Assessment of nanoparticle release and associated health effect of polymer-silicon composites
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Introduction

The fast advancement in the nanotechnology and manufacturing of engineering nanomaterials has inevitably caused concerns over their impact on environment and human health. Little information is currently available on possible release of nanomaterials or/and nanoparticles (NP) from conventional and novel products and associated health effect. The NEPHH project funded under the EU FP7 programme is aimed to assess the environment and health impact of polymer-silicon nanocomposites in comparison to conventional polymer composites from life cycle perspective. As part of the NEPHH project, this study focused on assessing the possible release of NP during the application stage of conventional and nanoproducts. NP release was monitored during physical processing of polymer-silicon composites, and the toxicity of both the released NP and the raw silica nanomaterials that were used as fillers in nanocomposites was assessed in vitro using human lung epithelial A549 cells.

Materials and methods

The components of some of the polymer composites tested in the NEPHH project are listed in table 1. The polyamide 6 (PA6) and Polypropylene (PP)-composites were used as matrixes which were reinforced with 5% of glass fibres (GF, Taiwan Glass Industry Corporation), SiO2 NP (Aerosil 200 or Aerosil 974, both 12 nm, amorphous, fumed, Evonik Degussa Polska Sp. z. o. o), organically modified montmorillonite (MMT, Dellite 43B and Dellite 72T, Laviosa Chimica Mineraria S.p.A), or foam glass crystal (FGC, provided by Tomsk Polytechnic University, Russia). The PA6 and PP neat polymers were also included as reference materials (REF). Among the reinforcement materials SiO2 NP and MMT are nanomaterials and the rest are microsized materials. The testing specimens with dimension of 100x100x10 mm3 were prepared by the compression moulding technique with a temperature of 250°C over the compression time of 5 min1. These specimens were subjected to drilling in a testing chamber. The release of NP from PA6 and PP group composites was comparatively quantified using a scanning mobility particle sizer (SMPS) during the 28 minutes of drilling. NP in the dust of the testing chamber were extracted using a two-step of filtration method.

The toxicity of the dust NP and the raw SiO2 NP at concentrations of 25 µg/ml, 50 µg/ml and 100 µg/ml was assessed in human lung epithelial A549 cells by membrane integrity (LDH) and viability (MTT) assays. Silica NP of size 7 nm (Si 7) and H2O2 (200 µM) were used as positive controls in these assays. In order to correlate the toxicity of NP with their size, all the testing NP were characterized for dispersion pattern and
size distribution in cell culture medium by dynamic light scattering and SEM assays before toxicity studies.

Table 1. Components the polymer materials tested in the NEPHH project

<table>
<thead>
<tr>
<th>Polymers</th>
<th>Reinforcement materials</th>
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<tr>
<td>Dellite 43B MMT Nanostructured</td>
<td>Delite 72T MMT Nanostructure</td>
</tr>
<tr>
<td>PA6</td>
<td>PA-MMT</td>
</tr>
<tr>
<td>PP</td>
<td>PP-MMT</td>
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Result and discussion

It was shown in figure 1 that under drilling, the PA6 group materials generated more airborne NP than the PP group (compare A with B) with the polymeric-silica nanocomposites generating the most. Interestingly, the polymer–MMT composite produced the lowest amount of NP, suggesting that the use of MMT as fillers could prevent the release of NP from final polymer products under certain application conditions. The level of NP release from PA6 group materials is in the order of PA6-SiO2>PA6-FGC>PA6-GF>PA6-REF>PA6-MMT. The concentration of NP in the air reduced subsequently after reaching peak concentration at 500000 NP/cm³ or higher, depending on the type of specimens. The airborne NP were also examined by scanning electron microscopy, suggesting that the released NP formed aggregates and were of polymer but not filler material origins.

![Figure 1. Monitoring of airborne NP using SMPS during 28 minutes of drilling](image)

**Figure 1. Monitoring of airborne NP using SMPS during 28 minutes of drilling.** The concentration of NP generated from PA6 group materials shown in A; the concentration of NP generated from PP group materials shown in B. The lines represent NP released from polymer composites with reinforcement material of foam glass crystal (FGC), glass fiber (GF), silica NP (SiO2), montmorillonite (MMT), or without reinforcement material (REF).
At concentrations up to 100 µg/ml, all the dust NP disperse well in culture medium with an average size less than 100 nm. In contrast, all the raw silica NP at 10-100 µg/ml exhibit a polydispersion pattern with particle size increasing in a concentration dependent manner. Figure 2 illustrates a concentration dependent dispersion pattern of Aerosil 200 in culture medium. Other silica NP studied also showed very similar behaviour in the culture medium.

**Figure 2.** Concentration dependent dispersion pattern of silica NP in culture medium. Aerosil 200 was dispersed in culture medium at concentrations as indicated. The dispersion pattern and size distribution were determined by DLS assay.

The cytotoxicity in dust NP was not detectable as assessed by the LDH assay in A549 cells at the concentrations tested for up to 48 h (figure 3 A and B) and was only detected by the MTT assay at 72 h (Figure 3 C and D). In contrast, the toxicity of raw SiO2 NP was detected by the LDH assay as early as 12 h (figure 4 A) and by the MTT assay at 24 h, 48h and 72 h (figure 4 B).

**Figure 3.** Cellular effect of NP from PP (A) and PA6 (B) materials. Cell membrane integrity (LDH) assay at 48 hour (A and B); cell viability assay at 72 hour (C and D).
Our study is consistent with previous work demonstrating that polymeric products can generate NP under certain application scenarios. This study further suggests that 1) the level of NP release from polymer composites could be altered by different reinforcement materials; e.g. nanostructured MMT could reduce the release while SiO2 NP could increase the release; 2) working with polymer composites under certain conditions could risk inhalation of high level of polymer NP, although which exhibit low toxicity; 3) raw nanomaterials appeared to be toxic in the chosen in vitro system. Further study of the effect of novel filler materials on NP release from final polymer products and the effect of released NP on environment and human health will inform design of safety materials and minimization of negative impact.

**Figure 4. Raw SiO2 NP toxicity assessment.** Cell membrane integrity (LDH) assay at 12 hours (A); cell viability (MTT) assay at 72 hours (B).

**Reference**


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