

The study of Jie Wang, Zhong-Lan Chen, Cheng-Gang Liang, Hui-Ying Yang, Xian-Fu Wu and Hui-Min Sun, named “Toxicological impacts of polypropylene nanoparticles in plastic-bottled injections on human umbilical vein endothelial cells”, is devoted to the problem of clarifying the molecular-cellular mechanisms of action micro- and nanoplastics on the human body, especially the cardio-vascular system. The work has several strengths and weaknesses.

The introduction contains detailed quantitative data on the characteristics of plastic nanoparticles, their routes of entry, accumulation, transport through the body and elimination from the body, which justifies the choice of the studied doses of plastic nanoparticles. Figure 1 complements the text excellently.

The overall research plan is consistent and well thought out. The results obtained complement each other and allow the authors to draw well-founded conclusions.

The list of materials contains a list of catalog numbers, which significantly simplifies the reproducibility of experimental data by other authors.

However, the work can be significantly improved and clarified to attract more attention from readers. I believe that the work can be published in the journal *Toxins* after peer review.

Main comments:

The authors clearly indicate the impossibility of studying NPs from plastic bottles and the alternative use of commercially available NPs. I think that the title can simply be left as "polypropylene nanoparticles" or make a softer accent "plastic-bottled-like polypropylene nanoparticles". I think the word "injections" can be omitted from the title.

It is advisable to add information on the number of passages for the cell lines on which the experiments were performed. For endothelial cells, especially HUVEC, the passage number is critical for obtaining adequate results.

Brief information about healthy volunteers is also desirable to provide: total number, age, gender distribution, what details of the anamnesis were checked before the study.

For endothelial cells, special surface treatment for attachment is important. It is advisable to detail whether such treatment was done (e.g. pretreatment with gelatin or use of commercially available pretreated glassware).

Due to the high diversity of ROS, it is desirable to specify which forms are registered. The technical documentation states that this is especially superoxide and hydroxyl radicals. Please add clarification to the text. It would also be desirable to clarify which ROS pool is measured: extracellular, intracellular or total.

It is important to add information about the lipopolysaccharide used. The immunogenicity of LPS depends significantly on the species and strain of the producer bacteria. Some LPS may not cause an immune response, while others may cause hyperactivation of cells. It is very important to indicate the species name of the bacteria and the strain, preferably with the serotype.

Please clarify at what temperature and for how long were the ELISA samples stored?

Using Student's t-test and one-way ANOVA with Tukey's test gives adequate results only in the case of normal distribution of values in the compared samples. The authors need to add information about the test of normality of distribution of sample values. In case the normality test (for example, the Shapiro-Wilk test) is not passed, then the authors will need to recheck the statistical hypotheses using more adequate methods of nonparametric statistics, for example, the Mann-Whitney U test or Kruskal-Wallis ANOVA with post-hoc Dunn's test.

Figure 3. In the title, the word “necrosis” should be replaced with the more general “cell death”. Since this method analyzes the ratio of cells in apoptosis and necrosis. The authors extracted only one third of the information from their results. I suggest that the authors analyze the cytometry data in a classical way and determine the proportions of living cells (Q3), cells in early (Q4) and late apoptosis (Q2), necrosis (Q1). I believe that adding these data will strengthen the work and help with the discussion of the results of molecular methods. In addition, 2D dot plots in panels D-E appear insufficiently compensated (presence of a diagonal “tail of dots” at 45° angle). Please check your compensation matrix or change the gating according to the cloud shapes and recalculate results.

Please add description of sample preparation for SEM in the materials and methods section.

Figure 4. Panels *Ab* and *Bf* It is advisable to increase so that they are easier to read. Red letters are not very clearly readable on a gray background, perhaps the color should be changed. If there are examples of microphotographs of fluorescence microscopy, it is also advisable to provide them and add a color scale of brightness for fluorescence. I think that this will make the results more clear.

Line 282. The results demonstrated by the authors show the prevalence of cell death through apoptosis (with the majority of cells being in the early apoptosis phase, see Fig3 Q4). Annexin - V - FITC is a specific marker of apoptosis. The proportion of necrotic cells also increases, but less pronouncedly.

Line 288: The conclusion at the end of the subsection looks correct, but it could be strengthened by adding a quantitative assessment of the ratio of cells in apoptosis and necrosis.

Figures S 1 and S 2 provide insufficient information about LC3B fluorescence. The signal appears to be either absent or too low. In the first case, a positive control must be added to compare the experimental values obtained. In the second case, image post-processing (the same everywhere) must be performed to improve the signal display.

Figure 6H. The font is difficult to read. It is advisable to increase the size of the panel.

Figure 7. It is also necessary to specify the incubation time with LPS for each cytokine. If it is also 72 hours, it is advisable to add shorter incubations, for example, 4 or 24 hours, depending on the dynamics of LPS-induced cytokine secretion known from the literature. To obtain the most adequate results, in the case of PP-NPs, it is also critical to use their own incubation times for each cytokine, corresponding to their peak concentrations. In three days, all inflammatory reactions could have already passed. It is advisable to supplement measurements with adequate time points for each cytokine, if this is methodologically possible.

Figure 8. It seems that the figure does not always indicate statistical differences where they exist (e.g. panels b and c are LPS variants). Please recheck.

The discussion indicates a zeta potential value of less than 5 mV modulo. These values are typical for unstable colloids, in which NPs quickly aggregate with each other and precipitate. The authors should discuss this issue in more detail. Next comes a detailed description of the NPs dispersion procedure. Am I right in understanding that this is a natural consequence of aggregation? If so, it would be desirable to state this idea more clearly.

Do I understand correctly that 0.01% Tween 80 was also added to the control samples of cultures without NPs? If so, this clarification should be added in the materials and methods section.

The authors provided detailed theoretical data for potential extrapolation *in vivo*, however, I think it would be better to use a softer formulation in the introduction, for example, “potentially *in vivo*”

Since the study used the blood of healthy volunteers, it is necessary to provide information on obtaining written informed consent from the study participants, and the number of the conclusion of the bioethics commission with the date of receipt. It is strictly necessary to indicate whether the requirements of the Declaration of Helsinki were observed when working with volunteers.

Minor:

Authors are encouraged to check and accept all edits to the text (example, line 126) at the next stages of editing.

Line 132: Extra space in annexin V - fluorescein

Best regards