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## Study of the toxicity of inhaled ultrafine engineered powder: example of boehmite nanoparticles

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Boehmite nanoparticles (NP) can be used as a vector for vaccines, replacing conventional adjuvants but also in industry as abrasives, catalysts, substrates for electronic circuits, refractory materials. Thus, the boehmite NP constitutes a good model of study taking into account its various applications: in the industry as well as in the nanomedicine field.

This important use of boehmite NP makes particular interest of the evaluation of its potential toxicity. Some toxicological works have been yet performed, the health risk induced by boehmite NP is still not completely characterized. Nevertheless, some authors have shown from *In vitro* experiments that the boehmite can cause an inflammation characterized by production of IL-8 and a certain cytotoxicity associated with a LDH release. As a result, the boehmite NP must be considered as potential risk factors for health.

This work aims to develop a multidisciplinary approach to highlight the correlation between the toxicity of alumina engineered NP and their physico-chemical characteristics. Accuracy of measurements depends on cell production after contact with particles, but also depends on the ability of biomolecules to get adsorbed on the NP (Val, 2009). Therefore, mechanisms of biomolecules adsorption on NP must be fully understood to avoid misinterpretation of data.

Transmission electron microscopy and X-ray diffraction measurements showed that these NP had a crystallite size of 10nm. *In vitro* biological effects were evaluated on macrophages (RAW 264.7 cell line) after a 24 hours exposure to alumina NP. Inflammatory response was evaluated using TNF- $\alpha$  production in the cell culture supernatant. Biomolecules-NP interactions were evaluated on acellular medium measuring both affinity and amount of adsorbed TNF- $\alpha$  on alumina NP after 24 hours (ELISA kit, R&D Systems®).

Results showed an important inflammatory response (*e.g.* a TNF- $\alpha$  production about 400pg/mL for a dose of 120 $\mu$ g/10<sup>6</sup> cells) as compared to positive (quartz, 2000pg/mL) toxicity control. Moreover, we demonstrated a significant adsorption of TNF- $\alpha$  on alumina NP. Indeed, 75pg of TNF- $\alpha$  were added to 12  $\mu$ g of NP in cell culture medium. The mass measured after 24 hours incubation was only 65 pg, so 2.88pg of adsorbed TNF- $\alpha$  meaning that 10% of artifacts.

Thus adsorption artefacts must be taken into account when evaluating biological responses in order to reliably compare NP with different physico-chemical characteristics.

Val S, 2009. Carbon black and titanium dioxide nanoparticles induce pro-inflammatory responses in bronchial epithelial cells: Need for multiparametric evaluation due to adsorption artifacts. *Inhalation Toxicology*, 21, 115-122.