# Work package 5: In vitro methods for genotoxicity

## The objectives of the work package are the following:

To establish robust methodology using alternative tests to screen in vitro genotoxicity of MNs in pulmonary, oral and dermal cell systems. These assays will be applied to all MNs assessed. On the basis of the results obtained, a ring tests will be performed using the most promising approaches.

To generate in vitro genotoxicity data on MNs: Production of in vitro genotoxicity data on MNs using standard tests and modified assays utilizing specific cell models.

To perform a round robin test on in vitro testing of MNs : Based on in vitro genotoxicity and physical/chemical characterisation data obtained, a ring test on selected MNs will be carried out using the most promising in vitro assays

## Work progress

Several issues are still unclear in the in vitro genotoxicity testing of nanomaterials (MNs). The basic questions include (a) how well in vitro assays can be used for revealing the genotoxic potential of MNs, (b) which assays are suitable for this task, and (c) which modifications are needed in the commonly used tests when MNs are studied. WP5 aims at establishing a robust methodology using alternative tests to screen in vitro genotoxicity of MNs in pulmonary, oral and dermal cell systems (16 HBE, BEAS 2B, A549, Caco-2, reconstructed human epidermis, primary human lymphocytes etc.). These assays will be applied to all MNs assessed (only titanium dioxides will be tested in the dermal systems). On the basis of the results obtained, a round robin test will be performed using the most promising approach. During the first 12 months of the project, WP5 concentrated on setting up the in vitro methodology for the three categories of MNs (TiO2, SiO2 and carbon nanotubes) to be tested in WP5. The general principles of testing were agreed upon among WP5 partners. These concern for example: dose selection, treatment times, cytotoxicity assessment, negative and positive controls, specific details of the genotoxicity endpoints chosen ( DNA damage, micronuclei, and gene mutations), final choice of cell lines, and test protocols for each cell type. Details of the dispersion protocol provided by WP4 were discussed within WP5 and with WP4. Each laboratory tested the dispersion protocol and the ultrasonication devices available, according to WP4 instructions. The in vitro experimentation were started with cytotoxicity assessment used for dose finding, and the actual genotoxicity tests of the MNs begun

## Deliverables:

In vitro genotoxicity testing strategy for nanomaterials including database.

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## All the Associated and collaborating partners are involved on WP3

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## Collaborating partners working on WP5

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