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Titanium Dioxide: Inhalation Toxicology and Epidemiology

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Titanium dioxide (TiO_2) is manufactured worldwide in large quantities for use in a wide range of applications and is normally considered to be toxicologically inert. Findings of tumours in the lungs of rats exposed chronically to high concentrations of TiO_2, but not in similarly exposed mice or hamsters, suggest that the tumorigenic response may be a rat-specific phenomenon but nonetheless raises concerns for potential human health effects. With the limited toxicological understanding of species differences in response to inhaled TiO_2 and a similarly limited amount of epidemiological information with respect to TiO_2 exposure in the workplace, a consortium of TiO_2 manufacturers in Europe (under the European Chemistry Industry Council; CEFIC) and in North America (under the American Chemistry Council; ACC) initiated a programme of research to investigate inter-species differences as a result of exposure to TiO_2 and to conduct detailed epidemiological surveys of the major manufacturing sites. The toxicology studies exposed rats, mice and hamsters to pigment-grade TiO_2 (PG-TiO_2, 0, 10, 50 and 250 mg m^{-3}) or ultrafine TiO_2 (UF-TiO_2, 0, 0.5, 2 and 10 mg m^{-3}) for 90 days and the lung burdens and tissue responses were evaluated at the end of the exposure period and for up to 1 year after exposure. Results demonstrated clear species differences. Rats and mice had similar lung burdens and clearance rates while hamsters showed high clearance rates. At high lung particle burdens, rats showed a marked progression of histopathological lesions throughout the post-exposure period while mice and hamsters showed minimal initial lesions with recovery apparent during the post-exposure period. Lung neutrophil responses, a sensitive marker of inflammatory changes, reflected the development or recovery of the histopathological lesions. The use of surface area rather than gravimetric lung burden provided closer correlates of the burden to the biological effect across both TiO_2 types. The epidemiological investigations evaluated the mortality statistics at 11 European and 4 US TiO_2 manufacturing plants. They concluded that there was no suggestion of any carcinogenic effect associated with workplace exposure to TiO_2.

Keywords: epidemiology; inhalation; titanium dioxide; toxicology

INTRODUCTION

Titanium dioxide (TiO_2) is manufactured worldwide in large quantities for use in a wide range of applications. It is most widely used as a white pigment. This is due to its high refractive index and reflectance combined with its ease of dispersion in a variety of media and non-reactivity towards those media during processing and throughout product life.

The two main processes for making TiO_2 pigments are the sulphate process and the chloride process. The sulphate process was the first to be developed on a commercial scale in Europe and the USA around 1930. It was the primary process until the early 1950s, when the chloride process was researched and developed. Currently, the chloride process accounts for ~60% of the world's TiO_2 pigment production. Pure TiO_2 is extracted from its mineral feedstock by reaction with either sulphuric acid or chlorine, then it is milled and treated to produce a range of products that are designed for specific end uses. The majority of TiO_2 products are based on the crystal type rutile with a primary particle size range of 200–300 nm.

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In this context it is called 'pigment grade' (PG). At this particle size TiO_2 pigments offer maximum opacity as well as impart whiteness and brightness to the paints, coatings, papers and plastic products in which they are used. These TiO_2 pigments are also used in many white or coloured products including foods, pharmaceuticals, cosmetics, ceramics, fibres, rubber products, to mention only a few.

One of TiO_2 properties is its efficient absorption of ultraviolet light which makes it a very effective sunscreen for use in cosmetics. Usually its opacity is not required in this application, so very low particle size material (size 10–20 nm) is used and this is commonly called 'ultrafine' or UF. It should be acknowledged that in most environments the primary particles of both types of TiO_2 are significantly aggregated at equilibrium. At any particular time the particle size range is dependent on the nature of the environment and the intense energy that has been imparted to the particles through processing. The final product is of a particle size that could become airborne and inhaled.

The fact that TiO_2 is highly insoluble, non-reactive with other materials, thermally stable and non-flammable has led to it being considered to pose little risk to respiratory health. This is supported by the toxicological database on TiO_2 and the fact that TiO_2 has been used traditionally for many years as a 'negative control' dust in many *in vitro* and *in vivo* toxicological investigations. However, this view was challenged when lung tumours were found in the lungs of rats after lifetime exposure to very high concentrations of pigment grade TiO_2 (PG-TiO_2; Lee *et al*., 1985). Similar studies on a range of insoluble dusts, including carbon black, diesel exhaust and ultrafine TiO_2 (UF-TiO_2), also resulted in lung tumours in rats at the end of a lifetime exposure (Mauderley *et al*., 1987; Heinrich *et al*., 1995; Nikula *et al*., 1995), while in contrast, albeit in a more limited number of studies, no tumours were seen in similarly exposed mice and hamsters (Heinrich *et al*., 1986, 1995; Heinrich, 1996; Muhle *et al*., 1998). These apparent species differences suggest that the experimentally induced lung tumours might be a rat-specific, threshold phenomenon, dependent upon lung overloading accompanied by chronic inflammation to exert the observed tumorigenic response. The relevance of this phenomenon to human exposures remains questionable, but despite this, when the International Agency for Research on Cancer (IARC, 1996) evaluated the carcinogenic risk of carbon black, they decided that there was sufficient evidence in experimental animals for the carcinogenicity of carbon black and classified it as a category 2B carcinogen (possibly carcinogenic to humans). This raised the possibility that other insoluble dusts, including TiO_2, might be classified similarly in the future unless data become available to show that the rat lung tumours are indeed a rat-specific phenomenon and so of little or no relevance to man. Further essential support for this position would be the lack of any adverse health findings in epidemiological surveys of workers exposed to TiO_2. However, epidemiological information was largely limited to a single mortality and cancer incidence study of workers at two TiO_2-producing plants in the USA (Chen and Fayerweather, 1988). The investigators reported significantly reduced mortality due to lung cancer in workers employed from as early as 1935 and lung cancer incidence (over a more recent time period) that was similar to that observed in other workers employed by this chemical company.

It was clear that existing toxicological and epidemiological data for TiO_2 exposure were insufficient to provide the necessary information on potential long-term health effects. Therefore, a consortium of the TiO_2 manufacturers in Europe (under the European Chemistry Industry Council; CEFIC) and in North America (under the American Chemistry Council; ACC) initiated a programme of research to investigate inter-species differences as a result of exposure to TiO_2 and to conduct detailed epidemiological surveys of the major manufacturing sites. The toxicological studies were designed to compare the development and possible progression of the lung response of rats, mice and hamsters exposed to a range of concentrations of PG-TiO_2 or UF-TiO_2 over a period of 90 days with subsequent retention of some exposed animals for up to 1 year. Their aim was to establish early species-specific responses of the lungs to particulate exposure and overloading with the expectation that findings from short-term exposures would be consistent, by extrapolation, with those seen after lifetime exposure. Detailed results from these studies have been published (Bermudez *et al*., 2002, 2004; Hext *et al*., 2002) and show distinct species differences in lung responses, particle distribution and clearance rates. The intention here is to provide a comparative overview of the key results, focussing on the relationship between effects and the lung burdens of TiO_2 expressed as surface area rather than gravimetric. While the latter has been the traditional approach, it has been recognized for many years that for inhaled insoluble particulates, the relationship between the actual lung burden and the extent and severity of pathological responses observed shows a good linear correlation with surface area (e.g. Oberdörster, 1996; Lison *et al*., 1997; Tran *et al*., 2000) across a range of particle types and sizes, including PG- and UF-TiO_2 (Höhr *et al*., 2002). It will be shown that by using surface area as the principle comparator of effects, results from the studies described here for the two TiO_2 types can be combined and ranked in a dose–response relationship.

With respect to epidemiology, initial feasibility studies were conducted in Europe and the US to

evaluate the possibility of conducting a mortality study at the major manufacturing sites owned by the TiO_2 manufacturers in each region. These concluded that the occupational and exposure records at most of the manufacturing sites were of sufficient quality to permit a large-scale mortality investigation with adequate power to detect a modest increase in lung cancer risk. Separate multicentre studies were then commissioned in Europe and the US, and the two study groups collaborated closely to ensure that results from both regions could be pooled if required. The European study was led by researchers from the Karolinska Institute, Stockholm, Sweden, IARC, Lyon, France and the Institute of Occupational Medicine, Edinburgh, UK. The US study was performed by researchers from the International Epidemiology Institute, MD, USA. This paper reviews the results from these studies and the limited information from other epidemiological studies.

The overall aim of this paper is to bring together as overviews the results from the extensive programmes of work commissioned by the TiO_2 manufacturers to investigate the toxicology and epidemiology of TiO_2.

TOXICOLOGY STUDIES

Design

The toxicity studies were conducted at the CIIT Centers for Health Research, Research triangle Park, NC, USA. Full details of the study designs, conduct and results are reported by Bermudez *et al.* (2002, 2004). The aim here is to provide an inter-species comparison of the results obtained with the two TiO_2 types.

Test compounds were pigment grade TiO_2 supplied by the DuPont Company, Wilmington, DE, USA, and was from the same batch of material used by Lee *et al.*, (1985), and ultrafine TiO_2 (Degussa P25), supplied by Degussa AG, Hanau, Germany.

Test species were female B6C3F1/CrlBR mice, CDF (F344)/CrlBR rats and Lak: LVG (SYR) BR hamsters, obtained from Charles River Breeding Laboratories, Wilmington, MA, USA. They were exposed for 6 h per day, 5 days per week for 13 weeks to 0, 10, 50 or 250 mg m^{-3} PG-TiO_2 or 0, 0.5, 2 or 10 mg m^{-3} UF-TiO_2 in 1 m^3 H-1000 stainless steel and glass inhalation chambers. Atmospheres were monitored regularly for gravimetric concentrations and aerosol particle size. 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 TiO_2 burdens, lung clearance rates, lung cell proliferation and histopathology, and for lung lavage. The lavage fluid was analysed for markers of inflammation (total and differential free cell counts, cell and fluid phospholipid content, lactate dehydrogenase, γ-glutamyl transpeptidase, glutathione and total protein).

RESULTS

Relationships of lung burdens and biological responses to exposures

Lung burdens for each species and at each time point for both TiO_2 types, expressed as mg TiO_2 g^{-1} dried lung, are shown in Fig. 1. At the end of the exposure period the mice had accumulated the greatest lung burdens at the two highest concentrations of PG-TiO_2 compared with rats, while for UF-TiO_2, lung burdens were essentially similar for mice

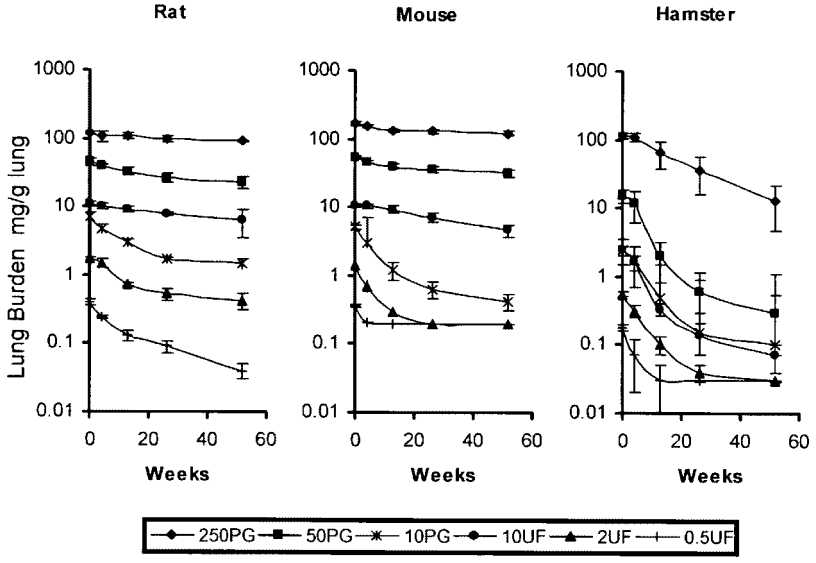

Fig. 1. Comparison of the lung TiO_2 burdens of rats, mice or hamsters expressed as mg TiO_2 g^{-1} lung.

and rats at all concentrations. Clearance half-lives (Table 1) show that overload of the lung clearance mechanisms (evidenced by considerable increase in clearance half-life) was achieved at the two highest PG-TiO_2 concentrations and highest UF-TiO_2 concentration in both species, with some indication of an effect at 10 mg m^{-3} PG-TiO_2 and 2 mg m^{-3} UF-TiO_2 in the rat. Results for hamsters were totally different from these other species. While the initial lung burden of PG-TiO_2 at 250 mg m^{-3} was similar to rats, it can be seen from Fig. 1 that there was subsequently a very rapid clearance, and at all lower concentrations and for UF-TiO_2 exposures, initial burdens were substantially lower than for rats and mice and clearance was equally rapid. This is reflected in the clearance half-lives for hamsters (Table 1), which show a minimal overload at the highest PG-TiO_2 concentration and normal clearance rates at all other concentrations of both PG- and UF-TiO_2.

Ranking of lung burdens according to weight (Fig. 1) follows the order of test atmosphere concentrations for each type of TiO_2. At the 10 mg m^{-3} concentration used for both PG- and UF-TiO_2, the rat and mouse lung burdens were slightly greater for UF-TiO_2 but clearance half-lives were markedly greater. Such differences were not apparent for hamsters. Thus for rats and mice, there is a discrepancy in the rankings of lung burden expressed as weight (Fig. 1), and rankings of concentrations according to clearance rates (Table 1). If however, lung burdens are plotted as surface area of the lung burden (Fig. 2; specific surface areas of 8.5 and 49.7 m^2 g^{-1} for PG-TiO_2 and UF-TiO_2, personal communication from CIIT to P.M.H.) then ranking of burden and ranking of clearance half-lives is similar. Moreover, the burdens for 50 mg m^{-3} PG-TiO_2 and 10 mg m^{-3} UF-TiO_2 are matched closely by their clearance half-lives. For rats, this similarity applies also to the 10 mg m^{-3} PG-TiO_2 and 2 mg m^{-3} UF-TiO_2 exposures.

Table 1. Comparison of lung clearance rates following exposure to PG-TiO_2 and UF-TiO_2 (data from Bermudez *et al*., 2002, 2004)

Test material	Concentration (mg m^{-3})	Clearance half life (days)		
		Rat	Mouse	Hamster
PG-TiO_2	250	838	621	110
	50	324	417	40
	10	100	50	40
UF-TiO_2	10	395	319	39
	2	132	40	37
	0.5	63	48	33

This relationship between clearance half-life and the surface area of the lung burden rather than weight is seen also for markers of the biological responses of the lungs to exposure to TiO_2. Most exposure-related responses of the lungs were of an inflammatory nature; neutrophil numbers in lung lavage fluid and histopathological changes were considered to be markers of this. Full details of both are reported by Bermudez *et al*. (2002, 2004) and some initial comparisons between PG-TiO_2 and UF-TiO_2, based on gravimetric data, were made by Hext *et al*. (2002). When comparing neutrophil responses, there is again a closer relationship of effect to lung burden expressed as surface area rather than gravimetric (Fig. 3). For rats and mice, intra-species similarities of neutrophil responses can be seen for exposures to 50 mg m^{-3} PG-TiO_2 and 10 mg m^{-3} UF-TiO_2, matching both the lung burdens surface areas and clearance half-lives. It should be noted that the overall severity of responses differed between these two species, i.e. the response was substantially greater in rats at all equivalent atmospheric concentrations. Hamsters again show marked differences from rats

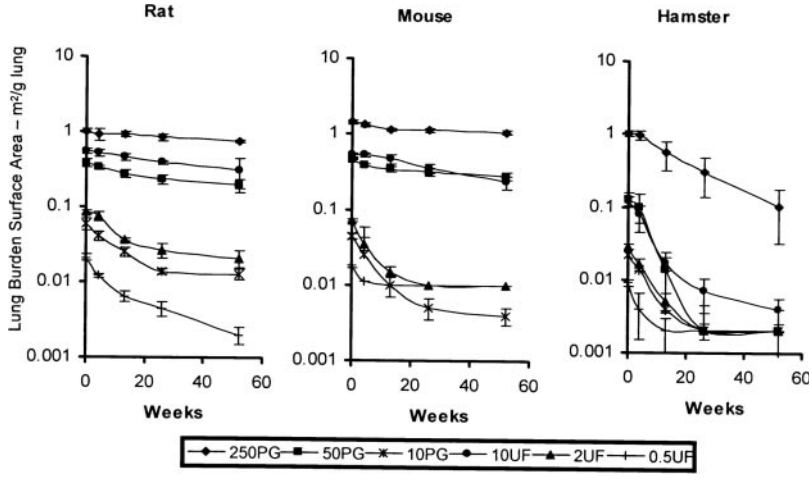

Fig. 2. Comparison of the lung TiO_2 burdens of rats mice or hamsters expressed as surface area (m^2 g^{-1}).

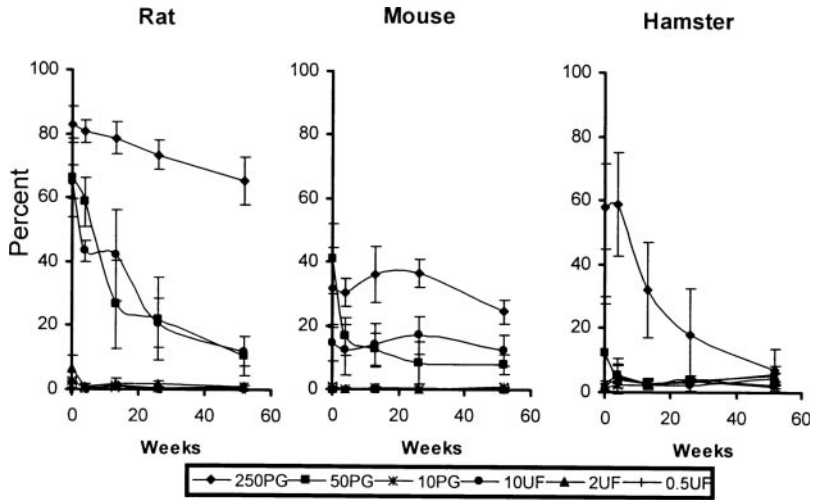

Fig. 3. Comparison of the neutrophil responses in bronchoalveolar lavage fluid in rats, mice or hamsters, expressed as percent of control.

and mice, the rapid clearance of particles from the lungs at 250 mg m^{-3} PG-TiO_2 being reflected in the rapid diminution of the neutrophil response and virtual lack of response at all other exposures to both PG-TiO_2 and UF-TiO_2.

A similar pattern of response-relationship is apparent in the histopathological findings. Within the individual species and for separate exposures to PG-TiO_2 or UF-TiO_2, the microscopic changes seen in the lungs at the end of the exposure and their regression or progression throughout the subsequent observation period were closely associated with concentration. However, as for parameters considered already (lung burden/clearance, neutrophil response), when findings from both forms of TiO_2 are combined for comparative purposes, there is an apparent relationship between the severity of any effects observed and the surface area of the lung burden rather than weight.

Species differences in histopathological responses

The main aim of the studies was to compare the lung response of different experimental species to exposure to the two types of TiO_2. Bermudez *et al.* (2002, 2004) describe in detail the microscopic pathology of all three species exposed to PG-TiO_2 or UF-TiO_2 respectively. Rats exposed to PG-TiO_2 differed substantially from mice and hamsters in that not only were significant epithelial changes seen in the lungs of rats exposed to 250 mg m^{-3} at the end of the exposure period, but also these progressed substantially during the post-exposure phase. In contrast, mice or hamsters, despite initial high lung burdens, showed only minimal initial responses and regression was clearly evident throughout the post-exposure period. While this might be attributable to the rapid clearance of lung burden in the hamster, the lack of any substantial clearance in the mouse points to a marked species difference in biological response between rats and mice. At the lower exposure concentrations of PG-TiO_2, the initial lung effects in rats were consistent with the observations made at 250 mg m^{-3} except that they diminished in incidence and severity with time after exposure. Inter-species differences consistent with those described above for 250 mg m^{-3} exposures were also observed.

Rats exposed to 10 mg m^{-3} UF-TiO_2 showed effects broadly consistent with exposure to 250 mg m^{-3} PG-TiO_2, i.e. microscopic changes at the end of exposure that continued to progress in severity following cessation of exposure. However, while this was observed throughout the entire post-exposure period with PG-TiO_2, the severity of the majority of the responses to UF-TiO_2 peaked ~13 weeks after exposure and then showed marked resolution or consolidation by the end of this period (52 weeks). Exposure of rats to lower concentrations of UF-TiO_2 resulted in minimal effects, which were of little or no consequence to the microscopic pathology of the lung. In mice, the only indications of exposure to UF-TiO_2 at any concentration were particle aggregates and particle-laden macrophages in the lungs. While some of these findings persisted until the end of the observation period, there were no tissue responses of any consequence at any concentration, despite the highest concentration causing a degree of overload in the lungs. Hamsters, as a consequence of their very rapid clearance rates of particles from the lung, showed little evidence of exposure to 10 mg m^{-3} UF-TiO_2 and no indications at lower concentrations.

Overall, the rat was the greatest responder at each concentration and clearly, in both rats and mice, the responses were consistent with the surface area of the lung burden rather than weight.

EPIDEMIOLOGY STUDIES

Results of the research programme of the TiO_2 manufacturers

European multicentre study. The results of the European multicentre study were reported by Boffetta *et al*. (2004). The study included workers employed in 11 plants producing TiO_2 in six European countries (Finland, France, Germany, Italy, Norway, UK). Overall, the study investigators identified 27 522 TiO_2 exposed workers first employed between 1927 and 2001. Workers who were first employed after 1990, or employed for less than one year in total, or who worked in non-production jobs, were excluded from analyses leaving a total of 15 045 workers (14 359 men and 686 women). Of the 11 plants 7 had only produced TiO_2 using the sulphate process and 2 had only produced TiO_2 using the chloride process. One plant operated both sulphate and chloride processes and the other plant currently using the sulphate process had operated a chloride process for a short period. A follow-up for mortality was conducted in all countries. The period covered by the mortality follow-up ranged from 27 years in Italy (1972–1999) to 47 years in the UK (1954–2001). A total of 3.3% of cohort members were lost to follow-up and 0.7% had emigrated. The cause of death was unknown for 5.9% of deceased cohort members. Cancer registration information was also obtained for workers from the two Scandinavian countries.

Two experienced occupational hygienists performed a comprehensive assessment of exposure. The exposure assessment was carried out at the level of occupational title for each plant for discrete time periods throughout the history of plant operations. Exposures to respirable TiO_2 dust, sulphuric acid mist, hydrochloric acid, asbestos and welding fumes were assessed and indices of cumulative exposure calculated by combining estimates across the entire occupational history of a worker. Exposure reconstruction was based on person sample measures mainly collected during the 1990s. Two factories had measurements from the late 1980s and one factory had measurements from 1990. Information on smoking status was collected for 37.6% of workers included in the analyses.

Figure 4 shows estimates of the average respirable TiO_2 dust concentration by calendar year and factory. Average estimated exposures fell at most factories over the study period to current typical levels of 0.2–0.4 mg m^{-3}. The median cumulative exposure of workers was 1.98 mg m^{-3} years (interquartile range 0.26–6.88 mg m^{-3} years).

There were 2619 male deaths during the period of follow-up and 33 female deaths. The standardized mortality ratio (SMR) for all causes was significantly decreased in both genders: among men it was 0.87 [95% confidence interval (CI) 0.83–0.90] and among women it was 0.58 (95% CI 0.40–0.82). Country-specific SMR for all causes in men ranged from 0.81 in Finland to 0.97 in France. Deaths due to all malignant neoplasms were also fewer than expected (SMR = 0.98; 95% CI 0.91–1.05). The only cause of death with a statistically significant increased SMR was lung cancer (SMR = 1.23; 95% CI 1.10–1.38). Mortality from ischaemic heart disease, liver cirrhosis and external causes was significantly below expectation. Mortality from non-malignant respiratory diseases was reduced in the whole cohort (SMR = 0.89; 95% CI 0.77–1.02), though not significantly.

There was little evidence that the increased mortality from lung cancer in the SMR analysis was indicative of a carcinogenic risk linked to employment in the industry. Lung cancer death rates did not increase with cumulative exposure to

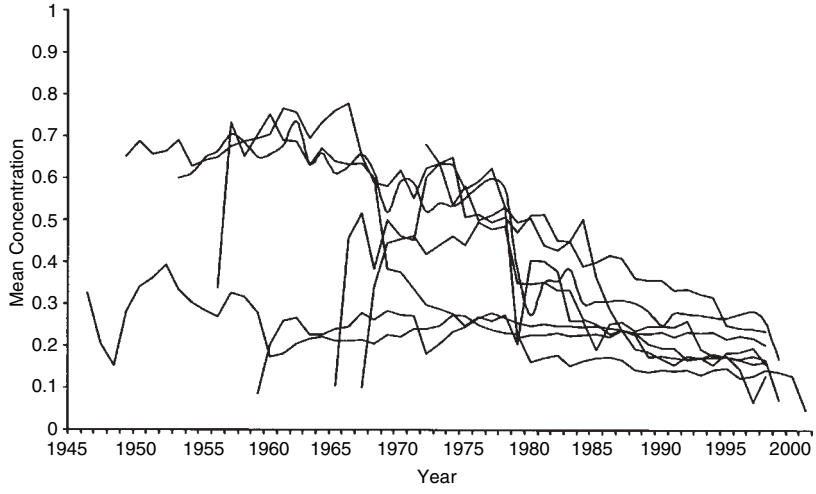

Fig. 4. Average estimated TiO_2 dust concentration (mg m^{-3}) by calendar year and factory.

TiO_2 dusts or with duration of employment in TiO_2 manufacturing plants. In addition, many of the regions where the factories happen to be sited have a higher lung cancer death rate than the national rate for their country (approximately one-fifth higher on average for workers in this study) and lung cancer mortality was close to expected when the expected number of deaths were calculated using local mortality rates (Boffetta, personal communication). The analysis of smoking habits was limited by the relatively small proportion of workers with known data, covering mainly the recent period of follow-up, but it suggested for all countries other than France and the UK, a higher prevalence of smoking among TiO_2 workers than in the respective national populations. Lung cancer mortality was not associated with exposure to sulphuric acid mist, asbestos or welding rod fumes in the factory work place.

US multicentre study. The results of the US multicentre study were reported by Fryzek *et al.* (2003). The study included workers employed at four TiO_2 manufacturing plants in the US. Overall, the study investigators identified 5713 workers employed on or after 1 January, 1960 for at least six months who were included in the study cohort. Among these, 1472 worked exclusively in administration or in other jobs that did not involve exposure to TiO_2. The remaining 4241 workers were the focus of this analysis and were followed until 31 December, 2000. More workers were employed in chloride plants (53%) than in sulphate plants (40%), with 7% not able to be categorized. Vital status information was found for 4194 of the 4241 (99%) workers in the study cohort. Of the 533 deceased workers, cause of death information was found for 511 (96%).

Exposure assessment was conducted by industrial hygienists with expertise in historical exposure reconstruction. A combination of walk-through surveys, interviews with knowledgeable long-term employees and historical industrial hygiene measurements taken at the plants were used to assign exposure levels to study subjects based on their job history. In contrast to the European study, only the long-term area samples for total TiO_2 dust were used. However, the US and European study investigators collaborated to ensure comparability between the two studies. Exposure variables representing average exposure per year, years exposed and cumulative amount exposed were created for TiO_2 and subjects were categorized into low, medium and high categories of exposure. Thirty-five per cent of the workforce had worked in one of the jobs with the highest potential for TiO_2 exposure i.e. packing, micronizing or internal recycle. Smoking information was abstracted from medical records for 2503 workers across all four plants from 1960 onward.

The median values for long-term area samples for total TiO_2 dust fell from 4.6 mg m^{-3} between 1976 and 1980 to 1.1 mg m^{-3} between 1996 and 2000. Packing, micronizing or internal recycle workers had a median exposure of 3.0 mg m^{-3} compared to a median exposure of 0.3–0.9 mg m^{-3} for other jobs.

SMRs were calculated for all workers as well as separately by type of plant (sulphate and chloride). It was stated that SMRs for women did not differ appreciably from those for men and only analyses for both sexes combined were presented. The SMR for all causes of death was significantly less than expected (SMR = 0.8; 95% CI 0.8–0.9), with the all-cause SMR for sulphate plants higher (SMR = 0.9; 95% CI 0.8–1.0) than that for chloride plants (SMR = 0.6; 95% CI 0.5–0.7). The number of lung cancers was close to expected (SMR = 1.0; 95% CI 0.8–1.3), with little variation by type of plant (sulphate: SMR = 1.1 95% CI 0.7–1.6; chloride: SMR = 0.9; 95% CI 0.6–1.3). No significant increases were seen for any cause of death by type of plant. Workers with the highest TiO_2 exposure (packing, micronizing or internal recycle workers) had a similar mortality pattern i.e. significantly lower than expected deaths for all causes with no excess for lung cancers.

No trends of increasing SMRs for malignant or non-malignant lung disease with increasing duration of employment were evident. Internal analyses showed that relative risks of all cause mortality and mortality due to lung cancer and non-malignant respiratory disease fell with increasing cumulative exposure. The investigators concluded that the data indicate that workers at the US plants have not experienced increased risks of lung cancer or other significant adverse health effects as a result of their occupational exposures to TiO_2.

Other relevant studies

Chen and Fayerweather (1988) studied mortality and cancer incidence among 1576 male employees of the DuPont Company who had been exposed to TiO_2 for more than one year in two TiO_2-producing plants in the USA. The cohort was observed from 1935 through 1983 for mortality and 1956 through 1985 for cancer incidence. Information on deaths among active and pensioned employees was obtained from the DuPont company mortality registry. Cancer incidence information was available for cancers diagnosed during the period that workers were employed by DuPont. The sulphate process was used to manufacture TiO_2 from 1935 to 1974 and the chloride process after 1948. A cumulative exposure index to TiO_2 and a time-weighted average exposure were calculated for each individual based on monitoring data available after 1975 and estimated exposures before then. The median time-weighted average

exposure of workers (cumulative exposure index divided by duration of exposure) was estimated to be 10 mg m^{-3}. Smoking histories were available for workers still employed in 1984. Observed numbers of deaths were compared with US rates and observed numbers of cancers were compared with company rates.

Mortality from all causes was significantly lower than expected (SMR = 0.85; 95% CI 0.74–0.97) and mortality due to all malignant neoplasms was lower than expected (SMR = 0.76; 95% CI 0.54–1.05). For lung cancer, nine deaths were observed, with 17.3 expected (SMR = 0.52; 95% CI 0.24–0.99). No increase was found in mortality from other cancers. Mortality from non-malignant respiratory diseases was lower than expected. Overall cancer incidence was slightly higher than expected, but lung cancer incidence was close to expected (8 observed, 7.7 expected). The mortality experience of the cohort is currently being updated and the cohort is being expanded to include workers at another DuPont TiO_2-producing plant.

Boffetta *et al*. (2001) analysed the risk of lung cancer among residents in Montreal, Canada. They included 857 histologically confirmed cases of lung cancer diagnosed during 1979–1985 among men aged 35–70, and a group of controls comprising 533 randomly selected healthy residents and 533 cases of cancers from organs other than the lung. Exposure to TiO_2 and other titanium compounds was assessed by a team of industrial hygienists on the basis of a detailed occupational questionnaire. Thirty-eight cases and 40 controls were classified as exposed to TiO_2 (mainly painters and motor vehicle refinishers and mechanics). The odds ratio was 1.1 (95% CI 0.7–1.8). No clear trend was apparent according to estimated frequency, level or duration of exposure. The odds ratio was 1.3 (95% CI 0.3–4.9) for medium or high exposure for at least 5 years. Few subjects were classified as exposed to other titanium compounds.

Two studies have addressed non-malignant respiratory effects in TiO_2 exposed workers. Garabrant *et al*. (1987) performed a cross-sectional study of 209 titanium metal production workers, including 78 workers involved in the reduction process who were exposed to titanium tetrachloride vapour, titanium oxychloride and TiO_2 particles. The other workers included workers exposed to a mixed aerosol of titanium, sodium chloride and hydrochloric acid and maintenance workers who worked in all areas of the plant. A non-significant reduction in lung function (forced expiratory volume in one minute) was found for reduction process workers. The authors noted that titanium tetrachloride probably represents the greatest acute hazard in the reduction area. Pleural disease with plaques and pleural thickening was observed in eight of the 78 reduction process workers (10.3%) and in 28 out of 131 other workers (21.4%), suggesting no association with TiO_2 exposure.

Chen and Fayerweather (1988) also performed a chest X-ray study of 398 workers at the same plants in the US that they studied for mortality. They reported 19 cases of pleural abnormalities (thickening/plaques) in 336 exposed workers (5.7%), as compared with 3/62 among unexposed workers at the same plants (4.8%). The odds ratio for chest X-ray abnormality associated with TiO_2 exposure adjusted for smoking status was 1.4 (not statistically significant). No case of lung fibrosis was observed.

DISCUSSION

Toxicology

The main aim of the toxicology studies was to compare the lung response of three experimental species to exposure to the two types of TiO_2 with a view to establishing differences between the rat and the other two species in line with the differences seen in the tumorigenic responses to lifetime inhalation exposure. The results clearly achieved this. In general, the rat and mouse showed similarities with respect to lung burdens and clearance rates across both TiO_2 types and concentrations (Fig. 1; Table 1). In contrast, the hamster showed very rapid clearance of deposited particles, even at initial retained lung burdens which produced clear overload in the rat and mouse. Since the clearance half-lives in the hamster at low exposure, non-overload, concentrations did not differ significantly from those in the rat or mouse at similar concentrations, this implies a greater capacity for clearance by the hamster compared with the other two species, rather than a shorter clearance half-life. A similar difference in apparent clearance capacity for inhaled toner between the hamster and the rat was shown by Oberdörster (1995).

Despite the similarities in lung burden and clearance for rats and mice, there was a marked difference in the biological responses to the accumulated particulate. At the highest PG-TiO_2 concentration where a lung overload situation had clearly been demonstrated, the rat showed a substantial cellular and pathological inflammatory response at the end of the exposure period with progression in severity of the pathological findings over the subsequent one-year holding period. The mouse showed a marked cellular response (in terms of the percent of neutrophils in lung lavage fluid), which persisted throughout the holding period, but was less severe than that seen in the rat. The initial pathological response was minimal and regressed following cessation of exposure. Similar differences between the rat and mouse were apparent when considering the lower concentrations of PG-TiO_2 and results for UF-TiO_2.

The cellular and pathological responses in the hamster lungs were comparable with those seen in the mouse but resolved more rapidly or were not even evident, which is in line with the rapid clearance rate seen for this species. Overall, the rat lung is clearly more responsive pathologically to the presence of high burdens of insoluble particles than the other two species. Furthermore, even when exposure has ceased, the induced lesions continue to develop in extent and severity over very extended time periods, a phenomenon not seen in either the mouse or hamster. Clearly, these results continue to raise questions over the relevance of findings in the overloaded rat lung to potential health effects from human exposures and further comments are made later in this discussion.

When the results of the basic parameters of particulate lung burdens and biological responses are compared, there are clear quantitative discrepancies. For example, 10 mg m^{-3} UF-TiO_2 exposure to the rat resulted in higher lung burdens and greater pathological changes than those associated with 10 mg m^{-3} PG-TiO_2 exposure. It was such discrepancies in the past that led to the consideration that ultrafine particles are more toxic than fine particles (i.e. those equivalent to PG-TiO_2). Conversely, when lung burdens are expressed as the surface area per unit lung weight, there is a far closer correlation to biological effect (Oberdörster, 1996; Lison *et al*., 1997; Tran *et al*., 2000) and this was clearly observed in these studies across both TiO_2 types and species responses. Rankings of the effects and severities of the three key parameters of lung burdens, clearance half-lives and biological responses against surface area all demonstrate a good correlate. For instance, rats exposed to 10 mg m^{-3} UF-TiO_2 showed effects broadly consistent with exposure to 250 mg m^{-3} PG-TiO_2, i.e. microscopic changes at the end of exposure that progressed in severity following cessation of exposure. However, unlike the response to exposure to 250 mg m^{-3} PG-TiO_2, where lesions developed increasing severity throughout the non-exposure period, the responses to UF-TiO_2 subsequently began to resolve or consolidate during the later stages. This can be explained in terms of the lung burdens—the surface area of the lung burdens at the end of exposure to 250 mg m^{-3} PG-TiO_2 or 10 mg m^{-3} UF-TiO_2 were 1.016 and 0.545 m^2 g^{-1}, respectively. While in both cases the initial burdens were adequate to initiate overload related pathological responses, that from exposure to 10 mg m^{-3} UF-TiO_2 in conjunction with the limited subsequent clearance, appears to have been insufficiently high to maintain the persistent and progressive response seen at 250 mg m^{-3} PG-TiO_2. A similar relationship exists between the responses to 10 mg m^{-3} UF-TiO_2 and 50 mg m^{-3} PG-TiO_2. The initial surface area of the lung burden from exposure to 10 mg m^{-3} UF-TiO_2 was ~40 % greater than that from exposure to 50 mg m^{-3} PG-TiO_2, The burden of the latter was adequate to induce some lung responses but was insufficient to maintain or progress these during the non-exposure period. These dose–response relationships provide additional evidence for the biological response of the lung to insoluble particles to be associated with the surface area of particles and not simply weight of particle accumulated. This is entirely logical as an insoluble solid reacts with or interfaces with a biological tissue at its external surface only.

While the main aim of the toxicology studies has been achieved, as stated earlier, some consideration of the relevance to human exposures is warranted. It must be recognized that although this paper concentrates on TiO_2, there are no detailed human data on clearance or the microscopic pathology of inhaled TiO_2. However, if TiO_2 is considered to be a member of a generic group of insoluble, low toxicity dusts, many of which have also been tested in animal studies and shown to have similar pulmonary effects in rats, then human data relevant to these materials may be used for comparison with the toxicology results considered here. Normal particle clearance from the human lung has a half-life of ~400 days (Bailey *et al*., 1985), considerably longer than that for the low concentration exposures in the experimental species tested here. This does not necessarily predispose humans to excessive loading of the lung since, as apparent with the hamsters, other factors may also play crucial roles in the quantities of materials cleared. Without doubt, particulate overloading of the human lung can occur and documented examples of this are associated with the coal mining industry. Past exposures to dust in this occupation have resulted in the development of pneumoconiosis, with good correlation between dust exposure (concentration), duration and coal workers pneumoconiosis (Attfield and Morring, 1992). Additionally, King *et al*. (1956) showed a relationship between the severity/grading of diagnosed pneumoconiosis and lung dust burden, i.e. high lung burdens correlated in general with more severe disease. This study found, on average, 35 g of dust in colliers' lungs. Stöber *et al*. (1967) reported similarly high dust burdens in the lungs of miners together with extended clearance half-lives. However, overloading of human lungs and reduced clearance rates are not associated with the excessive inflammation and subsequent development of pulmonary tumours which appears to be the typical response of the rat lung to such conditions. This indicates that there are distinct differences in the tissue responses of rat and human lungs to high burdens of low toxicity particulates. In this context, Nikula *et al*. (1997) compared the anatomical patterns of particle retention and the lung tissue responses between rats and cynomolgus monkeys following chronic exposure to diesel exhaust and coal dust. There was no significant

difference between diesel exhaust-exposed monkeys and rats in the relative amount of retained particulate materials but a very important difference was that rats retained a greater portion of the particulate material in the lumina of alveolar ducts and alveoli than monkeys; and monkeys retained a greater portion of the particulate material in the interstitium than rats. Rats, but not monkeys, had significant alveolar epithelial hyperplastic, inflammatory and septal fibrotic responses to the retained particles. The authors concluded that the results suggest that intrapulmonary particle retention patterns and tissue reactions in rats may not be predictive of retention patterns and tissue responses in primates exposed to poorly soluble particles at concentrations representing high occupational exposures. Additionally, they comment that the pulmonary responses of the rats were severe compared to the primate, where the insult to the lungs was handled without adverse consequences. In a subsequent study, Nikula *et al*. (2001) evaluated the influence of exposure concentration or dose on the distribution of particulate material within the lungs of rats and humans. The rats had been exposed for 24 months to diesel exhaust at 0.35, 3.5 or 7.0 mg soot m^{-3}. The human subject groups included (i) non-smokers who did not work as miners; (ii) non-smoking coal miners who worked under the current USA standard of 2 mg dust m^{-3} for 10–20 years; and (iii) non-smoking coal miners who worked under the former standard of <10 mg dust m^{-3} for 33–50 years. The distribution of retained particles within the lung compartments was markedly different between the two species. In all three groups of rats, 82–85% of the retained particulate material was located in the alveolar and alveolar duct lumina, primarily in macrophages. In humans, 57, 68 and 91% of the retained particulate material was located in the interstitium of the lung in the three study groups, respectively. The authors concluded: 'these results show that chronically inhaled diesel soot is retained predominantly in the airspaces of rats over a wide range of exposures, whereas in humans, chronically inhaled particulate material is retained primarily in the interstitium. In humans, the percentage of particles in the interstitium is increased with increasing dose (exposure concentration, years of exposure and/or lung burden). This difference in distribution may bring different lung cells into contact with the retained particles or particle-containing macrophages in rats and humans and, therefore, may account for differences in species response to inhaled particles'. These two publications (Nikula *et al*., 1997, 2001) demonstrate significant species differences in lung responses to inhaled particulates between rats and primates, including humans. They provide some evidence to suggest that not only does the rat differ from other experimental species with respect to the pulmonary response to high doses of low toxicity particulates, but that this difference extends also to primates and humans and there is no reason to consider that TiO_2 would be any different.

Epidemiology

The existing epidemiological information available for TiO_2 has been considerably strengthened by the European and US multi-centre studies. Together with the earlier study of Chen and Fayerweather (1988), the three cohort studies include a total of 20 862 workers from 17 factories in 7 countries. The power to detect an increased risk of lung cancer is high because of the large numbers of lung cancer deaths (328 deaths) that would have been expected to have been observed during the follow-up periods of workers in the three studies.

The small increase in lung cancer mortality seen in the European multicentre study was not observed in either of the US studies. Indeed, there was a statistically significant deficit of lung cancer mortality in the study of Chen and Fayerweather (1988). It is possible that the excess of lung cancer in the European study is an exposure related effect that was not detected in the US studies because of reasons such as power or differences in exposure. However, this does not seem likely as none of the studies reported lung cancer mortality or lung cancer incidence related to cumulative exposure to TiO_2 dust.

Workers in the US multicentre study may have had lower exposures than workers in the European study because on average, they started work in the TiO_2 industry more recently. However, the study by Chen and Fayerweather (1988) includes some of the earliest workers in the TiO_2 industry. It is difficult to compare average exposures to TiO_2 in the three studies as different exposure assessment methodologies were used. Both multicentre studies used comprehensive historical information about plant operations, ventilation and job descriptions as well as industrial hygiene measures to estimate exposure levels. Exposure assessment in the US multicentre study was based on long-term area samples whereas the investigators of the European study used personal sampling data. It is not possible to directly compare the average cumulative exposure to TiO_2 of workers in the two multicentre studies but it is unlikely that there is a significant difference. The other US study reported a median cumulative exposure of workers to TiO_2 of 10 mg m^{-3} years (Chen and Fayerweather, 1988). This is about 50% higher than the estimated median cumulative exposure of workers in the European study (assuming that approximately 30% of total dust was respirable). However, if this is a real difference, it is at odds with the deficit of lung cancer deaths observed by Chen and Fayerweather (1988).

The US and European multicentre studies also differ in the type of process used to produce TiO_2.

Most European workers had been engaged in the production of TiO_2 using the sulphate process compared to fewer than half of the workers in the US multicentre study. Fryzek *et al*. (2003) reported separate results for workers in sulphate and chloride plants. All cause mortality and all cancer mortality was significantly reduced among workers in chloride plants but mortality from these causes was only slightly lower than expected among workers in sulphate plants. However, mortality due to lung cancer was not related to the type of process. Many of the workers in the other US study had experience of both types of process: the chloride process replaced the sulphate process over the period of study at the two plants included in the study.

The US studies do have lower power than the European study (the US multicentre study is less than a third of the size of the European study). Nevertheless, the two US studies included almost 6000 workers in total and have the power to detect small increases in the risk of lung cancer.

In the animal studies, effects were seen at much lower doses of exposure to ultrafine TiO_2 than pigmentary TiO_2. In rats, microscopic histopathological changes were observed following cessation of exposure to 10 mg m^{-3} UF-TiO_2 that were broadly consistent with exposure to much higher levels of PG-TiO_2 (250 mg m^{-3}). Some of the dust arising from the manufacture of pigmentary TiO_2 will contain small amounts of ultrafine particles and the industry is currently attempting to quantify this. However, the ultrafine particles are known to agglomerate before release in the workplace atmosphere and hence it is unlikely that exposure to a true ultrafine dust explains some of the variations in lung cancer mortality between studies and factories. The epidemiology studies provide little information to evaluate and assess the health risks associated with the manufacture of ultrafine particles, as this process did not start until the 1990s.

Regional variations in the background levels of lung cancer mortality appear to provide the best explanation for the small excess of lung cancer mortality seen in the European study. The weight of evidence from the three epidemiological studies, taken together with understanding of mechanism derived from the animal research programme, strongly postulates a very low risk to human health from exposures in the TiO_2 manufacturing industry.

CONCLUSIONS

The toxicology studies set out to establish whether the rat was oversensitive to high lung burden of insoluble dust compared with the mouse or hamster. It is considered that the results show that this is the case for both pigment grade and ultrafine TiO_2.

The epidemiology studies investigated whether there was a link between increased incidence of lung cancer and exposure to TiO_2 dust. In all the studies the overall conclusion was the same: 'The results of the studies do not suggest a carcinogenic effect of TiO_2 dust on the human lung'.

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