

# Yudhiakuari Sincihu

## 2-No\_prominent\_SOD\_and\_CAT\_lung\_v

 Yudhiakuari Sincihu2

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**Original Article****No Prominent SOD and CAT Lung Expression of Rats Due to Exposure to Low-Density Polyethylene in The Air****Kusuma S. Lestari**<sup>1</sup> , **Yudhiakuari Sincihu**<sup>2</sup> , **Mohd Talib Latif**<sup>3</sup> , **Saliza Mohd. Elias**<sup>4</sup> , **Troef Soemarno**<sup>5</sup>, **Soedjajadi Keman**\*,<sup>1</sup> <sup>1</sup>Department of Environmental Health, Faculty of Public Health, Universitas Airlangga, Surabaya Indonesia<sup>2</sup>Faculty of Medicine, Widya Mandala Surabaya Catholic University, Surabaya Indonesia<sup>3</sup>Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Selangor Malaysia<sup>4</sup>Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Selangor Malaysia<sup>5</sup>Faculty of Medicine, Universitas Hang Tuah, Surabaya Indonesia**ARTICLE INFO****Article history**

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**KEYWORDS**Airborne microplastics  
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Microplastics in rat blood  
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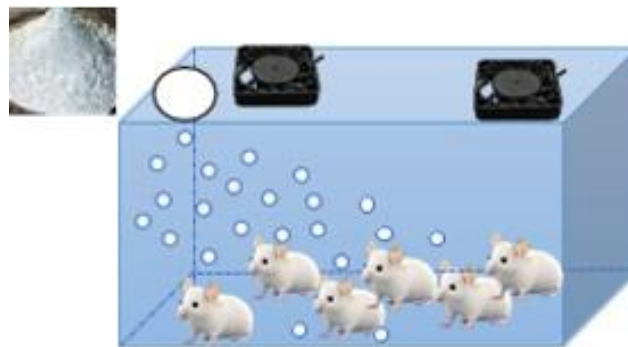
Microplastics are plastic particles less than 5 mm in diameter that contaminate the environment. Humans and animals can inhale microplastics measuring 2  $\mu\text{m}$  to 1 mm in the air. There has been limited research on inhalation exposure to low-density polyethylene (LDPE) microplastics. This study aimed to investigate the Reactive Oxygen Species (ROS) in the Wistar strain of *Rattus norvegicus* due to exposure to LDPE in the air. This study is an experimental study using a post-test control group design on the Wistar strain of *Rattus norvegicus*. Five experimental groups (X1 to X5) were each given LDPE microplastic particles with concentrations of 1 mg/L, 1.25 mg/L, 2.5 mg/L, 3.75 mg/L, and 5 mg/L for 28 days. This result showed the SOD and CAT enzymes were not present or active. There was no difference among groups of both SOD and CAT enzymes. The microplastic particle in the blood (/mL) was identified in each intervention groups which was  $6.80 \pm 2.58$  (X1),  $17.67 \pm 9.02$  (X2),  $35.83 \pm 12.22$  (X3),  $41.17 \pm 14.72$  (X4), and  $57.50 \pm 6.47$  (X5) while in control group was  $1.80 \pm 1.30$  with fragments being the most dominant form of microplastics. It can be concluded that ROS was not seen due to the dose, chamber condition, and exposure technique should be intratracheal. The greater the dose of LDPE exposure, the greater the average microplastics in the blood of the Wistar strain of *Rattus norvegicus* in both the control and intervention groups.

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## GRAPHICAL ABSTRACT

**Introduction**

Microplastics refer to plastic particles less than 5 mm (from 1  $\mu\text{m}$  to 5 mm) in diameter, which are of particular concern due to increased production and disposal of plastic products, as well as low rates of plastic degradation [1-5]. It is estimated that approximately 67.8 million metric tons of plastic will be in the natural environment or landfills by 2050 [4]. Primary microplastics are particles derived from commercial products, such as cosmetic products, and microfibers from clothing or fabrics. On the other hand, secondary microplastics come from plastic fragmentation processes [6,7]. Studies on microplastics are commonly conducted in a variety of environments, including water (water), soil (agriculture), food (seafood, milk, honey), drinking water, and table salt [8]. Recently, attention has also begun to be directed to the atmosphere. Studies on the presence of microplastics in the air in 2015 attracted attention because at the time there was little research investigating them [9]. Afterwards, several studies on airborne microplastics were conducted in various countries, including the United States, United Kingdom, Germany, France, Australia, China, India, and Vietnam, between 2016 and 2020 [9-25]. Microplastics are defined as synthetic solid particles or polymer matrices with regular or irregular shapes involving fibres, fragments, and filaments in the air [1,5,10]. Studies in China have compared

microplastic concentrations in northern and southern cities, and the results show that fragments are the main form of microplastics in the air (88.2%). The types of microplastics in the atmosphere stand out as the dominant are polymers polyethylene (PE), polyester, and polystyrene (PS) [26]. while the other types can be found involve polyamide (PA), polypropylene (PP), polyethylene terephthalate (PET), polyvinyl chloride (PVC), polyurethane (PUR), and polyacrylonitrile (PAN) [10]. Atmospheric particulate matter consists of a mixture of solids and liquids in the air that come from natural or anthropogenic sources such as soil, ocean, combustion, and the biosphere [2]. Microplastics in the air contribute to overall particulate matter pollution in a region. Increased concentrations of synthetic microplastics in terrestrial and aquatic ecosystems during winter suggest that rainfall can potentially affect microplastic content in the atmosphere. The concentration and characteristics of microplastics in the atmosphere are influenced by wind direction and distance from their source, while climatic factors such as wind speed and temperature affect the displacement of particulate matter, including microplastics [10]. Microplastics in the air contribute to air pollution [8,10]. Breathing polluted air is very detrimental and can cause death in humans and animals. Among various pollutants in the atmosphere, microplastics in the air are a new issue that

has recently been identified, attracting the attention of scientists, non-governmental organizations, and the public media [4]. Research conducted by Simon Wieland compares microparticles and microplastics to understand more deeply the mechanism of toxicity [27]. Studies on animal inhalation have shown that the harmful consequences of reactive oxygen species (ROS) activity might differ based on the particle's composition and source of emission [28]. All aerobic organisms produce ROS naturally, mostly due to mitochondrial electron transport. ROS are necessary for the physiological control of important signalling pathways that are involved in cell division, growth, proliferation, and survival [29].

Furthermore, the suppression of superoxide dismutase (SOD) catalase (CAT), glutathione peroxidases (GPx), and total glutathione (GSH) indicated that inhaling PS caused oxidative stress in mice. The chemical components that differ throughout polymer types—unreacted monomers and plastic additives, for example—should be regarded as crucial factors in determining the toxicity caused by microplastics [30-32]. The presence of microplastics in the air harms the environment and poses a risk to the health of living things. This study aimed to investigate the ROS on the Wistar strain of *Rattus norvegicus* due to exposure to low-density polyethylene (LDPE) in the air as a result of exposure to inhalation of LDPE microplastics.

## Materials and Methods

This research is a type of quantitative analytical research with a laboratory experimental approach using experimental animals. This research method involves inhalation intervention of microplastics exposure in experimental animals with a post-test control group design. The study design began by dividing experimental animals into six groups, namely the control group, the intervention group X1, X2, X3, X4, and X5.

## Microplastic exposure materials

Microplastics exposure consists of LDPE particles derived from LDPE powder. LDPE powder is filtered using a 1250 mesh sieve to obtain particles with a size of  $< 10 \mu\text{m}$ . Furthermore, microplastics diameter examination was carried out using Nikon Eclipse Ci-L- DS-F12-L3 binocular microscope at 400 magnifications, to ascertain the size of the microplastics diameter. LDPE powder is also analysed using Fourier Transform Infrared (FTIR) spectroscopy to detect polymer types and other material content.

## Animal

The experimental unit used in this study was rat of the Wistar strain of *Rattus norvegicus*, which was accompanied by a health certificate. The study's inclusion criteria included rat aged 2 months, male, and weighing between 150 and 175 grams. Meanwhile, the exclusion criteria involved Wistar strain *Rattus norvegicus* rats that were undergoing the disease, as identified through examination by a veterinarian. The drop-out criteria in this study referred to the Wistar strain of *Rattus norvegicus* that experienced death (characterized by the absence of vital signs of life) during the intervention. By applying the Lemeshow formula for replication of experimental units, 6 research samples were obtained in each group. A random allocation technique was used in this study, in which the Wistar strain of *Rattus norvegicus* that met the study's inclusion criteria (with homogeneous characteristics) was randomly assigned into six groups using a computerized random number generator, consisting of one control group and five intervention groups. Researchers did not know the allocation of the Wistar strain of *Rattus norvegicus* to which group (single binding). Therefore, all groups were considered equal before intervention was

given. This step was taken to maintain internal validity and prevent bias in this study.

#### *Rat care and exposure*

This study was conducted at the Biochemistry Laboratory, Department of Physiology and Biochemistry, Faculty of Medicine, Universitas Airlangga. Determination of the concentration of exposure to microplastics in the air refers to the OECD Guide for Chemical Testing, Part 4 Test Number 412: Toxicity of Subacute Inhalation: A 28-Day [32]. Study with particle exposure was carried out for 6 hours every day, 5 days a week, for 28 days. There were five intervention and one control group; each intervention involved six experimental animals placed in each inhalation full chamber body equipped with a thermohygrometer. In this space, optimal air circulation is required, therefore there is one fan that carries air in and one fan that carries air out, and is assisted by a blower every time exposure is given to help disperse LDPE in the chamber. After receiving intervention, the experimental animal is transferred to the cage. The X1 intervention group had a particle size of < 10  $\mu\text{m}$  at a dose of 1 mg/L, the X2 intervention group had a particle size of < 10  $\mu\text{m}$  at a dose of 1.25 mg/L, the X3 intervention group had a particle size of < 10  $\mu\text{m}$  at a dose of 2.5 mg/L, the X4 intervention group had a particle size of < 10  $\mu\text{m}$  at a dose of 3.75 mg/L, and the X5 intervention group had a particle size of < 10  $\mu\text{m}$  at a dose of 5 mg/L. The doses based on the number concentration of atmospheric microplastic was reported in Beijing to be 5,650  $\text{m}^3$  is equivalent to 5.65 mg/L [15]. This was the highest concentration microplastics among another research. It also refers to the OECD Guide for Chemical Testing [32]. The real exposure in the environment, particularly indoors, is described by this research approach. Experimental animals were placed in an inhalation full chamber body measuring 80 cm x 40 cm x 40 cm. For 28 days, temperature and humidity observations were

carried out to ensure that the physical environmental conditions in the chamber were following standards. Temperature and humidity measurements are carried out using a thermohygrometer installed in each inhalation full chamber body.

#### *Rat termination and blood sampling*

After 28 days, the animals were anesthetized using ketamine by injection of 50-100 mg/kg body weight. After anesthesia, the experimental animal was placed in the supine position, and then the lower ribs of the mouse were identified. Approximately, the heart's location could be found about 1 cm above it. The skin layer of the chest wall was gradually opened, and if a heartbeat was seen, a 3cc needle syringe would be inserted into the ventricle at a 45° angle. Blood was drawn quickly, keeping the syringe stable to prevent blood clots.

#### *Preparation and examination of microplastics in Rats blood*

Blood was added 10% KOH as much as 1 cc, agitated slowly, and incubated at 50 °C for 2 days in a water bath. Furthermore, 67% nitric acid was added as much as 1 cc which has been heated to 48 °C. The mixed solution was then centrifuged at 2300 rpm for 2 minutes or until the supernatant appears clear and separated from the rest of the organic tissue. The supernatant was filtered using an S-PAK filter membrane measuring 0.45  $\mu\text{m}$ . Filter paper was dried at 40 °C for 24 hours. Microplastic observations were conducted using a Nikon Eclipse E100 binocular microscope at 40x and 100x magnifications. Microplastic was measured three times by an expert and two assistants. The findings were recorded in the results form.

#### *Hematoxylin eosin staining*

Eosin hematoxylin staining used Mayer's Hematoxylin and Eosin for an

immunohistochemical staining comparison. The glass of the object that has been deparafined was washed with water for 15 minutes. It was given a lugol solution for 10 minutes, and rinsed with water 5 times dipped in. After that, hypo solution was added for 3 minutes, and then Mayer's Hematoxylin was added for 15 minutes and it was washed with warm water for 20 minutes and eosin was added, and it was kept for 2 minutes. Next, the object glass was dehydrated twice each with 95% and 100% ethanol for 2 minutes. Purification with xylol was carried out twice each for 2 minutes. The tissue slide was then covered with a glass cover and adhesive, and observed under a microscope.

#### Immunohistochemical Staining Procedure

The procedure of immunohistochemical staining (SOD bronchiolus expression and CAT bronchiolus expression) was carried out according to the reagent protocol. SOD and CAT bronchiolus expression used SOD-1 (24): sc-101523 Santa Cruz Biotechnology, Inc. and catalase (H-9): sc-271803 Santa Cruz Biotechnology, Inc. Expression Immunohistochemical slide assessment of SOD and CAT in bronchiolus of Wistar strain of *Rattus norvegicus* was conducted using a binocular light microscope with 400x magnification. In addition, in each of the nine visual areas, the mean number of bronchiolus expressing SOD and CAT was determined sequentially and noted on the examination sheet.

#### Statistical analysis

The table shows the results as mean  $\pm$  standard deviation (SD). The Dunnett's test was used to determine the significant difference between microplastic-treated and control groups. All statistical analyses were conducted with SPSS 19.0 software. A difference was considered statistically significant at  $p < 0.05$ .

#### Animal ethics

This study has obtained ethical approval with number No: 2.KEH.111.07.2023 issued by the Animal Ethics Commission of the Faculty of Veterinary Medicine, Universitas Airlangga, Animal Care and Use Committee (ACUC). ACUC uses animal ethical guidelines from the Indonesian Ministry of Health [33].

#### Results and Discussion

Temperature and humidity observations were recorded twice, before and after intervention, in an inhalation full-body chamber over a 28-day period. Observations during the day showed higher temperatures than in the morning, while humidity during the day was lower than in the morning. On day 9, one Wistar strain of *Rattus norvegicus* in the control group was found to have died of seizures, and on day 17, one Wistar strain of *Rattus norvegicus* in intervention group 1 was also found dead. Therefore, two Wistar strains of *Rattus norvegicus* were considered to drop out, bringing the number of samples to 34.

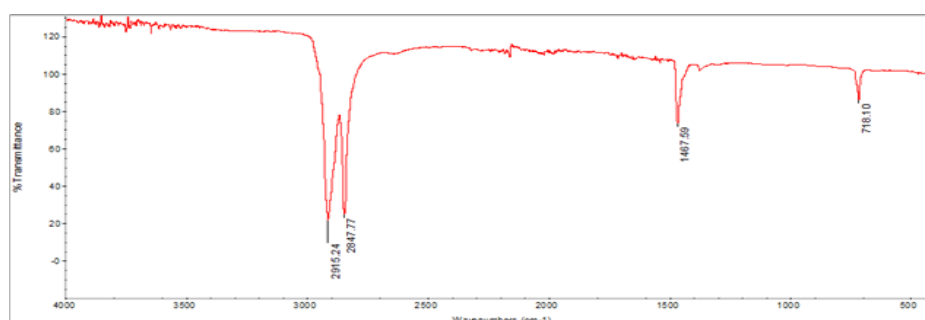


Figure 1: FTIR result

From the results of the FTIR examination (Figure 1), it can be seen that the peaks are 2915.24  $\text{cm}^{-1}$ , 2847.77  $\text{cm}^{-1}$ , 1467.59  $\text{cm}^{-1}$ , and 718.10  $\text{cm}^{-1}$ . The functional groups at these peaks are successively the functional groups O–H (carboxylic acid), O–H (carboxylic acid), alkane compounds, and alkyne compounds. This spectrum shows that the plastic polymer was LDPE.

**Table 1:** Mean  $\pm$ SD microplastics particles levels in rat blood (in Particle/mL)

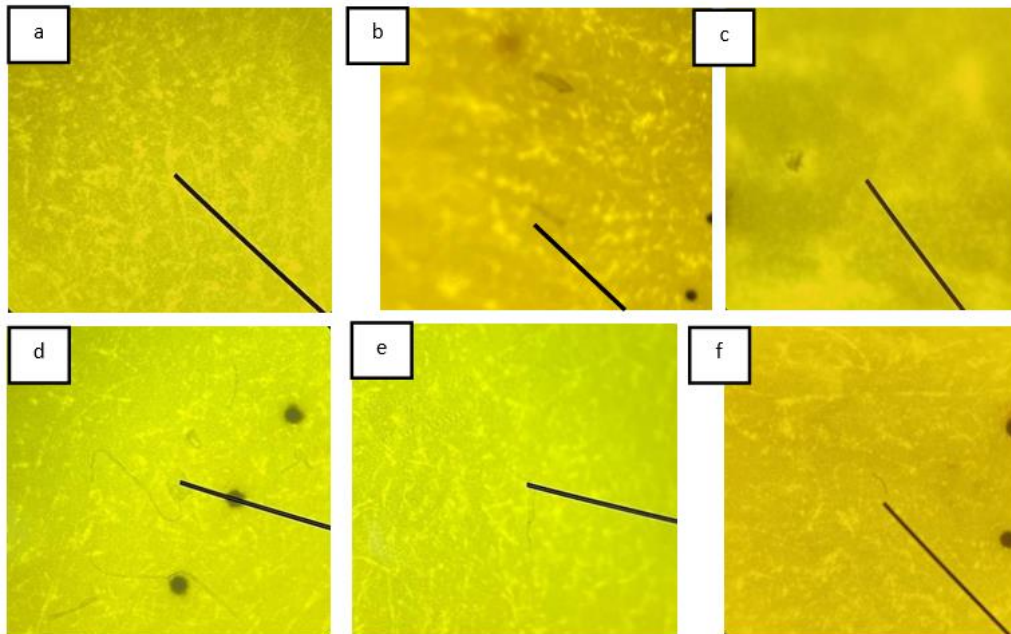
Group	Microplastics Particle
Control	1.80 $\pm$ 1.30
Intervention 1 (X1)	6.80 $\pm$ 2.58
Intervention 2 (X2)	17.67 $\pm$ 9.02
Intervention 3 (X3)	35.83 $\pm$ 12.22
Intervention 4 (X4)	41.17 $\pm$ 14.72
Intervention 5 (X5)	57.50 $\pm$ 6.47

From the results of microplastics examination in the blood (Table 1), the average in the control group was lowest compared to the intervention group (1-5). The higher the dose of inhaled microplastics exposure in the intervention group, the higher the average microplastics in the blood. Inhaled microplastics exposure in experimental animals was detected in the blood of microplastics in all groups. In the control group, a relatively small amount of microplastics was found, indicating that microplastics exposure in the environment is inevitable. Possible sources of such exposure may come from ingestion or inhalation unrelated to the LDPE to which they were exposed. Exposure can occur through direct ingestion of microplastics, licking rat fur containing LDPE microplastics as they gather, or inhaling airborne microplastics. The presence of microplastics in the environment has been known since 2015 based on research [9]. As a follow-up study, in France, indoor and outdoor air was evaluated to detect the presence of fibres in the air [34]. Several studies of airborne microplastics were conducted in various countries, such as the United States, United Kingdom, Germany, France, Australia, China, India, and Vietnam, between 2016-2020 [10]. The microplastics used in the study were LDPE, a synthetic polymer. Microplastics in the air can consist of natural polymers and synthetic polymers.

Natural polymers mainly include cellulose and proteins. Types of microplastic identified in the atmosphere, including synthetic types, include PVA (polyvinyl acetate), PUR (polyurethane), PTFE (teflon), PET (polyethylene terephthalate), PE (polyethylene), PP (polypropylene), and PS (polystyrene), PES (polyester), PAN (polyacrylonitrile), PAA (poly N-methyl acrylamide), RY (rayon), EVA (ethylene vinyl acetate), EP (epoxy resin), ALK (alkyd resin), and natural (cotton and wool), with forms such as fragments, foam, films, granules, fibers, and microbeads [4,35]. Microplastics pollution in the air is not only determined by the type and intensity of emissions, but also influenced by meteorological conditions (rainfall, rain, or snow), climate, and site topography, all of which play a major role in the spread and deposition of microplastics. Research identifies that the distribution of microplastics types, such as polyethylene (PE), polystyrene (PS), polypropylene (PP), polyvinyl chloride (PVC), and polyethylene terephthalate (PET), varies according to climate [16].

The most common polymers among indoor microplastics particles were polyester (28.4%), polyamide (PA, 20.54%), and polypropylene (PP, 16.3%). In contrast, outdoor microplastics were dominated by polyethylene (PE, 26.8%), polystyrene (PS, 17.8%), and polyester (17.2%). In particular,

the polymer composition in fibre microplastics differs from fragmentary microplastics. Fiber microplastics in the air mostly consist of polyester (44.9%), PA (22.4%), and PS (7.0%), while fragment microplastics are dominated by PE (27.8%), PP (17.8%), and PS (17.2%) [11].

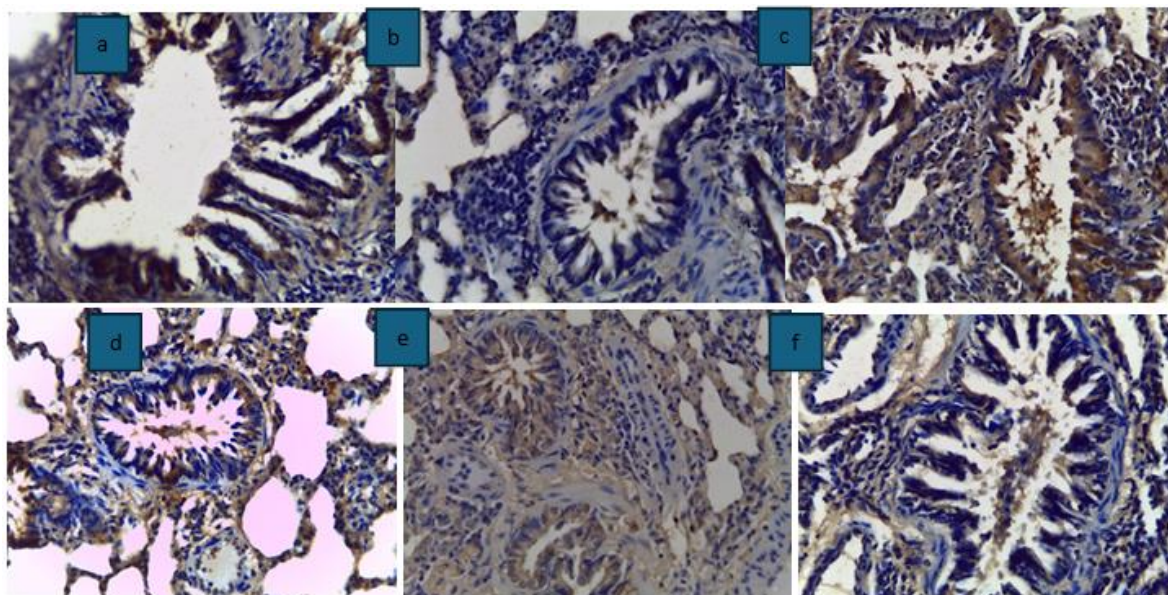


**Figure 2:** Polymer composition in the control and intervention 1-5 group (magnification 100x). (a). Pellet in Control Group; (b) Fragment in Intervention Group 1; (c) Fragment in Intervention Group 2; (d) Fragment and Filament in Intervention Group 3; (e) Fragment in Intervention Group 4; and (f) Fragment in Intervention Group 5

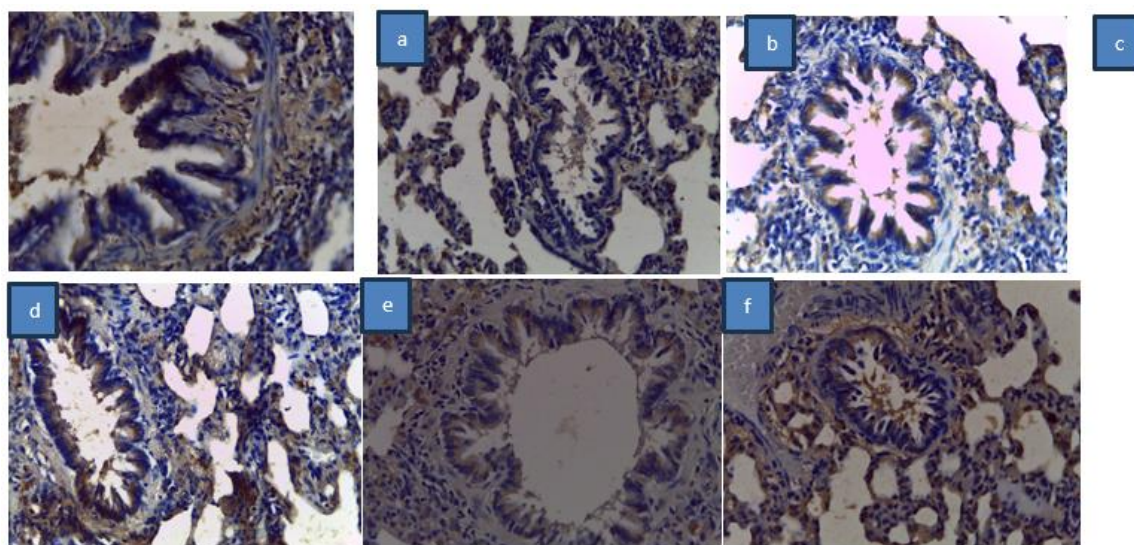
In Figure 2, it can be seen that the majority of microplastics types in all intervention groups are fragments. Mostly the microplastic form observed was fragment. This form is most common because fragments are a type of secondary microplastic originating from pieces of plastic with strong polymer properties such as polypropylene polyethylene polystyrene [34,35]. This is in accordance with the type of microplastic used in this research, namely LDPE. Forms of microplastics in the air include fragments, foam, films, granules, fibres, and microbeads [4]. Fibres and fragments are the most commonly reported forms in some studies [9,13,35-37]. Other results show that the most dominant form is only fibres [38].

The health risks of airborne microplastics depend on their abundance and properties, such as size, shape, and chemical composition. Although most airborne microplastics reported in the literature tend to be more than tens of microns in size, some recent studies show a predominance of smaller microplastics [5,11,39-40]. Microplastics have the potential to turn into smaller pollutant particles in the air, such as nanoplastics or femtoplastics, which are affected by temperature, humidity, and sun exposure [41]. This situation can be a health risk because at that size, microplastics can be easily inhaled compared to the size of microplastics. Exposure to microplastics in the air can also occur through the skin and ingestion due to atmospheric impact deposition. In addition to potential risks to

human health, it should also be noted that [4,42-43].  
 there are potential risks to the environment



**Figure 3:** SOD Immunohistochemistry in the control and intervention 1-5 group (magnification 400x). (a) Control Group, (b) Intervention Group 1, (c) Intervention Group 2, (d) Intervention Group 3, (e) Intervention Group 4, and (f) Intervention Group 5



**Figure 4:** CAT Immunohistochemistry in the control and intervention 1-5 group (magnification 400x). (a) Control Group, (b) Intervention Group 1, (c) Intervention Group 2, (d) Intervention Group 3, (e) Intervention Group 4, and (f) Intervention Group 5

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In this research method, it describes the actual exposure in the environment, especially indoors. Research on exposure to LDPE microplastics in the air is limited. Data on toxicity from inhaled exposure in previous studies were not yet available, so exposure doses were referred to in the OECD guidelines "OECD Guidelines for the Testing of Chemicals, Section 4 Test No. 412: Subacute Inhalation Toxicity: 28-Day Study." Based on OECD guidelines, the limit concentrations for gases, vapors, and aerosols are 20000 ppm, 20 mg/L and 5 mg/L, respectively [32]. The maximum dose used in this study was 5 mg/L and the minimum was 1 mg/L. The dosages were calculated using the number concentration of microplastics in the air, which was 5,650 m<sup>3</sup> (or 5.65 mg/L) in Beijing [15]. This study found that increasing the dose of inhaled exposure contributed to an increase in the concentration of microplastics in the blood. Research on oral LDPE exposure to the Wistar strain of *Rattus norvegicus* shows that the average microplastic gets higher with increased blood exposure dose [36]. In the blood, microplastics are degraded through oxygen-dependent and independent mechanisms through oxidative processes and inflammatory mediators [37]. A study conducted in Korea on female rats that were 6 days pregnant, using the method of exposure through the intratracheal during pregnancy, aimed to evaluate its effect on the organs of neonates aged 7 days. The results showed that no toxicity was detected in organs examined using immunohistochemical techniques [38]. There was no difference among groups of both SOD and CAT enzymes. This is similar to the result that there was no SOD and CAT expression due to LDPE exposure, it may cause by the doses received being divided by others in one chamber, whole-body chamber condition, and exposure technique should be intratracheal. Inhalation toxicity of polystyrene micro(nano)plastics using modified OECD TG 412 indicated there was no concentration-response (several endpoints in

physiological, serum biochemical, hematological, and respiratory function after 14 days of exposure [44]. The lack of SOD (superoxide dismutase) and CAT (catalase) expression can have significant health implications, particularly in the context of oxidative stress and cellular damage. These two enzymes play crucial roles in protecting cells from reactive oxygen species (ROS), which are highly reactive molecules that can damage cellular structures, proteins, lipids, and DNA [45-47]. Oxidative stress (SOD and GSH), apoptosis, and hastened the fibrosis process on polystyrene [48,49], on PVC [50], on polyethylene [51].

## Conclusion

No detectable presence or activity of the enzymes SOD and CAT due to LDPE exposure for 28 days. There was no difference among groups of both SOD and CAT enzymes. Exposure to LDPE microplastic inhalation led to the detection of microplastics in the blood, both in the control and treatment groups. There is an increase in the average microplastics in the blood with an increase in the exposure dose. Mostly the microplastic form observed was a fragment. Further research is needed by one chamber for one animal intratracheally with an LDPE size of less than 10 µm.

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## Author Contribution:

Kusuma S. Lestari, Yudhiakuari Sincihu, and Troef Soemarno carried out the experiment. Kusuma S. Lestari, Yudhiakuari Sincihu, Mohd Talib Latif, and Saliza Mohd. Elias wrote the manuscript with support from Soedjajadi Keman. Soedjajadi Keman helped supervise the project.

## Conflict of Interest

There was no conflict of interest

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