Interreg
Italy - Croatia
NET4mPLASTIC

EUROPEAN UNION

NET4mPLASTIC Project

Work Package 4.4

Deliverable 4.4.1

DETERMINATION OF THE FATE OF MICROPLASTICS WITHIN CELLS

June, 27, 2022 – Version 1.0

CONTRIBUTING PARTNERS
PP 0 – UNIVERSITY OF FERRARA [UNIFE], DEPARTMENT OF TRANSLATIONAL MEDICINE Ilaria Conti (UNIFE), Luca Maria Neri (UNIFE), Carolina Simioni (UNIFE), Daniela Milani (UNIFE) Authors are grateful for helpful collaboration to: PP 5 – VETERINARY PUBLIC HEALTH INSTITUTE OF ABRUZZO AND MOLISE REGIONS [IZSAM] Nadia Barile (IZSAM), Sara Recchi (IZSAM), Eliana Nerone (IZSAM), Federica Pizzurro (IZSAM), Gianfranco Diletti (IZSAM), Ersilia Di Pancrazio (IZSAM), Luana Candelori (IZSAM), Daniela Ciavarelli (IZSAM) PP 6 – TEACHING INSTITUTE FOR PUBLIC HEALTH [TIPH] Lina Velcic (TIPH), Itana Bokan (TIPH), Barbara Kvartuč (TIPH). PP 8 – UNIVERSITY OF SPLIT - FACULTY OF CIVIL ENGINEERING, ARCHITECTURE AND GEODESY [UNIST-FGAG] Roko Andricevic (UNIST-FGAG), Toni Kekez (UNIST-FGAG), Marin Spetic (UNIST-FGAG), Petra Simundic (UNIST-FGAG).

Contact Information:

https://www.italy-croatia.eu/web/netformplastic

Contact persons:

Ilaria Conti, PhD (Temporary Researcher – Type A): ilaria.conti@unife.it

Luca Maria Neri, Full Professor (Coordinator of Work Package 4.4 and Full Professor at University of Ferrara): luca.neri@unife.it

Institutions:

UNIFE: University of Ferrara - Department of Translational Medicine, Ferrara, Italy.

This document was produced as final deliverable 4.4.1 for NET4mPLASTIC Project in work package 4.4 (WP 4.4) – European Regional Development Fund 2014-2020 Interreg V-A Italy-Croatia CBC Programme.

NET4mPLASTIC: New Technologies for macro and MicroPlastic Detection and Analysis in the Adriatic Basin.

Project Application ID: 10046722 – CUP F76C19000000007

Project duration: 01.01.2019 – 30.06.2022

Programme: 2014-2020 Interreg V-A Italy-Croatia CBC Programme.

Disclaimer

This document has been produced with the financial assistance of the 2014-2020 Interreg V-A Italy-Croatia CBC Programme.
University of Ferrara (UNIFE) has taken care of the preparation of the following deliverable to ensure that the presented data is as accurate as possible within the scope of the project. However, they are not responsible for the decisions or actions taken based on the content of this document.

SUMMARY

This document refers to Deliverable 4.4.1, which is related to edit "Determination of the fate of microplastics within cells". *In vitro* experimental researches were performed and scientific literature was analyzed in order to evaluate the uptake of microplastics (MPs) by human cell lines and their distribution within the cells.

The main aims of the Deliverable 4.4.1 were:

- Analysis of internalization of MPs by different human cell lines.
- Analysis of the influence of parameters, such as MPs concentration, size and exposure time, in the interaction between MPs and human cells.

INDEX

1. Introduction 5
2. Materials and Methods 6
  2.1 Microplastics beads 6
  2.2 Scanning Electron Microscopy (SEM) 6
  2.3 Cell lines and treatments 6
  2.4 Transmission Electron Microscopy (TEM) 7
  2.5 Fluorescent assay 7
  2.5.1 Light Microscopy 7
  2.5.2 Confocal Microscopy 8
3. Results 8
  3.1 PS-beads characterization 8
  3.2 Assessment of PS-beads within human cell lines 8
  3.3 Interaction between PS-beads and human cell lines as consequence of exposure time and beads' concentration and size 9
4. Discussion 9
5. Conclusions 10
6. References 11

1. Introduction

Since their easy production, cheapness and adaptability to several life activities, plastic materials are globally spread and highly integrated in human life [Rodrigues M.O. et al.; 2019]. In 2020, plastic production reached 367 million tons in the world, of which 55 million tons were produced in Europe [PlasticsEurope; 2020]. A manufacture trend that is increasing in the world but decreasing in Europe during the last years (i.e. in 2018 the world plastic production achieved 359 million tons, of which 61.8 million tons in Europe) [PlasticsEurope; 2019], due to the European policies adopted to counteract plastic pollution. Notwithstanding the great use of plastic objects, awareness is arising due to plastic impact on environment and potential damage to wildlife [Conti I. et al.; 2021]. Plastic products have different lifespan, ranging from 1 to 50 years, and result from the combination of several compounds other than the plastic polymer chains. Plasticizers, catalysts, initiators, solvents, diluents, elastomers, stabilisers and anti-oxidant are added during the plastic polymerization process in order to increase the reaction yield and obtain a high stability of the output products [Hahladakis J.N. et al.; 2018]. Hence, the set of the different plastic materials (e.g. polystyrene [PS], polyethylene [PE], etc…) together to the several added compounds influences the post-consumer plastic waste treatment.

The “Conversion Market & Strategy GmbH” evaluated that, in Europe during the 2020, 29.5 million tons of post-consumer plastic waste were collected. Of them, more than one third (34.6%) was recycled and 42% was sent to energy recovery; while, still over the 23.4% of collected plastic waste was leaked in landfills [PlasticsEurope; 2020]. Large plastic items and plastic particles smaller than 5 mm, i.e. microplastic [MPs], have been detected in marine environment, in soil and in atmosphere [Galafassi S. et al.; 2019]. Moreover, MPs can be intentionally produced at the microscale (primary MPs), such as ingredients in scrubs (i.e. personal care products), or result from the fragmentation of plastic litter (secondary MPs) [Napper I.E. et al.; 2015 – Andrady A.L.; 2011].

Humans are potentially daily exposed to plastic contamination. Other than in air and in personal care products, MPs have been detected in foodstuffs, such as, seafood, sugar, salt, honey, beer and drinking water [Rhodes C.J.; 2018]. Inhalation and ingestion have been identified as the two main routes of MPs intake by human [Wright S.L., Kelly F.J.; 2017], although the current uptake of MPs and any associated human exposure risk has not been totally clarified. Moreover, recent researches have described the presence of MPs in human blood, placenta and stool [Leslie H.A. et al.; 2022 – Ragusa A. et al.; 2021; Schwabl P. et al.; 2019].

However, several *in vitro* studies showed contradictory results regarding the accumulation of plastics in human cell lines. Yan-Yang Lu et al. showed a time- and a concentration-dependent interaction of PS-particles in human umbilical vein endothelial cells [HUVECs], which differed between the two studied beads’ sizes (100 and 500 nm diameter): only the 100 nm PS-beads were up-taken by HUVEC and aggregated into the cellular cytoplasm, while the 500 nm PS-beads were bound to the cell surface but not internalized [Lu Y.Y. et al.; 2022]. Whereas, another study

observed the internalization of 460 nm PS-particles by both human peripheral blood mononuclear cells [PBMCs] and human dermal fibroblasts cells [HDFs], localizing themselves mostly within the cytoplasm of the cells [Hwang J. et al.; 2020]. Wung et al. showed the internalization of both 0.1 and 5 µm PS-beads by human colon cancer CaCo-2 cells where they co-localized with the cellular lysosomes [Wung B. et al.; 2019]. Finally, a size-dependent PS-beads internalization was noticed by Wang Q. et al. in CaCo-2 cells treated with five different PS-particles sizes (300 nm, 500 nm, 1 µm, 3 µm and 6 µm). The percentages of up-taken MPs, from the smallest to the biggest particles, were 73%, 71%, 49%, 43% and 30%, respectively [Wang Q. et al.; 2020].
However, the previously described results obtained by microscopy (light, confocal and/or electron microscopy) and flow cytofluorimetry analysis are not of easy interpretation. Moreover, the PS-particles concentrations used within the in vitro studies are probably not comparable to what human are exposed to [Lim X.; 2021].
In this deliverable, we deeply investigated the internalization of PS-beads with different sizes by three human cell lines. In particular, we analyzed the potential MPs uptake by HCT-116, A549 and Mahlavu cell lines (respectively colorectal, lung and hepatocellular carcinoma), in order to elucidate the fate of plastic particles within human body.

2. Materials and Methods

2.1 Microplastics beads

Two different types of PS-MPs dispersions in distilled H_2O (10% solid content), that were purchased from Sigma-Aldrich, were used in this study: i) fluorescent labelled PS-MPs with diameters of 0.1 – 1 – 2 µm (product number: L9904, L9654 and L9529, respectively). The excitation/emission wavelength were nearly 500 nm/540 nm. Ii, corresponding to fluorescein (FITC), unlabelled PS-MPs with diameters of 0.1 – 0.3 – 0.6 - 1.1 – 3 µm (product number: LB1, LB3, LB6, LB11 and LB30, respectively). At the beginning of the cellular experiment, PS-MPs samples were sterilized by 70% ethanol treatment for 10 min at +4°C followed by two washes with phosphate buffer saline [PBS] to remove the remaining ethanol.

2.2 Scanning Electron Microscopy (SEM)

SEM was used to assess the morphology and the dimension of the unlabelled PS beads. Beads were diluted 1:100 in distilled H_2O and spotted on slides. After drying, they were covered by gold and examined using a Scanning Electron Microscope (EVO 40, Zeiss).

2.3 Cell lines and treatments

Three different cell lines were exposed to PS-MPs: HCT-116 (human colorectal carcinoma), A549 (human lung carcinoma) and Mahlavu (human hepatocellular carcinoma) (Fig. 1). All the three cell lines were maintained in DMEM medium (Lonza) supplemented with 10% inactivated FBS (Sigma-

Aldrich), 1% Penicillin/Streptomycin (Lonza) and 1% L-Glutamine (Lonza) at 37°C and 5% CO_2. Cells were treated with different concentrations of fluorescent labelled or unlabelled PS-MPs (100 – 500 - 1,000 – 2,000 - 5,000 – 10,000 – 20,000 beads/mm^2 of well plate) for 24 and 48 h and then collected for further analysis.

2.4 Transmission Electron Microscopy (TEM)

Firstly, TEM was used to assess the morphology and the dimension of the unlabelled PS beads. Beads were diluted 1:100 in distilled H_2O and spotted on copper grids. After drying, they were examined using a Transmission Electron Microscope (EM 910, Zeiss).
Then, TEM was performed to evaluate the internalization of PS-MPs and determine the intracellular localization of plastic particles in Mahlavu cell line. Mahlavu cells were plated in 6-wells culture plates at a cell density of 2 x 10^5 cells/well; the following day, cells were treated with 2 mL of fresh medium containing 1,000 or 10,000 PS-MPs/mm^2 for 48h. Afterwards, cells were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer for 2h at room temperature (RT), washed at least three times with 0.1 M phosphate buffer for 1 h at RT, post-fixed with 1% osmium in 0.1 M phosphate buffer for 1 h at RT and washed at least three times with the same buffer for 5 min at RT. Then, the samples were dehydrated through a graded ethanol and acetone series, followed by embedded in Durcupan resin. Cell ultrathin sections were cut using a glass knife in an ultra-microtome (Reichert Ultracut S Leica). Finally, sections were observed using the Transmission Electron Microscope (EM 910, Zeiss).

2.5 Fluorescent assay

2.5.1 Light Microscopy

Firstly, fluorescence microscopy was used to assess the morphology and the dimension of the fluorescent labelled PS-MPs. Beads were diluted 1:100 in distilled H_2O and spotted on slides. After drying, they were fixed with 4% paraformaldehyde in PBS for 30 min at RT, washed three times with PBS for 5 min at RT, once with H_2O for 2 min at RT. After the addition of the mounting slide medium (2.3% 1,4-diazabicyclo[2.2.2]octane [DABCO] in PBS/glycerol 1:1), the PS-MPs were examined using a fluorescent microscope (Nikon DS-Qi2).
To analyze the internalization of PS-MPs and determine the intracellular localization of plastic particles, cells were plated either on pre-coated (with Poly-L-lysine 0.1%) or not cover-slips in 6-wells culture plates at different cell density for the 24 and 48 h respectively, in order to obtain non confluent cells at the day of MPs exposure (HCT-116 and A549: 2 x 10^5 cells/well and 1 x 10^5 cells/well, Mahlavu: 1 x 10^5 cells/well and 5 x 10^4 cells/well, respectively for the 24 and 48 h PS-MPs administration). The following day, cells were treated with 2 mL of fresh medium containing 100 – 500 – 1,000 – 2,000 - 5,000 - 10,000 – 20,000 PS-MPs/mm^2. After 24 and 48 h, cells were washed three times with PBS for 5 min at RT to remove both the residual medium and the not internalized MPs and were fixed with 4% paraformaldehyde in PBS for 30 min at RT. Following the permeabilization with 0.2% saponin in PBS for 45 min at RT, cells were stained with Phalloidin iFluor-555 (Abcam) or Phalloidin Texas-Red (Sigma), diluted 1 : 1,000 in 1% bovine serum albumin/PBS for 1 h at RT to label the actin filaments of the cytoskeleton. The cell nuclei were stained with 0,5 µg/mL

DAPI (Sigma) for 1 min at RT. After cell dehydration with a grade ethanol series and addition of the mounting slide medium (2.3% DABCO in PBS/glycerol 1:1), fluorescent images were obtained using the fluorescent microscope (Nikon DS-Qi2).

2.5.2 Confocal Microscopy

Confocal microscopy observation was performed to evaluate PS-beads internalization and to better understand their distribution within the cells. Confocal images were obtained using a Laser Scanning Confocal Microscope (LSM 510, Zeiss). Image processing was performed using Fiji software (ImageJ).

3. Results

3.1 PS-beads characterization

SEM images were used to examine the morphology and dimension of unlabelled PS-MPs (Fig. 2). Within each PS particles type, they had quite homogeneous sizes showing an average diameter of 0.09 ÷ 0.17, 0.20 ÷ 0.35, 0.58 ÷ 0.65, ~ 1.2 and 2.9 ÷ 3.5 µm respectively for the 0.1 – 0.3 – 0.6 - 1.1 – 3 µm theoretical diameters. Moreover, smaller particles were identified within the 1.1 and 3 µm PS-MPs samples. 0.2 µm beads were observed within the 1.1 µm PS-MPs in a percentage of 2% compared to the expected beads. While numerous 0.2 ÷ 0.4 µm diameter particles were evaluated within the 3 µm PS-MPs sample. Furthermore, unlabelled PS-MPs were also assessed by TEM analysis: plastic beads appeared as "black dots" with quite homogeneous sizes within each particles type. Several particles with a diameter smaller than 0.2 µm were identified within the 3 µm PS-MPs samples, confirming the SEM observation (Fig. 3).
Fluorescent labelled PS-MPs were characterized by light microscopy to evaluate their dimension and the fluorescent appearance (Fig. 4). The PS-beads showed different sizes according to their diameters and were quite homogeneous within the same type.

3.2 Assessment of PS-beads within human cell lines

Mahlavu cells were treated with the three size fluorescent labelled PS-beads at the concentration of 1,000 beads/mm^2 for 48 h. In particular, green fluorescent beads were detected in 1 and 2 µm PS-MPs treated Mahlavu cells, when compared to untreated cells [ctrl]. Within the cells, beads were localized in the cytoplasm and especially, in the perinuclear region. Moreover, the highest number of PS-MPs in relation to the cells was observed for the 1 µm PS-MPs (Fig. 5).
HCT -116 cells showed a similar behaviour (data not shown).
To further assess the internalization of the plastic particles, 1 µm PS-MPs treated Mahlavu were analyzed at confocal microscopy, showing PS-MPs beads localized inside the cells (Fig. 6).
TEM analysis was performed in order to clarify the distribution of the PS-MPs within the cells. Mahlavu cells were treated either with fluorescent labelled or with unlabelled PS-MPs (ranging from 0.1 to 3 µm) for 48 h, then processed for TEM visualization (Fig. 7). In particular, several 0.1 µm PS-MPs treated cells showed clusters of black (dark) rounded particles within their cytoplasm, compatible with plastic beads, while, a 0.3 µm particle compatible with plastic bead was detected

only in one cell. The 0.6 and 1.1 µm PS-MPs were not observed within the cells by TEM analysis. Moreover, in 3 µm PS-MPs treated Mahlavu dark rounded particles of about 0.1 µm diameter were observed. They could be identified as the smaller plastic particles observed within the 3 µm sample also by SEM analysis, as probably resulting from 3 µm beads fragmentation.

3.3 Interaction between PS-beads and human cell lines as consequence of exposure time and beads' concentration and size

Once determined the presence of PS-MPs beads inside the human cells, we wanted to explore if different exposure parameters (such as MPs concentrations and exposure time) to the different sizes PS-MPs beads may influence their uptake by the cells. Moreover, we wanted to examine if cells, derived from different organ or apparatus, may display a different response to the PS-MPs exposure.

Mahlavu and HCT-116 cells were treated with increasing concentration of 1 µm PS-MPs (100 – 500 – 1,000 – 2,000 – 5,000 beads/mm^2) for 48 h. The obtained results showed a dose-dependent uptake of PS particles by cells from 100 to 5,000 beads/mm^2 in both the cell lines. Moreover, the MPs-cells interaction changed between Mahlavu and HCT-116 cells, showing a major uptake of PS-MPs by Mahlavu compared to HCT-116 cells (Fig. 8).

On the basis of these results, A549 cells were then exposed to the different fluorescent labelled PS-MPs at a concentration of 5,000 - 10,000 - 20,000 beads/mm^2 for 24 – 48 h and observed with light fluorescence microscopy. Green fluorescent 1 and 2 µm PS-MPs were observed in A549 cell line at all the different beads concentrations (Fig. 9-10-11-12).

The PS-particles uptake raised as MPs concentration increased and a larger uptake of the 1 µm PS-beads compared to the 2 µm size was observed (Fig. 12).

For the 1 µm PS-MPs within the same concentration, increased plastic beads uptake was observed at 48 h when compared to the 24 h exposure time. Differently, concerning the 2 µm size, a similar increase was observed in the 5,000 beads /mm^2 treated cells, but not in cells exposed to 10,000 or 20,000 beads/mm^2 (Fig. 10-11).

Detection of 0.1 µm beads was not possible/made difficult due to technical limitations; it will be overcome in the near future (data not shown).

Pictures of PS-MPs treated A549 cells are representative also for HCT-116 cell line.

In comparison to the other cell lines, Mahlavu cells showed a greater 1 and 2 µm PS-MPs uptake, with a dose-dependent increase also for the 2 µm PS-MPs number (Fig. 13 – 14).

Moreover, following the treatment with the highest concentration of plastic MPs (i.e. 20,000 beads/mm^2), 100% of Mahlavu cells resulted positive for the uptake of both the 1 and 2 µm PS-MPs already at 24 h of exposure (Fig. 13-14-15).

4. Discussion

Several *in vitro* studies have observed the accumulation of micro and nanoplastics within human cells [Hesler M. et al.; 2019]. However, their conclusions on plastic particles accumulation within

cells appears questionable due to the poor supporting data. Moreover, as mentioned previously, the plastic concentrations used in the in vitro experiments are probably not comparable to what human are exposed to. For example, Hwang J. et al. used for his *in vitro* study MPs concentrations up to 500 µg/mL for each particle size (from 460 nm to 1 µm), which is a much larger amount of MPs than the author's hypothesized human intake of plastic in a year (i.e. 19 µg/mL per year) [Hwang J. et al.; 2020]. Moreover, particles with diverse diameters weight differently: on a volume basis 1 µm particles are approximately 1,000 times smaller than 10 µm particles [Goodman K.E. et al.; 2021].

Here, we exposed different human cell lines to PS-MPs with different diameters but maintaining fixed the number of particles per mm^2 of the well. In this way, cells were treated with the same numbers of PS-MPs independently from the volume/dimension and weight of the beads. Three human cell lines (Mahlavu, HCT-116 and A549) were exposed to both unlabelled- and fluorescent labelled PS-MPs in a range from 0.1 to 3 µm diameter at a concentration from 100 to 20,000 beads/mm^2 for 24 and 48h. Analysis at light fluorescence microscopy showed uptake of both 1 and 2 µm PS-MPs by all the three cell lines in both a dose and time-dependent manner and they distributed within the cytoplasm and in the perinuclear region. Between the two dimensions, cells appeared to uptake more the 1 µm beads than the 2 µm PS-MPs. Besides, some of the 1 µm PS-MPs were internalized by Mahlavu cells as evaluated by confocal microscopy.

Among the three cell lines, Mahlavu appeared to be more susceptible to MPs uptake probably due to the greater dimension of these cells in comparison to HCT-116 and A549 cells.

5. Conclusions

Plastic particles can be internalized by living cells via both passive membrane penetration and active endocytosis as consequence of their dimensions. On the other hand, internalized plastics can be excreted from cells via energy-free penetration and energy-dependent lysosomal exocytosis [Liu L. et al.; 2021].

In this deliverable, we investigated the internalization of PS-MPs with different sizes and at different concentrations by three human cell lines (colorectal, lung and hepatocellular carcinoma) that could represent the human tissue mainly exposed to human plastic intake. The uptake of PS-MPs was evaluated at single-cell level highlighting the internalization of beads by cells in a dose- and time-dependent manner (especially for the 1 µm beads) but with differences among the three cell lines. Here, a major uptake of beads was identified for the hepatocellular carcinoma cells.

Further studies will be necessary to clarify and complete the set of *in vitro* experiments useful to determine the potential internalization of all the dimensions of PS-MPs by the three cell lines.

6. References

- Rodrigues M.O., Abrantes N., Gonçalves F.J.M., Nogueira H., Marques J.C., Gonçalves A.M.M., “Impacts of plastic products used in daily life on the environment and human health: what is known?” Environ. Toxicol. Pharmacol., vol 72 (2019).
- PlasticsEurope 2020, “Plastics – The Facts 2020; An analysis of European plastics production, demand and waste data” (2021).
- PlasticsEurope 2019, “Plastics – The Facts 2019; An analysis of European plastics production, demand and waste data” (2021).
- Conti I., Simioni C., Varano G., Brenna C. Costanzi E., Neri L. M., “Legislation to limit the environmental plastic and microplastic pollution and their influence on human exposure” Environ. Pollut., vol 288 (2021).
- Hahladakis J.N., Velis C.A., Weber R., Iacovidou E., Purnell P., “An overview of chemical additives present in plastics: migration, release, fate and environmental impact during their use, disposal and recycling”, J. Hazard Mater., vol 344, pp 179–199 (2018).
- Galafassi S., Nizzetto L., Volta P., “Plastic sources: a survey across scientific and grey literature for their inventory and relative contribution to microplastics pollution in natural environments, with an emphasis on surface water”, Sci. Total Environ. vol 693 (2019).
- Napper I.E., Bakir A., Rowland S.J., Thompson R.C., “Characterisation, quantity and sorptive properties of microplastics extracted from comsetics” Mar. Pollut. Bull., vol 99, pp 178-185 (2015).
- Andrady A.L., “Microplastic in the marine environment”, Mar. Pollut. Bull., vol 62, pp 1596-1605 (2011).
- Wright S.L., Kelly F.J., “Plastic and human health: a micro issue?” Environ. Sci. Technol., vol 51, pp 6634–6647, (2017).
- Rhodes C.J., “Plastic pollution and potential solutions” Sci. Prog., vol 101, pp 207–260 (2018).
- Leslie H.A., van Velzen M.J.M., Brandsma S.H., Vethaak A.D., Garcia-Vallejo J.J., Lamoree M.H., “Discovery and quantification of plastic particle pollution in human blood” Environ Int., vol 163, (2022).
- Ragusa A., Svelato A., Santacroce C., Catalano P., Notarstefano V., Carnevali O., Papa F., Rongioletti M.C.A., Baiocco F., Draghi S., D'Amore E., Rinaldo D., Matta M., Giorgini E., “Plasticenta: First evidence of microplastics in human placenta”, Environ. Int., vol 146 (2021).
- Schwabl P., Köppel S., Königshofer P., Bucsics T., Trauner M., Reiberger T., Liebmann B. “Detection of various microplastics in human stool: a prospective case series”, Ann. Intern. Med., vol 171, pp 453-457 (2019).
- Lu Y.Y., Li H., Ren H., Zhang X., Huang F., Zhang D., Huang Q., Zhang X., “Size-dependent effects of polystyrene nanoplastics on autophagy response in human umbilical vein endothelial cells”, Journ. Hazard. Mat., vol 421 (2022).
- Hwang J., Choi D., Han S., Jung S.H., Choi J., Hong J., “Potential toxicity of polystyrene microplastic particles”, Sci. Rep., vol 10 (2020).
- Wu B., Wu X., Liu S., Wang Z., Chen L., “Size-dependent effects of polystyrene microplastics on cytotoxicity and efflux pump inhibition in human Caco-2 cells”, Chemosphere, vol 221, pp 333-341 (2019)
- Wang Q., Bai J., Ning B., Fan L., Sun T., Fang Y., Wu J., Li S., Duan C., Zhang Y., Liang J., Gao Z., “Effects of bisphenol A and nanoscale and microscale polystyrene plastic exposure on particle uptake and toxicity in human Caco-2 cells”, Chemosphere, vol 254 (2020).
- Lim X, “Microplastics are everywhere — but are they harmful? Scientists are rushing to study the tiny plastic specks that are in marine animals — and in us”, Nature, vol 593 (2021).

- Hesler M., Aengenheister L., Ellinger B., Drexel R., Straskraba S., Jost C., Wagner S., Meier F., von Briesen H., Büchel C., Wick P.,Buerki-Thurnherr T. Kohl Y., “Multi-endpoint toxicological assessment of polystyrene nano- and microparticles in different biological models in vitro”, Toxicology in Vitro, vol 61 (2019).
- Goodman K.E., Hare J.T., Khanis Z.I., Hua T., Sang Q.X.A., “Exposure of human lung cells to polystyrene microplastics significantly retards cell proliferation and triggers morphological changes”, Chem. Res. Toxicol., vol 34, pp 1069-1081 (2021).
- Liu L., Xu K., Zhang B., Ye Y., Zhang Q., Jiang W., “Cellular internalization and release of polystyrene microplastics and nanoplastics”, Science of The Total Environment, vol 779 (2021).