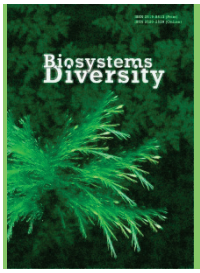

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The effect of polystyrene foam in different doses on the blood parameters and relative mass of internal organs of white mice

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Due to their durability, versatility and economy, plastic products are widely used in all spheres of human life. Despite the inertness of polymers, recent studies show the ability of microplastic to overcome natural tissue barriers, accumulate in the animal's body, affect metabolism and change the intestinal microbiota, negatively affecting it. In a 42-day experiment, changes in the internal organs' relative mass, blood biochemical and morphological parameters of white mice were established under the influence of different doses of polystyrene foam in their diet. Four groups of white mice consumed crushed polystyrene foam particles (10%, 1% and 0.1% by weight of the feed, control group without the addition of polystyrene foam). At the end of the experiment, the morphofunctional state of the internal organs was determined by the organ mass index and blood biochemical parameters. Adding crushed polystyrene foam to the feed in an amount of 1% causes a significant decrease in the mass index of the heart and stomach, 10% – only the heart, and 0.1% – does not affect this indicator. Polystyrene foam had a significant effect on blood biochemical parameters, regardless of the dose, causing an increase in the activity of aspartate aminotransferase against the background of a decrease in the activity of alkaline phosphatase. The content of total bilirubin, urea, urea nitrogen and cholesterol decreased, and the concentration of creatinine and total protein increased (due to the albumin fraction). The use of crushed polystyrene foam in mice did not cause significant changes in the blood morphological composition, except for a dose-dependent increase in the number of monocytes. In the future, it is planned to determine histological, histochemical and immunohistochemical changes in the organs of laboratory animals under the influence of plastic in a laboratory experiment.

Keywords: microplastic; organ mass index; blood biochemical parameters; albumins; globulins; blood enzymatic activity.

Introduction

Polystyrene foam is a lightweight synthetic material formed by foaming polystyrene under pressure, consisting of hydrogen and carbon atoms. This material was synthesized in 1951 in Germany. Since then it has been widely used in many areas of human life, in particular in construction as a heater. Whereas the raw material for its production is ordinary polystyrene, it was believed that polystyrene foam was also safe for humans. However, studies in recent years show that due to the widespread use of various types of plastic, a significant part of it ends up in trash. In the period 1951–2018, about 6.3 billion tons of plastic were produced, of which only 9% was recycled, 12% burned, and the vast majority ended up in the environment. It takes a long time for complete plastic decomposition to take place, so its particles enter the food chains of various biosystems, in particular, in fresh and marine waters. Particularly dangerous in this regard are microplastics, a new type of environmental pollution consisting of particles less than 5 mm in diameter (Moore, 2008; Barnes et al., 2009).

Microplastic pollution is considered the second most important scientific problem in ecology, along with the problem of climate change and the thinning of the atmosphere's ozone layer (Amaral-Zettler et al., 2015). Aquatic biological resources are the most contaminated with microplastics: oceans, seas, inland lakes, and even polar regions (Moore, 2008; Engler, 2012; Obbard et al., 2014; Eerkes-Medrano et al., 2015). Small plastic fragments in the ocean are likely to sink to the bottom (Barnes et al., 2009; Woodall et al., 2014), while non-sinking large particles end up on the coastline, where a number of processes (UV-radiation, temperature changes, microbial degradation, abrasion and leaching of plasticizers) are subject to degradation and, as a result of wind and wave-erosion, increase the impact of microplastics on ecosystems both in water and on land (Andrady et al., 2003; Andrady, 2011, 2017; Urbanek et al., 2018). Recent studies show that the distribution of microplastics in soils is constantly increasing, and this is becoming a new threat to terrestrial ecosystems (Horton et al., 2017). Entering the ground, plastic particles have a direct impact on the animals living there. Walton et al. (2017) found the toxicity of polyvinyl chloride (PVC) particles of different sizes to *Enchytraeus crypticus* Westheide & Graefe, 1992, which was manifested by a decrease in their ability to survive and reproduce (Lahive et al., 2019).

The danger of plastic to aquatic organisms is that the size of its microparticles can be similar to the size of the food of many aquatic organisms, and they can consume it instead of their regular food (Cole et al., 2013; Reilly et al., 2017; Steer et al., 2017). This is dangerous since plastic particles have no nutritional value, can cause traumatic damage to tissues and organs, have a toxic effect, cause internal damage (block the movement of food through the intestines), reduce the enzymatic activity of the digestive glands, cause oxidative stress, and reduce the body growth rate and even affect reproductive function (Wright et al., 2013; Jeong et al., 2016; Sussarellu et al., 2016; Jovanović, 2017; Rodriguez-Seijo et al., 2017). As a result of the ingestion of plastic micro- and nanoparticles, they penetrate into some tissues and the circulatory system (Avio et al., 2015; Grigorakis et al., 2017; Jabeen et al., 2018). In this regard, thorough research is needed on the impact of microplastics on the animals' bodies, because they can affect human health through various food chains. Since aquatic animals, in particular shellfish and fish, are often eaten by humans, this definitely poses a threat to our health. Therefore, the study of the influence of different types of plastic on the bodies of mammals, and their metabolic processes becomes an urgent task. There is also a report in the literature about the absence of any negative effect of microplastics on aquatic animals, a conclusion about this was made based on the analysis of results of some toxic biomarkers (Kaposi et al., 2014).

Not only the plastic itself but also the substances that are used during its manufacture or as a result of its decomposition (biodegradation) can pose a danger to human health. Polystyrene itself in its pure form is inert and non-toxic. In its manufacture, harmful substances are used (stabilizers, salts of heavy metals, technological impurities, etc.). Therefore, when released into the environment, these substances can pose a danger to living organisms and humans (Anon, 2007; Koch & Calafat, 2009). Polymer additives such as phthalates, bisphenol A, brominated antipyrines, triclosan, and organotin compounds can enter various body tissues and pose health risks (Moriyama et al., 2002; Moore, 2008; Andrady, 2011; Muncke, 2011; Engler, 2012; Pan et al., 2021).

Talsness et al. (2009), Halden, (2010) and Proshad et al. (2018) report the ability of phthalates to act as antiandrogens; Bisphenol A (BPA) has estrogen-like activity. Polybrominated diphenyl ether (PBDE) and tetrabromobisphenol A (TBBPA) are capable of disrupting thyroid hormone homeostasis, and PBDEs also exhibit antiandrogenic activity.

Styrene is a polystyrene component that poses a danger to human life and the environment. It can penetrate food from dishes made of polystyrene. It was determined that volatile styrene monomers were found in eggshells after two weeks of storage in polystyrene containers in supermarkets. The content of ethylbenzene and styrene was seven times higher in dishes made from such eggs compared to foods prepared using fresh eggs (Matiella & Hsieh, 1991).

Because polystyrene food utensils are ubiquitous, traces of styrene have been found in 100% of human tissue samples and 100% of breast milk samples tested. Long-term exposure to small amounts of styrene can affect the central nervous system, gastrointestinal tract, blood formation, allergic, cytogenetic (chromosomal and lymphatic abnormalities) and carcinogenic manifestations (Santos-Burgoa et al., 1992; Galloway, 2015; Farrelly & Shaw, 2017).

The ways in which plastic particles enter the tissues of living organisms and their subsequent migration have been studied relatively well, especially in aquatic animals. Microplastic causes histological damage to various intestinal tissues (Paul-Pont et al., 2016; Rodriguez-Seijo et al., 2017), and causes physical injuries in fish (Pedà et al., 2016; Jovanović, 2017). Various aspects of penetration, distribution and influence on the metabolic processes of the mammalian organism have not been studied enough. There are separate reports that in laboratory animals, polystyrene particles caused intestinal microbiota dysbiosis and impaired lipid metabolism in the liver (Lu et al., 2018), reduced mucus secretion in the intestine, thereby disrupting the function of the intestinal barrier (Jin et al., 2019). Also, by using fluorescent microplastic particles of different sizes, the distribution, accumulation, and tissue specificity of these substances in the body of laboratory mice were determined (Deng et al., 2017).

The purpose of our study is to establish the effect of different doses of crushed polystyrene foam in the diet of white miceon the organ mass index and blood biochemical parameters in a laboratory experiment.

Materials and methods

The research protocol was agreed upon with the ethical local committee of Dnipro State Agrarian and Economic University, and the experiment itself was carried out on the basis of a veterinary clinic and laboratories of this university. All procedures on animals were carried out in accordance with international recommendations and the national legislation of Ukraine for the humane treatment of vertebrate animals. To withdraw animals from the experiment, ether was used for anaesthesia, introducing animals into a state of sleep in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental or other Scientific Purposes (Strasbourg, 1986; Kyiv, 2001) and guided by the requirements of the Law of Ukraine No. 3447-IV dated February 21, 2006 "On protection of animals from cruel treatment".

For the experiment, 24 white laboratory mice, aged 3 weeks, with an average weight of 50 g, were divided into four groups of 6 animals in each. Within 10 days before the experiment, the animals were adapted to the upkeep and diet. The control group consumed a standard diet balanced for key nutrients (Table 1).

In the experimental groups, different amounts of crushed polystyrene foam were added to the standard diet – 0.1%, 1.0% and 10.0% by weight of the feed (Fig. 1). The grain mixture and crackers were ground in a mill to the state of flour, other dry components of the diet were added and granules were formed. Green grass and carrots were given separately. Animals had unlimited access to food and water.

Table 1
Composition of the experimental animals' diet

Product	Quantity, g
Grain mixture (wheat : barley : corn – 3 : 1 : 1)	5.0
Wheat bread (crackers)	1.3
Oatmeal	2.0
Powdered milk	2.0–4.0
Fishmeal	0.2
Feed yeast	0.1
Bone meal	0.2
Green Grass	2.0
Root crops (carrots)	2.0

During the experiment, the animals' general condition was observed, and the amount of consumed food and water was taken into account. Blood sampling from the carotid artery was performed by decapitating the animals for further biochemical and morphological studies (Lieshchova et al., 2018, 2020). Biochemical blood tests included: the determination of total protein – by the biuret method, globulins and protein coefficient – were calculated, and albumins – by reaction with bromocresol green. To determine the activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), a kinetic method based on the Warburg optical test was used, for alkaline phosphatase (AP) – enzymatic with p-nitrophenyl phosphate (pNPP), and for glucose – glucose oxidase methods were used. Total bilirubin and urea were determined enzymatically with uricase using an automatic biochemical analyser Miura 200 (I.S.E. Srl, Rome, Italy), and High Technology kits of reagents (High Technology Inc., North Attleborough, MA, USA), PZ Cormay S.A. (Cormay Diagnostics, Lublin, Poland) and Spinreact S.A. (Spinreact, Girona, Spain). Determination of the erythrocytes and leukocyte count in the stabilized mice blood was carried out in an automatical haematology analyser BC-2800Vet (Mindray, Shenzhen, China). For the leukogram, blood smears were prepared according to Pappenheim, followed by their staining according to Romanovsky-Giemsa (Bilan et al., 2019; Brygadyrenko et al., 2019).

Internal organs (heart, liver, lungs, spleen, stomach, small intestine, kidneys, testis) were taken from white mice by anatomical preparation. Their absolute mass was determined by weighing them with an AB224 (Metrinco, China, 2021) analytical balance with an accuracy of 0.0001, followed by the determination of the organ mass index.

Data analysis was carried out using the Statistica 6.0 program (StatSoft Inc., USA). The data are given in the tables as $x \pm SD$. Differences between the values in the control and study groups were determined using the Tukey test, where the differences were considered significant at $P < 0.05$ (adjusted for Bonferroni).

Results

The addition of polystyrene foam to the diet in the amount of 1% and 10% by weight of the feed caused a tendency to decrease in the relative mass of the heart (up to 84.9% and 84.6% of the level of the control group, respectively). Also, the addition of polystyrene foam in the amount of 1% and 10% caused a tendency to decrease the relative mass of the stomach (up to 73.7% and 82.0%, respectively, Table 2).

More pronounced changes occurred in the biochemical composition of the animals' blood (Table 3). The supplementation of the diet of mice with polystyrene foam in the amount of 1% and 10% by weight of the feed led to a slight increase in the total protein content above the physiological norm (up to the level of 108.1% and 110.7% of the control). Interestingly, even the lowest studied concentration, 0.1% polystyrene foam in the diet, significantly (above the physiological norm) increased the albumin concentration in the blood: to the level of 167.5%, 183.1%, and 181.9% of the physiological norm for 0.1%, 1% i 10% of the feed mass, respectively. This led to a doubling of the protein coefficient in relation to the physiological norm. Under the influence of polystyrene foam, nitrogen metabolism in the mice body was changed: in the experimental groups, in

relation to the control, the concentration of creatinine doubled and the concentration of urea and urea nitrogen decreased by two to four times.

Due to the activity of aspartate aminotransferase, the de Ritis ratio (AST/ALT) significantly increased in all three experimental groups up to 153.8–168.3% of this indicator in the control group. At the same time, the activity of alkaline phosphatase sharply decreased (to 29.8–40.3% of the level of the control group).

Under the influence of adding even the lowest dose of the studied polystyrene foam concentrations (0.1% of the feed mass) to the mice diet, the content of total bilirubin significantly decreased to a level of 29.5% of the control group level. The supplementation of polystyrene foam to the mice's diet also reduced the concentration of cholesterol in the blood up to 70.7–80.7% of the control group level in all three experimental groups of animals. Less pronounced changes were observed in the blood morphological composition (Table 4). Under the action of polystyrene foam, the content of platelets rose to 126.6–180.2% of the control group level in all experimental groups. At the same time, attention is drawn to a two to a three-times lower content of leukocytes. The leukocyte formula changed due to an increase in the number of band neutrophils in the blood of the animals of the experimental groups compared to the control group. It should be noted that these indicators did not go beyond the reference values of the norm.

Table 2

Relative mass (%) of internal organs of white mice during the polystyrene foam consumption (x ± SD, n = 6, duration of the experiment – 42 days)

Organ	Standard diet without polystyrene (control group)	Standard diet +0.1% crushed polystyrene	% relative to control group	Standard diet +1% crushed polystyrene	% relative to control group	Standard diet +10% crushed polystyrene	% relative to control group
Heart	0.652 ± 0.089^{a}	0.568 ± 0.073^{a}	87.1	0.554 ± 0.021^{b}	84.9	0.552 ± 0.071^{ab}	84.6
Liver	5.46 ± 0.49^{a}	6.40 ± 0.44^{a}	117.1	6.01 ± 0.31^{b}	110.1	6.43 ± 0.99^{b}	117.7
Lungs	0.695 ± 0.039^{a}	0.725 ± 0.079^{ab}	104.3	0.691 ± 0.065^{a}	99.5	0.862 ± 0.172^{b}	124.1
Spleen	0.337 ± 0.048^{a}	0.353 ± 0.165^{a}	104.5	0.310 ± 0.048^{a}	91.8	0.327 ± 0.087^{a}	97.1
Stomach	1.61 ± 0.15^{a}	1.57 ± 0.28^{ab}	97.8	1.19 ± 0.15^{b}	73.7	1.32 ± 0.17^{ab}	82.0
Small intestine	10.57 ± 1.04^{a}	9.58 ± 0.30^{a}	90.7	9.69 ± 0.72^{a}	91.6	8.88 ± 0.23^{a}	84.1
Right kidney	0.697 ± 0.084^{a}	0.738 ± 0.058^{a}	105.9	0.652 ± 0.073^{a}	93.6	0.604 ± 0.026^{a}	86.7
Left kidney	0.718 ± 0.078^{a}	0.714 ± 0.048^{a}	99.5	0.683 ± 0.077^{a}	95.1	0.624 ± 0.074^{a}	87.0
Testis	0.380 ± 0.051^{a}	0.347 ± 0.033^{a}	91.1	0.353 ± 0.025^{a}	92.7	0.368 ± 0.054^{a}	96.8

Note: different letters indicate values which reliably differed one from another within one line of table according to the results of comparison using Tukey test with Bonferroni correction.

Table 3

Blood biochemical parameters of white mice during consumption of polystyrene foam (x ± SD, n = 6, duration of the experiment – 42 days)

Parameters	Standard diet without polystyrene (control group)	Standard diet +0.1% crushed polystyrene	% relative to control group	Standard diet +1% crushed polystyrene	% relative to control group	Standard diet +10% crushed polystyrene	% relative to control group
Total protein, g/L	54.4 ± 2.2^{a}	53.4 ± 7.5^{a}	98.2	58.8 ± 2.6^{a}	108.1	60.2 ± 5.2^{ab}	110.7
Albumins, g/L	16.6 ± 1.2^{a}	27.8 ± 2.1^{b}	167.5	30.4 ± 1.4^{b}	183.1	30.2 ± 2.6^{b}	181.9
Globulins, g/L	37.8 ± 2.6^{a}	27.8 ± 1.5^{b}	73.5	30.4 ± 3.6^{ab}	80.4	30.0 ± 3.0^{ab}	79.4
Protein coefficient, U	0.440 ± 0.061^{a}	0.980 ± 0.040^{b}	222.7	1.060 ± 0.049^{b}	240.9	0.980 ± 0.040^{b}	222.7
Urea, mmol/L	11.90 ± 3.45^{a}	6.12 ± 1.81^{ab}	51.4	4.96 ± 0.51^{b}	41.7	5.92 ± 0.38^{b}	49.7
Blood urea nitrogen, mg/100 g	37.1 ± 10.2^{a}	11.7 ± 3.5^{b}	31.6	9.5 ± 1.0^{b}	25.6	11.4 ± 0.7^{b}	30.6
Creatinine, μmol/L	21.7 ± 2.5^{a}	42.2 ± 7.0^{b}	194.8	45.6 ± 2.4^{b}	210.5	45.4 ± 16.8^{b}	209.6
AST, U/L	118 ± 5^{a}	207 ± 42^{b}	175.4	170 ± 24^{b}	144.3	221 ± 49^{b}	187.4
ALT, U/L	58.6 ± 9.2^{a}	66.0 ± 13.2^{a}	112.6	49.8 ± 7.7^{a}	85.0	63.6 ± 11.0^{a}	108.5
De Ritis ratio (AST/ALT), U	2.08 ± 0.34^{a}	3.20 ± 0.60^{b}	153.8	3.44 ± 0.40^{b}	165.4	3.50 ± 0.62^{b}	168.3
Alkaline phosphatase, U/L	63.6 ± 6.4^{a}	25.7 ± 8.1^{b}	40.3	23.0 ± 3.9^{b}	36.2	19.0 ± 5.2^{b}	29.8
Total bilirubin, μmol/L	8.88 ± 0.35^{a}	2.62 ± 0.71^{b}	29.5	3.78 ± 0.97^{b}	42.6	2.52 ± 0.45^{b}	28.4
Cholesterol, mmol/L	2.80 ± 0.33^{a}	2.02 ± 0.34^{b}	72.1	2.26 ± 0.08^{b}	80.7	1.98 ± 0.23^{b}	70.7

Note: see Table 2.

Table 4

Blood morphological and functional parameters of white mice during the polystyrene supplementation (x ± SD, n = 6, duration – 42 days)

Parameter	Standard diet without polystyrene (control)	Standard diet +0.1% crushed polystyrene	% relative to control group	Standard diet +1% crushed polystyrene	% relative to control group	Standard diet +10% crushed polystyrene	% relative to control group
Hemoglobin, g/L	150.6 ± 5.9^{a}	135.2 ± 1.7^{ab}	89.8	156.6 ± 6.6^{ab}	104.0	142.0 ± 11.4^{ab}	94.3
Hematocrit, %	43.6 ± 2.7^{a}	44.4 ± 3.0^{ab}	101.9	51.5 ± 4.5^{ab}	118.1	47.2 ± 4.5^{ab}	108.3
Erythrocytes, 10^{12}/L	9.18 ± 0.33^{a}	7.83 ± 0.33^{ab}	85.3	9.49 ± 0.66^{ab}	103.3	8.44 ± 0.68^{ab}	91.9
Thrombocytes, 10^{9}/L	295 ± 68^{a}	503 ± 72^{b}	170.5	532 ± 67^{b}	180.2	374 ± 107^{ab}	126.6
Leukocytes, 10^{9}/L	10.76 ± 1.62^{a}	4.16 ± 0.10^{bc}	38.6	6.18 ± 1.20^{b}	57.4	3.26 ± 0.23^{c}	30.3
Leukocytic formula							
Eosinophils, %	0.00 ± 0.00^{a}	0.67 ± 0.52^{ab}	–	0.83 ± 0.41^{b}	–	0.67 ± 0.52^{ab}	–
Basophils, %	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	–	0.0 ± 0.0^{a}	–	0.0 ± 0.0^{a}	–
Neutrophils, %:							
– young	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	–	0.0 ± 0.0^{a}	–	0.0 ± 0.0^{a}	–
– band	0.83 ± 0.75^{a}	2.00 ± 0.63^{ab}	240.0	2.17 ± 0.75^{ab}	260.0	2.33 ± 0.82^{b}	280.0
– with segmented nuclei	23.0 ± 8.0^{ab}	21.8 ± 3.1^{ab}	94.8	26.8 ± 4.4^{a}	116.5	17.6 ± 3.9^{b}	76.5
Lymphocytes, %	75.8 ± 7.9^{a}	72.6 ± 3.1^{a}	95.8	63.4 ± 6.1^{a}	83.6	77.2 ± 4.1^{a}	101.8
Monocytes, %	0.33 ± 0.52^{a}	3.00 ± 0.63^{b}	900.0	6.83 ± 3.06^{b}	2050.0	3.17 ± 1.60^{b}	950.0

Note: see Table 2.

Noteworthy is the sharp increase in the number of monocytes in the blood of animals of the experimental groups compared with the control. Moreover, when adding 0.1% and 10% polystyrene foam to the diet, this indicator increased by 900% and 950% compared with the control, reaching and even slightly exceeding the upper limit of the reference range. The addition of 1% crushed polystyrene foam to the feed caused a sharp increase in the number of monocytes by 20–50% relative to the control group, which is almost 2.3 times higher than the standard values for this animal species.

Discussion

Certainly, plastic, especially its small particles, is potentially dangerous for the mammalian organism, since it can affect them both directly and through symbiotic microorganisms (Lu et al., 2018; Jin et al., 2019). The danger of microplastic for the body is associated with its potentially harmful effects due to the formation of reactive oxygen species and the development of oxidative stress and inflammation (Jeong & Choi, 2019).

The main routes of plastic entry into the body of an animal are respiratory and through the digestive system. Entering the body, plastic causes mechanical damage with the subsequent development of inflammation. Often, microplastics, adsorbing various substances and pathogenic microorganisms, transfer them to the internal environment of the macroorganism, thereby contributing to the toxic effects and even the occurrence of infections (Prata et al., 2021). In a laboratory experiment on *Eisenia andrei* (Bouche, 1972), when exposed to various concentrations of microplastics for 28 days, no significant effect on survival, the number of young animals, and the final weight of adults was found. However, histopathological analysis of the earthworms' intestines indicated damage to their tissues and an increase in immune system responses (Rodriguez-Seijo et al., 2017).

Microparticles of polyvinyl chloride, polystyrene and polyethylene terephthalate (PET) are most commonly found in wild animals tissues (Haave et al., 2021), while domestic animals are exposed to PET and polycarbonate (PC), which are more commonly found in their feces (Zhang et al., 2019). Microplastics have been found in the digestive system of mollusks (Reguera et al., 2019), fish (Neves et al., 2015), birds (Carlin et al., 2020; Hoang & Mitten, 2022), and marine mammals (Nelms et al., 2019). The fact that plastic microparticles overcome natural tissue barriers and enter the internal environment is indicated by their detection in animal organs, for example, in the liver of fish (Su et al., 2019), the stomach, intestines, liver, muscles of wild animals (otters, birds, fish) living in the coastal regions of Norway (Haave et al., 2021), as well as in the kidneys, liver, lungs, intestines and blood clots of domestic animals (cats, dogs) (Prata et al., 2022).

Analyzing the change in the stomach's relative mass, we see that when 1% and 10% of crushed polystyrene foam were added to the diet, the stomach's relative mass was significantly decreased. Since polystyrene foam added to the feed has no nutritional value and passes through the gastrointestinal tract in transit, this probably leads to a decrease in the synthesis of digestive juices and a rapid evacuation of the contents. When ingested with food, microplastics accumulate in the intestine, and their smallest particles can overcome the intestinal barrier and enter the circulatory system (Hussian et al., 2001). In the presence of diseases accompanied by damage to the intestinal tissue integrity, the transport of microplastics will be significantly higher due to changes in tissue permeability due to inflammation (Schmidt et al., 2013). Microplastics 1–20 μm in size have been found in blood clots of domestic cats. Most microplastic particles found in the tissues of the ileum, lungs, kidneys, and liver of domestic cats and dogs were 1–10 μm in size (Prata et al., 2022). There is an assumption that only microplastic particles smaller than 20 μm can enter the interstitial tissue of various organs through the vascular wall (FAO, 2017). Both small and medium-sized particles can accumulate in the kidneys and are more commonly found in the periglobular afferent arterioles found in dogs (Slack et al., 1981).

The transport mechanism of microplastics through the biological barriers of the intestine is known, and it depends on its size (FAO, 2017). The absorption process in the intestine occurs by paracellular transport, the size of which is limited by the mechanism of intercellular adhesion (Volkheimer, 1977; Fiorentino et al., 2015). Microparticles ≥100 μm are also known to enter the lymphatic system directly through Peyer's patches (Eldridge et al., 1990; FAO, 2017). Once in the circulatory system, plastic particles can accumulate in small capillaries, causing mechanical blockage and aggregation of blood cells (Yamaoka et al., 1993). In the future, they will somehow interact with phagocytes (Herzlinger et al., 1981). In our study, an increase in the number of blood monocytes in all experimental groups (especially when 1% polystyrene was added to the feed) was revealed, which significantly exceeded the physiological norm. It is with participation in the excretion of microplastic particles by macrophages that we attribute a sharp increase in the content of monocytes in the blood of experimental mice that received different doses of polystyrene foam with food.

An increase in the number of monocytes in the blood of laboratory mice is also caused by the addition of different plastic types (polyvinyl chloride, polyethylene, polystyrene) to the bedding (Lieshchova, 2019). Plastic particles (polyurethane) affect macrophages that originate from monocytes, causing a stimulating effect and prolonging the life of these cells in the connective tissue that forms around the implants (Anon, 2007).

Research results have shown that polystyrene microparticles can affect the health of a macroorganism by changing its microbiota, which in turn can lead to impaired intestinal barrier function (Bhatia et al., 2014; Jin et al., 2017). According to Lu et al. (2018), the composition of the intestinal microbiota changes at the levels of type and genus in the caecum of laboratory mice under the action of polystyrene microparticles. Microplastic causes a decrease in the secretion of intestinal mucus, in particular mucin, the main component that protects the intestinal mucosa and prevents the penetration of bacteria (Linden et al., 2008). Functional products of the intestinal microbiota (including amino acids) are important modulators that play a major role in the development of obesity, insulin resistance, and type II diabetes mellitus (Ley et al., 2005; Neis et al., 2015). Jin et al. (2019) in an experiment on mice found that the effect of 5 μm polystyrene microparticles directly changes the levels of arginine and tyrosine, and also affects the exchange of bile acids between the liver and intestines, which is another mechanism of influence on the intestinal microbiota.

As a result of the impact of crushed polystyrene foam on the mice's intestinal microbiota, which was confirmed in our previous study (Bilan et al., 2022), we also predicted a negative effect on metabolic processes in the body of laboratory animals. In this study, we determined the effect of different doses (0.1%, 1%, 10%) of crushed polystyrene foam added to the normal diet on the morphological and functional parameters of some internal organs in laboratory mice during a 42-day experiment. Crushed polystyrene foam pieces, entering the animal's body through the gastrointestinal tract, have a toxic effect on internal organs. This was reflected in the changes, first of all, in the blood biochemical parameters. Significant changes were observed in the work of parenchymal organs – the liver and kidneys. Despite the absence of changes in the relative mass of these organs, the indicators of their functional state have undergone changes. Polystyrene foam, regardless of the dose, causes a violation of protein metabolism, in particular, an increase in the blood total protein content due to the albumin fraction, with a change in the protein coefficient. At the same time, we found a change in the concentration of urea and blood urea nitrogen, which are also the final metabolites of protein metabolism. A decrease in the content of total blood bilirubin, cholesterol concentration and a change in the activity of blood plasma enzymes also indicate violations of liver function during the consumption of crushed polystyrene foam.

The liver is an organ that plays a key role in lipid metabolism (Nguyen et al., 2008; Mylostyva et al., 2022; Skliarov et al., 2022). Polystyrene microparticles induce abnormal lipid metabolism in the mouse liver, which Lu et al. (2018) associate with intestinal dysbiosis. Compare et al. (2012) confirm a strong relationship between the gut microbiota and liver health through individual metabolites excreting microorganisms. However, microplastic particles can also have a direct effect on organ tissues, since it has been proven that they can accumulate in them (Deng et al., 2017). In an experiment using fluorescent and native polystyrene microplastics with a diameter of 5 and 20 μm, its accumulation in the liver, kidneys, and intestines was shown. The nature of the distribution and the way microplastics migrate depend on their size. The toxic effect on tissues where microplastics accumulate has been established on the basis of biochemical markers and metabolic profiles. Polystyrene microplastics cause disturbances in energy and lipid metabolism and oxidative stress, as well as changes in the concentration of neurotoxicity biomarkers (Deng et al., 2017).

The results of our study show that crushed polystyrene foam added to the feed in various doses has a systemic toxic effect on the model animals' body, both directly, causing organs' parenchymal dystrophy (liver, kidneys, myocardium), and indirectly due to changes in the composition of the intestinal microbiota, thereby violating his barrier function. Polystyrene

foam particles, causing mechanical irritation and damage to the mucous membrane tissues of the digestive tract, contributed to the massive penetration of autotoxins through the wall into the internal body environment. This is confirmed by a sharp increase in the activity of AST, a marker of the liver functional state and myocardium parenchyma, in all groups of mice. At the same time, a decrease in the activity of alkaline phosphatase may indicate that degenerative processes in the parenchyma of organs pass from compensatory ones into the stage of decompensation, which is associated with the onset of the development of irreversible morphological changes. This is confirmed by a sharp decrease in the liver functional ability indicators – the content of total bilirubin, urea, urea nitrogen, cholesterol and kidneys – an increase in the creatinine level.

Conclusion

Crushed polystyrene, entering the body, affects the internal organs, causing, depending on the dose, morphofunctional changes and metabolic disorders. Adding crushed polystyrene foam to the feed in an amount of 1% causes a significant decrease in the mass index of the heart and stomach, 10% – only the heart, and 0.1% – does not affect this indicator. When exposed to crushed polystyrene, regardless of the dose, in the mice's blood AST activity sharply increased with a simultaneous decrease in alkaline phosphatase activity. The content of cholesterol, total bilirubin, urea and urea nitrogen decreased, as well as and the creatinine content also increased.

In the mice's blood, the level of monocytes increased in a dose-dependent manner. The maximum increase in this indicator according to the control and the reference range was established by adding 1% crushed polystyrene foam to the diet.

The results of this study suggest that polystyrene foam, entering the body through the gastrointestinal canal, has a systemic toxic effect, both directly accumulating in tissues and affecting metabolism, and indirectly, due to a violation of the barrier function of the mucous membrane due to traumatic tissue damage.

The authors declare no conflict of interest.

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