The penetration of nanoplastics through inhalation, digestive tract, or by contact with the skin in healthy and disease mice, with allergic contact dermatitis, colitis, or asthma.

Plastic particles have become a new type of pollution, because it is used globally and its biodegradation is limited. Plastics are easily absorbed by organisms, especially marine ones. The number of reports of the presence of plastic in atmospheric air, food and water samples is growing dramatically. We still cannot assess how many and what kind of plastic particles are found in living organisms. Plastic particles with a size of 1 micrometer (μm) up to 5 millimeter (mm) are called microplastics. Fragmentation to a size below 1 μm is also possible. Nanoplastics are particles ranging in size from 1 nanometer (nm) to 100 nm. This means that nano- and mikroplastics can potentially enter the human body through the respiratory and digestive systems, as well as contact with the skin**.**

Many studies are conducted on cells, outside the body (*in vitro*), show that plastic can be toxic, cause inflammation, change gene expressions, or decrease the cell viability. *In vitro* studies also show that the effect depends on the type, size, charge, concentration, and duration of exposure to plastic. However, the health impact of nano- and microplastics on humans is not clearly understood. Therefore, it is very important to check whether nanoplastic can get into the human body and whether it can contribute to the worsening of disease states.

The aim of this project is to investigate whether, in natural conditions (*in vivo*), nanoplastic overcomes the barrier of the skin and endothelium in the respiratory and digestive tract. Another goal is to investigate whether skin, respiratory and digestive disease conditions may contribute to greater plastic penetration and whether consequently higher concentrations in tissues may be found.

Experimental mice will be used in the project. Polystyrene nanoplastics, sizes 25 and 50 nm, with carboxylic acid groups (COOH), amino groups (NH_2) or none group will be used. Visualization of plastic in tissue samples is challenging, so all of the particles will be labeled with fluorochrome, which can be seen in a high-resolution fluorescence microscope (confocal microscope).

In order to test the penetration of nanoplastic through the skin, mice will have their backs shaved, and then a patch containing fluorescent nanoplastic particles will be attached for a certain period. To test whether the nanoplastic makes the disease worse, allergic contact dermatitis will be induced in mice, by contact with the allergen, and then a nanoplastic will be applied to the same site of skin.

To test the uptake of the nanoplastic by the respiratory system, the mice will be inhaled in a special chamber with air containing the fluorescent nanoplastic. To test how disease affects the absorption of plastic, we will induce asthma in mice and then inhaled mice with plastic.

To assess whether the plastic is absorbed in the digestive tract, the animals will be fed a suspension of fluorescent nanoplastic directly into the stomach via an intragastric probe. In the next stage, we will cause inflammatory bowel disease, ulcerative colitis, and subsequently we will administer intragastric plastic to mice.

In these experiments, mice will receive different concentrations of plastic for different periods of time. Data on their well-being will collect. We will take samples of blood, urine, feces, biopsies of the skin, lungs, intestines, and then analyze them in a confocal microscope. We'll also take lymph nodes, and see if the nanoplastic has activated the immune cells.

The research hypothesis assumes that the nanoplastic will penetrate the skin, digestive and respiratory systems. In addition, we expect that this will depend on the dose of plastic and the time of exposure. In addition, we assume that skin damage, asthma, and inflammatory bowel disease will increase plastic penetration and worsen the disease condition.