Long term inhalation study
Deliverable 4.1

Introduction

Long-term exposure to biopersistent, poorly soluble nanomaterials and possible carcinogenicity induced thereof, has been identified as one of the major data gaps for regulatory decision process in the field of nanomaterials. Only two nanomaterials, titanium dioxide and carbon black, have been tested so far in rodent inhalation carcinogenicity studies. In these studies lung tumours in rats were obtained at higher doses.

There is a current scientific controversy, whether the lung tumours detected in these chronic rat inhalation studies only appear at high exposure concentrations (i.e. so-called dust 'overloading' of the lungs) associated with inflammation. According to the overload hypothesis, in lower (and real-life) exposure levels there is no dust overloading and no inflammation in the lung and consequentially no tumour risk in case an exposure threshold is not exceeded.

The limited data as well as the uncertainty of the role of inflammation and overload in the process of tumour development when exposed to poorly soluble nanomaterials, hinder any regulatory decision on how to evaluate the long-term risk of nanomaterials.

Task 4.1 of the NANoREG project (long term inhalation study) serves to experimentally verify the 'overload' hypothesis for two well characterised nanomaterials (CeO_2 and $BaSO_4$) which represent the industrial relevant group of poorly soluble, low toxicity particles (PSLT). It is aimed at getting insight into the mechanistic hypothesis, i.e. the role of inflammation and / or overload and allow a proper future risk evaluation. The results can be cross-read to other PSLT nanomaterials. For the first time, relevant results for low dose exposures will be available for nanomaterials for which the mode of possible carcinogenic action is determined exclusively by dust toxicity. As the study focuses on investigating a putative inhalation carcinogenicity of PSLT nanomaterials in low dose exposures, this study offers relevant information not only for occupational but also for environmental and consumer health.

Deliverable D4.1 reports on the tests carried out like test design, exposure levels, body weight development, lethality rate and macroscopic observations. Further results will be reported in a later phase in deliverables D4.5 [M36, Lung burden and particle detection and quantification in olfactory bulbs - chronic exposure], D4.6 [M36, Organ burden, feces analyses and particle detection pattern in other organs after chronic exposure], and D4.7 [M42, Histopathological evaluation (all organs) Immunohistochemical detection of local and systemic genotoxicity].

Description of Work

The chronic inhalation study was carried out according to OECD TG 453 under GLP (Good Laboratory Practice) with OECD depository material. It started with 100 rats per dose group. 50 animals per dose group were sacrificed after 24 months. The remaining animals are kept exposure-free till natural death or till month 30. Animals of each group which die during the exposure or post-exposure period are examined as well.

Based on a 28 day range-finding study with nanoscaled CeO_2 (NM212) and $BaSO_4$ (NM220),the following concentrations were selected: 3 mg/m³: as high concentration with expected toxic effects (TG04), 1 mg/m³: as mid concentration (TG03), 0.3 mg/m³: as a second low concentration(TG02) and 0.1 mg/m³

(TG01): as low concentration and expected NOAEL. For $BaSO_4$ a high concentration of 50 mg/m³ was selected (TG05).

Satellite groups were sacrificed after 12 months (chronic group with 10 animals per dose for histopathology) and after 3 months, 12 months, and 24 months (for kinetic/organ burden evaluations). The results have been or will be reported in deliverables mentioned before.

Main Results

As shown in the figure below, exposure to both nanoparticles up to 24 months did not lead to body weight reduction.

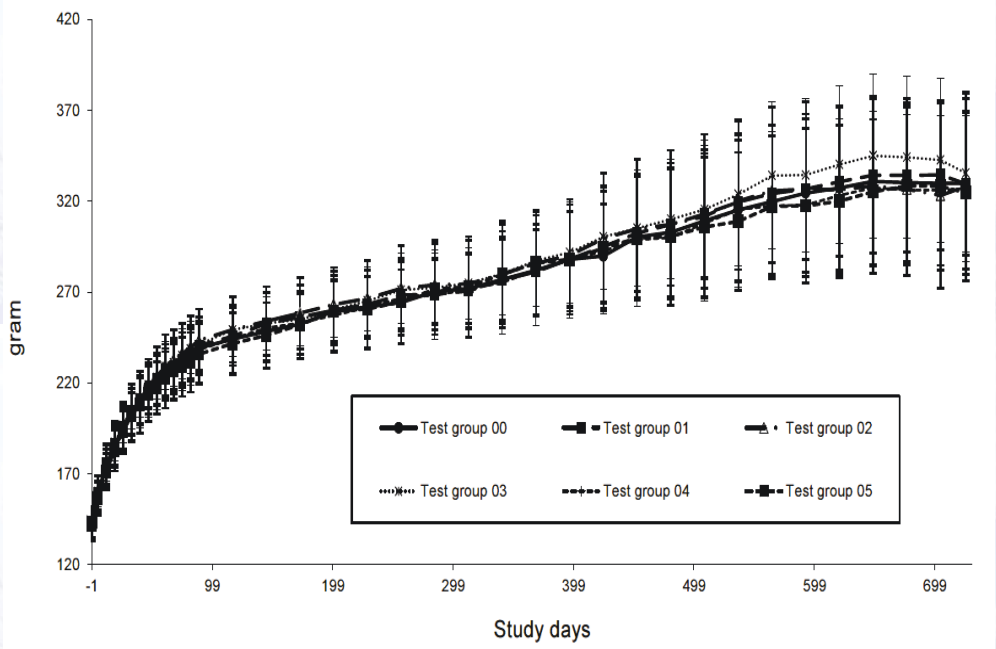

Figure 3 Body weight development during the 24-month exposure phase

The mortality rates were in an acceptable range.

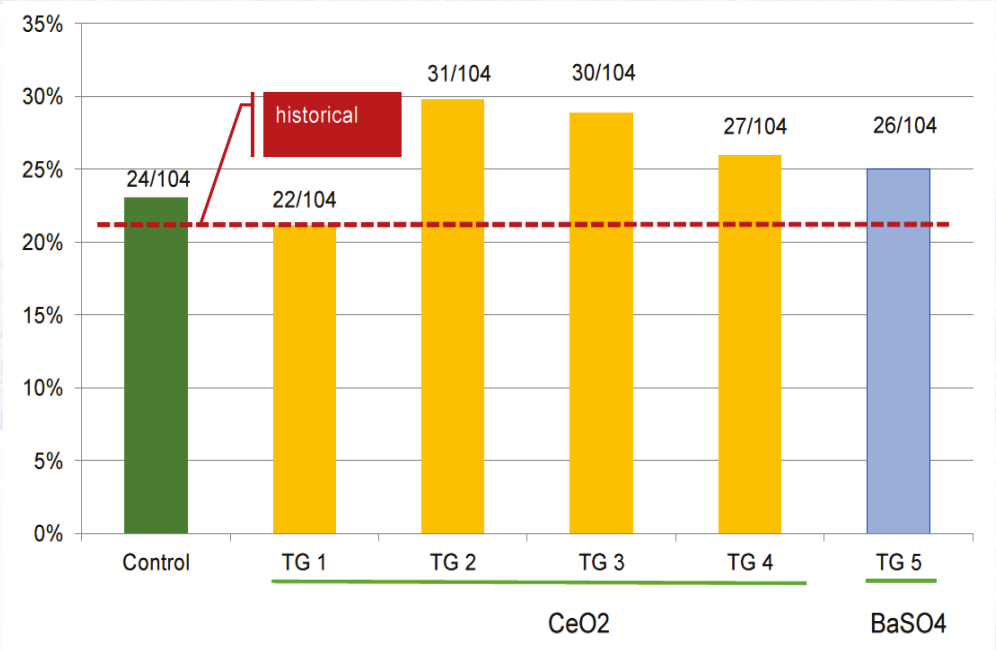

Figure 4 Lethality rate in the different study groups up to study day 734

Macroscopically evident tumours were not detected after 24 months of exposure.

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