGICAL PARAMETERS IN JUVENILE WISTAR RATS

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ABSTRACT

Microplastics (MPs) represent an increasing environmental and biological concern, with their potential effects on early development and physiological homeostasis still not fully elucidated. The present study aimed to evaluate the effects of chronic juvenile exposure to 1 µm and 5 µm polystyrene microplastics (PS-MPs) on growth, organ indices, and endocrine profile in Wistar rats. A total of 36 juvenile Wistar rats (18 males and 18 females) were orally exposed to PS-MPs (0.1 mg/day) from postnatal day (PND) 21 to PND 61. Body weight dynamics were monitored throughout the experimental period, while at the end of exposure organ indices and serum levels of testosterone and estradiol were assessed. Exposure to PS-MPs was associated with delayed body weight gain, with more pronounced effects observed following exposure to 1 µm particles. Organ-specific and sex-dependent alterations were observed, predominantly affecting brain, lung, and spleen indices, as well as reproductive organs, with effects varying according to both sex and particle size. In addition, significant changes in serum sex hormone levels were detected, characterized by decreased testosterone in males and decreased estradiol in females. In conclusion, juvenile exposure to PS-MPs was associated with sex- and size-dependent alterations in growth dynamics, organ indices, and endocrine status in Wistar rats, suggesting potential systemic physiological effects of microplastics during early development.

Key words: polystyrene microplastics (PS-MPs), juvenile Wistar rats, growth dynamics, organ indices, sex hormones.

Introduction

Microplastics (MPs), defined as plastic particles smaller than 5 mm, are increasingly recognized as persistent environmental contaminants with important implications for animal health (Wright & Kelly, 2017). They originate either as primary MPs, intentionally manufactured for industrial and commercial applications, or as secondary MPs, formed through the degradation of larger plastic materials under mechanical and environmental processes (Boucher & Friot, 2017;

Roy *et al.*, 2022). Due to the extensive production, widespread use, and environmental persistence of plastic materials, MPs are now broadly distributed across terrestrial and aquatic ecosystems, resulting in continuous exposure of domestic, farm, laboratory, and wild animals.

MPs vary considerably in size, shape, and polymer composition, with fibers, fragments, and spherical particles being the most frequently reported forms. Among polymer types, polyethylene, polypropylene, and polystyrene are commonly detected in environmental and biological samples (Erni-Cassola *et al.*, 2019; Çebi & Uncumusaoğlu, 2024). Animals are exposed to MPs primarily through oral ingestion, as contaminated feed, water, and bedding materials represent major sources of intake in both natural and experimental settings (Carr *et al.*, 2016; Skilbeck, 2022; Maganti & Akkina, 2023). Following ingestion, MPs may cross biological barriers and accumulate in multiple organs, including the liver, kidneys, spleen, lungs, reproductive organs, and brain (Popa & Tăbăran, 2025). Experimental studies in mammalian models have demonstrated that MP exposure may disrupt physiological homeostasis, resulting in altered growth performance, metabolic disturbances, changes in liver and kidney function, inflammatory responses, and endocrine alterations, including disturbances in sex hormone regulation and reproductive function (Beyzaei *et al.*, 2025; Popa & Tăbăran, 2025). In addition, plastic-associated additives and adsorbed environmental contaminants may interfere with endocrine and immune regulation, thereby affecting growth, development, and reproductive function (Ullah *et al.*, 2023). The severity of these effects depends on multiple factors, including particle size, polymer type, concentration, exposure duration, and species-specific susceptibility. Despite the growing attention toward MP toxicity, limited information is available regarding the effects of MP exposure during critical periods of postnatal development. The juvenile period represents a particularly sensitive stage in mammalian life, characterized by rapid somatic growth, organ maturation, and metabolic programming. In laboratory rodents, the early post-weaning phase involves ongoing development of the gastrointestinal, immune, endocrine, and nervous systems, rendering animals more vulnerable to environmental stressors (Abramova *et al.*, 2021). Exposure to environmental contaminants during this developmental window may result in persistent alterations in growth performance, organ development, and physiological function (Ghasemi *et al.*, 2021).

From a veterinary and experimental toxicology perspective, parameters such as body weight gain, food and water intake, relative organ weights, and circulating sex hormone levels are fundamental indicators of growth, developmental status, endocrine function, and systemic toxicity (OECD, 2008). Therefore, the present study aimed to evaluate the effects of chronic juvenile exposure to differently sized polystyrene microplastics (PS-MPs) on growth dynamics, organ indices, and serum sex hormone levels in Wistar rats during the early post-weaning period. In addition, potential sex-dependent differences in the response to PS-MPs exposure were assessed. The findings may contribute to a better understanding of environmental risk factors for early postnatal development, endocrine regulation, and animal health following exposure to MPs.

Materials and Methods

Experimental animals

A total of 36 Wistar rats (18 males and 18 females), 21 days old, with initial body weights of 40–45 g (males) and 35–40 g (females) were used. Animals were housed under controlled environmental conditions (22 ± 1°C, 50 ± 5% humidity, 12 h light/dark cycle) with ad libitum access to

standard laboratory chow and drinking water. The study was conducted in accordance with Directive 2010/63/EU and approved by the institutional Ethics Committee (No. 425/24.02.2025).

Experimental design

Animals were randomly allocated into three experimental groups (n = 6 per sex per group): control (Co), 1 µm PS-MPs (G1), and 5 µm PS-MPs (G5). Red fluorescent spherical polystyrene microplastics (PS-MPs) with nominal sizes of 1 µm and 5 µm were obtained from MagSphere Inc. (Pasadena, CA, USA). According to the manufacturer's certificate of analysis, the particles were standardized and physicochemically characterized with respect to size, morphology, and fluorescence properties. Prior to administration, PS-MPs were suspended in purified drinking water and sonicated for 30 min to ensure homogeneous particle dispersion. Control animals received clean purified water. PS-MPs were administered via drinking water at a target dose of 0.1 mg/animal/day from postnatal day (PND) 21 to PND 61. The selected exposure protocol and dose were based on previously published experimental studies investigating the biological and reproductive effects of PS-MPs in rodents (Kanzova *et al.*, 2026a; Kanzova *et al.*, 2026b).

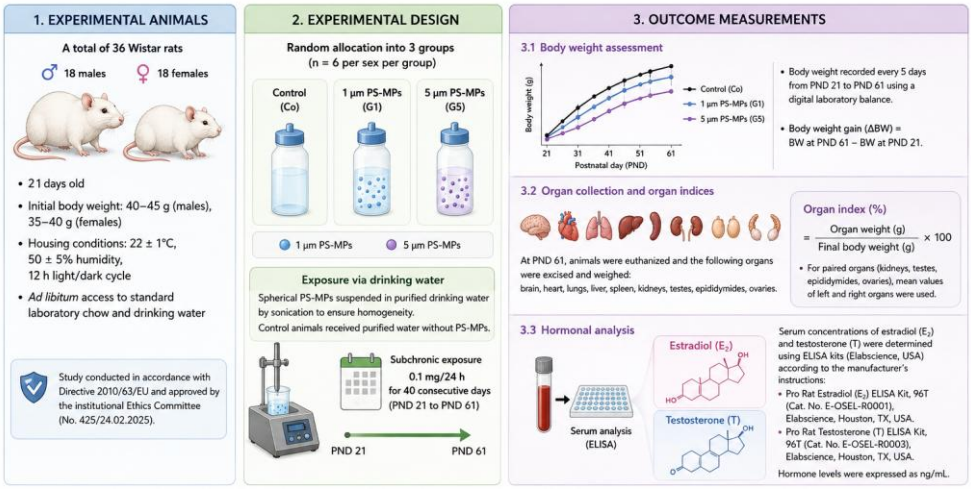


Figure 1: Schematic representation of the experimental design, exposure protocol, and outcome assessments.

Body weight assessment

Body weight was recorded every 5 days from PND 21 to PND 61 using a digital laboratory balance. Body weight at PND 61 was used for statistical analysis.

Organ collection and organ indices

At the end of the experimental period, animals were euthanized, and organs (brain, heart, lungs, liver, spleen, kidneys, testes, epididymides, and ovaries) were excised and weighed.

Organ indices were calculated using the formula:

$$\text{Organ index (\%)} = \frac{\text{Organ weight (g)}}{\text{Final body weight (g)}} \times 100$$

For paired organs (kidneys, testes, epididymides, ovaries), mean values of left and right organs were used.

Hormonal analysis

Serum concentrations of estradiol and testosterone were determined using ELISA kits (Elabscience, USA) according to the manufacturer's instructions:

- Pro Rat Estradiol (E2) ELISA Kit, 96T (Cat. No. E-OSEL-R0001), Elabscience, Houston, TX, USA.
- Pro Rat Testosterone (T) ELISA Kit, 96T (Cat. No. E-OSEL-R0003), Elabscience, Houston, TX, USA

Hormone levels were expressed as ng/dL

Statistical analysis

Data are presented as mean ± standard deviation (Mean ± SD), except for body weight dynamics, which are presented as mean ± standard error of the mean (Mean ± SEM). All endpoint data (final body weight, organ indices, and hormone levels measured at PND 61) were analyzed using two-way ANOVA with sex (male/female) and treatment group (control, 1 μm, 5 μm) as factors, followed by Tukey's post hoc test for multiple comparisons. Statistical significance was set at $p < 0.05$. In all tables and figures, intergroup comparisons are presented, with statistically significant differences indicated where applicable.

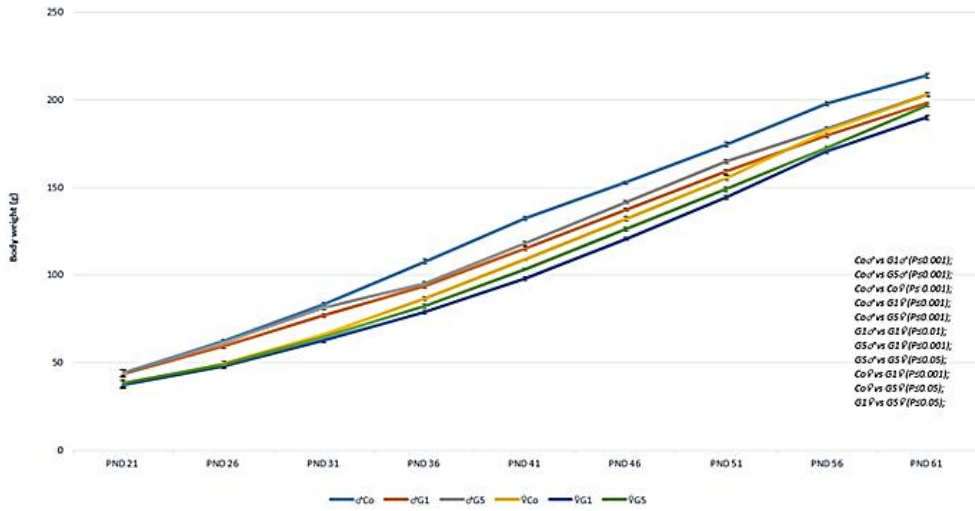
Results and Discussion

The increasing environmental contamination with MPs has raised significant scientific interest regarding their potential impact on growth and physiological development of organisms, particularly during the juvenile period, which is characterized by intensive somatic and functional maturation processes. The present study investigated the effects of PS-MPs with sizes of 1 μm and 5 μm on growth dynamics, organ indices, and sex hormone profiles in juvenile Wistar rats.

At the beginning of the experiment, all animals across the groups exhibited comparable body weights, with mean values of 43.96 ± 0.14 g in male rats and 37.66 ± 0.27 g in female rats. Monitoring of body weight from PND 21 to PND 61 revealed a progressive increase in all experimental groups throughout the observation period. As early as approximately PND 36, corresponding to the 15th day of exposure, a more pronounced reduction in growth rate was observed in the treated animals compared with their respective controls, and this trend persisted until the end of the experiment. Although statistical analysis revealed significant differences at PND 61 ($P \leq 0.001$), lower body weight values in the exposed groups were evident throughout nearly the entire experimental period (Figure 2).

In male animals, the lowest final body weight was observed in G1♂ (198.41 ± 1.36 g), followed by G5♂ (202.97 ± 1.26 g), compared with Co♂ (213.83 ± 1.90 g). A similar trend was observed in female animals, where final body weight values in G1♀ (189.94 ± 1.20 g) and G5♀ (196.86 ± 0.83 g) were lower than in Co♀ (203.16 ± 1.35 g). These findings are consistent with accumulating evidence in the literature indicating that exposure to MPs in juvenile animals may disrupt normal growth and developmental processes, leading to reduced growth rate and dysregulation of metabolic homeostasis (Lu *et al.*, 2018; Zhang *et al.*, 2024).

In addition, experimental studies suggest that MPs may induce inflammatory responses in the gastrointestinal tract, disrupt intestinal barrier integrity, and modulate mucin secretion, which has been proposed as a potential indirect mechanism affecting nutritional status and metabolic processes (Li *et al.*, 2020; Sun *et al.*, 2021).



Note: Data are presented as mean ± SEM. Statistically significant intergroup differences are indicated in the figure.

Figure 2: Body weight dynamics in male and female animals exposed to polystyrene microplastics (PS-MPs) during the period PND21–PND61.

At the end of the 40-day exposure period, analysis of organ indices revealed the presence of sex- and organ-specific differences among the investigated groups (Table 1). The most pronounced changes in male animals were observed in the brain index, with values in G1♂ (0.88 ± 0.04%) and G5♂ (0.85 ± 0.05%) being significantly higher compared with Co♂ (0.74 ± 0.06%) ($P \leq 0.01$ and $P \leq 0.05$). In addition to within-group differences, the brain index in Co♀ and G1♀ was also significantly higher compared with Co♂ ($P \leq 0.01$), suggesting the presence of sex-related differences.

For the heart index, limited but statistically significant differences were observed between specific groups, including G1♂ and G5♂ ($P \leq 0.01$), indicating a possible size-dependent response. The lung index showed the most pronounced alterations in female animals, with G5♀ (0.62 ± 0.05%) exhibiting significantly lower values compared with all other groups ($P \leq 0.001$). In male animals, a reduction was also observed in G1♂ compared with Co♂ ($P \leq 0.001$).

The liver and kidney indices did not show statistically significant intergroup differences. In contrast, the spleen index in exposed male groups was significantly reduced compared with Co♂, with the most pronounced effect observed in G5♂ (0.33 ± 0.02%). In addition, the spleen index in both control and exposed female groups also differed significantly from Co♂, further highlighting the presence of sex-specific differences.

Regarding reproductive organs, a statistically significant increase in the testicular index was observed in G1♂ (0.57 ± 0.05%) compared with Co♂ (0.46 ± 0.03%) ($P \leq 0.01$), whereas the epididymis and ovary indices did not show significant intergroup differences.

Table 1: Organ indices (% of body weight) in control and microplastic-exposed Wistar rats

Group	n	Brain	Heart	Lungs	Liver	Spleen	Kidneys (paired)	Testes (paired)	Epididymis (paired)	Ovaries (paired)
♂Co	6	0.74 ± 0.06	0.41 ± 0.04	1.02 ± 0.09	5.29 ± 0.37	0.55 ± 0.05	0.50 ± 0.02	0.46 ± 0.03	0.11 ± 0.03	–
♂G1	6	0.88 ± 0.04	0.38 ± 0.02	0.82 ± 0.07	5.01 ± 0.59	0.41 ± 0.03	0.47 ± 0.04	0.57 ± 0.05	0.10 ± 0.01	–
♂G5	6	0.85 ± 0.05	0.45 ± 0.03	0.93 ± 0.11	4.91 ± 0.13	0.33 ± 0.02	0.48 ± 0.04	0.51 ± 0.07	0.10 ± 0.01	–
♀Co	6	0.88 ± 0.04	0.46 ± 0.03	0.92 ± 0.05	5.29 ± 0.15	0.45 ± 0.04	0.46 ± 0.07	–	–	0.13 ± 0.22
♀G1	6	0.88 ± 0.08	0.42 ± 0.02	0.92 ± 0.20	5.07 ± 0.06	0.39 ± 0.07	0.48 ± 0.06	–	–	0.04 ± 0.01
♀G5	6	0.83 ± 0.06	0.42 ± 0.03	0.62 ± 0.05	5.24 ± 0.26	0.39 ± 0.01	0.47 ± 0.08	–	–	0.05 ± 0.01
Statistically Significant Difference		*Co♂ vs G1♂ (P≤0.01); Co♂ vs G5♂ (P≤0.05); Co♂ vs Co♀ (P≤ 0.01); Co♂ vs G1♀ (P≤0.01);*	*G1♂ vs G5♂ (P≤0.01); G1♂ vs Co♀ (P≤0.001); Co♀ vs G1♀ (P≤0.01); Co♀ vs G1♀ (P≤0.001);*	*Co♂ vs G1♂ (P≤0.001); Co♂ vs G5♀ (P≤0.001); G1♂ vs G5♀ (P≤0.001); G5♂ vs G5♀ (P≤0.001); Co♀ vs G5♀ (P≤0.001); G1♀ vs G5♀ (P≤0.001);*	*ns*	*Co♂ vs G1♂ (P≤0.001); Co♂ vs G5♂ (P≤0.001); Co♂ vs Co♀ (P≤ 0.01); Co♂ vs G1♀ (P≤0.001); Co♂ vs G5♀ (P≤0.001); G1♂ vs G5♂ (P≤0.01); G5♂ vs Co♀ (P≤0.001);*	*ns*	*Co♂ vs G1♂ (P≤0.01);*	*ns*	*ns*

Notes: Organ index (%) = (organ weight / body weight) × 100. Brain, heart, lungs, liver, and spleen represent single organs. Paired organs (kidneys, testes, epididymis, ovaries) were calculated as the mean of both sides. Co = control, G1 = 1 µm MPs, G5 = 5 µm MPs, ♂ = male, ♀ = female. Statistically significant differences between groups are indicated in the table; ns indicates not significant.

The observed changes in organ indices suggest that PS-MP exposure induces organ-specific and sex-dependent physiological responses. The most pronounced alterations in the present study were observed in the brain, lung, and spleen indices, indicating differential sensitivity of individual organs to the exposure. Similar effects have been reported in other experimental models, where PS-MP accumulation in various organs was associated with oxidative stress, metabolic disturbances, and changes in organ indices (Deng *et al.*, 2017). The observed sex-related differences are consistent with evidence indicating that the toxicological effects of PS- MPs may be both size-dependent and sex-specific, with smaller particles exhibiting a higher potential for tissue penetration and accumulation (Zhang *et al.*, 2024). In addition, MPs have been linked to alterations in the gut microbiota, induction of inflammatory processes, and disruption of intestinal barrier function, which may indirectly contribute to the observed metabolic and organ-level changes (Li *et al.*, 2020).

Exposure to PS-MPs during the early postnatal period was associated with alterations in the hormonal profile in both sexes. In male animals, a statistically significant decrease in serum testosterone levels was observed in the exposed groups G1♂ (3.02 ± 0.46 ng/dL) and G5♂ (3.28 ± 0.42 ng/dL) compared with Co♂ (4.63 ± 0.31 ng/dL) ($P \leq 0.001$). In female animals, the exposed groups showed lower estradiol levels compared with the control group, with the most pronounced reduction observed in G1♀ (3.85 ± 0.27 ng/dL) compared with Co♀ (4.68 ± 0.30 ng/dL) ($P \leq 0.001$). In addition, statistically significant differences were identified between male and female groups, suggesting a sex-specific endocrine response to the exposure.

The observed hormonal changes are consistent with previous experimental studies reporting impaired reproductive function and disruption of sex steroid balance following PS-MP exposure. Reduced testosterone levels and adverse effects on the male reproductive system have also been documented in other experimental models (Hou *et al.*, 2021; Jin *et al.*, 2021). At the same time, alterations in the female hormonal profile, including decreased estradiol and progesterone levels, as well as changes in morphology and receptor expression in the reproductive system, have been reported following MP exposure in female animals (Wang *et al.*, 2023; Amran *et al.*, 2023).

Table 2: Effects of polystyrene microplastic (PS-MP) exposure on serum testosterone and estradiol levels in male and female Wistar rats

Group	Parameters			
	Testosterone (ng/dL)	Estradiole (ng/dL)	*Testosterone (p-values)*	*Estradiole (p-values)*
Co♂	4.63±0.31	3.86±0.36	*Co♂ vs G1♂ (P≤0.001); Co♂ vs G5 (P≤0.001); Co♂ vs Co♀ (P≤ 0.001); Co♂ vs G1♀ (P≤0.001); Co♂ vs G5♀ (P≤0.001); G1♂ vs Co♀ (P≤0.001); G1♂ vs G1♀ (P≤0.001); G1♂ vs G5♀ (P≤0.001); G5♂ vs Co♀ (P≤0.001); G5♂ vs G1♀ (P≤0.001); G5♂ vs G5♀ (P≤0.001); Co♀ vs G1♀ (P≤0.01); Co♀ vs G5♀ (P≤0.001);*	*Co♂ vs G1♂ (P≤0.01); Co♂ vs Co♀ (P≤ 0.001); G1♂ vs G1♀ (P≤0.01); G5♂ vs Co♀ (P≤0.01); Co♀ vs G1♀ (P≤0.001); Co♂ vs G2 (P≤0.01);*
G1 ♂	3.02±0.46	4.45±0.36		
C5 ♂	3.28±0.42	4.02±0.25		
Co♀	1.18±0.24	4.68±0.30		
G1♀	1.92±0.35	3.85±0.27		
C5♀	1.50±0.26	4.07±0.18		

Note: Data are presented as mean ± SD. Statistically significant differences are indicated in the table.

In conclusion, juvenile exposure to PS-MPs was associated with sex- and size-dependent alterations in growth dynamics, organ indices, and endocrine parameters in Wistar rats, as reflected by changes in body weight development, organ indices, and serum sex hormone levels. These findings suggest that particle size and sex may modulate the biological response to microplastic exposure during early developmental stages, highlighting the need for further investigation of their long-term physiological effects.

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