2016-09

Evaluation of the minimum inhibition concentration (MIC) assay for environmental assessment of nanomaterials

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<http://hdl.handle.net/10026.1/10551>

10.1016/j.toxlet.2016.06.1934

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being more active compared to pellet UFP. With the exception of the higher effect observed with beech logwood UFP in THP-1 cells, the ability of soft or hard woods to induce the release of IL-8 was similar. The higher activity of beech logwood UFP observed in THP-1 cells could not be explained by higher uptake or to endotoxin contamination. We observed a qualitatively different protein adsorption profile, with less proteins bound to beech logwoods UFP compared to conifer UFP or DEP, which may provide higher intracellular availability of components, i.e. levoglucosan and galactosan, toward which THP-1 were more responsive compared to A459 cells.

On a weight base, UFP-induced IL-8 release was similar or lower compared to DEP, arguing against a higher biological activity of UFP compared to particles with higher size range.

Acknowledgment: This project was funded by Fondazione Cariplo (Project TOBICUP – Ref. 2013-1040).

<http://dx.doi.org/10.1016/j.toxlet.2016.06.1931>

P17-012

Effects of short SWCNTs and MWCNTs on pulmonary and pleural inflammation

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Recently, the development of industrial products using manufactured nanomaterials and their many applications in information-communications, the environment, and energy have been enthusiastically pursued on a worldwide scale. The toxicity of carbon nanotubes (CNTs) has been studied with a view toward of relationships between the physical properties. However, little research has been conducted to investigate the pulmonary and pleural inflammation caused by short-fiber single-walled CNTs (SWCNTs) and multi-walled CNTs (MWCNTs). We performed to characterize differences in rat pulmonary and pleural inflammation after intratracheal instillation with doses of 0.15 or 1.5 mg/kg of either short-sized SWCNTs or MWCNTs. Data from bronchoalveolar lavage fluid analysis, histopathological findings, and transcriptional profiling of rat lungs obtained over a 90-day period indicated that short SWCNTs caused persistent pulmonary inflammation. Additionally, the short MWCNTs markedly impacted alveoli immediately after instillation, with the levels of pulmonary inflammation following MWCNT instillation being reduced in a time-dependent manner. MWCNT instillation induced greater levels of pleural inflammation than did short SWCNTs. SWCNTs and MWCNTs translocated in mediastinal lymph nodes were observed, suggesting that SWCNTs and MWCNTs underwent lymphatic drainage to the mediastinal lymph nodes after pleural penetration. Our results suggest that short SWCNTs and MWCNTs induced pulmonary and pleural inflammation and that they might be transported throughout the body after intratracheal instillation. The extent of changes in inflammation differed following SWCNT and MWCNT instillation in a time-dependent manner. This study is based on results obtained from a project commissioned by the New Energy and Industrial Technology Development Organization (NEDO), Japan.

<http://dx.doi.org/10.1016/j.toxlet.2016.06.1932>

P17-013

Comparative toxicity of metribuzin based pesticides in nano- and macrodisperse forms

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Objectives: Comparative toxicological investigation of metribuzin herbicide formulations in nano- and macro-disperse forms.

Materials and methods: The tested formulations were in tow dispersed forms with particles size < 2 µm (nanofarm) and > 50 µm (macrofarm). 12-month toxicodynamics study was performed on white male rats divided into the groups: three groups received the studied substance at doses of 0.75, 5.0, 16.7 mg/kg of body weight in nanofarm, three groups (the same doses) in macro-disperse form and control group.

Results: The following changes of biochemical values were the most significant in nano-form: lactatdehydrogenase activity decrease (dose 16.7 mg/kg, 1 month), triglyceride content decrease (5 mg/kg, 9 months; 5, 16.7 mg/kg, 12 months), cholesterol content increase (5, 16.7 mg/kg, 3 months). Macro-form intake showed no such changes.

Triiodothyronine (T3) content decrease was noticed when studying hormonal status, this decrease was marked in macro-disperse form during 1, 3, 6 months; in nanofarm – during 1, 3, 6, 9 months; thyroxin (T4) content increase was noticed for 0.5, 1, 3, 6, 12 months in macro-disperse form and for 0.5, 1, 3, 6 months in nanofarm.

Assessment of central nervous system functional state, changes of body weight and absolute and relative masses of the internals showed no changes.

Conclusions: After multiple oral exposures to metribuzin based pesticide formulations in nano- and macro-disperse form, polytropic effect at the two highest doses was observed (impact on blood system, liver, endocrine system). At the lowest dose there no impact was observed. It is demonstrated that nano-formulation has increased toxicity in comparison with macro-formulation.

<http://dx.doi.org/10.1016/j.toxlet.2016.06.1933>

P17-014

Evaluation of the minimum inhibition concentration (MIC) assay for environmental assessment of nanomaterials

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The use of consumer and industrial nano-containing products may likely cause nanomaterials to be released into the environment. The minimum inhibition concentration (MIC) assay allows the determination of the minimum dilution of a test suspension that gives complete inhibition of microbial growth. This test is commonly used in pharmaceutical regulatory testing but does not allow optimal organism growth. In this study, the MIC assay was used to screen toxicity of various nanomaterials: nanodiamonds, cadmium telluride quantum dots, titanium (IV) dioxide, multi-walled carbon nanotubes and copper (II) oxide to *Escherichia coli* K-12 (Guyer). Cells were exposed to a dilution series of the test suspensions in 96-well plates ($n = 6$ plates/treatment). Nanoparticle tracking analysis revealed natural particle aggregation of all test suspensions.

Ultrasound (sonication) was not used while preparing these test suspensions, to prevent the detachment of the nanomaterial surface coatings from the pristine materials. The magnitude of growth inhibition was chosen as the test end-point. Under these exposure conditions, only cadmium telluride quantum dots were observed to be statistically significantly toxic to the organism (ANOVA, $p < 0.05$). Overall, the MIC assay was found to provide a consistent reproducible output across the different materials tested. This was possible by correcting for the turbidity occurring to nanomaterials, thus determining the fraction of turbidity that corresponds to microbial growth. Further nanotoxicity research is on-going to improve the ecotoxicological usefulness and sensitivity of the MIC assay.

<http://dx.doi.org/10.1016/j.toxlet.2016.06.1934>

P17-015

Time evolution of the silicon-based quantum dots nano-bio interactions is critical for the effect on model biological membranes

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The increased number of reports on biological use of quantum dots (QDs) in diagnostic, drug delivery and cellular imaging applications has triggered various questions concerning their interactions with cells in the physiological environment. In this way, it is of great importance to follow the progressive events triggered at the interface between particles and cell surface lipid bilayer to obtain a relevant analysis on model biological membranes. Herein, we studied the changes over time of the silicon-based QDs physico-chemical parameters in various biological media and investigated the time evolution of changes in protein secondary structure and biocorona composition, and its effect on cell membrane organization and integrity. Using a novel combination of multidisciplinary approaches (dynamic light scattering, laser Doppler velocimetry, Fourier transform infrared spectroscopy, UV-Vis spectroscopy, gel electrophoresis, hemolysis assay, confocal microscopy, Langmuir-Blodgett trough), the present study revealed the dynamic interactions between QDs and proteins which promoted the formation of QDs-protein corona interface responsible for an enhanced affinity for membrane phospholipids, attenuating the cell membrane damage and QDs toxicity. Besides the QDs incorporation among the phospholipids, which increased the stiffness of the monolayer, QDs induced time-dependent changes in the amide I/amide II ratio of bovine serum albumin, favoring random coils instead of α -helix in its secondary structure. These data provides useful information for future molecular dynamics simulation and for mapping interactions between QDs and biological membranes at atomic level which will improve considerably the design strategies for QDs-based drug delivery systems and for preventing QDs toxicity.

<http://dx.doi.org/10.1016/j.toxlet.2016.06.1935>

P17-016

Relation of protein corona and proteomic changes

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Due to the rising number of applications of silver nanoparticles in food-associated consumer products knowledge about silver nanoparticles induced effects should be studied. In a biological environment, for example after uptake into a cell, nanoparticles interact with biopolymers like proteins to form complex surfaces, impacting the interaction between nanoparticles and cellular structures. Thus, the identity of the proteins that bind to the silver nanoparticles, also termed protein corona, is of interest because it has an impact on cellular functions.

We used 1D-SDS-PAGE and LC-ESI-MS/MS to analyze the proteomic response of an in vitro model of enterocytes (Caco-2 cells) exposed to silver nanoparticles for 24 h, as well as the protein corona of these silver nanoparticles after incubation in Caco-2 cell lysate. Ultracentrifugation was used to separate nanoparticles from unbound proteins.

After expression analysis 105, 54 and 30 proteins were identified as regulated due to silver species incubation and compared with the corona proteins of silver nanoparticles and silver ions. The interacting proteins fit well with observed proteomic alterations in Caco-2 cells in response to treatment with silver nanoparticles, like oxidative stress, mitochondrial dysfunction and cell contacts.

The results of this study show that the proteins of the corona of silver nanoparticles and proteins that interact with silver ions are involved in cellular processes that are affected by silver species. For this reason, we assume that the protein corona results in proteomic changes that are related to typical cellular changes after nanoparticle uptake.

<http://dx.doi.org/10.1016/j.toxlet.2016.06.1936>

P17-017

Activation of autophagy protects against mesoporous silica nanoparticles-induced NF- κ B dependent inflammation in macrophagy

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Mesoporous silica nanoparticles (MSNs) are a new drug carrier system with the pore size and spacing in nanoscale (2–50 nm). MSNs have been widely used for drug delivery, drug targeting, gene transfection, and cell tracking. The increasing applications of MSNs require safety study about their adverse effects and the related mechanism of toxicity.

We have reported that MSNs induced inflammation by activating NF- κ B signaling pathway, which resulted in acute kidney toxicity, and even renal interstitial fibrosis. Herein, the present study investigated MSNs-induced cytotoxicity and cellular-uptake in RAW 264.7 cell, NF- κ B activation and autophagy induced by