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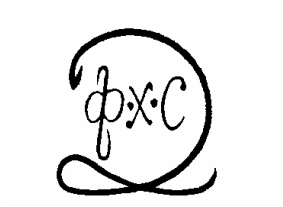

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DOES EXPOSURE TO A SINGLE DOSE OF MICROPLASTIC REPRESENTS A HEALTH RISK?

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ABSTRACT
Worldwide pollution with plastic debris represents tremendous environmental issue. Small particles originated from plastic bottles exert various effects in organisms when exposed chronically, while the effects of a single exposure are completely unknown. Thus, to test their potential health impact, male Wistar rats were exposed by oral gavage to a single dose of microplastic particles (MP) derived from polyethylene terephthalate (PET) bottles (1.4, 35 or 125 mg/kg with median diameter of 85 μm). Food and water intakes were monitored, and neurological and clinical tests were conducted. Obtained results point to lower food and water intakes in groups that received two higher MP doses indicating to interference with normal digestion. None of three used MP doses provoked neurological and clinical impairments either due to short-term exposure and/or lack of MP cumulative effect. Overall, presented results indicate that exposure to a single dose of MP can initiate health issues.

INTRODUCTION
Even though the environmental pollutants are recognized as a major threat to a human health, little is achieved in global reduction of their omnipresence. One of these hazardous materials is biochemically inert polyethylene terephthalate (PET), widely used due to its low-cost production and long durability. Under certain conditions, PET can be fragmented into smaller pieces, including microplastic particles (MP) that are smaller than 5 mm. It appears that particles less than 150 μm in diameter could penetrate tissues and accumulate in organs, including brain [1].

Numerous studies reported that fishes exposed to MP exert alterations in behaviour (such as hypoactivity, erratic movements, seizures), body weight and length along with histological changes of organs and tissues [2]. In contrast to the relatively abundant MP studies in aquatic organisms, only few of them have been conducted to investigate the impact of these particles in mammals. They revealed that exposure to MP results in their uptake and presence in the gut, liver and kidneys of the mice [3] and could lead to decrease in body, liver, and body fat weights, disorders in energy metabolism, inflammation and neurotoxicity [2].

Given that a behavioural disruptions (such as alterations regarding activity levels, potential anxiogenic effects, depression-like behaviours and altered cognitive functions) can arise as a consequence of organism's chronic exposure to small sized particles [1], it is of great importance to test whether a single ingested dose of MP, that correlates to daily ingested dose in humans [4], could impact victuals consumption, sensory-motor functions and factors of acute toxicity in rats. The parameters of interest were assessed 24 h after a single MP exposure to provide new insights of potential health risks upon acute MP ingestion.

METHODS
Microplastic particles were produced by filing PET bottles of worldwide famous soft drink brand which were thoroughly washed with distilled and Milli-Q water. Produced MP sawdust was sifted through laboratory sieve with mesh size of 160 μm and MP images were captured by optical microscope B-500MET (Optika, Ponteranica, Italy). Furthermore, FTIR (ATR) measurements were

performed on Nicolet Nexus spectrometer (Thermo Fisher Scientific, Waltham, USA) to identify chemical composition of samples and Malvern Mastersizer laser diffraction particle size analyser (Malvern, Worcestershire, UK) was used for additional characterisation of MP.

Research procedures conducted on animals were approved by the Ethical Committee for the Use of Laboratory Animals of VINČA Institute of Nuclear Sciences - National Institute of the Republic of Serbia, University of Belgrade, Republic of Serbia (protocol authorization number 323-07-03460/2021-05) and were in accordance with European Communities Council Directive (2010/63/EU).

Young adult male *Wistar* rats (300–350 g, n = 4 per group) were randomly divided in five groups: intact animals, used as controls (I); rats that received 2,5 ml of Milli-Q (Q); rats that received MP in dose 1.4 mg/kg (P1)/35 mg/kg (P2)/125 mg/kg (P3). Microplastic particles were dispersed in 2.5 ml of Milli-Q and the dose was calculated by multiplying human dose to correction factor (Km, 6.2 for rats) [3], [4]. All treatments were applied in a single dose by oral gavage. The food and water intakes were measured 24 h following the treatments.

Serious of non-invasive neurological and clinical tests were conducted 24 h after the treatment to evaluate potential sensory-motor deficits and possible signs of acute toxicity [5], [6]. The neurological test included scoring of following sensory-motor parameters: consciousness (0–1 point), respiration (0–1), spontaneous activity (0–3), forepaw outstretching (0–2), climbing (0–1), visual placing (0–1), cage grasp (0–1), gait posture (0–2), geotaxis (0–1), hearing and pacing/circling (0–1). Total score of neurological tests was expressed as the sum of average scores of all investigated parameters and graded on the scale of 0 to 15. The clinical test assessed: agitation, convulsion, piloerection, sleepiness and lethargy. Parameters were valued as – (no effect), + (mild effect), ++ (moderate effect) and +++ (major effect). In pre-treatment control testing, none of the animals exhibited any physiological deficit. All assessments were performed by the researcher blinded to experimental setup.

Obtained data were analysed by One-way analysis of variance followed by Tukey's *posthoc* test using GraphPad Prism 6 Software (San Diego, USA). Results are presented as a percentage of the mean of the values in I group ± SEM (standard error of the mean). p value of 0.05 is considered significant.

RESULTS AND DISCUSSION

Beside magnitude of organism exposure to small size particles, a variety of factors could influence their neurotoxicity and it appears that it may be also strongly affected by particle characteristics such as size and shape.

Images obtained by the optical microscope revealed that MP are of irregular shape with higher aspect ratios (whiskers, bundles of whiskers) (**Figure 1A**). Compared with data, FTIR spectra confirmed that samples are PET MP and laser diffraction analysis showed that specimens are composed of MP with median diameter of 85 μm (d10 = 35 μm and d90 = 170 μm) (**Figure 1B**).

Chemically inert MP larger than 150 μm, most likely, when ingested cannot be absorbed by surrounding tissues. Before reaching the intestinal epithelium, MP pass through different compartments of the gastrointestinal tract which might affect MP surface parameters and physicochemical properties. However, the particles' stability minimizes the possibilities of enzymatic or chemical MP degradation and probably, no major MP decomposition occurs during ingestion. Nonetheless, it is considered that particles less than 150 μm in diameter could be uptaken by epithelial lining of the gut by phagocytosis and further transported around the body by lymphatic and circulatory systems possibly reaching, among other tissues, the brain [1].

According to the obtained results, no change in any investigated parameter was observed between I and Q groups, indicating that oral gavage treatment *per se* does not affect investigated parameters. Comparing to I/Q groups, rats in P2 and P3 groups were consuming less food and water

($p < 0.05$ for P2 and $p < 0.01$ for P3, respectively) pointing out that MP treatment with higher doses decreased consumption of victuals (**Figure 2A** and **2B**). These findings might indicate that certain amount of MP was retained in digestive tract and animals had a false sense of satiation. Food and water intakes are usually positively correlated, and being highly controlled processes under the influence of hormones, enzymes and various regulatory mechanisms, their reduction in this study might point to health issues.

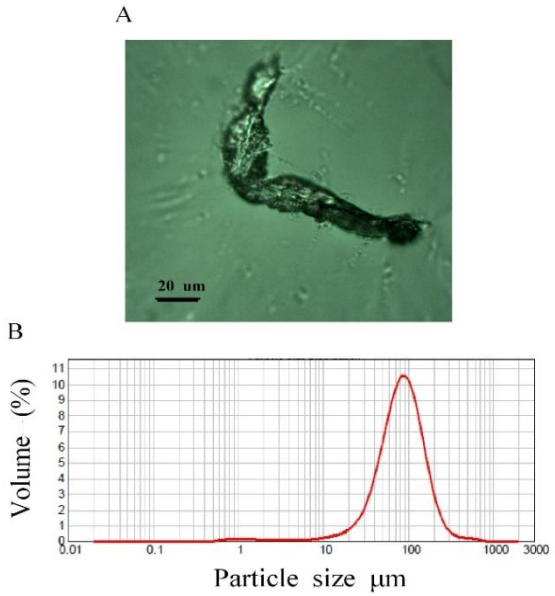

Figure 1. Microplastic particles characterisation **A)** Specimens are of irregular shape with higher aspect ratios (whiskers, bundles of whiskers) **B)** Specimens are composed of microplastic particles with median diameter of 85 µm (d10 = 35 µm and d90=170 µm).

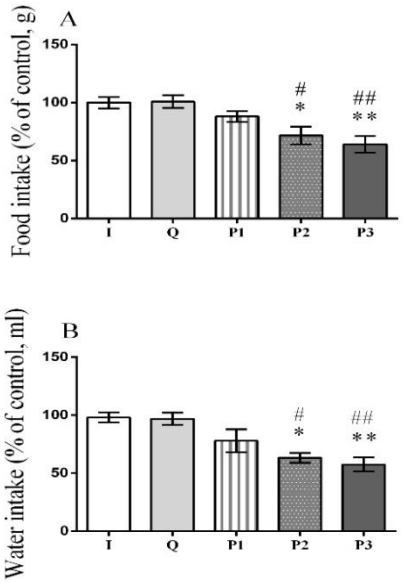

Figure 2. Food and water intake of rats 24 h after receiving single dose of microplastic **A)** Food intake **B)** Water intake. Data are presented as % of control ± SEM of n = 4 animals per group. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test (*$p < 0.05$, **$p < 0.01$ P2/ P3 vs I and $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ P2/P3 vs Q).

Considering the size of particles used in this study, there might be a possibility that at least some of the MP were uptaken and transported within the body, reaching various organs and tissues. Thus, batteries of relevant neurological and clinical tests were used to examine whether an acute MP exposure represents a health risk. Although results of neurological testing revealed subtle decrement of sensory-motor functions in groups that received MP comparing to I and Q groups, these changes were not sufficient to make statistical difference (**Table 1**). Moreover, no changes were detected among these 5 groups regarding clinical scoring, either (**Table 1**) since rats were not anxious, sleepy, lethargic nor displayed sudden, irregular movements. Observed lack of MP impact on neurological and clinical scoring could be attributed to acute exposure of rats to MP and absence of cumulative effect. However, taking all results into account, there is a possibility that proposed MP specimens might be transported throughout the body inducing health issues. Thus, additional investigations are needed to elucidate mechanism of MP acting in mammals with emphasis on clarification whether and by what terms single dose of MP provokes health issues.

Table 1. Results of neurological (assessed by alterations in sensory-motor functions) and clinical tests scoring 24 h after treatments

Experimental group/test	**I**	**Q**	**P1**	**P2**	**P3**
Sensory- motor functions	100 ± 0.25	100 ± 0.25	98.31 ± 0.29	96.6 ± 0.48	94.92 ± 0.71
Clinical signs	-	-	-	-	-

CONCLUSION

Since MP have pervaded the ecosystems, exposure to these particles and their accumulation will probably only increase with time. Although acute MP exposure had no effect on sensory-motor functions and did not induced acute toxicity, due to reduced water and food intakes that are reported in current study, it can be assumed that MP might lead to health issues. Further research regarding pathologic mechanisms at cellular and tissue levels, as well as on the long-term effects of tissue accumulation, are necessary.

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