P17-07
(Eco)toxicity assessment of commercial engineered nanomaterials for plastic industry in zebrafish

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International awareness towards safe and sustainable nanostructured materials have conducted to the development of pioneering and fangled green and smart nanosolutions, and have boosted a remarkable expansion potential for multiple industry sectors. Yet, current uncertainty in the regulatory framework for the commercialization of these nano-sized structures, the barely available literature on their potential effects towards human health and environmental safety, and the scarce information on their specific properties have limited their use in the industry.

Our research group has screened the *in vivo* nanotoxicity of calcium carbonate, antimony tin oxide and titanium dioxide nanoparticles. All these engineered nano-sized structures are already commercially incorporated in materials developed in the industrial sector of plastics improving their barrier, mechanical and conductive properties. The above-mentioned nanoparticles were characterized for their biocompatibility via *in vivo* zebrafish embryo toxicity (ZET) protocol and the results were contrasted with the ones from zebrafish acute toxicity testing in order to assess the ability to substitute for the acute fish test in nano EHS (environmental health and safety) investigation [1].

Fertilized zebrafish eggs were monitored via continuous waterborne exposure to the different nanoparticles at the nominal concentrations of 0.01–100 mg mL^{-1} for 80 h postfertilization, permitting a fast-track screening of the embryonic lethality, developmental delay signals, phenotypical malformations, spontaneous movements and free-swimming patterns, heart rate and hatching. F0 parental zebrafish (i.e. adult fish) were further waterborne exposed to the same nanoparticles and at the same nominal concentrations range for 1 week.

Adult zebrafish acute toxicity testing results were accurately extended by *in vivo* ZET data that allowed for a sensitive and early warning signal of nanotoxicity. Inversely to titanium dioxide nanoparticles, calcium carbonate and antimony tin oxide nanoparticles did not cause significant (sub-)lethality to zebrafish. Yet, all nanoparticles tested induced-developmental cardiotoxicity.

Reference

Lin, S., Zhao, Y., Nel, A.E., Lin, S., 2013. Zebrafish: an *in vivo* model for nano EHS studies. Small 9 (910), 1608–1618.

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P17-09
The membrane toxicology of rods and spheric zinc oxide nanoparticles

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It is assumed that the key role in the cytotoxicity of nano-sized ZnO compared to other metallic nanoparticles is played exactly Zn^{2+} due to ion-shedding effect, which is capable in a high concentration to trigger an apoptosis. On the other hand, the shape of the nanomaterial can also contribute to these processes under interaction with the components of cell membrane.

The aim of work – to conduct a comparative analysis of the membrane effects of nano-sized ZnO in different shapes: rods (ZnO NRs) and spherical particles (ZnO NPs), after their interaction with human lymphocytes.

Materials and methods: Lymphocytes were isolated from heparinized peripheral blood of healthy donors. The morphology of the nanostructured ZnO was studied by scanning electron microscopy (SEM). PI test is used for cells viability assessment. Fluorescent labeling with TMA-DPH, laurdan, N-(1-pyrenyl)-maleimide, fluorescamine allow examining the conformation of cell membrane components.

Results and discussion: It was shown that incubation of isolated lymphocytes with 1–100 μg/ml ZnO NRs and ZnO NPs during 20 and 40 h leads to a dose-dependent increase of count cells in a late apoptotic phase but the highest cytotoxity was revealed for ZnO NPs. Study of physical state of membrane lipids after exposure of lymphocytes to ZnO NRs revealed a decrease of lipid microviscosity of the hydrophilic membrane area but increase it in hydrophobic area. The effects of ZnO NPs on membrane lipid components were not significant. Investigations of the membrane proteins conformation state revealed rise of protein NH_2-groups level on the membrane surface only after lymphocytes exposure to ZnO NRs. At that time, level of the membrane protein SH-groups after action of ZnO NPs was increased, otherwise interaction of ZnO NRs with cells lead to oxidation of thiol groups. According to the results of SEM, the geometric size of the spherical ZnO NPs did not exceed 50 nm, the diameter of the ZnO NRs was 50–80 nm and the length did not exceed 500 nm. So, the obtained results of toxicological tests can be partly explained a possibility of spherical ZnO NPs to enter into cell while the more probable mechanisms for interaction with cell of NRs are electrostatic interaction or membrane puncturing.

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