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Study on the toxicity of inhaled alumina nanoparticles: impact of physicochemical properties and adsorption artifacts on the measurement of biological responses.

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Abstract. This work aims at developing a multidisciplinary approach to highlight the correlation between the toxicity of alumina engineered nanoparticles (NP) and their physicochemical 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 [1]. That’s why, mechanisms of biomolecules adsorption on NP must be fully understood to avoid misinterpretation of data.

1. Introduction
Aluminum hydroxide is a material used in various applications such as abrasive materials, refractories, substrates for electronic circuits...

At each step of the manufacturing process (synthesis, collection and purification of the powders), workers could be exposed to fine dust suspended in the atmosphere. Inhalation of some types of particles may cause effects on the health such as reduced lung function, respiratory symptoms and increased risk of tumors. Therefore, it is important to evaluate intrinsic toxicity of fine and ultrafine particles of aluminum hydroxide particles.
2. Materials and methods

2.1. Physicochemical characterization
We work on an aluminium hydroxide powder. The physicochemical characterizations were done by laser granulometry (Malvern Mastersizer 2000), XRD (D5000, Siemens) and TEM (JEOL 2010). The specific surface area of powders was determined by the BET method using a nitrogen sorption apparatus (MicroMetrics) and the Photon Correlation Spectroscopy (Zetasizer Nano S, Malvern) was performed in solutions of different pH (pH3.5, pH7 and pH9.4).

2.2. Toxicity assessment
To assess the biological activity of aluminum hydroxide powder, cells from the RAW 264.7 macrophage cell line were incubated with aluminum hydroxyde nanoparticles.

Three parameters were examined:
- The lactate dehydrogenase (LDH) release: LDH is an enzyme found in the cytoplasm of living cells. In case of alteration of the plasma membrane, LDH is released into the environment.
- The Tumor Necrosis Factor alpha (TNF-α) production: TNF-α is a cytokine secreted by macrophages. It reflects the activation of macrophage and acute phase of the inflammatory response.
- The Reactive Oxygen Species (ROS) production, reflecting the oxidative stress [2-3].

The interactions can be established between these cells and nanoparticles in specific experimental conditions. This study will highlight the correlation between the level of toxicity induced by nanoparticles and their physicochemical properties.

Moreover, biomolecules-nanoparticles interactions were evaluated on a cellular medium measuring both affinity and amount of adsorbed TNF-α on aluminum hydroxyde nanoparticles, at equilibrium state (ELISA kit, R&D Systems®).

3. Results and conclusion
The different physicochemical characterizations show coherent results, the crystallites size is 10nm. Photon correlation spectroscopy shows that the aggregation of particles in water increases with pH.

Biological activity of aluminum hydroxide was less important than that of the positive control of toxicity (quartz) but also, and unexpectedly, less important than that of cells alone. To determine if this phenomenon was linked with a cytokine adsorption process, we added 75 pg of TNF-α to aluminum nanoparticles, and after a 24h incubation we found 65 pg of TNF-α in cell culture medium, suggesting a significant adsorption of TNF-α on aluminum hydroxide nanoparticles (13%).

In conclusion, cytokine adsorption on nanoparticles could represent an important artifact to take into account for an accurate evaluation of biological responses, especially in order to reliably compare nanoparticles with different physicochemical characteristics.

References