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Cellular Uptake and Nuclear Accumulation of Polystyrene Nanoplastics in 3T3 Fibroblasts and Hepatocytes of *Rattus norvegicus*

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Abstract | Polystyrene nanoplastics (PSNPs) are emerging contaminants that pose potential risks to cellular health due to their small size and ability to penetrate biological barriers. This study investigates the cellular uptake mechanisms and intracellular distribution of PSNPs using *Rattus norvegicus* hepatocytes and 3T3 fibroblast cell cultures. Fluorescence microscopy analysis demonstrated substantial PSNP accumulation within both cell types, with distinct intracellular localization patterns. In hepatocytes, PSNPs were predominantly observed in the cytoplasm and occasionally within the nucleus, suggesting active endocytosis and potential nuclear transport. In 3T3 fibroblasts, PSNPs were found not only in the cytoplasm and around the nucleus but also within the nuclear compartment, highlighting their ability to traverse the nuclear envelope. Quantitative analysis revealed significant morphological changes, increased cell and nucleus diameters, and a higher percentage of necrotic cells in PSNP-exposed groups compared to controls ($p < 0.05$). The observed cellular stress responses may induce oxidative stress, membrane disruption, and apoptosis, supported by morphological and fluorescence evidence indicating interactions with proteins, lipids, and nuclear components.. These findings highlight the potential health risks associated with PSNP exposure, highlighting the need for further research into their long-term biological impacts and the mechanisms underlying their cellular interactions.

Keywords | Nanoplastics, Cellular health, Cellular-transport, Hepatocytes, 3T3-fibroblast

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INTRODUCTION

Plastic pollution has emerged as a critical environmental challenge, with macro- and microplastics fragmenting into even smaller particles, known as nanoplastics, through physical, chemical, and biological degradation (Allen *et al.*, 2022). These nanoscale fragments have infiltrated ecosystems and food chains, raising growing concerns about their potential health risks (Hayati *et al.*, 2024). Nanoplastics exist as both homogeneous and heterogeneous aggregates,

exhibiting unique physicochemical properties that enhance their interactions with biological systems (Liu *et al*., 2019).

The International Organization for Standardization (ISO) defines nanoparticles, including nanoplastics, as materials with external dimensions between 1 and 100 nm (Kiran *et al*., 2022; Nguyen *et al*., 2019). However, the International System of Units (SI) extends the nanoplastic definition to include plastic fragments up to 1 µm (1000 nm). Due to their high surface area, nanoplastics can adsorb environmental contaminants and migrate through biological membranes. Once inside living organisms, they may accumulate in cells, potentially triggering toxic effects (Triwahyudi *et al*., 2023).

The ability of nanoplastics to penetrate cellular structures poses a growing health concern. Studies suggest that these particles enter cells via endocytosis or passive diffusion, allowing them to reach intracellular compartments and interact with critical biomolecules (Hayati *et al*., 2024). Their presence within the body has been linked to oxidative stress, inflammation, and disruptions in cellular function, which could contribute to liver dysfunction, respiratory distress, skin disorders, and reproductive health issues (Pitt *et al*., 2018; Zhang *et al*., 2020). However, despite increasing concerns, research on nanoplastics' long-term health effects remains in its early stages (Saputra *et al*., 2025).

To understand the biological risks, both in vitro and in vivo models are essential in toxicological studies (Bijukumar *et al*., 2023). In vitro models, such as 3T3 fibroblast cell cultures, are widely employed due to their sensitivity, reproducibility, and relevance in assessing cellular stress responses, morphological changes, and nanoparticle uptake (Bhardwaj and Webster, 2015), while *Rattus norvegicus* provides a relevant physiological model due to its biological similarities to humans (Khan *et al*., 2019). Due to their widespread use and slow degradation, polystyrene contributes significantly to global plastic waste, ultimately fragmenting into micro- and nano-sized particles that are highly persistent in the environment (Gilani *et al*., 2023; Lv *et al*., 2024; Zhang *et al*., 2024).

PSNPs, with a nanoscale size of <100 nm, can directly interact with lipid membranes and cellular proteins, modulating biological processes at the molecular level (Lv *et al*., 2024). Upon internalization, their excretion is limited, potentially leading to accumulation in the digestive tract. Some particles may traverse the intestinal epithelium, enter the bloodstream, and distribute across various tissues and organs (Li *et al*., 2024). However, their intracellular dynamics and long-term biological impacts remain largely unexplored, underscoring the need for further research. Understanding the mechanisms of PSNP-cell interactions is crucial for assessing their toxicological impact.

This nanotoxicology study aims to provide deeper insights into the biological impacts of nanoplastics, particularly PSNPs, on cellular systems and their potential to disrupt physiological functions. Research on PSNP transport mechanisms into cells has identified key pathways, including endocytosis, protein corona formation, and direct lipid membrane interactions. However, significant knowledge gaps remain in comprehensively understanding these processes and their cellular consequences, necessitating further investigation into PSNP-related health risks. This study investigates the transport mechanisms of PSNPs into cells, focusing on primary pathways such as endocytosis and membrane interactions. Experimental analyses explore the biological effects of PSNP exposure, including oxidative stress induction, structural disruptions, and inflammation, which may lead to cellular dysfunction. By examining cellular uptake dynamics and assessing the potential long-term impacts of PSNPs on biological systems, this research aims to provide critical insights into their broader implications for overall health.

MATERIALS AND METHODS

PSNP Characterization

PSNPs used in this study were obtained from Sigma-Aldrich (USA). As specified by the manufacturer, the PSNPs are carboxylate-modified, monodisperse polystyrene spheres with an average diameter of 100 nm. The particle size distribution was determined by dynamic light scattering (DLS), with a polydispersity index (PDI) of less than 0.1, indicating a narrow size distribution and uniformity. The zeta potential of the PSNPs is approximately –40 mV in aqueous suspension, reflecting a stable surface charge that promotes dispersion and prevents aggregation in cell culture medium. This characterization The PSNPs were stored and handled according to the supplier's guidelines, and no visible signs of agglomeration were observed during experimental procedures. The PSNPs were stored and handled in accordance with the supplier's guidelines, and no visible agglomeration was observed during experimental procedures. These physicochemical characteristics have also been confirmed in previous independent studies, which reported similar size, charge, and dispersion profiles (Shorny *et al*., 2023).

Cell Culture (In Vitro Experiment)

Mouse embryo fibroblast cells (3T3) were obtained from the Parasitology Laboratory, Universitas Gadjah Mada, Indonesia. The cells were cultured in T225 flasks using Dulbecco's Modified Eagle Medium (DMEM) (Gibco, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, USA), 1% penicillin-streptomycin solution, and 0.5% fungizone to maintain optimal growth conditions. The cell cultures were maintained at 37°C in a 5% CO_2 atmosphere.

For the experiment, the 3T3 cells were divided into two groups: a control group without PSNP and a treatment group exposed to PSNP at a concentration of 100 µL/mL. After 48 hours of treatment, the cells were collected and prepared for confocal microscopy analysis to assess PSNP uptake and intracellular distribution. All experiments were conducted in triplicate.

Animal Study (In Vivo Experiment)

The in vivo study was conducted using adult Wistar rats (*Rattus norvegicus* L.), weighing 200 g, obtained from the Animal Laboratory, Faculty of Pharmacy, Universitas Airlangga. The experimental animals were maintained under controlled conditions at a temperature of 25°C ± 2°C with a 12-hour light-dark cycle. Rats were provided ad libitum access to drinking water and were fed 80%–90% of their standard daily food intake. All experimental procedures, including animal handling and treatment protocols, were conducted in accordance with ethical guidelines and were approved by the Research Ethics Committee, Faculty of Veterinary Medicine, Universitas Airlangga (1157/HRECC.FODM/X/2023). Each experimental group was conducted in triplicate.

Experimental Design

The rats were randomly assigned to two groups: a control group and a treatment group. The control group received distilled water, while the treatment group was administered PSNPs (Sigma-Aldrich, USA) orally at a dose of 0.5 mL (10 µL/kg) twice daily for 30 consecutive days. The selected PSNP concentration was based on previous dose-response studies and our own preliminary experiments, which demonstrated that this dosage consistently induced measurable biological effects without causing excessive cytotoxicity. The PSNP suspension was freshly prepared and thoroughly mixed before each administration to ensure uniformity. At the end of the 30-day treatment period, the rats were euthanized following ethical guidelines, and liver samples were carefully excised under sterile conditions. The collected liver tissues were immediately fixed in 10% neutral-buffered formalin for histological analysis.

Preparation of Confocal Microscopy Slides

The preparation of confocal microscopy slides involved several steps. Cultured cells were fixed with 4% formaldehyde. A 5 µL suspension of the fixed cells was placed onto a glass slide and evenly spread to create a uniform monolayer. Following fixation, the slides were stained with 4',6-diamidino-2-phenylindole (DAPI) (Sigma-Aldrich, USA) and Nile Red (Sigma-Aldrich, USA). After staining, the slides were thoroughly rinsed to remove excess dye and then examined using a confocal microscope for high-resolution imaging (Tarafdar *et al*., 2022).

Histological and Confocal Microscopy Analysis

Histological evaluation was conducted to observe liver tissue morphology, detect structural changes, and assess the toxic effects of PSNP. The liver tissue was fixed in 10% formalin solution for 48 hours. Following fixation, the samples underwent a series of graded washing steps to remove residual fixative and prepare the tissue for staining. The processed liver sections were stained with 4′,6-diamidino-2-phenylindole (DAPI) to visualize nuclei and Nile Red to highlight lipid components, allowing for a detailed assessment of cellular architecture and potential lipid accumulation. Imaging was conducted using a laser scanning confocal microscope (Olympus) to obtain high-resolution fluorescence images. Additionally, 3D image analysis was performed using Thermo Scientific HCS Studio 2.0 to enhance visualization and quantification of structural changes within the liver tissue. The cell diameter was calculated by averaging the longest and shortest diameters of cells. The accumulation of PSNPs was quantified by measuring red lipid fluorescence intensity using ImageJ software, providing a precise assessment of PSNP uptake and distribution within the liver tissue. Measurements were conducted on 100 cells per sample, with each analysis performed in triplicate.

Statistical Analysis

Data were analyzed using GraphPad Prism 10.3.1 with one-way ANOVA ($p < 0.05$) to compare control and NP-treated groups. Normality and variance were checked with Shapiro-Wilk and Levene's tests. Quantitative histological data were processed with ImageJ, and results were expressed as mean ± SD, with Tukey's post hoc test for significant differences. Descriptive analysis was used for qualitative observations.

RESULTS AND DISCUSSION

PSNP Accumulation in 3T3 Fibroblasts and *Rattus norvegicus* Hepatocytes

Exposure to PSNPs significantly increased their accumulation in both 3T3 fibroblast cells and *Rattus norvegicus* hepatocytes (Figure 1). Fluorescence imaging demonstrated minimal red fluorescence in the control groups, indicating negligible PSNP presence (Figure 1A). In contrast, PSNP-exposed cells exhibited a substantial increase in red fluorescence, suggesting pronounced nanoparticle accumulation. Quantitative analysis (Figure 1B) reinforced these findings, showing significantly higher PSNP accumulation in treated groups compared to controls ($p < 0.05$), as indicated by different letter annotations. These results highlight the capacity of PSNPs to accumulate within both cell types, suggesting their potential for intracellular retention and associated biological effects.

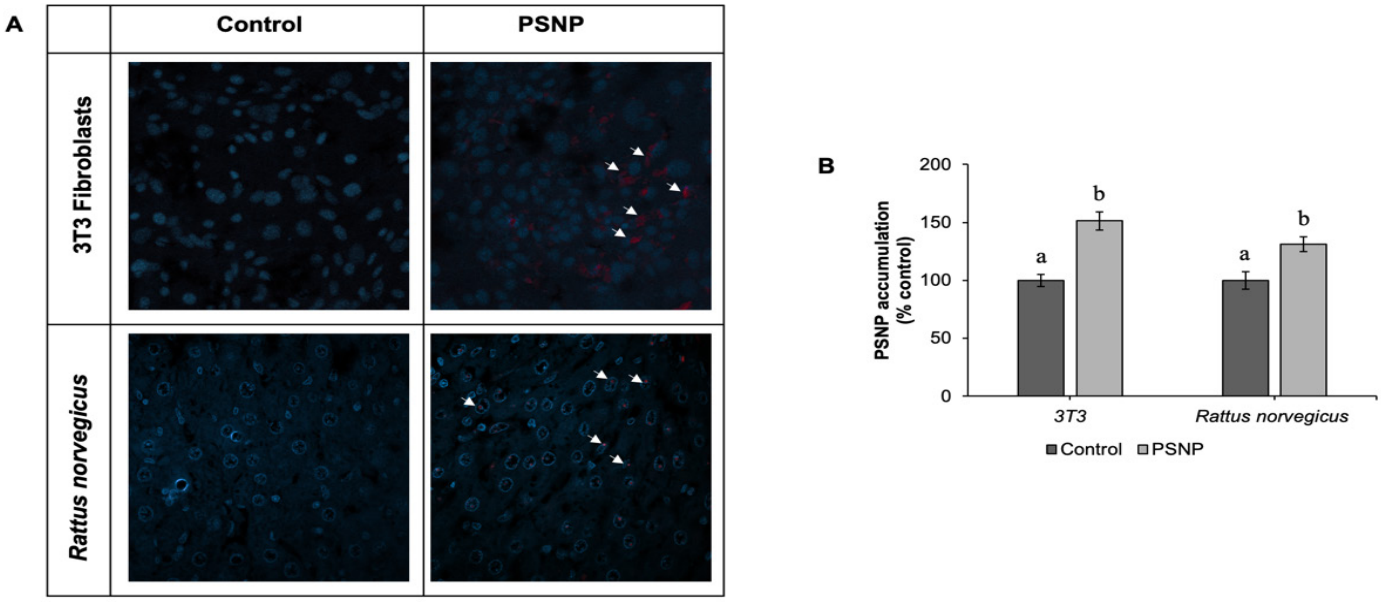

Figure 1: Accumulation of PSNPs in 3T3 fibroblasts and *Rattus norvegicus* hepatocytes. **A:** Representative fluorescence images of 3T3 fibroblasts and *Rattus norvegicus* hepatocytes under control conditions and after exposure to PSNPs. Minimal red fluorescence is observed in the control groups, whereas PSNP-exposed cells show a marked increase in red fluorescence, indicating significant nanoparticle accumulation. White arrows indicate regions of PSNP accumulation. Images were captured at 40× magnification, and at least 100 cells were analyzed per group. **B:** Quantification of PSNP accumulation expressed as a percentage of the control. Data are shown as mean ± SEM from three independent experiments. Error bars represent standard error of the mean. Different letters (a, b) denote statistically significant differences between groups ($p < 0.05$).

The observed uptake of PSNPs is consistent with previous studies demonstrating nanoparticle internalization in various cell lines, including fibroblasts and hepatocytes (Deng *et al.*, 2017; Wang *et al.*, 2022). Nanoparticles can enter cells through diverse pathways such as clathrin-mediated endocytosis, caveolae-dependent uptake, and macropinocytosis, influenced by factors including size, surface charge, and composition (Mazumdar *et al.*, 2021). The interaction of PSNPs with 3T3 fibroblasts is particularly noteworthy, as fibroblasts are pivotal in tissue maintenance and repair. Disruptions to fibroblast function could potentially impair wound healing and compromise extracellular matrix integrity (Dong *et al.*, 2024). Meanwhile, the accumulation of PSNPs in hepatocytes raises additional concerns, given the liver's critical role in detoxification and metabolic homeostasis. The increased PSNP retention in hepatocytes suggests a potential risk of nanoparticle-induced hepatotoxicity, emphasizing the need for further investigation into the mechanisms of PSNP-mediated liver toxicity (Das *et al.*, 2024).

Morphological Changes in Cells Due to PSNP Exposure

Exposure to PSNPs led to significant morphological and structural changes in both 3T3 fibroblasts and *Rattus norvegicus* hepatocytes. Confocal microscopy imaging (Figures 2A and 2B) revealed marked differences between control and PSNP-exposed cells. In the control group (Figure 2A), cells displayed a uniform structure with well-defined nuclei (white arrows) and minimal red fluorescence, indicating normal lipid distribution. In contrast, PSNP-exposed cells (Figure 2B) exhibited a substantial increase in red fluorescence (yellow arrows), suggesting enhanced lipid accumulation or alterations in membrane composition. Additionally, the cytoplasmic distribution appeared irregular, with significant variations in cell shape and size, indicative of potential cellular stress responses. Quantitative analysis (Figures 2C and 2D) corroborated these observations, showing that both cell and nucleus diameters were significantly increased in PSNP-treated groups compared to controls ($p < 0.05$), and a substantial increase in necrotic cells was observed, highlighting the cytotoxic effects of PSNP exposure.

Our findings offer strong indirect evidence suggesting that PSNPs likely interact with both cellular proteins and lipids. Confocal microscopy revealed pronounced cytoplasmic disorganization, enlarged cell and nuclear diameters, irregular morphology, and intense Nile Red fluorescence localized predominantly in the cytoplasm and near the nuclear envelope. These fluorescence patterns strongly suggest PSNP accumulation, as well as lipid remodeling or droplet formation. Such changes align with established literature indicating lipid perturbation, membrane destabilization, and oxidative stress induced by nanoplastic exposure (Hayati *et al.*, 2023). Such disruptions may compromise

membrane fluidity, affect signaling pathways, and initiate inflammatory responses (Itri *et al*., 2014). Together, these observations provide sufficient evidence to support our current conclusions while also highlighting directions for deeper mechanistic exploration in future studies.

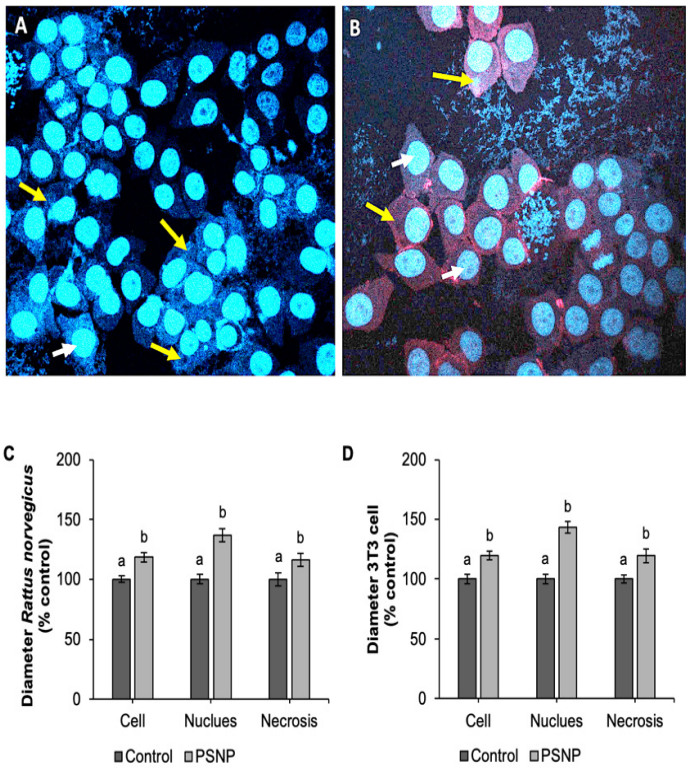

Figure 2: Morphological Changes in 3T3 Cells and PSNP Distribution After PSNP Exposure, Stained with DAPI and Nile Red, 100x Magnification. **A:** Confocal microscopy image of control 3T3 fibroblast cells stained with DAPI (blue) to visualize nuclei and Nile Red (red) for lipid distribution. White arrows indicate intact nuclei, while yellow arrows highlight normal lipid distribution in the cytoplasm. **B:** Confocal microscopy image of PSNP-exposed 3T3 fibroblast cells showing increased red fluorescence intensity (yellow arrows), indicating lipid accumulation or alterations in membrane composition. White arrows highlight nuclear changes, suggesting potential stress responses. **C:** Quantification of cell diameter, nucleus diameter, and necrosis in *Rattus norvegicus* hepatocytes under control and PSNP-exposed conditions. **D:** Quantification of cell diameter, nucleus diameter, and necrosis in 3T3 fibroblast cells under control and PSNP-exposed conditions. Data are presented as mean ± SEM. Different letters (a, b) indicate statistically significant differences ($p < 0.05$) between groups.

Our results also align with previous reports showing that micro- and nanoplastics can interfere with lipid homeostasis, induce oxidative damage, and destabilize cytoskeletal structures (Hayati *et al*., 2023). The irregular cytoplasmic distribution observed in PSNP-treated cells implies potential disruptions in actin filament dynamics and cytoskeletal integrity. Prior research has shown that nanoplastics can destabilize the cytoskeleton, resulting in altered cell morphology, impaired intracellular transport, and compromised cellular function (Schirinzi *et al*., 2017). The increase in cell and nucleus size may reflect cytoskeletal reorganization or swelling due to osmotic imbalance and stress signaling, indicating that PSNPs affect not only structural stability but also intracellular homeostasis and fibroblast function.

Importantly, the enhanced red fluorescence and visible changes in membrane structure suggest that PSNPs may interact directly with cellular lipids. Nanoplastics are known to integrate into lipid bilayers or alter membrane fluidity, potentially impairing membrane integrity, signal transduction, and lipid metabolism (Khan and Jia, 2023). The intensified Nile Red signal in PSNP-exposed cells may reflect not only the presence of the nanoparticles themselves but also a PSNP-induced accumulation of lipids or formation of lipid-rich domains. These disruptions may promote lipid peroxidation, further exacerbating oxidative stress and cytotoxicity (Nurbani *et al*., 2025).

Furthermore, intracellular accumulation of PSNPs could initiate inflammatory responses and activate apoptotic signaling pathways, ultimately compromising cell viability and proliferation (Khan and Jia, 2023). The significant rise in necrotic cells in PSNP-treated samples supports the hypothesis of PSNP-induced cytotoxicity. Previous studies have linked nanoplastic exposure to oxidative stress, mitochondrial dysfunction, and the activation of apoptosis, which may progress to necrosis if cellular damage is irreversible (Chen *et al*., 2025; Lee *et al*., 2022; Mahmud *et al*., 2024).

While this study did not include direct biochemical assays to confirm specific PSNP interactions with cellular proteins or lipids, the combined morphological, quantitative, and fluorescence-based evidence strongly supports the likelihood of such interactions. These observations are in agreement with a growing body of research on nanoplastic-induced cellular damage through mechanisms involving protein corona formation, membrane destabilization, and oxidative stress (Wang *et al*., 2023). Future studies incorporating proteomic and lipidomic analyses, as well as targeted assays for oxidative markers and cytoskeletal dynamics, will be essential to fully characterize the molecular mechanisms underlying PSNP-induced toxicity.

High-Resolution Analysis of PSNP Localization in 3T3 Cells

To further investigate PSNP accumulation within 3T3 cells, confocal microscopy analysis at 400x magnification was performed. Figure 3 illustrates the distinct morphological differences between control and PSNP-exposed cells, stained with DAPI (blue) to highlight nuclei and Nile Red

(red) to visualize PSNP presence. In the control group (Figure 3A), 3T3 cells exhibited normal morphology, with a round, well-defined nucleus and a uniformly distributed cytoplasm, indicative of healthy cellular conditions. In contrast, PSNP-exposed cells (Figure 3B) exhibited intense red fluorescence signals, predominantly localized in the cytoplasm and around the nuclear envelope (highlighted by red asterisks), indicating considerable nanoparticle internalization and accumulation.

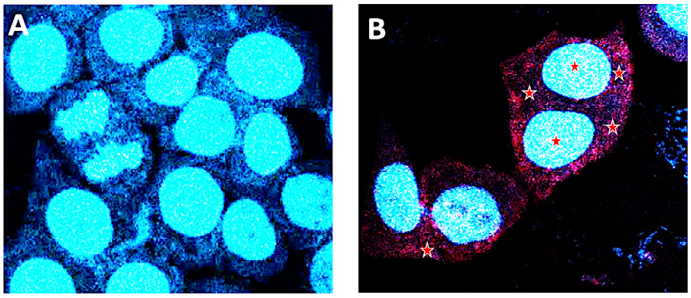

Figure 3: Nanoplastic Distribution in 3T3 Cells. Confocal Microscopy Analysis with DAPI and Nile Red Staining, 400x Magnification. **A:** Control Group: Displays normal 3T3 cell morphology with round nuclei and uniformly distributed cytoplasm, indicative of healthy cellular conditions. **B:** PSNP-Exposed Group: Demonstrates significant nanoplastic accumulation (red asterisks) within the cytoplasm and around the nucleus, as evidenced by the intensified red fluorescence, suggesting potential cellular stress and interaction with intracellular components.

The intensified red fluorescence and its perinuclear localization suggest that PSNPs are not only taken up by cells but also trafficked through the cytoplasm, potentially accumulating in vesicular structures such as endosomes or lysosomes. This distribution pattern aligns with earlier studies showing that nanoplastics can enter eukaryotic cells via endocytic pathways, including clathrin-mediated endocytosis and micropinocytosis (Viršek *et al*., 2017). Once internalized, PSNPs may evade lysosomal degradation due to their chemical stability and accumulate in intracellular compartments, leading to functional disruptions.

The close proximity of PSNPs to the nuclear envelope further raises concerns regarding possible interactions with nuclear components. Such interactions may compromise nuclear membrane integrity, interfere with nucleocytoplasmic transport, and influence processes such as DNA replication, transcription, and ribosome biogenesis. Disruption in these regulatory pathways could ultimately impact gene expression, promote genomic instability, or trigger stress-induced signaling cascades. Although nuclear entry was not directly confirmed in this specific image set, the fluorescence pattern observed is suggestive of near-nuclear or intranuclear localization, warranting further investigation.

Furthermore, the altered fluorescence intensity and distribution patterns in PSNP-treated cells may reflect changes in lipid metabolism or membrane composition. Nile Red, a lipophilic dye, preferentially binds to lipid-rich regions; therefore, increased fluorescence may result from lipid droplet accumulation, membrane remodeling, or PSNP association with lipid bilayers. This interpretation is consistent with previous studies demonstrating that nanoplastics can disturb membrane dynamics, alter lipid raft organization, and impair membrane-associated signaling pathways (Ji *et al*., 2024; Kumah *et al*., 2023; Lai *et al*., 2022). Collectively, these observations suggest that PSNP exposure may induce changes in lipid composition and membrane integrity, contributing to cytotoxic effects through both physical accumulation and biochemical disruption.

Structural Changes in Hepatocytes Following PSNP Exposure

The animal study using *Rattus norvegicus* revealed significant structural changes in hepatocytes following exposure to PSNPs, as illustrated in Figure 4. Fluorescent staining with DAPI (blue) and Nile Red (red) was utilized to assess hepatocyte morphology and PSNP distribution. In the control group (Figure 4A), hepatocytes displayed normal structural characteristics, with well-defined, round nuclei (white arrows) and uniform cytoplasmic distribution (yellow arrows). The absence of red fluorescence indicated no PSNP accumulation, suggesting healthy cellular conditions. With early-stage PSNP exposure (Figure 4B), red fluorescence began to appear within specific cytoplasmic regions, indicating initial PSNP infiltration. Although the nuclei remained intact, mild PSNP presence in the cytoplasm suggested the onset of cellular exposure to nanoplastics. As PSNP exposure progressed (Figure 4C), more pronounced red fluorescence was observed, demonstrating increased PSNP accumulation within both the cytoplasm and perinuclear regions. The red-stained spots were more abundant, highlighting the extensive uptake of PSNPs by hepatocytes. The appearance of red signals near the nuclei also suggested a potential translocation of particles toward the nuclear envelope.

In the advanced PSNP exposure stage (Figure 4D), red fluorescence was highly intensified, indicating significant PSNP accumulation within the hepatocyte cytoplasm and nucleus. The nuclear structural changes and the widespread red fluorescence suggest considerable cellular stress and possible functional disruptions due to PSNP exposure. The presence of PSNPs in the nucleus raises concerns about potential impacts on cellular processes, including gene expression regulation and genomic stability. PSNPs can penetrate biological membranes, including the plasma and nuclear membranes (Tarafdar *et al*., 2022). The fluorescence patterns observed in this study show a gradual infiltration

of PSNPs into hepatocytes, starting from the cytoplasm (Figures 4B and 4C) and eventually reaching the nucleus (Figure 4D). Nanoplastics typically enter cells through endocytosis mechanisms, particularly macropinocytosis and clathrin-mediated endocytosis (Liu *et al*., 2021). The detection of PSNPs within the nucleus suggests either disruption of the nuclear envelope or active transport through the nuclear pore complex. The accumulation of PSNPs within the nucleus poses a significant risk, as it may induce DNA damage and increase the likelihood of genetic mutations, potentially disrupting hepatocyte function and liver metabolism (Haldar *et al*., 2023). Previous studies have shown that prolonged PSNP exposure can trigger oxidative stress, promote inflammatory responses, and impair organelle function within liver cells (Hu and Palić, 2020).

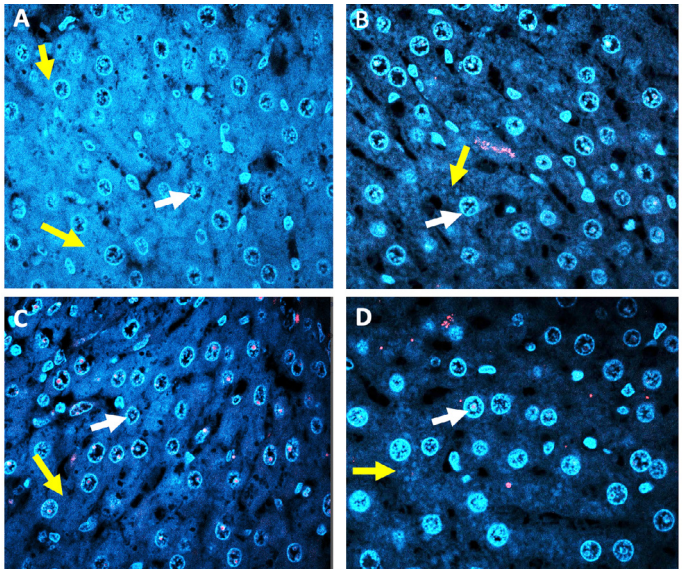

Figure 4: PSNP Accumulation in *Rattus norvegicus* Hepatocytes: Fluorescence Staining with DAPI and Nile Red. **A:** Control group showing normal hepatocyte morphology. **B:** Early PSNP exposure with mild red fluorescence indicating initial cytoplasmic accumulation. **C:** Progressive PSNP accumulation with increased red fluorescence in the cytoplasm and near the nucleus. **D:** Advanced PSNP infiltration showing intense red fluorescence within the cytoplasm and nuclei, indicating cellular stress and potential disruption. White arrows indicate hepatocyte nuclei, and yellow arrows mark the cytoplasm. Fluorescence staining with DAPI (blue) visualizes the nuclei, while Nile Red (red) highlights PSNP presence.

Given the liver's critical role in detoxification and blood filtration, it is a primary target for nanoparticle accumulation (Campanale *et al*., 2020). PSNP-induced hepatotoxicity can manifest through alterations in hepatocyte morphology, increased intracellular vacuole formation, nuclear deformation, and enhanced apoptotic activity. Additionally, PSNP exposure can elevate reactive oxygen species (ROS) production, leading to oxidative stress, lipid peroxidation, and mitochondrial dysfunction (Deng *et al*., 2017; Saputra *et al*., 2025). Another critical observation is the disruption of liver metabolism, evidenced by changes in detoxification enzyme expression, which could impair hepatocyte function and overall liver health. The findings of this study confirm that PSNPs can infiltrate the cytoplasm and nucleus of *Rattus norvegicus* hepatocytes, as demonstrated by fluorescence staining with Nile Red and DAPI. The ability of PSNPs to reach the nucleus underscores their potential to cause cellular damage, disrupt liver function, and pose a substantial toxicological risk to the health of exposed organisms.

Intracellular Distribution of PSNPs and Potential Cellular Impact

Figure 5 illustrates the structural characteristics of *Rattus norvegicus* hepatocytes following PSNP exposure, highlighting the intracellular distribution of PSNP within the cytoplasm and nucleus. DAPI staining (blue) reveals round or oval hepatocyte nuclei with bright blue fluorescence, while Nile Red staining (red) indicates PSNP accumulation as red fluorescent dots. The presence of PSNP in both the cytoplasm and nucleus suggests successful internalization and potential for subcellular disruption.

The detection of PSNP within the nucleus is particularly concerning, as it indicates that nanoplastics can penetrate the nuclear membrane, likely through endocytosis or active transport via the nuclear pore complex (Dehghanian *et al*., 2023). This raises the possibility of direct interactions with nuclear components such as chromatin, transcription factors, or DNA repair machinery. Such interactions may impair gene regulation, disrupt nucleolar activity, or induce genomic instability (Misteli and Soutoglou, 2009). Although our study did not directly assess gene expression or DNA integrity, the close proximity of PSNPs to the nucleus and nucleolus strongly suggests a potential risk of DNA damage, altered transcription, and impaired ribosomal biogenesis. In addition to nuclear effects, the accumulation of PSNPs in the cytoplasm confirms active cellular uptake, likely through macropinocytosis or clathrin-mediated endocytosis. This internalization can trigger oxidative stress, inflammation, and mitochondrial dysfunction-factors known to indirectly influence gene expression by activating stress-responsive transcription pathways or epigenetic modifications (Mahmud *et al*., 2024). Furthermore, the presence of PSNPs in the extracellular space suggests their ability to migrate through tissues and enter systemic circulation, which could facilitate distribution to other vital organs. This observation aligns with previous reports that nanoplastics can cross biological barriers and accumulate in metabolically active tissues (Lai *et al*., 2022).

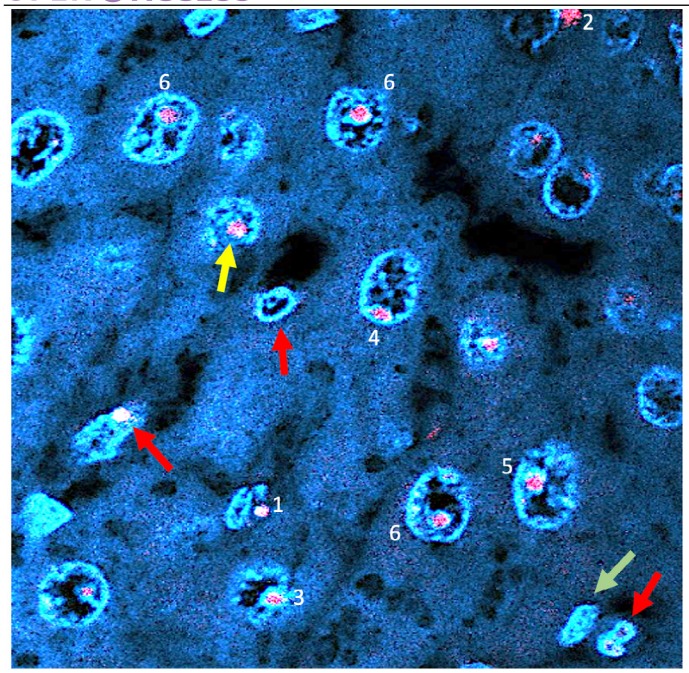

Figure 5: PSNP Distribution and Infiltration in *Rattus norvegicus* Hepatocytes. Confocal microscopy images showing hepatocyte nuclei (blue, DAPI) and PSNP accumulation (red, Nile Red). PSNP particles are observed in the cytoplasm (yellow arrows) and within the nucleus (red arrows), indicating successful cellular internalization and nuclear infiltration. The numbered labels (1–6) represent sequential stages of PSNP intracellular progression: (1) Extracellular localization: PSNPs are initially observed in the extracellular matrix surrounding hepatocytes. (2) Initial cytoplasmic uptake : PSNPs begin to accumulate in the cytoplasm, likely through endocytotic mechanisms. (3) Nuclear envelope interaction : PSNPs interact with and penetrate the nuclear membrane, entering the nucleus. (4) Intracellular dispersion : PSNPs are dispersed within the nucleoplasm, suggesting widespread nuclear distribution. (5) Directed movement toward nucleoli : PSNPs exhibit directed migration toward the nucleolar region. (6) Nucleolar accumulation : PSNPs accumulate near or within the nucleolus, potentially affecting nucleolar structure or function. Green arrows highlight regions of intense PSNP clustering, possibly indicating agglomeration or retention zones within or near nucleolar areas.

Given the liver's critical role in detoxification and metabolic regulation, PSNP accumulation could significantly impair normal liver function, leading to hepatotoxic effects, including oxidative stress, lipid peroxidation, and mitochondrial dysfunction (Allameh *et al*., 2023). The progressive stages of PSNP infiltration (steps 1 to 6 in Figure 5) indicate not only physical accumulation within cells but also potential biochemical interactions with nuclear components. The proximity of PSNP to the nucleolus (step 6) raises concerns about possible disruptions to ribosomal biogenesis and alterations in cellular protein synthesis. Moreover, PSNP-induced oxidative stress may impair gene transcription, lead to cell cycle arrest, and activate pro-inflammatory pathways, potentially contributing to hepatocyte dysfunction and liver tissue necrosis (Shi *et al*., 2021). The ability of PSNPs to reach the hepatocyte nucleus has significant implications for human health and ecosystems. Accumulation in critical organs such as the liver and penetration into the nucleus suggest that PSNPs could similarly affect other vital organs. The liver's primary role in metabolism and detoxification makes it especially vulnerable to metabolic disturbances caused by PSNP exposure. These particles could lead to hepatotoxicity, disrupt energy homeostasis, and interfere with normal liver function (Wang *et al*., 2024).

The long-term effects of PSNP accumulation in the body could contribute to chronic cellular damage and increase the risk of liver disease, cancer, and endocrine disorders (Amereh *et al*., 2020; Chiang *et al*., 2024). This study demonstrates that PSNPs can enter hepatocytes and infiltrate the nucleus, potentially causing oxidative stress, disrupting gene expression, and inducing other hepatotoxic effects. The mechanisms for PSNP entry include endocytosis and passive diffusion, with biological impacts linked to DNA damage, inflammation, and metabolic disturbances (Mahmud *et al*., 2024; Rajendran and Chandrasekaran, 2023).

CONCLUSIONS AND RECOMMENDATIONS

PSNPs can effectively penetrate cell membranes, accumulate in the cytoplasm, and reach the nucleus in both 3T3 fibroblast cells and *Rattus norvegicus* hepatocytes. Their intracellular presence is associated with pronounced morphological alterations, cytoplasmic disorganization, and evidence of cellular stress, suggesting interference with vital metabolic and structural functions. These effects likely occur via active endocytic pathways and possible nuclear transport mechanisms. The accumulation of PSNPs within critical subcellular compartments highlights their potential to disrupt membrane integrity, induce oxidative stress, and interfere with nuclear processes, raising important concerns about genotoxicity and long-term cellular health. These findings underscore the urgent need for further research into the chronic impacts of nanoplastic exposure, particularly studies that investigate molecular toxicity mechanisms, including inflammation, oxidative damage, and genetic instability.

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NOVELTY STATEMENTS

This study provides new insights into the intracellular behavior of PSNPs, demonstrating their ability not only to accumulate within the cytoplasm but also to penetrate the nuclear membrane of both 3T3 fibroblast cells and *Rattus norvegicus* hepatocytes. This nuclear localization of PSNPs, particularly in hepatocytes, is a novel finding that highlights potential risks to cellular and genetic stability.

AUTHOR'S CONTRIBUTIONS

Febriyansyah Saputra, Alfiah Hayati, Adiibtia Septiani, Manikya Pramudya, Raden Joko Kuncoroningrat Susilo, Vuanghao Lim and Bayyinatul Muchtaromah: Conceptualized and designed the study and participated in the investigation and the drafting of the paper as well as, analyzed and interpreted the data and revised the paper critically for intellectual content and approved the final version of the paper to be published. All authors agree to be accountable for all aspects of the work.

Conflict of Interest

This study has no conflict interest between all of authors

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