**Safety evaluation of manufactured nanomaterials: comparison of genotoxic effects of multi-walled carbon nanotubes in two human cell lines**

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**Abstract**

Nanotechnologies are developing very rapidly and presently, nanomaterials are increasingly used in a wide range of applications in science, industry and biomedicine. While a lot of effort has been put in the development of new manufactured nanomaterials (NMs) and in many innovative applications of nanothecnologies, comparatively less research has been performed to evaluate their safety for humans and the environment. Sound information about hazard is lacking for the vast majority of nanomaterials, especially related to chronic exposure to low doses that is likely to occur through consumer products. The genotoxic effects of NMs, which may be linked to carcinogenic effects, are of special concern because cancer has a long latency period; thus, these late effects can be less obvious and more difficult to predict than the acute effects.

Multi-walled carbon nanotubes (MWCNT) are NMs that have been widely applied in structural composites, energy appliances and electronics [1]. The same physicochemical properties that have rendered them attractive for those purposes might also underlie relevant biological effects with impact on human health and the environment. Size, surface properties, agglomeration state, biopersistence and dose are likely to influence cell responses to MWCNT, presenting a challenge to the assessment of their potential hazards. In particular, the similarity, in size and shape, between MWCNTs and asbestos fibers has raised concerns about their potential genotoxic and carcinogenic effects. The potential genotoxic effects of several MWCNT have been characterized *in vitro* and *in vivo*, but discrepant results have been reported, showing either absence [2,3] or induction of genomic instability [4-7]. These inconsistent results may be related to differences in the physicochemical properties of the NMs studied and to other variables inherent to the *in vitro* test systems and exposure conditions. Therefore, the use of standardized methods and well characterized NMs has been recommended, to allow the comparison of the genotoxic effects obtained for a given NM in different laboratories or the genotoxic potential of several NMs [8].

In the context of an EU Joint Action (NANOGENOTOX), aimed at establishing a robust methodology for the safety evaluation of the manufactured nanomaterials, the objective of the present work was to compare the potential genotoxic effects of two thin and short MWCNT (NM-402 and NM-403; JRC repository) in a human type-II alveolar epithelial cell line (A549 cells) and in primary human lymphocytes.

Dispersions of each NM were freshly prepared according to a standardized protocol [9] and cells were exposed to several NMs concentrations. Concurrent control cultures were also analyzed: vehicle control, positive and nanosized controls (mitomycin C and ZnO, respectively). The *in vitro* micronucleus assay, a validated method accepted for regulatory purposes [10], was selected to assess chromosome structural and numerical changes.

Considering alveolar cells exposure to NM-402, a dose-response relationship was obtained for the frequency of micronucleated cells with the two highest concentrations being able to significantly increase the frequency of micronuclei, comparatively to the vehicle control. However, equivocal results were obtained in lymphocytes, with a single concentration yielding micronuclei induction. In contrast, NM-403 failed to produce micronucleation in alveolar cells but was able to significantly induce micronuclei in lymphocytes at the same dose-range. Positive controls yielded positive results in both cell types.

In conclusion, a differential response was observed for two closely related MWCNT in two human cell systems, which might be explained by differences in the uptake capacity or sensitivity of the tested cell types or by structural differences of MWCNT, including surface activity and transition metals present as impurities. This study illustrates the difficulty of implementing hazard-grouping strategies based on the similarity of the NMs physicochemical properties and hypothesized mode of action. It highlights the importance of investigating the toxic potential of each NM individually until the main characteristic(s) that determine NMs genotoxicity is(are) uncovered. Most of all, interconnections between the pace of technological change and safety has to be guaranteed, in order to benefit from innovation while protecting public health and the environment.

Co-funded by EU Grant Agreement 2009 21 01 (NANOGENOTOX), in the framework of the EU Health Programme and INSA.

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