Comparison of the Pulmonary Responses to Inhaled Pigmentary and Ultrafine Titanium Dioxide Particles in the Rat, Mouse and Hamster

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Groups of female rats, mice and hamsters were exposed to 10, 50 or 250 mg/m3 pigment grade titanium dioxide (PG-TiO$_2$) or to 0.5, 2 or 10 mg/m3 ultrafine titanium dioxide (UF-TiO$_2$) for 6 h/day, 5 days/week for 13 weeks. At the end of the exposure period, and after holding periods of 1, 3, 6 and 12 months, a range of investigations were made to assess lung burdens, indicators of pulmonary inflammation, oxidant damage, cellular proliferation and pathological responses. Findings in rats and mice exposed to 50 and 250 mg/m$^3$ PG-TiO$_2$ or 10 mg/m$^3$ UF-TiO$_2$ were consistent with the development of lung overload, i.e. accumulation of TiO$_2$ in the lungs, reduced clearance rates and pulmonary inflammation. Pulmonary lesions in rats exposed to 250 mg/m$^3$ PG-TiO$_2$ continued to develop in severity throughout the post-exposure period, while those exposed to 50 mg/m$^3$ PG-TiO$_2$ or 10 mg/m$^3$ UF-TiO$_2$ showed diminution. Mice had similar lung burdens to rats throughout the study, but in contrast, the inflammatory and histological responses were less severe and diminished with time. Hamsters exposed to 250 mg/m$^3$ PG-TiO$_2$ had similar initial lung burdens to the rat but demonstrated a high clearance rate post-exposure, with an accompanying reduction in the inflammatory and histopathological findings. Hamsters exposed to 10 mg/ml UF-TiO$_2$ had accumulated only ~20% of the lung burden seen in the rat and this was reflected in the limited changes observed in the measured parameters. These studies demonstrate marked species differences in the response to pulmonary overload, and will help in understanding the events and mechanisms responsible for the tumours seen in rats after lifetime exposure to dusts.

Keywords: titanium dioxide; lung; overload; rat; mouse; hamster

INTRODUCTION

Titanium dioxide (TiO$_2$) is manufactured in large quantities throughout the world for use in a wide range of applications, including fillers and pigments. It is considered normally to be toxicologically inert and has often been the choice for a ‘negative control’ in studies investigating the toxicological effects of biologically reactive dusts, such as crystalline silica. However, pulmonary tumours were seen (Lee et al., 1965) in a chronic inhalation study in which rats were exposed to very high concentrations (250 mg/m$^3$) of pigment grade (PG) TiO$_2$, and similar tumours were seen in rats exposed to ultrafine (UF) TiO$_2$ (Degussa P25; Heinrich, 1994). It is now well established that lifetime exposure of rats to high concentrations of a range of other insoluble dusts results in the development of similar tumour types. This has been attributed to overload of the lung with particulate material and the subsequent tissue responses that may result in tumorigenesis, via a non-genotoxic mechanism, towards the end of the lifetime of the rat (Hext, 1994). A limited number of studies in mice and hamsters suggest that these species do not form tumours under such circumstances (Heinrich, 1996). Since results from experimental animals may cause concern regarding similar effects in humans, may affect classification and labelling of materials and may have implications for occupational hygiene, the TiO$_2$ manufacturers of Europe and North America initiated a programme of work to address these issues. This was composed of an investigation of the epidemiology of workers in manufacturing plants and the differences in pulmonary responses between experimental species (rat, mouse and hamster) exposed
to PG- or UF-TiO₂ particles. Parameters investigated in the toxicology study were designed to compare cellular, biochemical and histological changes with the lung burden of TiO₂ at a number of time points following subchronic inhalation. An overview of the results from the investigations on lung burdens, inflammatory responses and histopathology are reported here.

**MATERIALS AND METHODS**

**Exposures**

Groups of female F334 rats, B6C3F1 mice and Syrian golden hamsters were exposed to 10, 50 or 250 mg/m³ PG-TiO₂ (DuPont) or to 0.5, 2 or 10 mg/m³ UF-TiO₂ (Degussa P-25) for 6 h/day, 5 days/week for 13 weeks in 1 m³ stainless steel and glass inhalation chambers.

At the end of the exposure period and after holding periods of 1, 3, 6 and 12 months, groups of five of each species were removed for assessment of lung and lung-associated lymph node TiO₂ burdens and lung clearance rates, and a number of biological parameters, including markers of inflammation in bronchoalveolar lavage fluid, cell proliferation and histopathology.

**RESULTS**

**Lung burdens**

Following exposure to 250 mg/m³ PG-TiO₂, similar lung burdens (expressed as mg/g dried lung) were measured in each species (Fig. 1) and similarly in rats and mice exposed to 50 mg/m³. Hamsters exposed to 50 mg/m³ had a markedly lower lung burden (~30% that of the rat). Analysis of lung burdens at subsequent time points (Fig. 1) shows low clearance rates with increasing retention time in rats and mice exposed to 50 and 250 mg/m³ PG-TiO₂ (clearance half-lives of 324 and 838, respectively, for rats and 417 and 621 days for mice), consistent with the development of lung overload. In contrast, hamsters exposed to 250 mg/m³ PG-TiO₂ showed a rapid clearance of the high lung burden (clearance half-life 100 days) with clearance half-lives of 40 days at the two lower concentrations.

Rats and mice exposed to 2 and 10 mg/m³ UF-TiO₂ showed similar initial lung burdens to each concentration (Fig. 1). Subsequent clearance half-lives for exposure to 2 mg/m³ were 132 and 40 days for rats and mice, respectively, and for exposure to 10 mg/m³ were 319 and 395 days, respectively—indicative of overload at 10 mg/m³. Initial lung burdens in the hamster were substantially lower than those of the rat and mouse (Fig. 1; ~22% that of the rat), and the subsequent clearance rates for hamsters at all three exposure concentrations ranged from 33 to 39 days.

**Markers of lung inflammation/oxidative stress**

The overall pattern apparent from these parameters was that all three species develop an initial inflammatory response to high lung burdens of TiO₂. This was greatest and more persistent in the rat over the duration of the post-exposure period. The rapid clearance of TiO₂ from the lungs of hamsters was accompanied by a similarly faster reduction in inflammatory markers compared with the rat and mouse. This is exemplified by Fig. 2, which shows lung neutrophils as a percentage of the total lung cell count for exposure to both PG- and UF-TiO₂.

**Histopathology: PG-TiO₂**

Significant differences in histopathological findings were apparent between the three species. The microscopic pattern of particle retention in rat lungs differed noticeably from that in mice. Rats had a predominantly centriacinar pattern of accumulation whereas mice had a more panacinar distribution. The retention pattern in hamsters was more difficult to discern due to the rapid clearance, but it appeared to have features similar to both the rats and mice.

Although mice had the least and rats had the most interstitial accumulation of particles and particle-laden macrophages, all three species had a pattern of particle retention that is best characterized as alveolar sequestration. Rats exposed to the medium and high concentrations had less free particle accumulation in the alveolar lumens but had more clustering of particle-laden macrophages than mice. Unlike mice, rats frequently had particle-laden macrophage accumulations in subpleural regions, with associated epithelial proliferative changes. Hamster changes resembled mice initially at early time points, with concentration of particles and associated epithelial changes in central lobar areas, but with clearance there was intense aggregation of heavily particle-laden macrophages into focal lesions, as was noted in rats, but with the absence of the tissue responses to such aggregates described later in the rat.

Epithelial change in all species was concentrated in areas of heavy dust accumulation and correlated with the markers of inflammation. Neutrophilic infiltration was most prominent in rats. Hypertrophic and hyperplastic epithelial changes were seen in immediate proximity to aggregates of inflammatory cells and particle-laden macrophages. With the exception of rats exposed to 250 mg/m³, the epithelial changes diminished in incidence and severity with the reduction in pulmonary inflammation that occurred during the post-exposure period. In rats exposed to 250 mg/m³, epithelial proliferative changes were associated with metaplastic alteration and septal fibrosis, and became more severe post-exposure (Fig. 3a). Such lesions were not seen in similarly exposed mice or hamsters (Fig. 3b,c).
Histopathology: UF-TiO₂

Preliminary evaluation of the tissues indicate that changes indicative of overload occurred only in the rat and mouse exposed to 10 mg/m³. At lower concentrations and in the hamster such changes were not apparent. At the end of the exposure period in rats exposed to 10 mg/m³ there was hypertrophy, slight proliferation of Type II cells and some septal thickening, but little metaplasia. At the 13 week time point these lesions had progressed, with more interstitial thickening and epithelial proliferation, including metaplasia of alveolar epithelium along and subjacent to the duct. At subsequent time points there was a diminution of the epithelial response and focal clustering of particle-laden macrophages, mostly alveolar intraluminal, but with more aggregation in...
interstitial areas. At the final time point, tight clusters of particle-laden macrophages were seen in some alveoli and substantial interstitialization of such macrophages was apparent. Occasional small foci of epithelial hyperplasia and hypertrophy and minimal metaplasia were seen, with minimal interstitial fibrosis associated with areas of the latter. There was, in general, little epithelial or fibroproliferative reaction in regions of intraluminal or interstitial aggregation of particle-laden macrophages.

In mice exposed to 10 mg/m³ UF-TiO₂, particle-laden macrophages and aggregates of free particles were seen in centriacinar regions with no epithelial response. Interstitialization of these macrophages...
Fig. 3. Comparison of lung histopathology in the rat (a), mouse (b) and hamster (c) exposed to 250 mg/m³ PG-TiO₂ for 13 weeks and retained for 52 weeks post-exposure.
was apparent at the 13 week time point, but again no epithelial response was observed. At the final time point intraluminal accumulations of particle-laden macrophages were still apparent, but again in the absence of an epithelial response. In hamsters, the rapid clearance of particles during the exposure phase resulted in the appearance of limited numbers of particle-laden macrophages in the alveoli adjacent to and along the alveolar ducts and interstitialized free particles. By the 26 week time point there were virtually no particles or particle-laden macrophages discernible.

**DISCUSSION**

Marked species differences were seen in the response of the lung to exposure to PG- and UF-TiO$_2$ and in patterns of particle retention, especially between rats and mice. Overall, findings in rats and mice exposed to 50 and 250 mg/m$^3$ PG-TiO$_2$ or 10 mg/m$^3$ UF-TiO$_2$ were consistent with the development of lung overload, i.e. accumulation of TiO$_2$ in the lungs, reduced clearance rates and pulmonary inflammation. Pulmonary lesions in rats exposed to 250 mg/m$^3$ PG-TiO$_2$ continued to develop in severity throughout the post-exposure period, while those exposed to 50 mg/m$^3$ PG-TiO$_2$ or 10 mg/m$^3$ UF-TiO$_2$ showed diminution. Mice had similar lung burdens to rats throughout the study, but in contrast, the inflammatory and histological responses were less severe and diminished with time. Hamsters exposed to 250 mg/m$^3$ PG-TiO$_2$ had similar initial lung burdens to the rat but demonstrated a high clearance rate post-exposure, with an accompanying reduction in the inflammatory and histopathological findings. Hamsters exposed to 10 mg/m$^3$ UF-TiO$_2$ had accumulated only ~20% of the lung burden seen in the rat and this was reflected in the limited changes observed in the measured parameters.

The picture to emerge from these results is that over the duration of these studies the rat lung produces a marked tissue response to the presence of high concentrations of accumulated dust and that this not only persists but becomes more severe with time. Further progression would be expected to result in the lesions reported in the lungs of rats chronically exposed to dusts and from which tumours may emerge occasionally. In contrast, the tissue response in the mouse and hamster at similar lung burdens was minimal, reduced with time, and is considered unlikely to lead to neoplastic change when extrapolated to lifetime exposure, which is supported by the negative finding of the study where mice were exposed lifetime to TiO$_2$ (Heinrich, 1994). Additionally, the rapid clearance rate in the hamster virtually precluded the development of marked overload in this species except under extremes of exposure.

While results have been considered above in terms of atmospheric concentration and lung mass of retained PG- or UF-TiO$_2$, expression of the lung burden as surface area provides a better correlate with the observed tissue responses and allows direct comparison to be made between the two forms of TiO$_2$: for example, the tissue response to 50 mg/m$^3$ PG-TiO$_2$ was similar to that for 10 mg/m$^3$ UF-TiO$_2$, and when expressed as surface area (using 8 m$^2$/g PG and 49 m$^2$/g UF) the lung burdens are also similar. This is entirely consistent with the other studies with insoluble dusts, where the best inter-study correlate of dose and response comes from surface area of the lung burden.

**REFERENCES**


