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S. Manixay, S. Delaby, F. Gaie-Levrel, Marlène Wiart, C. Motzkus, et al.. In vivo evaluation of the potential neurotoxicity of aerosols released from mechanical stress of nano-TiO₂ additived paints in mice chronically exposed by inhalation. NANOSAFE 2016, Commissariat à l'Energie Atomique et aux Energies Alternatives (CEA). Grenoble, FRA., Nov 2016, Grenoble, France. 10.1088/1742-6596/838/1/012025 . hal-01676669

HAL Id: hal-01676669

<https://hal.science/hal-01676669>

Submitted on 2 Jun 2020

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To cite this article: S Manixay *et al* 2017 *J. Phys.: Conf. Ser.* **838** 012025

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***In vivo* evaluation of the potential neurotoxicity of aerosols released from mechanical stress of nano-TiO₂ additived paints in mice chronically exposed by inhalation**

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Abstract. Engineered Nanomaterials (ENM) provide technical and specific benefits due to their physical-chemical properties at the nanometer scale. For instance, many ENM are used to improve products in the building industry. Nanoscaled titanium dioxide (TiO₂) is one of the most used ENM in this industry. Incorporated in different matrix, cement, glass, paints... TiO₂ nanoparticles (NPs) provide the final product with anti-UV, air purification and self-cleaning properties, thanks to their photocatalytic activity. However, ageing processes of such products, as photocatalytic paints, during a mechanical stress have been shown to release TiO₂ NPs from this matrix associated with sanding dust. Thus, workers who sand painted walls could be exposed to TiO₂ NPs through inhalation. As inhalation may lead to a translocation of particulate matter to the brain via olfactory or trigeminal nerves, there is an urgent need for evaluating a potential neurotoxicity. In order to provide new knowledge on this topic, we developed a dedicated experimental set-up using a rodent model exposed *via* inhalation. The aerosol released from a mechanical stress of photocatalytic paints containing TiO₂ NPs was characterized and coupled to an exposition chamber containing group of mice free to move and chronically exposed (2 hours per day for 5 days a week during 8 weeks).

1. Introduction

Engineered Nanomaterials such as titanium dioxide (TiO₂), carbon nanotubes, silica dioxide (SiO₂) or silver (Ag), are increasingly used in the construction products, e.g. cement, wet mortar and concrete, coatings and paints [1-3]. It is assumed that the majority of nanomaterials are most likely safe, at least at concentrations for which workers are exposed. However, many questions are still open concerning the impact of these nanomaterials on workers' health when handling these products. As an example, the degradations induced by the abrasion mechanisms of nanocomposite materials could induce the release of nanoparticles into the environment for which chemical nature, size and concentrations are mostly unknown [4-6]. In this context, the most common route of exposure to nanoparticles is inhalation and the nanoparticle sources are aerosols of which nano-aerosols generated by activities in the construction industry [7]. Nanoscaled TiO₂ is one of the most used ENM in this industry and an emblematic use concerns photocatalytic paints [8]. Incorporated in the paints matrix, TiO₂ nanoparticles (NPs) provide the final product with anti-UV, air purification and self-cleaning effects properties, thanks to their photocatalytic activity [9]. However, ageing processes of these products during a mechanical stress have been shown to release TiO₂ NPs from their matrix associated with sanding dust [5, 10]. Thus, workers who sand painted walls could be exposed to TiO₂ NPs in



combination to paint matrix through inhalation. Although some experimental studies underlined the potential hazardous effects of inhaled nanoparticles like lung inflammation or affection of the cardiovascular system, to the best of our knowledge, none investigated a potential impact on the nervous system [11-13]. However, since nanoparticles inhaled through the nose can translocate to the brain directly *via* the nerve endings of olfactory neurons, there is a crucial need to study their potential neurotoxicity, especially in case of chronic exposure [14, 15].

We thus developed a dedicated experimental set-up using a rodent model exposed *via* inhalation, with the aim of determining if chronic exposure through inhalation to sanding dusts containing TiO₂ NPs may impact brain functions. The objective of this paper is to report the study strategy and the key elements.

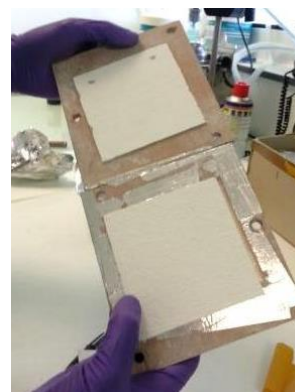
2. Materials and Methods

2.1. Nanocomposite materials

Homemade nano-TiO₂ enriched paint (5% in mass) was used compared to nano-TiO₂ NPs alone (Degussa-P25: composed of rutile (80%) and anatase (20%) crystallites, primary particles with a mean diameter approximately of 21 nm and a specific surface of 50 +/- 15 m²/g), as well as paint matrix alone. The paints (enriched or not) are applied on a 10x10cm plate of stainless steel (Figure 1).

The dry paint weighs 5.4 g for the homemade nano-TiO₂ enriched paint (with a standard deviation of 0.4g) and represents the nanomaterial tested in our study. A painted plate allowed generating sufficient number of particles in the aerosol form for a 30 minutes exposure. Abrasion tests were performed using a rotating abraser sander to mimic the mechanical abrasion process (Figure 2).

Figure 1: Illustration of painted plates used in the study



2.2. Physico-chemical characterization of the generated aerosol

Aerosol productions from the mechanical stress applied to these nanocomposite materials were characterized. The particle size distributions and the associated concentrations and morphologies (free or agglomerated nanoparticles or nanoparticles embedded in polymer matrix) of aerosolized particles were measured with on-line (FMPS, ELPI, APS, CPC, TEOM) and off-line measurements (electronic microscopy on TEM grid). Repeatability and reproducibility measurements were performed in accordance with the ISO 5725-2 standard in order to compare the aerosol production between the set-up characterization and the exposition phases.

2.3. Mice exposure

In order to investigate the potential neurotoxicity of these sanding dusts containing TiO₂ NPs, a dedicated experimental set-up developed by our group was used to chronically expose wild type mice *via* inhalation. The generations of aerosol during the mechanical solicitation of paint dust with or without TiO₂ NPs were characterized. The set-up developed in this project permits a secured and entirely controlled exposure to the aerosol. Coupled to an exposition chamber containing a group of 30 female mice (C57Bl6 mice, 6-week-old) free to move, it allowed performing chronic exposure during 2 hours per day for 5 days a week for 8 weeks. All procedures were carried out in complete compliance with the guidelines laid down by the French Ethical Committee (Decree 87-848) and

European Community Directive 86/609/EEC. The complete inhalation study was approved by National Committees on the Ethics of Animal Experiments (ANSES/ENVA/UPEC (n°15-068) and CELYNE). Animal experiments were performed in the ANSES animal facilities which have the relevant approval to carry out animal experiment (C 69 387 0801) by licensed people working in the animal experiment unit (license numbers AB: 69 387 531, LL: 69 387 191).

In order to investigate a time-dependent effect, groups of 5 mice were studied after 1, 2, 3, 4 and 8 weeks of exposure. Several parameters were collected *in vivo* such as weight, general appearance, evolution of locomotor performances using rotarod tests and *in vivo* brain imaging by Magnetic Resonance Imaging (MRI) at 0, 2, 4 and 8 weeks of exposure. Other parameters are collected post-mortem, such as histopathological studies, performed on brain sections. Some brains were dedicated to elemental content analysis (Ti brain mapping). Since the data collection and related analyses are still under progress, only the project strategy is presented in this paper.

3. The Release NanoTox project

The graphical summary of our study principle is given in Figure 2.

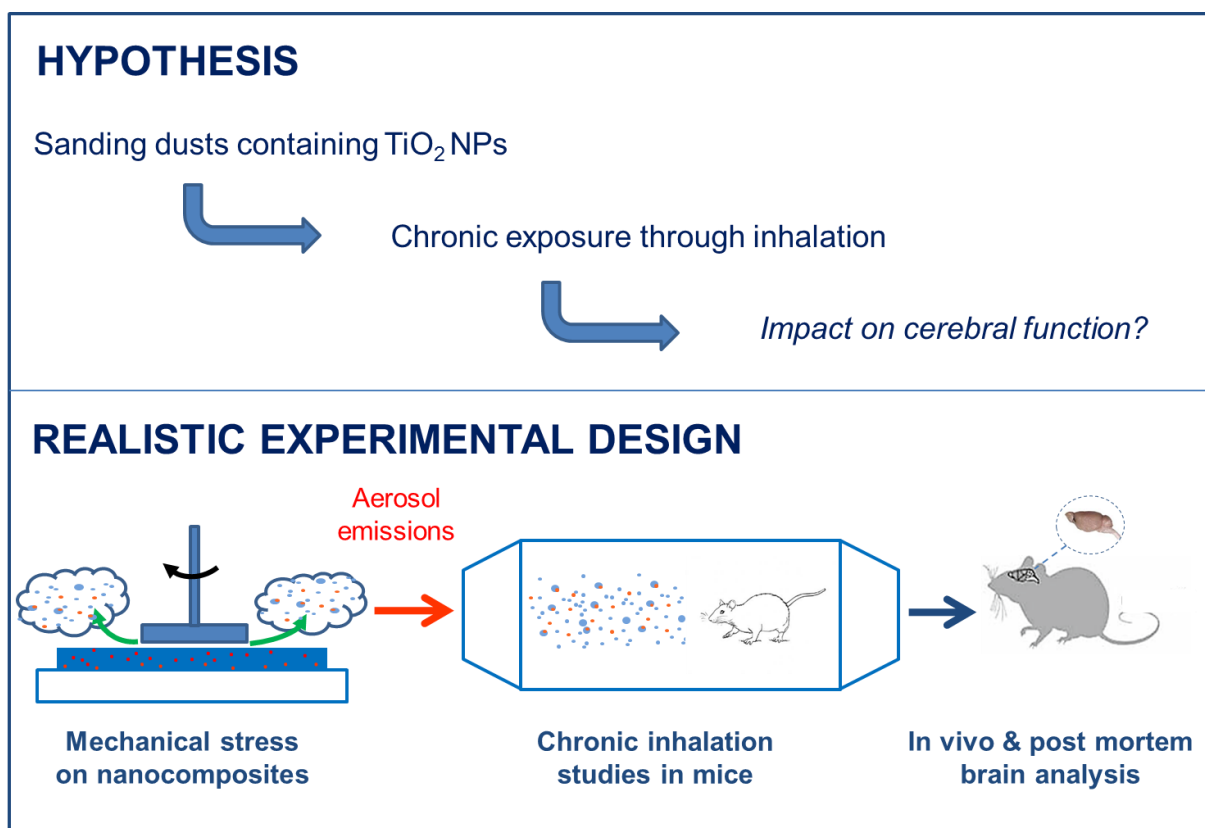


Figure 2: Principle of the Release NanoTox study

The emitted aerosols, produced under realistic conditions, were first characterized, and then used for *in vivo* studies to evaluate their potential neurotoxicity in mice after chronic inhalation exposure. This experimental design mimics a realistic scenario of workers' exposure.

MRI analysis gives access to potential morphological and functional abnormalities that can further be related to other parameters recorded *in vivo* such as the rotarod tests performances, both being suitable to detect a neurotoxic effect. *Post mortem* histological assessment of potential neuroinflammation and

neuronal loss will also allow interpreting, in an appropriate manner, a potential negative effect on the brain function.

The work plan composed of several tasks and sub-tasks is presented in the Figure 3.

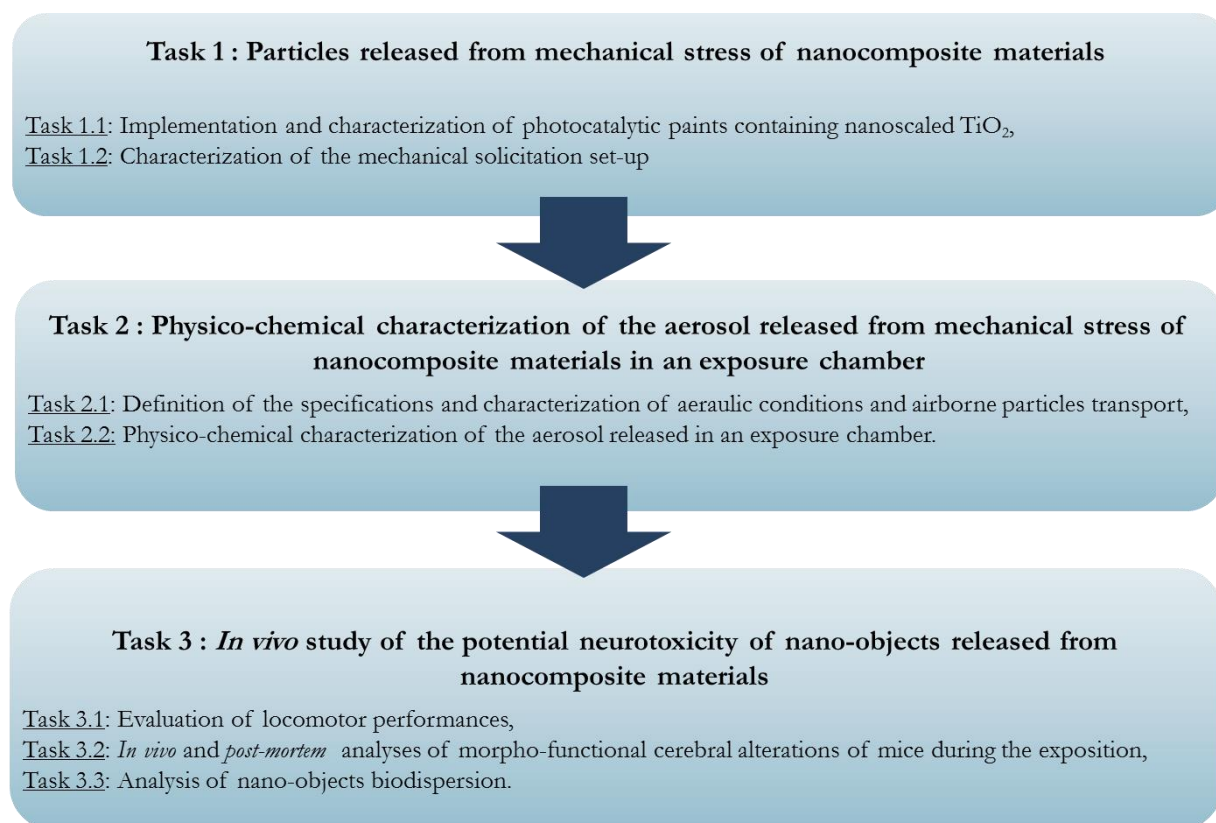


Figure 3: Workplan of the Release NanoTox project

4. Conclusion

The main originality of the Release NanoTox project consists in the convergence work on particle release from nanocomposite materials and the study of the *in vivo* neurotoxicity of nano-objects. Indeed, to date and to the best of our knowledge the work on toxicity is focused on the impact of nano-objects only. As it has been shown that the wear of a nanocomposite material leads to the production of polymorphic particles in size and composition, with a majority of particles consisting of nanoparticles encapsulated in the matrix material, it is necessary to assess their potential toxicity compared to the added NM alone. Moreover, the chemical composition of the nanocomposite material's matrix is a factor that is not taken into consideration in toxicology studies. Similarly, no study has focused on the neurotoxic effect of particles from nanocomposite materials. Our study will bring new knowledge and should contribute to clarify these important questions for the workers' health.

Acknowledgements

This work was founded by a grant from ANSES (EST-2014/1/178). Stéphane Manixay was supported by a grant from ANSES (EST-2014/1/178).

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