TITANIUM DIOXIDE

Titanium dioxide was considered by a previous Working Group in October 1988 (IARC, 1989). Since that time, new data have become available, and these have been included in the present monograph and taken into consideration in the evaluation.

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 13463–67–7, titanium dioxide; 1317–70–0, anatase titanium dioxide; 1317–80–2, rutile titanium dioxide
Chem. Abstr. Name: Titanium dioxide
IUPAC Systematic Name: Titanium dioxide
Synonyms: CI: 77891; dioxotitanium oxide; E 171; NCI-CO4240; Pigment White 6; titania; titanic oxide; titanium oxide; titanium (IV) oxide; titanium peroxide

1.1.2 *Molecular formula and relative molecular mass*

TiO_2 Relative molecular mass: 79.90

1.1.3 *Chemical and physical properties of the pure substance*

Description: Fine white powder (Windholz, 1983)
Crystal structure
Four naturally occurring titanium dioxide polymorphs exist: rutile, anatase, brookite and titanium dioxide(B) (Banfield & Veblen, 1992). Anatase and rutile are tetragonal, brookite is orthorhombic and titanium dioxide(B) is monoclinic. In all four polymorphs, titanium is coordinated octahedrally by oxygen, but the position of the octahedra differs between polymorphs. The structure of rutile is the most dense and its unit cell is the smallest. Anatase has four formula units per unit cell with a = 0.379 nm and c = 0.951 nm; rutile has two with a = 0.459 nm and c = 0.296 nm; brookite has eight with a = 0.917 nm, b = 0.546 nm and c = 0.514 nm; and titanium

dioxide(B) has eight with a = 1.216 nm, b = 0.374 nm, c = 0.651 nm and β = 107.29° (Banfield & Veblen, 1992). Only the structures of rutile (titanium dioxide-rutile) and anatase (titanium dioxide-anatase) are reported in commercial products.

Density of ideal minerals: Anatase, 3.79 g/cm^3; rutile, 4.13 g/cm^3; brookite, 3.99 g/cm^3; and titanium dioxide(B), 3.64 g/cm^3 (Banfield & Veblen, 1992)

Refractive index: Anatase, 2.561, 2.488; rutile, 2.605–2.616, 2.890–2.903; and brookite, 2.583, 2.700 (Phillips & Griffen, 1981)

Hardness on Moh's scale: Anatase, 5.5–6; rutile, 6–6.5; and brookite, 5.5–6 (Harben & Kuzvart, 1996)

Solubility: Soluble in sulfuric acid and alkalis; insoluble in water (Weast, 1985)

Spectroscopy: X-Ray diffraction patterns for anatase and rutile are available from the International Center for Diffraction Data (2005), which maintains the powder diffraction file.

Chemical composition: Natural rutile, anatase and brookite contain impurities of up to ≈2% that include iron, chromium, vanadium, aluminium, niobium, tantal, hafnium and zirconium (Heaney & Banfield, 1993) and account for slight variations in density, colour and indices of refraction. Since most commercial titanium dioxide is manufactured from natural material by dissolution of the parent mineral and reprecipitation as fine particles with the structure of anatase or rutile (referred to as titanium dioxide-anatase or titanium dioxide-rutile), most but not all of these chemical impurities are generally removed.

Other characteristics: Titanium dioxide is an ultraviolet (UV)-activated catalyst, and organic polymers that are in contact with it degrade under UV radiation. Anatase is 10 times more active than rutile and responds to slightly different wavelengths (Braun, 1997).

1.1.4 *Technical products and impurities*

Trade names for titanium dioxide include Aeroxide, A-Fil Cream, Atlas white titanium dioxide, Austiox, Bayertitan, Calcotone White T, Comet, Cosmetic White C47–5175, Cosmetic White C47–9623, C-Weiss 7, Flamenco, Hitox, Hombitan, Hombitec, Horse Head A-410, Horse Head A-420, Horse Head R-710, Kemira, KH 360, Kronos titanium dioxide, Levnox White RKB, Pretiox, Rayox, Runa RH20, Rutile, Rutil RC, Rutiox, Tichlor, Tiofine, TiO2 Hombitan, Tiona T.D., Tioxide, Tipaque, Ti-Pure, Ti-Select, Titafrance, Titan, Titania, Titandioxid, Titanium White, Titanox, Titanox 2010, Trioxide(s), Tronox, Tytanpolr, Unitane products (various), UV-Titan, 1700 White and Zopaque.

(a) Particle size

Titanium dioxide particles are referred to as primary, aggregates or agglomerates. Primary particles are single crystals that are bound by crystal planes. Aggregates are

sintered primary particles that are connected by crystal faces. Agglomerates are multiple primary particles and aggregates that are held together by van der Waal's forces.

Scattering of light by titanium dioxide is maximized in particles that are 0.2–0.3 μm in diameter, and most commercial products that are used as pigments have modal primary particle sizes within this range. The range of ultrasonically dispersed primary particles and aggregates is narrow, and generally ranges from <0.1 to 0.5 μm (Braun, 1997; Linak *et al*., 2002; Swiler, 2005). A recent study showed that commercial pigments contain almost no particles <0.1 μm. This range may not apply to bulk material, which contains aggregates that are not broken down during industrial use (Braun, 1997).

Non-pigmentary titanium dioxide is composed of either uncoated manufactured titanium dioxide (both titanium dioxide-anatase and titanium dioxide-rutile) or ground natural rutile. In general, these products contain coarser particles than pigmentary titanium dioxide (Linak *et al*., 2002).

Ultrafine titanium dioxide particles (nanoparticles) range in size from 1 to 150 nm (Linak *et al*., 2002), with a modal primary particle size of 10–50 nm. They are generated by sol-gel synthesis and the wide variation in their morphology and size is controlled by the pH of the gel.

Primary particles generally form aggregates and agglomerates and are not normally found as discrete particles. In commercial products, the particle size of pigmentary and ultrafine material is approximately equal because of aggregation and agglomeration (American Chemistry Council, 2005).

Titanium dioxide has also been produced as engineered nanomaterials, which may be equidimensional crystals or sheets and are composed of either titanium dioxide-rutile or titanium dioxide-anatase. A tubular structure has been produced from scrolling layers of titanium dioxide-anatase, which results in fibres with an outer diameter of about 6 nm and an inner tube of about 3 nm (Barnard *et al*., 2005). Non-scrolled nanofibres have also been produced from titanium dioxide-anatase and titanium dioxide(B) with diameters of 20–100 nm and lengths of 10–100 μm (Pavasupree *et al*., 2005).

(b) Types of titanium-dioxide pigment

According to the American Society for Testing and Materials (ASTM, 1988) D476–84 standard, four types of titanium dioxide pigment exist (Schurr, 1981; Fisher & Egerton, 2001):

Type I (94% titanium dioxide min.) is a titanium dioxide-anatase pigment that chalks [forms a layer of loose pigment powder on the surface of weathered paint film] freely and is used in white interior and exterior house paints.

Type II (92% titanium dioxide min.) is a titanium dioxide-rutile pigment that has a medium resistance to chalking and is used in varying amounts in all types of interior paints, enamels and lacquers.

Type III (80% titanium dioxide min.) is also a titanium dioxide-rutile pigment that has a medium resistance to chalking and is used principally in alkyd and emulsion flat-wall paints.

Type IV (80% titanium dioxide min.) is another titanium dioxide-rutile pigment that has a high resistance to chalking; it is used in exterior paints and has excellent durability and gloss retention.

The Japanese grading system, the JIS K5116–1973, specifies four grades of titanium dioxide-rutile, three of which contain at least 92% titanium dioxide and the fourth contains a minimum of 82%. The type of coating in each grade is also specified (Fisher & Egerton, 2001).

(c) Extenders, impurities and coatings

Titanium dioxide extenders were used in commercial pigments in the past, but are not generally employed now. Calcium sulfate (Braun, 1997) and barium sulfate (Fisher & Egerton, 2001) were commonly used during the early years of production, and other materials that may have been used as extenders for white pigment include calcium carbonate, alumina, silica and kaolin (Linak *et al*., 2002).

Titanium dioxide-anatase pigments may contain titanium dioxide-rutile. Before coating, titanium dioxide-anatase produced by the sulfate process contains both phosphorous and sulfate that are concentrated at the particle surface. In addition, uncoated titanium dioxide-anatase pigments retain about 0.3% niobium pentoxide and 0.3% phosphorus pentoxide from the ore and up to 0.2% alumina that is added during manufacture (Braun, 1997).

Prior to coating, titanium dioxide-rutile pigments that are produced by chlorination contain about 1% alumina, which is concentrated at the surface of the particles (Braun 1997), but not titanium dioxide-anatase.

With the exception of non-pigmentary titanium dioxide such as ground rutile and titanium dioxide-anatase that are used as food additives, all commercially produced titanium dioxide is coated by a variety of oxides and oxyhydrates by aqueous precipitation techniques. These coatings improve dispersibility, dispersion stability, opacity, durability and gloss. They form a barrier between the titanium dioxide and organic substances, such as those found in paints, and prevent contact catalysis. In some cases, organic or silicone treatments may be added after initial coating. Titanium dioxide-rutile pigments generally contain 1–15% of coatings and titanium dioxide-anatase pigments contain 1–5% of coatings. The most common coatings are composed of oxyhydrates and oxides of aluminium and silicone. Oxides and oxyhydrates of zirconium, tin, zinc, phosphorous, cerium and boron are also used (Linak *et al*., 2002). Table 1.1 (American Chemistry Council, 2005) gives the types of coating that are used in decreasing order of importance.

The thickness of these coatings is variable but may be only a few atom layers. They are generally coherent over the surface of the titanium dioxide particle (American Chemistry Council, 2005), but some titanium oxide and titanium hydroxide may also be present on the surfaces (Braun, 1997). The thinness of the coatings precludes most techniques of structural analysis and their atomic structure therefore remains largely unknown (Braun, 1997). The composition (but not necessarily the atomic structure) of the

alumina coatings are γ-AlOOH (bohemite), α-AlOOH (diaspor) and γ-$Al(OH)_3$ (hydrargillite). The silica coatings may be fluffy, and consist of polymerized silicic acid or a dense, true shell of glass. Ultrafine titanium dioxide is also coated; examples of coatings are given in Table 1.2.

Coating with alumina and silica can more than double the surface area (Braun, 1997). The surface area of untreated pigment ranges from 8 to 10 m^2/g, while treated pigment surface areas generally span 8–19 m^2/g and matt-finish pigments (that have high levels of alumina) can extend up to 35 m^2/g. Surface areas of the ultrafine products are in the range of 35–100 m^2/g (American Chemistry Council, 2005).

Titanium dioxide-coated surface and pigments are hydrophilic; those coated with silicones are not used as pigment because they are hydrophobic.

Table 1.1. Types of coating used for common grades of titanium dioxide pigment (normally titanium dioxide-rutile)

Surface treatment type	Composition, range (wt %)	Application
Alumina/TMP	Al_2O_3, 1.0–5.5 Total carbon, <0.3	Paint/coatings
Alumina/zirconia/TMP	Al_2O_3, 1.0–5.0 ZrO_2, 0.3–1.0 Total carbon, <0.3	Paint/coatings
Alumina/silica/siloxane	Al_2O_3, 1–6 SiO_2, 0.3–3 Total carbon, <0.3	Plastics
Alumina/silica/TMP	Al_2O_3, 1.0–6.0 SiO_2, 0.5–13.0 Total carbon, <0.3	Paint/coatings/plastics
Alumina/TME	Al_2O_3, 1.0–3.5 Total carbon, <0.3	Paint/coatings
Alumina/zirconia/TME	Al_2O_3, 1.0–5.0 ZrO_2, 0.3–1.0 Total carbon, <0.3	Paint/coatings
Alumina/silica/TME	Al_2O_3, 1.5–5.0 SiO_2, 1.5–3.5 Total carbon, <0.3	Paint/coatings
Alumina/silica/silane	Al_2O_3, 1.0–6.0 SiO_2, 0.3–3 Total carbon, <0.3	Plastics

From American Chemistry Council (2005)
TME, trimethylol ethane; TMP, trimethylol propane; wt, weight

Table 1.2. Relative proportion[a] of the production of common grades of ultrafine titanium dioxide used in sunscreens with different types of coating

Organic	Inorganic				
	None	Silica 5–25%	Alumina 1–25%	Silica 1–10% + alumina 5–15%	Sodium meta-phosphate 1–5%
None	2	4	3	4	1
Stearate 5–15% as carbon			16		
Butyl glycol dicaprylate 60% + stearate 5%		1			
Methicone max. 11%		1	1		
Dimethicone 1–10%			2	4	
Dimethicone/siloxane 2% as SiO_2			2		
Dimethicone/methicone copolymer 1–10%	2	2		4	
Simethicone 5% (as SiO_2) + water 13%	2				
Trimethylsiloxysilicone 1–10%				4	
Polyvinyl-pyrrolidone max. 3%			1		
Alkyl silane 2.7–3.7% as carbon	1				
Glycerin max. 1%			1		
Alginate 1–5%				1	

From American Chemistry Council (2005)
[a] 16=high, 1=low

1.1.5 *Analysis*

Exposure to particulates in occupational environments is generally determined gravimetrically. The behaviour of titanium dioxide in air and its deposition in the respiratory tract upon inhalation are important factors in human exposure, and are determined by the aerodynamic diameter of the particles. The aerodynamic diameter can be measured by impactors and is dependent upon the geometric diameter, [material] density and shape [factor] of the aggregates. Most commonly, the size distribution of airborne particles is expressed as the mass median aerodynamic diameter (MMAD) and the geometric standard deviation. Several dust fractions are often identified, namely, 'total' dust, inhalable dust and respirable dust.

Inhalable dust approximates the fraction of airborne material that enters the nose and mouth during breathing and is therefore available for deposition anywhere in the respiratory tract (International Standards Organization, 1995; Health and Safety Executive, 2000). The inhalable fraction depends on the prevailing movement of air around the exposed person and whether breathing is by the nose or mouth. It is, however, possible to define target specifications for sampling instruments that approximate the inhalable fraction and these are provided by the International Standards Organization (1995). In the United Kingdom, the standard sampling devices for measuring inhalable dust are the multiorifice sampler and the Institute of Medicine (IOM) sampler (Health and Safety Executive, 2000).

Respirable dust approximates the fraction of airborne material that penetrates the gas-exchange region of the lung. The respirable fraction varies between individuals; however, it is possible to define a target specification for sampling instruments that approximates the respirable fraction for an average person (International Standards Organization, 1995). Respirable dust is generally collected using a cyclone preselector (Health and Safety Executive, 2000).

The term 'total' dust refers to total particulates that are represented (in North America at least) by the material that is collected by a closed-face three-piece plastic sampling cassette that holds a 37-mm filter (Eller & Cassinelli, 1994). The term 'total' dust is not equivalent to all airborne dust; in fact, measurements of inhalable dust by the IOM sampling head are 1.0–2.5 times higher than 'total' dust levels using a closed-face 37-mm filter cassette, depending on the aerodynamic diameter of the particles (Werner *et al*., 1996).

Analysis of different types of coatings is accomplished by transmission electron microscopy equipped with energy-dispersive X-ray spectroscopy.

1.2 Production and use

1.2.1 *Production*

The manufacture of pure titanium white for use as a pigment (anatase form) was first reported in 1923 in France. The growth of the production and use of titanium white pigments began in the early 1930s and continued until recently, but the rate has now decreased. In 2004, worldwide production was estimated at 4.4 million tonnes (Swiler, 2005).

(a) Sources

Titanium dioxide pigments are manufactured from a variety of ores that contain ilmenite ($FeTiO_3$), rutile, anatase and leucoxene ($TiO_2.xFeO.yH_2O$), which are mined from deposits located throughout the world. Titanium may also be recovered from slag produced during iron smelting and from synthetic rutile produced from ilmenite.

Large deposits of titanium dioxide occur in association with igneous rocks and as heavy mineral deposits in unconsolidated sands (Garnar & Stanaway, 1994; Chang, 2002). Major igneous deposits are found in Brazil, Canada, Norway, the Russian Federation and the Ukraine (Chang, 2002).

Important heavy mineral sands are found along the eastern and western coasts of Australia, the eastern coast of South Africa, the southeastern coast of the USA, the west coast of South Island, New Zealand, the eastern coast of China, the northeastern coast of Sri Lanka, at various locations along the southern coast of India, in coastal Malaysia and in alluvial deposits in Sierra Leone and China (Chang, 2002).

Anatase, brookite and titanium dioxide(B) are common minor constituents in soils and sediments, particularly those derived from titanium-rich rocks. Rutile is a common accessory mineral in a wide variety of crustal and mantle-derived rocks and in sediment and sedimentary rocks (Heaney & Banfield, 1993).

Ilmenite is found in beach sand in existing or fossil coastlines and is an important raw material in titanium dioxide production. Surface processes alter the ilmenite in these deposits to produce submicroscopic mixtures of minerals that include anatase, rutile and amorphous phases. Mixtures that contain as much as 90% titanium dioxide are referred to as leucoxene. Leucoxene is recovered from some deposits and treated separately. However, the quantities produced are small in comparison with those of ilmenite. The concentrates obtained from ilmenite sand, which are depleted of iron, are generally richer in titanium dioxide than those from the massive deposits. Other elements in these concentrates include magnesium, manganese and vanadium that are present in the ilmenite, and aluminum, calcium, chromium and silicon (Kischkewitz *et al*., 2002).

The second most commonly available ore is the buff-coloured mineral rutile, which contains about 95% titanium dioxide with smaller amounts of iron and other impurities. The rutile contained in primary rocks cannot be extracted. Only sands in which rutile is accompanied by zircon and/or ilmenite and other heavy minerals can be used as raw materials. Rutile sands are mostly found in Australia, Sierra Leone and South Africa. The importance of mineral rutile to the titanium dioxide industry is waning. In the 1970s, it accounted for 20% of the feedstock, but now accounts for less than 10% due to diminishing reserves (Kischkewitz *et al*., 2002; Linak & Inoguchi, 2005).

Anatase, like rutile, is a modification of titanium dioxide. The largest reserves of this mineral are found in carboniferous intrusions in Brazil. Techniques for preparation of the ore produce concentrates that contain 80% titanium dioxide, and further concentration to 90% titanium dioxide is possible by treatment with hydrochloric acid (Kischkewitz *et al*., 2002).

(b) Processing

There are five stages in the manufacture of pigmentary titanium dioxide. First, titanium dioxide ore is converted to either aqueous titanyl sulfate solution or anhydrous titanium tetrachloride. These intermediates are then converted to crystalline, size-specific pigmentary particles of titanium dioxide-rutile or titanium dioxide-anatase. The pigment

is coated, in some cases involving a grinding step, and then filtered, washed and dried. Finally, the pigment agglomerates may be ground to reduce their size without breaking the primary titanium dioxide particles (Braun, 1997).

Most ores are concentrated or otherwise processed to increase the titanium dioxide content before they are suitable as a raw material for pigment production. Impurities such as iron and alkaline earth elements colour the ores from buff to black and must be removed to obtain a clean white titanium dioxide pigment (Kischkewitz *et al*., 2002; Linak & Inoguchi, 2005).

Direct use of ilmenites has decreased due to their high iron content. A digestion process is employed to produce iron sulfate heptahydrate from ilmenite. When iron sulfate is not required as a product, metallurgical recovery of iron from iron-rich ilmenites and production of a titanium-rich slag are increasingly being used (Kischkewitz *et al*., 2002; Linak & Inoguchi, 2005).

Titanium dioxide pigment is produced from titanium mineral concentrates by either the chloride process or the sulfate process. In the sulfate process, ilmenite or titanium slag is reacted with sulfuric acid. Titanium hydroxide is then precipitated by hydrolysis, filtered and calcined. In the chloride process, rutile is converted to titanium tetrachloride by chlorination in the presence of petroleum coke. The titanium tetrachloride is oxidized by air or oxygen at about 1000°C, and the resulting titanium dioxide is calcined to remove residual chlorine and any hydrochloric acid that may have formed in the reaction. Aluminium chloride is added to the titanium tetrachloride to ensure that virtually all the titanium is oxidized into the rutile crystal structure. Although either process may be used to produce pigment, the decision to use one process instead of the other is based on numerous factors, including the availability of raw materials, freight and waste disposal costs. In finishing operations, the crude form of the pigment is milled to produce a controlled distribution of particle size and the surface is treated or coated to improve its functional behaviour in different media. Typical surface treatments include alumina, organic compounds (e.g. polyols, esters, siloxanes, silanes) and silica (Kischkewitz *et al*., 2002; Gambogi, 2003).

Each producer of titanium dioxide has its own purity requirements and hence places different values on certain physical properties. For example, Japanese producers tend to prefer ilmenite which has a higher ferrous oxide content but a lower titanium dioxide content than the ores generally favoured by European producers (Kischkewitz *et al*., 2002; Linak & Inoguchi, 2005).

(c) Capacity, production and consumption

In 2004, world production of titanium mineral concentrates had increased to 5.2 million tonnes from 4.6 million tonnes in 2000. Approximately 95% is used as feedstock for titanium dioxide and the remainder is used in titanium metal alloys. In 2004, the leading supplier of titanium feedstock was South Africa (25%), followed by Australia (21%), Canada (14%), China (8%), the Ukraine (7%) and Norway (7%) (Linak & Inoguchi, 2005).

Approximately 60 plant sites worldwide (outside of China) produce titanium dioxide, with an average annual capacity of 60 000 tonnes. Table 1.3 presents world titanium dioxide capacity by region and process for 1993, 1998, 2002 and 2005 (Linak & Inoguchi, 2005).

In recent years, most increases in capacity have been through the development of small plants in China and other less developed regions. Until recently, global capacity had been growing faster than demand, resulting in oversupply and erosion of prices. In real terms, prices have been decreasing on average by about 1% per year for the past 20 years (Linak & Inoguchi, 2005).

For environmental, economic and qualitative reasons, chloride process plants continue to be favoured over sulfate plants in industrialized countries, particularly for new production facilities. Operators of sulfate process plants have had to invest in waste acid recycling facilities to extend operating lives. In addition, the production of rutile pigment from the chloride process has increased (Linak & Inoguchi, 2005).

Titanium dioxide is used in more than 170 countries. The major exporting regions are North America and Australia, and most of the countries in the rest of the world are net importers. Table 1.4 presents world supply and demand for titanium dioxide in 1997, 2001 and 2004 (Linak & Inoguchi, 2005).

1.2.2 *Use*

Titanium dioxide is valued for its opacifying strength (commonly called hiding power) and brightness. Other important features of titanium dioxide pigments are excellent resistance to chemical attack, good thermal stability and resistance to UV degradation. Rutile pigment is more resistant to UV light than anatase, and is preferred for paints, plastics, especially those exposed to outdoor conditions, and inks. Anatase pigment has a bluer tone than the rutile type, is less abrasive and is used mainly in indoor paints and in paper, ceramics, rubber and fibres manufacture. Both rutile and anatase pigments can be made more resistant to photodegradation by coating the pigment particles, which also improves their dispersibility, dispersion stability, opacity and gloss. Usually alumina, silica, zirconia or a combination of these is used; silica is most effective in retarding the photoactivity of the pigment, while alumina is most effective in enhancing dispersibility and binder compatability. Generally, rutile pigments contain 1–15% coating and anatase pigments contain 1–5%. The higher levels of coating are given to pigments that are typically used for applications such as flat (low-gloss) paints (Linak & Inoguchi, 2005).

The major consumer industries for titanium dioxide pigments are mature sectors in high-resource countries where they are used for surface coatings, paper and paperboard and plastics. Therefore, consumption of titanium dioxide tends to parallel general economic trends. Paint and coating applications have the largest global use, and plastics and paper account for most of the remainder. World consumption of titanium dioxide by end-use in 2001 was: coatings, 55%; plastics and rubber, 24%; paper, 12%; printing inks, 3%; and other, 6%; that in 2005 was: coatings, 58%; plastics and rubber, 23%; paper, 11%;

Table 1.3. World capacity for titanium dioxide (thousand tonnes, gross weight)

Region	1993			1998			2002			2005		
	S	C	Total	S	C	Total	S	C	Total	S	C	Total
North America	202	1288	1488	178	1436	1614	134	1656	1790	80	1717	1797
Central and South America	55	0	55	60	0	60	60	0	60	96	0	96
Western Europe	875	317	1192	913	405	1318	925	472	1397	862	547	1409
Central and eastern Europe	195	0	195	203	0	203	217	0	217	234	0	234
Africa and Middle East	35	50	85	40	80	119	40	100	140	25	100	125
Japan	270	50	319	272	52	324	259	68	327	240	68	308
China	–	–	–	–	–	–	258	408	666	658	15	673
Oceania and other Asia	224	114	338	291	184	475	–	–	–	141	404	545
Total	1856	1819	3672	1957	2157	4113	1893	2704	4597	2336	2857	5187

From Linak & Inoguchi (2005)
C, chloride process; S, sulfate process

Table 1.4. World production and consumption of titanium dioxide (thousand tonnes, gross weight)

Region	1997		2001		2004	
	P	C	P	C	P	C
North America						
Canada	75	105	68	90	76	104
Mexico	102	37	124	65	124	64
USA	1340	1129	1340	1100	1511	1162
Central and South America						
Brazil	79	108	78	111	80	124
Other	0	60	0	60	0	85
Western Europe	1113	1099	1150	1100	1254	1183
Central and eastern Europe	136	125	155	155	155	155
Africa and Middle East						
Saudi Arabia	50	10	55	10	90	30
Other Middle East	0	60	0	65	0	120
South Africa	30	25	30	20	20	28
Other Africa	0	15	0	35	0	45
Japan	241	269	257	246	253	238
Oceania and other Asia						
Australia	160	40	181	66	200	40
China	102	170	147	256	350	540
India and Pakistan	50	70	44	77	52	82
Indonesia	–	–	–	–	0	49
Malaysia	–	–	50	28	50	15
Philippines	–	–	–	–	0	33
Republic of Korea	35	100	42	118	40	120
Singapore	–	–	41	16	45	30
Southeast Asia	77	145	–	–	–	–
Taiwan (China)	68	71	123	66	120	66
Thailand	–	–	–	–	0	71
Other	–	–	0	108	0	29
Total	3658	3638	3885	3792	4420	4423

C, consumption; P, production
From Linak & Inoguchi (2005)

and other, 8% (Linak & Inoguchi, 2005). Some other uses of titanium dioxide are in catalysts, ceramics, coated fabrics and textiles, floor coverings and roofing granules (Gambogi, 2005; Swiler, 2005).

Despite their lower price, anatase-grade pigments account for only 10% of total global production. About two-thirds of the total anatase supply is used in markets where quality is less important, such as paper, low-priced emulsion paints, or tiles and enamels. Only one-third of the anatase is used in applications for which its specific properties are highly valued, such as when a bluish tint is desired in some plastics. Anatase is also used because of its photocatalytic properties; total global demand for its use as an active material for the removal of nitrogen oxide compounds from waste gases of coal-fired power plants and for the cleaning of exhaust gases of diesel engines is 15 000 tonnes per year (Linak & Inoguchi, 2005; Swiler, 2005).

Traditionally, the industry has produced a wide variety of grades of titanium dioxide that are tailored for specific applications. In recent years, producers have introduced so-called 'multipurpose products' to try to reduce the number of grades needed in an effort to increase operating efficiency. For example, in the paint market, titanium dioxide manufacturers propose a universal product that is acceptable for use in flat (low-gloss) and enamel (high-gloss) coatings (Linak & Inoguchi, 2005).

Some products with coarse particle sizes are obtained at an intermediate step (before coating with inorganic oxides) in the manufacture of pigmentary titanium dioxide. Manufacturers propose a 'buff' titanium dioxide that is made by grinding rutile ore to yield a product with a 95% titanium dioxide content that can be used as a partial replacement for white titanium dioxide in formulations that are tinted with other colour pigments. Total estimated global production of pigment by this process is about 10 000 tonnes per year (Kischkewitz *et al*., 2002; Linak & Inoguchi, 2005).

Ultrafine grades of titanium dioxide (particle size, 1–150 nm), which transmit visible light but scatter UV radiation, are used as UV blockers in sunscreens and plastics, catalysts and colour pigment precursors and in electroceramics (Kischkewitz *et al*., 2002; Linak & Inoguchi, 2005).

Relatively small quantities of titanium dioxide are used for non-pigmentary purposes. The estimated global market is 110 000 tonnes per year, and the largest user sectors are enamels and ceramics (25–30%), glass and glass ceramics (25–30%), electroceramics (10–15%), catalysts and catalyst supports (10–15%) and welding fluxes (10–15%) (Kischkewitz *et al*., 2002; Linak & Inoguchi, 2005).

1.3 Occurrence and exposure

1.3.1 *Natural occurrence*

Titanium is the ninth most abundant element in the world, it is five times less abundant than iron but 100 times more abundant than copper. The chemical composition

of titanium dioxide is described in detail in Section 1.1.3 and its sources in Section 1.2.1(*a*).

1.3.2 *Occupational exposure*

On the basis of a National Occupational Exposure Survey, conducted in the USA between 1981 and 1983, the National Institute for Occupational Safety and Health (NIOSH, 1983) estimated that 2.7 million workers (2.2 million men and 0.5 million women) were potentially exposed to titanium dioxide. [This estimate is based on a survey of companies and did not involve measurements of actual exposure; for many workers, very low levels and/or incidental exposures to titanium dioxide may be incurred.]

No estimate of the number of workers currently exposed to titanium dioxide was available to the Working Group.

(a) Manufacture of titanium dioxide

The highest levels of exposure within a titanium dioxide manufacturing plant are generally observed in the milling and packing areas (Fryzek *et al*., 2003). In these areas, titanium dioxide is finely processed by micronizers, and dust from the bags used for shipment may be dispersed through the air during bagging by the packers. Lower, but consistent, exposure to titanium dioxide may be incurred by treatment operators, who are involved in the addition of special coatings to and treatments of titanium dioxide before the product is finally milled and packed. Although maintenance mechanics are not exposed to titanium dioxide on a daily basis, they may experience short periods of heavy exposure during routine maintenance and repair activities associated with precipitation of titanium dioxide and subsequent processes or post-oxidation steps. Minimal exposure to titanium dioxide is incurred by workers who are involved in the initial processing and refinement of the product. In addition, general labourers or helpers, laboratory workers who work mainly in the laboratories to monitor the product and workers who handle raw ore also have minimal exposure to titanium dioxide.

Fryzek *et al*. (2003) reported results from 914 personal full-shift or near full-shift air samples for 'total' titanium dioxide that were obtained from four plants between 1976 and 2000 (Table 1.5). Eighteen of these samples appeared to the authors to be unrealistically high and were limited to 50 mg/m^3. The highest exposures were observed for packers, micronizers and workers involved in shovelling spilled titanium dioxide into bags (*n*=686; mean, 6.0 mg/m^3). Exposure levels decreased over time from a mean of 13.7 mg/m^3 (*n*=21) in 1976–80 to 7.9 mg/m^3 (*n*=87) in 1981–85, 6.4 mg/m^3 (*n*=210) in 1986–90, 5.3 mg/m^3 (*n*=239) in 1991–95 and 3.1 mg/m^3 (*n*=357) in 1996–2000.

In seven titanium dioxide manufacturing plants in Europe, Boffetta *et al*. (2003) reported results from 1348 personal exposure measurements of titanium dioxide dust that were predominantly collected during routine measurement programmes. The results related to inhalable, respirable and 'total' dust measurements, which were converted to respirable dust levels using several conversion factors. To convert 'total' to inhalable dust,

a conversion factor of 1.2 was used, based on a study by Kenny *et al*. (1997). A factor of 0.3 was chosen to convert inhalable titanium dioxide dust measurements to respirable measurements, based on results from a study in the European carbon black manufacturing industry (Gardiner *et al*., 1992). Table 1.6 summarizes the results for these standardized levels of respirable titanium dioxide for the packing areas in these plants. The highest levels were observed in Factory 10, where the geometric mean (GM) respirable dust levels ranged from 7.99 mg/m^3 between 1970 and 1974 to approximately 1.3–2.2 mg/m^3 between 1980 and 1999. The authors mentioned that one of the possible reasons for the relatively high exposure levels in Factory 10 may reflect the conversion factors used rather than actual differences in exposure, and care should be taken when interpreting the differences in exposure between the factories.

Although not reported in the study by Boffetta *et al*. (2003, 2004), results from other areas in the titanium dioxide plants were also obtained. Table 1.7 includes results from inhalable and 'total' dust measurements that have been converted to respirable dust levels, and should therefore be interpreted with some care. Highest levels of exposure to respirable dust were found in the drying and milling (GM range, 0.19–2.12 mg/m^3) and packing (GM, 0.48–2.11 mg/m^3) areas, although high exposure levels were also observed for maintenance workers (GM, 0.62–2.24 mg/m^3), handymen (GM, 4.02 mg/m^3) and cleaners (GM, 5.02 mg/m^3). Exposure levels appear to have declined between 1970 and 2000, due to the implementation of control measures such as local exhaust ventilation, increased automation and isolation or segregation of personnel (Sleeuwenhoek, 2005).

To enable a quantitative exposure–response analysis, exposure reconstruction was undertaken for each occupational title at each plant for different time periods (Boffetta *et al*., 2003, 2004) using a method developed by Cherrie *et al*. (1996). The yearly estimated exposure to titanium dioxide dust by factory between 1950 and 1999 varied between 0.1 and 1.0 mg/m^3 (Boffetta *et al*., 2004). However, very high exposure levels were estimated (>7 mg/m^3) in several factories either for cleaning jobs during the end of the production process or for jobs that involved recycling of titanium dioxide dust. Jobs with the highest estimated exposure to titanium dioxide were recycling/blending, sweeper, cleaner, packing, drying, warehouseman and fitter/mechanic (Boffetta *et al*., 2003). The authors observed a decreasing trend in exposure, particularly in factories with the highest estimated exposures during the early production period. Although the highest exposure levels in the factory were in the order of 1.0 mg/m^3, average levels ranged up to 5.0 mg/m^3 for individual occupational titles (Boffetta *et al*., 2004).

Somewhat higher exposure levels were found in earlier studies. Reported concentrations of total dust ranged from 10 to 400 mg/m^3 during the grinding of titanium dioxide pigment, but documentation of these levels was not provided (Elo *et al*., 1972). Long-term exposures to titanium dioxide dust in a titanium pigment production factory occasionally exceeded 10 mg/m^3, and exposures greater than 10 mg/m^3 were common during the repair of production machinery (Rode *et al.*, 1981).

Table 1.5. Personal 'total' exposure to titanium dioxide in four titanium dioxide manufacturing plants in the USA by job category (1976–2000)

Job category	No.	Mean (mg/m^3)	SD	Median (mg/m^3)	GM (mg/m^3)
Packers, micronizers and addbacks	686	6.2	9.4	3.0	2.7
Ore handlers	21	1.1	1.1	0.9	0.6
Maintenance mechanics	59	2.5	6.9	0.7	0.7
Dry and wet treatment	117	2.0	7.6	0.3	0.4
Other exposed jobs	31	0.6	0.9	0.4	0.4

Adapted from Fryzek *et al*. (2003)
GM, geometric mean; No., number of samples; SD, standard deviation

Table 1.6. Exposure to titanium dioxide in packing areas in titanium dioxide manufacturing plants in Europe

Factory	Year	No. of measurements	GM (mg/m^3)	Interquartile range	Range
1	1995–99	55	1.33	0.46–3.31	0.10–19.86
	2000–02	9	0.68	0.20–2.74	0.13–4.17
3	1990–94	1	0.25	–	–
	1995–99	61	0.88	0.50–1.90	0.04–7.74
	2000–02	6	0.69	0.27–1.75	0.27–3.83
6	1990–94	6	1.24	0.61–2.47	0.47–5.14
	1995–99	13	2.51	1.63–4.31	0.72–9.72
8	1995–99	11	0.77	0.48–0.96	0.32–6.16
9	1985–89	12	1.57	0.96–2.44	0.72–4.64
	1990–94	16	2.00	1.44–3.08	0.64–3.39
	1995–99	18	1.31	0.80–1.99	0.40–4.24
10	1970–74	10	7.99	3.64–16.64	2.34–79.20
	1975–79	20	2.49	1.64–3.53	1.01–6.41
	1980–84	22	2.16	1.25–3.88	0.63–10.91
	1985–89	18	1.31	0.94–1.93	0.68–5.04
	1990–94	19	1.34	0.94–2.23	0.32–5.29
	1995–99	6	2.11	1.60–3.28	0.47–3.96
15	1985–89	76	0.47	0.31–0.70	0.02–3.54
	1990–94	92	0.45	0.29–0.66	0.06–4.94
	1995–99	37	0.63	0.32–1.57	0.04–4.89

Adapted from Boffetta *et al*. (2003)
GM, geometric mean

Table 1.7. Measurements of respirable dust (mg/m^3) from the white end[a] of the titanium dioxide manufacturing process in Europe (1970–2000)

Area	Plant	No.	GM	Interquartile range	Range
Moore filtration	1	8	0.11	0.06–0.54	<0.01–0.94
	8	8	0.28	0.16–0.64	0.08–0.80
Calcination	10	28	0.78	0.36–1.25	0.18–4.79
	15	4	1.01	0.40–3.18	0.39–3.68
Raymond mills and conveying	9	29	1.20	0.88–1.72	0.25–3.84
Surface treatment	1	59	0.66	0.29–1.31	0.05–17.30
	15	5	0.10	0.04–0.37	0.04–0.57
Drying and milling	3	30	0.44	0.12–1.62	0.02–10.80
	8	2	0.71	–	0.48–1.04
	9	46	2.12	1.40–3.82	0.49–7.76
	10	135	1.37	0.86–2.09	0.32–20.66
	15	6	0.19	0.08–0.89	0.02–2.35
Packing	1	64	1.21	0.45–2.97	0.10–19.86
	3	68	0.84	0.46–1.72	0.04–7.74
	6	19	2.01	1.25–4.26	0.47–9.72
	8	11	0.77	0.48–0.96	0.32–6.16
	9	46	1.59	0.96–2.57	0.04–4.64
	10	95	2.11	1.12–3.42	0.32–79.20
	15	205	0.48	0.30–0.70	0.02–4.94
Warehouse	3	38	0.29	0.15–0.53	0.04–4.89
	10	6	1.96	1.32–2.84	1.08–3.28
Forklift truck driver	15	12	0.45	0.24–0.97	0.14–2.14
Loader	15	13	0.29	0.15–0.35	0.10–4.98
Maintenance	1	32	0.62	0.14–1.59	0.04–9.07
	3	28	0.97	0.33–2.79	0.04–18.86
	10	47	2.24	1.30–3.38	0.54–10.19
White end	8	5	1.36	0.60–3.32	0.32–3.44
Handyman	10	44	4.02	2.54–7.35	0.72–20.16
Cleaner	10	9	5.02	3.40–8.71	1.15–9.68

Adapted from Sleeuwenhoek (2005)
GM, geometric mean
[a] White end, TiO_2 precipitation and all subsequent processes

(b) Particle concentration

Wake *et al*. (2002) reported the results of measurements taken with a P-trak, Portacount or scanning mobility particle sizer in a titanium dioxide manufacturing plant in the United Kingdom. The particle number concentrations in the bagging area ranged from 4.2×10^3 particles/cm^3 to 16.6×10^3 particles/cm^3 compared with 9.7–58.4×10^3 particles/cm^3 outside the plant on the same day, which indicated that exposure to ultrafine particles (not in conglomerates) is relatively low. [The report does not specify what method was used to count the airborne titanium dioxide particles or what size particles were included in these measurements.]

Various other exposure concentrations have been reported in the manufacture of titanium dioxide, such as ore and other dusts, sulfuric acid, sulfur dioxide, welding fumes, hydrochloric acid and asbestos.

(c) User industries

Titanium dioxide is used in various industries (see Section 1.2.2) and exposure may occur before and during the addition of titanium dioxide to matrices such as paints, coatings, plastics, rubber, ink and foodstuffs. The potential for exposure is greatly reduced in other parts of the process. Very little information is available on exposure to titanium dioxide in various user industries.

In the pulp, paper and paper product industry, Kauppinen *et al*. (2002) estimated that 70% of stock preparation departments had an exposure prevalence greater than 5% (i.e. more than 5% of the workforce was exposed); this proportion was 73% for on-machine coating of paper. The median level of exposure in these departments was assessed to be between 1.5 and 10 mg/m^3.

No significant exposure to primary particles of titanium dioxide is thought to occur during the use of products in which titanium dioxide is bound to other materials, such as in paints.

1.3.3 *Environmental exposure*

No information was available to the Working Group on environmental exposure to titanium dioxide.

1.4 Regulations and guidelines

Occupational exposure regulations and guidelines in several countries are presented in Table 1.8.

Current occupational exposure limits for titanium dioxide in the USA are based on the airborne mass fractions of either respirable or ‘total’ dust fractions, and may be the same for titanium dioxide and particles that are not otherwise regulated or classified, with limits ranging from 1.5 mg/m^3 for respirable dust excluding ultrafine particles (Federal Republic

Table 1.8 Occupational exposure standards and guidelines for titanium dioxide

Country or region	Concentration (mg/m^3)	Interpretation
Austria	6	TWA – ACC
Belgium	10	TWA – ACC
China	8 (T)	TWA
	10 (T)	STEL
	0 (T)	Ceiling
Canada		
Alberta	10 (T)	TWA
British Columbia	3 (R)	TWA
	10 (T)	TWA
	20 (T)	STEL
Ontario	10 (T)	TWA
Quebec	10 (T)	TWA; containing no asbestos and < 1% crystalline silica
Czech Republic	10	TWA – ACC
Denmark	6 (as Ti)	TWA
Finland	10	TWA
France	10	TWA
Germany	1.5[a] (R)	MAK (see also aerosol allowable concentrations)
Greece	10	TWA – ACGIH (from ACC)
Hong Kong	3 (R)	TWA
	10 (T)	TWA
Ireland	4 (R)	TWA
	10 (I)	TWA
Italy	10	TWA – ACGIH (from ACC)
Mexico	10	TWA
	20	STEL
Netherlands	10 (I)	TWA – ACC
	5 (R)	TWA – ACC
New Zealand	10 (I)	TWA; containing no asbestos and <1% free silica
Norway	5	TWA
Poland	10 (I)	TWA; containing no asbestos and <2% free crystalline silica
Portugal	10	TWA – ACGIH (from ACC)
South Africa	5 (R)	TWA
	10 (I)	TWA
Spain	10	TWA
Sweden	5 (T)	TWA
Switzerland	3	TWA
United Kingdom	4 (R)	TWA
USA		
ACGIH (TLV)	10 (A4)	TWA
NIOSH (REL)	(Ca)	lowest feasible concentration
OSHA (PEL)	15 (T)	TWA

From Direktoratet for Arbeidstilsynet (2002); SUVA (2003); American Chemistry Council (2003); ACGIH Worldwide (2005); Deutsche Forschungsgemeinschaft (2005); Health and Safety Executive (2005); INRS (2005); Työsuojelusäädöksiä (2005)

A4, not classifiable as a human carcinogen; ACC, American Chemistry Council; ACGIH, American Conference of Government Industrial Hygienists; Ca, potential occupational carcinogen; I, inhalable dust; MAK, maximum concentration at the workplace; NIOSH, National Institute of Occupational Health; OSHA, Occupational Safety and Health Administration; PEL, permissible exposure limit; R, respirable dust; REL, recommended exposure level; STEL, short-term exposure limit; T, total dust; TLV, threshold limit value; TWA, 8-h time-weighted average

[a] Excluding ultrafine or aggregates of ultrafine

of Germany maximum concentration value in the workplace) to 15 mg/m^3 for total dust (Occupational Safety and Health Administration, 2002). The National Institute for Occupational Safety and Health (NIOSH, 2005) currently has no recommended exposure limit for titanium dioxide in the USA and classifies it as a potential occupational carcinogen. [The Working Group is aware that the National Institute for Occupational Health is considering recommending exposure limits of 1.5 mg/m^3 for fine titanium dioxide and 0.1 mg/m^3 for ultrafine titanium dioxide as time-weighted average concentrations for up to 10 hour per day during a 40-hour work week. This recommendation would remove the current classification of titanium dioxide as an occupational carcinogen.]

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2. Studies of Cancer in Humans

Studies on compounds related to titanium dioxide such as titanium tetrachloride or titanium metal dust (Garabrant *et al*., 1987; Fayerweather *et al*., 1992) are not included in this monograph.

2.1 Case report

Yamadori *et al*. (1986) reported a papillary adenocarcinoma of the lung and titanium dioxide-associated pneumoconiosis in a male titanium dioxide packer with 13 years of potential dust exposure and a 40-year history of tobacco smoking.

2.2 Cohort studies (Table 2.1)

Chen and Fayerweather (1988) conducted an industry-based epidemiological study and described mortality and cancer incidence among 1576 male employees who had been exposed to titanium dioxide for more than 1 year in two plants in the USA. Information on cancer incidence was obtained from the company cancer registry, which was started in 1956. Information on deaths among active and retired employees was obtained from the company mortality registry, which was started in 1957. Vital status was determined for about 94% of the cohort, and death certificates were available for about 94% of those known to have died. Observed numbers of incident cases of cancer were compared with expected numbers based on company rates, and the observed numbers of deaths were compared with both company rates and rates in the USA. Mortality from all cancers was lower than expected. For lung cancer, nine deaths were observed, with 17.3 expected on the basis of national rates (standardized mortality ratio (SMR), 0.52 [95% confidence interval (CI), 0.24–0.99]) and 15.3 expected on the basis of company rates (SMR, 0.59 [95% CI, 0.27–1.12]). There was a slight excess of incident cases of cancer (38 observed, 32.6 expected; SMR, 1.17 [95% CI, 0.83–1.60]) due mainly to 10 cases of tumours of the genitourinary system versus 6.3 expected (SMR, 1.59 [95% CI, 0.76–2.92]); eight cases of lung cancer were observed whereas 7.7 were expected (SMR, 1.04 [95% CI, 0.45–2.05]). No increase in mortality from other cancers was observed. [The Working Group noted that details of exposure to titanium dioxide and other factors were not described, that cancer mortality and specific cancer sites were not reported in detail, that incident cases of cancer only in actively employed persons were used for both observed and company reference rates, and that the numbers of incident cases were compared only with company rates.]

In a nested case–control study conducted in a cohort of workers from the oldest and largest of the two plants, no increased risk for lung cancer was found with estimated

Table 2.1. Industry-based studies of titanium dioxide and cancer

Reference, location	Study population	Exposure assessment	Exposure categories	No. of cases/ deaths	SMR (95% CI)	Adjustment for potential confounders comments
Chen & Fayerweather (1988); Fayerweather *et al*. (1992), USA	1576 male wage-grade employees in two titanium dioxide production plants who worked for ≥1 year before 1 January 1984; mortality follow-up from 1935 through to 1983; incident cases of cancer in 1956–85 from company insurance records	Committees were established at the plants to estimate exposure to titanium dioxide for all jobs; a cumulative exposure index, duration and time-weighted average were derived and used in the analysis.	Lung cancer	**Deaths** 9 9 **Cases** 8 **Cases (from case–control study)** 16 10/6.3	 0.52 (0.24–0.99) 0.59 (0.27–1.12) 1.04 (0.45–2.05) 0.6 (CI not reported) [1.59 (0.76–2.91)]	Age, exposure to titanium tetrachloride, potassium titanate fibres, asbestos; unclear how the exposure history for controls in the nested case–control study was obtained; unclear if quantitative results from exposure monitoring or sampling were used; adjustment for smoking only made for case–control analyses.
			Genito-urinary cancers			
Fryzek *et al.* (2003), USA	Retrospective mortality cohort study of 3832 male and 409 female workers employed for ≥6 months at four titanium dioxide production industries on or after 1 January 1960; follow-up until December 2000	Exposure levels to titanium dioxide assessed by industrial hygienists and based on job history	*All causes* All exposures Packers, micronizers and Addbacks *Trachea, bronchus, lung cancer* All exposures Packers, micronizers and Addbacks *Urinary cancer* All exposures	**Deaths** 533 112 61 11 3	**SMR** 0.8 (0.8–0.9) 0.7 (0.6–0.9) 1.0 (0.8–1.3) 1.0 (0.5–1.7) 0.4 (0.1–1.3)	Sex, age, race, time period, state where the plant was located; not adjusted for smoking; 35% of workers employed in jobs with high potential exposure to titanium dioxide (packers, micronizers, add-backs)

Table 2.1 (contd)

Reference, location	Study population	Exposure assessment	Exposure categories	No. of cases/ deaths	SMR (95% CI)	Adjustment for potential confounders comments
Boffetta *et al.* (2004), Finland, France, Germany, Italy, Norway, United Kingdom	15 017 employees for at least 1 month in production of 11 European titanium dioxide industries (14 331 men); employment started from 1927–69 and ended 1995–2001; mortality follow-up 1950–72 until 1997–2001 (variable per country); 371 813 person–years.	Occupational hygienists reconstructed exposures for each occupational title; exposure estimates were linked with occupational history.	All causes/cancers	**Deaths** *All causes* 2619 men 33 women *All cancers* 807 men 18 women *Lung cancer* 307 men 1 woman	 0.87 [0.83–0.90] 0.58 (0.40–0.82) 0.98 (0.91–1.05) 0.96 (0.58–1.54) 1.23 (1.10–1.38) 0.80 (0.02–4.09)	Age/birth cohort, sex, calendar year; women were not included in most analyses (33 deaths only); national rates were used in comparisons.
			Exposure to respirable titanium dioxide dust (mg/m^3)–year *Lung cancer* 0–0.73 0.73–3.43 3.44–13.19 ≥13.20	 *Men* 53 53 52 53	 1.0 (reference) 1.19 (0.80–1.77) 1.03 (0.69–1.55) 0.89 (0.58–1.35) (*p* for trend=0.5)	
			Kidney cancer <4.0 4.0–13.9 ≥14		 0.45 (0.12–1.16) 1.15 (0.31–2.89) 1.18 (0.37–2.67) (*p* for trend=0.09)	Results for kidney cancer were based on 13 cases among men.

CI, confidence interval; SMR, standardized mortality ratio

exposure to either titanium dioxide or titanium tetrachloride (Fayerweather *et al*., 1992). [The Working Group noted important methodological limitations of this study, such as a lack of detailed information on exposure assessment, duration of exposure and type of follow-up.]

Fryzek *et al*. (2003) conducted a multicentre study in the USA that included 5713 workers employed on or after 1 January, 1960 for at least 6 months at four titanium dioxide manufacturing plants. Among these, 1472 worked exclusively in administration or in other jobs that did not involve exposure to titanium dioxide. The remaining 4241 workers were followed up until 31 December 2000 (average follow-up, 21 years; standard deviation, 11 years). More workers were employed in chloride plants (53%) than in sulfate plants (40%) and 7% could not be categorized. Nearly 2400 records of air sampling measurements of sulfuric acid mist, sulfur dioxide, hydrogen sulfide, hydrogen chloride, chlorine, titanium tetrachloride and titanium dioxide were obtained from the four plants. Most were area samples and many were of short duration. 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. Only the long-term area samples for total titanium dioxide dust were used. Exposure categories (defined by plant, job title and calendar years in the job) were created to examine mortality patterns for those jobs in which the potential for exposure to titanium dioxide was greatest. Exposure variables representing average exposure per year, years exposed and cumulative exposure were created for titanium dioxide and subjects were categorized into low, medium and high categories of exposure. A total of 914 full-shift or near full-shift personal samples for total titanium dioxide dust were used to estimate relative exposure concentrations between jobs over time (see Table 1.5). The number of expected deaths was based on mortality rates by sex, age, race, time period and the state in which the plant was located. Cox proportional hazard models that adjusted for the effects of age, sex, geographical area and date of first employment were used to estimate relative risks of exposure to titanium dioxide (i.e. average intensity, duration and cumulative exposure) in medium- or high-exposure groups versus the lowest exposure group. SMRs were calculated for all workers as well as separately by type of plant (sulfate and chloride). Information on vital status was found for 4194 of the 4241 (99%) workers in the study cohort. Of the 4241 workers (58% white, 90% male), 958 did not have adequate information on work history and were omitted from some plant analyses. Of the 533 deceased workers, information on cause of death was found for 511 (96%). Thirty-five per cent of the workforce had worked in one of the jobs with the highest potential exposure to titanium dioxide, i.e. packing, micronizing or internal recycling. Information on tobacco smoking was abstracted from medical records for 2503 workers across all four plants from 1960 onwards, but no individual adjustments were possible. 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

lower than expected (SMR, 0.8; 95% CI, 0.8–0.9); the SMR for all causes of death for sulfate plants was 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 that expected (SMR, 1.0; 95% CI, 0.8–1.3), with little variation by type of plant (sulfate plant: SMR, 1.1; 95% CI, 0.7–1.6; chloride plant: SMR, 0.9; 95% CI, 0.6–1.3). No significant increases were seen for any cause of death by type of plant, and no trends with exposure were observed. Workers with the highest exposure to titanium dioxide (packing, micronizing or internal recycling workers) had a similar pattern of mortality, i.e. significantly smaller number of deaths than that expected for all causes with no excess for lung cancer. No trend of increasing SMRs for malignant or non-malignant lung disease with increasing duration of employment was evident. Internal analyses showed that relative risks for mortality from all causes and mortality due to lung cancer and non-malignant respiratory disease decreased with increasing cumulative exposure. [This cohort was relatively young (about half were born after 1940) making the duration of exposure to titanium dioxide and the latency period for the development of lung cancer rather short. Moreover, the oldest company reports were not available for the authors to evaluate.]

In response to a letter by Beaumont *et al*. (2004), Fryzek *et al.* (2003) indicated no significant exposure–response relationships for mortality from lung cancer and cumulative exposure to titanium dioxide (i.e. 'low', 'medium' and 'high') with either a time-independent or a time-dependent exposure variable and a 15-year exposure lag (adjusted for age, sex, geographical area and date of first employment).

Boffetta *et al*. (2004) studied mortality from lung cancer among workers employed in 11 plants that produced titanium dioxide in six European countries (Finland, France, Germany, Italy, Norway and the United Kingdom). Overall, 27 522 titanium dioxide-exposed workers first employed between 1927 and 2001 were identified. Workers who were first employed after 1990, employed for less than 1 year in total or who worked in non-production jobs were excluded from analyses, which left a total of 15 017 workers (14 359 men and 686 women). Of the 11 plants, seven had only produced titanium dioxide using the sulfate process and two had only produced titanium dioxide using the chloride process. One plant operated both sulfate and chloride processes and the other plant that currently used the sulfate process had operated a chloride process for a short period. Follow-up for mortality was conducted in all countries and ranged from 27 years in Italy (1972–99) to 47 years in the United Kingdom (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. Two occupational hygienists performed a comprehensive assessment of exposure, which 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 titanium dioxide dust, sulfuric acid mist, hydrochloric acid, asbestos and welding fumes were assessed and indices of cumulative exposure were calculated by combining estimates across the entire occupational history of a worker. Exposure reconstruction was based on personal sample measurements that were mainly collected during the 1990s (see Section 1.3). Two factories had measurements from the

late 1980s onwards and one factory had measurements from 1990 onwards. Information on tobacco smoking status was collected for 37.6% of workers included in the analyses. During the period of follow-up, 2619 male and 33 female deaths occurred. The SMR for all causes of death was significantly decreased in both genders: 0.87 [95% CI, 0.83–0.90] among men and 0.58 (95% CI, 0.40–0.82) among women. The country-specific SMR for all causes of death in men ranged from 0.81 in Finland to 0.97 in France. The number of deaths due to all malignant neoplasms was similar to that 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), based on a fixed-effects statistical model. The SMRs varied from 0.76 (95% CI, 0.39–1.32) in Finland to 1.51 (95% CI, 1.26–1.79) in Germany. Because the heterogeneity between countries was of borderline significance (p-value=0.05), a random-effects model was also fitted and gave an SMR of 1.19 (95% CI, 0.96–1.48). There was no evidence of a significant difference in the SMRs for lung cancer according to job titles, or between the sulfate process (including no difference between the black and white ends) and the chloride process. Death rates from lung cancer did not increase with cumulative exposure to titanium dioxide dusts or with duration of employment in titanium dioxide manufacturing plants. In addition, many of the regions where the factories were located had a higher death rate from lung cancer than the national rate for their country, which implied that the SMR for lung cancer would have been lower if regional reference mortality had been used. The analysis of tobacco smoking was limited by the relatively small proportion of workers with known habits mainly during the recent period of follow-up but suggested that, for all countries other than France and the United Kingdom, titanium dioxide workers had a higher prevalence of smoking than the respective national populations. Mortality from lung cancer was not associated with exposure to sulfuric acid mist, asbestos or welding rod fumes in the factory workplace. A positive, non-significant dose–response relationship was suggested between estimated cumulative exposure to titanium dioxide dust and mortality from kidney cancer. No increase was found for this neoplasm in the SMR analysis: the SMRs for the three categories of estimated cumulative exposure to titanium dioxide dust were 0.45 (95% CI, 0.12–1.16), 1.15 (95% CI, 0.31–2.89) and 1.18 (95% CI, 0.37–2.67). Four deaths from pleural cancer were observed, one of which occurred in a worker with only 2 years of employment in the titanium dioxide production industry. Job information was totally lacking for one case and largely lacking for another; however, the remaining jobs in which these workers were employed did not obviously entail exposure to asbestos, although it should be noted that asbestosis was mentioned on the death certificate of one of them. Mortality from pleural cancer in this cohort did not seem to be increased compared with national rates. [Among the strengths of the European titanium dioxide study are the large size, the high follow-up rate and the detailed exposure assessment. The availability of data on tobacco smoking, although limited to slightly more than one-third of the cohort, provided some reassurance that tobacco smoking was unlikely to be a confounder. Besides the lack of adjustment for smoking, other limitations are possible exposure misclassification, which might have biased the results towards the null, the

exclusion of part of the early experience of the cohort from the analysis, which reduces the power of the study to detect an association, and the relatively recent beginning of operation of some of the factories that resulted in a follow-up period that was too short to allow the detection of an increase in risk for lung cancer.]

2.3 Community-based case–control studies (Table 2.2)

Siemiatycki (1991) conducted a hypothesis-generating case–control study in Montréal, Canada, that has been described in detail in the monograph on carbon black. More than 4000 subjects were interviewed and included patients with 20 different types of cancer and a series of population controls. A panel of industrial hygienists reviewed each job history reported by study subjects and assessed exposure to 293 substances. Results on associations between titanium dioxide and several sites of cancer were reported. Some indications of excess risk were found in relation to squamous-cell lung cancer (odds ratio, 1.6; 90% CI, 0.9–3.0; 20 cases) and urinary bladder cancer (odds ratio, 1.7; 90% CI, 1.1–2.6; 28 cases). No excesses were observed for any exposure to titanium dioxide for all lung cancer combined (odds ratio, 1.0; 90% CI, 0.7–1.5; 38 cases), for kidney cancer (odds ratio, 1.1; 90% CI, 0.6–2.1; seven cases) or for cancer at several other sites other than the urinary bladder (odds ratio, 1.7; 90% CI, 1.1–2.6).

Subsequently, Boffetta *et al*. (2001) undertook a new in-depth analysis of the relationship between titanium dioxide and lung cancer in the Montréal study. They included 857 histologically confirmed cases of lung cancer diagnosed during 1979–85 among men aged 35–70 years and a group of controls comprising 533 randomly selected healthy residents and 533 cases of cancer of organs other than the lung. In preparation for the new analysis, the industrial hygienists reviewed and modified some of the attributions of exposure to titanium dioxide. The analysis also used a slightly different categorization for considering subjects as exposed to titanium dioxide. Exposure was classified as 'substantial' when it occurred for more than 5 years at a medium or high frequency and level. Most workers who were classified as exposed to titanium dioxide were painters and motor vehicle mechanics and repairers with painting experience; the highly exposed cases mixed raw materials for the manufacture of paints and plastics that contained titanium dioxide. [The Working Group noted that exposure to paints that contain titanium dioxide may not entail exposure to titanium dioxide particles.] Thirty-three cases and 43 controls were classified as having been exposed to titanium dioxide, for which the odds ratio was 0.9 (95% CI, 0.5–1.5). Results of unconditional logistic models were adjusted for age, socioeconomic status, ethnicity, respondent status (i.e. self or proxy), tobacco smoking, asbestos and exposure to benzo[*a*]pyrene. No trend was apparent according to the estimated frequency, level or duration of exposure for which the odds ratio was 1.0 (95% CI, 0.3–2.7) for medium or high exposure for at least 5 years. Few subjects were classified as exposed to titanium dioxide fumes or to other titanium compounds, but the risk for lung cancer was non-significantly increased for exposure to these agents. Results did not depend on the choice of control group and no significant

Table 2.2. Community-based case–control studies of titanium dioxide and cancer

Reference, study location, period	Characteristics of cases and controls	Exposure assessment	Exposure categories	Exposed cases	Odds ratio (90% CI)	Adjustment for potential confounders and comments
Siemiatycki (1991), Canada, 1979–86	Urinary bladder, lung, squamous-cell lung; 3730 histologically confirmed cases of 20 different cancer types diagnosed from September 1979 to June 1985 in men aged 35–70 years; 533 randomly selected healthy residents and 533 cancer controls not matched	Industrial hygienists/ chemists evaluated occupational histories to estimate exposure	*Occupational exposure*	*Urinary bladder cancer*		Age, family income, ethnicity, respondent (self/proxy), smoking, coffee consumption; hypothesis-generating study (293 exposures were evaluated); substantial exposure was defined as ≥10 years in the industry or occupation up to 5 years before onset
			Any	28	1.7 (1.1–2.6)	
			Substantial	3	4.5 (0.9–22.0)	
				Lung cancer		
			Any	38	1.0 (0.7–1.5)	
			Substantial	5	2.0 (0.6–7.4)	
				Squamous-cell lung cancer		
			Any	20	1.6 (0.9–3.0)	
			Substantial	2	1.3 (0.2–9.8)	
Boffetta *et al.* (2001), Canada	Lung cancer; 857 incident cases from 1979 to 1985; men aged 35–70 years; 533 randomly selected healthy residents and 533 cancer controls not matched	Industrial hygienists based on occupational histories collected by Siemiatycki (1991)			**Odds ratio (95%CI)**	Age, family income, ethnicity, respondent (self/proxy), smoking
			Exposure group			
			Unexposed	821	1.0	
			Ever exposed	33	0.9 (0.5–1.5)	
			Non-substantial exposure	25	0.9 (0.5–1.5)	
			Substantial exposure[a]	8	1.0 (0.3–2.7)	
			Level of exposure			
			Low	25	0.9 (0.5–1.7)	
			Medium	6	1.0 (0.3–3.3)	
			High	2	0.3 (0.07–1.9)	
			Duration of exposure			
			1–21 years	17	1.0 (0.5–2.0)	
			≥22 years	16	0.8 (0.4–1.6)	

CI, confidence interval

[a] Substantial exposure: medium or high level frequency ≥5% for at least 5 years, occurring at least 5 years before the interview.

associations were found with exposure to titanium dioxide and histological type of lung cancer. [The main limitations of this study are the reliance on self-reported occupational histories and expert opinion rather than measurement of exposure. A strength of this study was the availability of lifetime smoking histories and other covariates.]

2.4 References

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3. Studies of Cancer in Experimental Animals

The Working Group identified an issue that relates to the interpretation of several of the inhalation and intratracheal instillation studies of titanium dioxide. A lesion that is frequently seen in rats that have been exposed by inhalation to a range of poorly soluble particles such as titanium dioxide has been described variously as 'proliferating squamous cyst', 'proliferative keratinizing cyst', 'proliferating squamous epithelioma', 'benign cystic keratinizing squamous-cell tumour' or 'cystic keratinizing squamous-cell tumour'. Various authors have included this lesion in tumour counts, but the neoplastic nature of this lesion has been debated (Kittel *et al*., 1993; Carlton, 1994; Mauderly *et al*., 1994; Boorman & Seely, 1995; Rittinghausen *et al*., 1997; Rittinghausen & Kaspareit, 1998); its relationship to pulmonary neoplasia is uncertain.

3.1 Oral administration

3.1.1 *Mouse*

Groups of 50 male and 50 female $B6C3F_1$ mice, 5 weeks of age, were fed diets containing 0, 2.5 or 5% titanium dioxide (size unspecified; anatase; purity, ≥98%) daily for 103 weeks. Mice were killed at 109 weeks of age, at which time no significant difference in survival was observed between treated and control males (32, 40 and 40 surviving animals in the control, low-dose and high-dose groups, respectively). In females, a dose-related trend in decreased survival was noted (P=0.001, Tarone test; 45, 39 and 33 survivors, respectively). No significant differences in body weights or incidence of tumours were observed between treated and control groups (National Cancer Institute, 1979).

3.1.2 *Rat*

Groups of 50 male and 50 female Fischer rats, 9 weeks of age, were fed diets containing 0, 2.5 or 5% titanium dioxide (size unspecified; anatase; purity, ≥98%) daily for 103 weeks. The rats were killed at 113 weeks of age, at which time no significant difference in survival was observed between treated and control groups of either sex (31, 37 and 36 surviving males and 36, 36 and 34 surviving females in the control, low-dose and high-dose groups, respectively). No significant differences in body weights or incidence of tumours were observed between treated and control groups (National Cancer Institute, 1979).

Groups of 50 male and 50 female Fischer 344 rats, 6 weeks of age, were fed diets containing 0, 1.0, 2.0 or 5.0% titanium dioxide-coated mica (flat platelets; longest

dimension, 10–35 μm; 28% titanium dioxide; 72% mica) for up to 130 weeks. There was no evidence of a carcinogenic effect (Bernard *et al*., 1990).

3.2 Inhalation exposure

3.2.1 *Mouse*

A group of 80 female Crl:NMRI BR mice, 7 weeks of age, was exposed by inhalation to ultrafine titanium dioxide (P25, Degussa, Germany; MMAD, 0.80 μm) for 18 hour per day on 5 days per week for up to 13.5 months (7.2 mg/m^3 for the first 4 months, then 14.8 mg/m^3 for 4 months and 9.4 mg/m^3 for 5.5 months) and then maintained in clean air for a further 9.5 months. A control group of 80 animals was maintained in clean air. The mortality rate was 50% in the titanium dioxide-treated group after 17 months versus 20% in the control group. After 23 months, the percentages of mice with adenomas/adenocarcinomas were 11.3%/2.5% in the titanium dioxide-treated group and 25%/15.4% in the controls. The lung tumour rate in the mice was not significantly influenced by exposure to titanium dioxide (according to the method of Hoel & Walburg) (Heinrich *et al*., 1995).

3.2.2 *Rat*

Groups of 50 male and 50 female Sprague-Dawley rats, 8 weeks of age, were exposed by inhalation to 0 or 15.95 mg/m^3 titanium dioxide (99.9% <0.5 μm; purity unspecified) for 6 hour per day on 5 days per week for 12 weeks. The rats were killed at 140 weeks. Average survival was 116 and 113 weeks for control and treated males, and 114 and 120 weeks for control and treated females, respectively. At the end of the study, 39 and 44 control and treated males and 45 and 45 control and treated females, respectively, were still alive. No significant differences in body weights or incidence of tumours were observed (lung and other respiratory tract tumours were benign; other neoplasms seen in the lung were metastases from tumours of other sites) between treated and control groups (Thyssen *et al*., 1978). [The Working Group noted the short duration of exposure.]

Groups of 100 male and 100 female CD rats, 5 weeks of age, were exposed by inhalation to 0, 10, 50 or 250 mg/m^3 titanium dioxide (rutile; 99% pure; MMAD, 1.5–1.7 μm; ~84% of dust particles <13 μm) for 6 hour per day on 5 days per week for 2 years, at which time all surviving rats were killed. No differences in mortality, body weights or clinical signs were observed. The incidence of lung tumours was increased in both male and female high-dose rats (adenomas: 2/79, 1/71, 1/75 and 12/77 (P<0.001) control, low-, mid- and high-dose males, respectively; 0/77, 0/75, 0/74 and 13/74 (P<0.001) females, respectively; squamous-cell carcinomas: 0/79, 0/71, 0/75 and 1/77 males and 0/77, 1/75, 0/74 and 13/74 (P<0.001) females, respectively). One anaplastic carcinoma occurred in a low-dose male (Lee *et al*., 1985a,b, 1986). Difficulty

was experienced in distinguishing between keratinizing squamous metaplasia and squamous-cell carcinomas (Trochimowicz *et al*., 1988). The 15 squamous-cell carcinomas reported (Lee *et al*., 1985a,b; 1986; Trochimowicz *et al*., 1988) were re-evaluated by Warheit and Frame (2006), who described 11 of the squamous-cell carcinomas as non-neoplastic pulmonary keratinizing cysts.

Groups of 50 male and 50 female SPF Fischer 344 rats, 8 weeks of age, were exposed to titanium dioxide (5.0±0.7 mg/m^3; 99.5% rutile; MMAD, 1.1 μm) for 6 hour per day on 5 days per week or air only (control) for 24 months then maintained in clean air for a further 1.5 months. No treatment-related effects on lifespan or causes of death were observed. No differences in tumour development were seen between the groups (one adenoma and one adenocarcinoma in treated animals and two adenomas and one adenocarcinoma in controls) (Muhle *et al*., 1989, 1995). [The Working Group noted the relatively low exposure concentration.]

A group of 100 female Wistar rats, 7 weeks of age, was exposed by inhalation to titanium dioxide (P25, Degussa, Germany; MMAD, 0.80 μm) for 18 hour per day on 5 days per week for up to 24 months (7.2 mg/m^3 for the first 4 months, then 14.8 mg/m^3 for 4 months and 9.4 mg/m^3 for 16 months) and then maintained in clean air for a further 6 months. A control group of 220 animals was maintained in clean air. After 30 months, 32/100 treated rats had lung tumours (20 benign squamous-cell tumours, three squamous-cell carcinomas, four adenomas and 13 adenocarcinomas) in contrast to only 1/217 controls (one adenocarcinoma). Lung tumour incidence was 19/100 when benign squamous-cell tumours were not included (Heinrich *et al*., 1995).

3.3 Intratracheal administration

3.3.1 *Mouse*

Groups of 24 and 22 female A/J mice, 20 weeks of age, received a single intratracheal instillation of a suspension of 0.5 mg titanium dioxide (>99.9% pure; size unspecified) in saline or saline alone (control), respectively, and were maintained until 105 weeks of age. No differences in the incidence of lung tumours (17/24 versus 19/22 controls) or tumour multiplicity (2.24±1.35 versus 1.42±0.77) were noted (Koizumi *et al*., 1993). [The Working Group noted the single administration of a low dose.]

3.3.2 *Rat*

Groups of 24 or 48 female SPF Wistar (HsdCpb:WU) rats, 8–9 weeks of age, received weekly intratracheal instillations under carbon dioxide anaesthesia of one of three types of titanium dioxide. The first type was P25: hydrophilic, majority anatase; mean particle size, ~0.025 μm; density, 3.8 g/mL; specific surface area, 52 m^2/g. The second type was P805 (AL 90 003-2): hydrophobic; mean particle size, 0.021 μm [data on T805 were available to the authors and the Working Group assumed that T805 was

very similar to P805]; density, 3.8 g/mL; specific surface area, 32.5 m^2/g. The third type was AL 23 203-3: hydrophilic, anatase; mean particle size, ~0.2 μm; density, 3.9 g/mL; specific surface area, 9.9 m^2/g. The dusts were suspended by ultrasonification in 0.4 mL 0.9% phosphate buffered sodium chloride solution, and Tween 80® was added (1.0%) as a detergent to improve the homogeneity of the dosed suspensions. A control group was maintained untreated. Table 3.1 summarizes the experimental groups and the doses instilled. Rats were inspected for clinical signs of morbidity and mortality twice per weekday and once a day on weekends. The experiment was terminated at 30 months unless rats were killed when moribund or diagnosed with a growing subcutaneous tumour. Because of acute toxicity, the number of animals exposed to the hydrophobic titanium dioxide was reduced. After death of the animals and before necropsy of the thoracic and abdominal cavity, lungs were insufflated *in situ* with formalin via the trachea. In particular, the surface of the lung was inspected and lesions were recorded. Lungs were embedded in paraffin and sections were stained with haematoxylin-eosin. All suspected tumour tissues that were taken from other sites were also examined for histopathological lesions, especially for tumours that might be primary tumours with lung metastases. Table 3.1 also summarizes the lung tumour incidence of each group. Statistically significant increases in benign and/or malignant lung tumours were observed with both types of hydrophilic titanium dioxide (Pott & Roller, 2005).

Table 3.1. Dose schedules and incidence of tumours in female SPF Wister rats after intratracheal instillation of titanium dioxide

Type of titanium dioxide	Dose instilled	No. of rats at start/at risk[a]	50% survival (weeks)[b]	Lungs with benign tumours[c] (%)	Lungs with malignant tumours[c] (%)	Lungs with total tumours[c] (%)	Lungs with metastases of other tumours (%)
P25, hydrophilic	5×3 mg	48/42	114	21.4	31.0	52.4	14.3
	5×6 mg	48/46	114	17.4	50.0	67.4	15.2
	10×6 mg	48/46	104	23.9	45.7	69.6	15.2
P805, AL90, hydrophobic	15×0.5 mg[d]	24/11	86	0.0	0.0	0.0	9.1
	30×0.5 mg[d]	48/15	114	6.7	0.0	6.7	6.7
AL23, anatase, hydrophilic	10×6 mg	48/44	108	15.9	13.6	29.5	11.4
	20×6 mg	48/44	113	38.6	25.0	63.6	2.3
No treatment	–	48/46	113	0.0	0.0	0.0	13.0

From Pott & Roller (2005)

[a] Number of rats examined that survived at least 26 weeks after the first instillation.

[b] Period after first instillation in which 50% of the animals died excluding rats that died immediately after anaesthesia.

[c] Primary lung tumour types diagnosed; benign: adenoma, epithelioma; malignant: adenocarcinoma, squamous-cell carcinoma; lungs with one or more malignant tumours may additionally have had benign tumours.

[d] The doses had to be reduced because of unexpected acute toxicity.

3.3.3 *Hamster*

Groups of 24 male and 24 female Syrian golden hamsters, 6–7 weeks of age, received intratracheal instillations of 0 (control) or 3 mg titanium dioxide ([purity unspecified]; particle size: 97% <5 μm; 51% <0.5 μm) in 0.2 mL saline once a week for 15 weeks. The animals were observed until spontaneous death. All control and treated hamsters died by weeks 110–120 and 70–80, respectively, after the beginning of the experiment. The respiratory tract and other organs with gross lesions were examined histopathologically. No respiratory tract tumours were found in the treated groups compared with two tracheal papillomas that were found in untreated controls (Stenbäck *et al*., 1976).

3.4 Subcutaneous injection

Rat

Groups of 20 male and 20 female Sprague-Dawley rats, 13 weeks of age, received a single subcutaneous injection into the flank of 1 mL saline (control) or 30 mg of one of three preparations of titanium dioxide (>99% pure, coated with antimony trioxide; >95% pure, coated with aluminium oxide; or >85% pure, coated with both compounds) in 1 mL saline. All rats were observed until spontaneous death, which occurred as late as 136, 126, 146 and 133 weeks in the control and three titanium dioxide-treated groups, respectively. No tumour was observed at the site of the injection in any group (Maltoni *et al.,* 1982). [The Working Group noted the inadequate reporting of the study.]

3.5 Intraperitoneal injection

3.5.1 *Mouse*

Groups of 30 or 32 male Marsh-Buffalo mice, 5–6 months of age, received a single intraperitoneal injection of 0 (control) or 25 mg titanium dioxide (purity, >98%; manually ground) in 0.25 mL saline, respectively. All survivors (10 control and 13 treated mice) were killed 18 months after treatment. No difference in the incidence of local or distant tumours was observed between treated and control animals (Bischoff & Bryson, 1982).

3.5.2 *Rat*

As part of a large study on various dusts, three groups of female Wistar rats [initial numbers unspecified] (9, 4 and 5 weeks of age, respectively) received intraperitoneal injections of titanium dioxide (P25, Degussa, Germany) in 2 mL 0.9% saline solution. The first group received a total dose of 90 mg/animal in five weekly injections; the second group received a single injection of 5 mg/animal; and the third group received three weekly injections of 2, 4 and 4 mg/animal. One concurrent group of Wistar rats (controls), 5 weeks of age, received a single injection of saline alone. Average lifespans

were 120, 102, 130 and 120 weeks, respectively. No intra-abdominal tumour was reported in 47 and 32 rats that were examined in the second and third groups; six of 113 rats (5.3%) examined in the first group had sarcomas, mesotheliomas or carcinomas of the abdominal cavity [numbers unspecified]. Two of 32 controls (6.3%) had abdominal tumours [tumour type not specified]. In a similar experiment with female Sprague-Dawley rats that received single intraperitoneal injections of 5 mg/animal titanium dioxide, 2/52 rats (3.8%) developed abdominal tumours [tumour type not specified] (average lifespan, 99 weeks). [Controls were not available for comparison in this last experiment] (Pott *et al*., 1987). [The Working Group noted the limited reporting of the study.]

Groups of female Fischer 344/Jslc rats [*n*=330; number of rats per group unspecified], 5 weeks of age, received intraperitoneal injections of one of several man-made mineral fibres, including titanium oxide (rutile) whiskers [fibre length, ~2.5 μm; fibre diameter, ~0.125 μm (estimated from a figure)]. The fibres were given in doses of 5, 10 or 20 mg with 1 mg of dust suspended in 1 mL saline before injection. The greatest volume administered in a week was 5 mL. The fibre concentration of titanium oxide whiskers was 639×10^3/μg. Two years after administration, peritoneal mesotheliomas were induced by silicon carbide whiskers (fibre concentration, 414×10^3/μg; cumulative incidence, 70–100%) and potassium titanate whiskers (fibre concentration, 594×10^3/μg; cumulative incidence, 20–77%) but not by titanium dioxide whiskers (Adachi *et al*., 2001). [The Working Group noted the inadequate reporting of the study.]

3.6 Administration with known carcinogens

Hamster

Groups of 24 male and 24 female Syrian golden hamsters, 6–7 weeks of age, received intratracheal instillations of 3 mg titanium dioxide ([purity unspecified]; particle size: 97% <5 μm; 51% <0.5 μm) plus 3 mg benzo[*a*]pyrene in 0.2 mL saline or 3 mg benzo[*a*]pyrene alone in saline (controls) once a week for 15 weeks. Animals were observed until spontaneous death; all control and treated hamsters had died by 90–100 and 60–70 weeks, respectively. In the 48 hamsters treated with titanium dioxide plus benzo[*a*]pyrene, tumours [number of tumours per sex unspecified] occurred in the larynx (11 papillomas, five squamous-cell carcinomas), trachea (three papillomas, 14 squamous-cell carcinomas, one adenocarcinoma) and lung (one adenoma, one adenocarcinoma, 15 squamous-cell carcinomas, one anaplastic carcinoma). Two papillomas occurred in the trachea of benzo[*a*]pyrene-treated controls. In the same study, ferric oxide (3 mg) and benzo[*a*]pyrene induced a similar spectrum of tumours to that induced by the combination with titanium dioxide (Stenbäck *et al*., 1976).

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4. Mechanistic and Other Relevant Data

The general principles of inhalation, deposition, clearance and retention of poorly soluble particles that have low toxicity are discusssed in the Monograph on carbon black in this volume.

4.1 Humans

4.1.1 *Deposition, retention and clearance*

Humans can be exposed to titanium dioxide via inhalation, ingestion or dermal contact. This section describes several case reports of pulmonary findings in humans exposed to titanium dioxide, a clinical study of absorption of titanium dioxide in the gastrointestinal tract and several studies that examined dermal effects and absorption of titanium dioxide from sunscreens.

The human pulmonary studies of titanium dioxide are largely limited to case reports of one or more highly exposed individuals that detail the location of large amounts of titanium dioxide in the tissues. Interpretation of these studies is complicated by co-exposures to other compounds (e.g. cigarette smoke and silica) and a lack of information regarding the estimated delivered pulmonary doses. Therefore, clearance kinetics following acute and chronic exposure to titanium dioxide are poorly characterized in humans relative to animals.

The autopsy of a 55-year-old man was conducted approximately four years after four years of 'heavy' exposure to titanium dioxide (rutile) (Rode *et al*., 1981). The surface of the lungs showed numerous white deposits (1–2 mm in diameter) beneath the intact pleura. Within the lungs, the same white pigment was found fairly evenly distributed among all lobes. The pigment was mainly distributed around the perivascular tissue, but small amounts were found in alveolar walls and in alveolar macrophages. Lymph nodes also contained large amounts of pigment.

Gylseth *et al*. (1984) reported the case of a 53-year-old nonsmoking male farmer who had a mixed dust pneumoconiosis and a lung tumour. The lobe that contained the tumour was removed and analysed. Mineral dusts were deposited in peribronchial and perivascular areas, within alveolar macrophages in the peripheral lung or as small granular accumulations in the interstitium. Dusty deposits were accompanied by local fibrosis. Of the fibres identified, 63% were rutile fibres (0.76–5.5 μm) and 37% were amphibole asbestos (0.7–9 μm).

Yamadori *et al*. (1986) reported the case of a 53-year-old man with pneumoconiosis due to approximately 13 years of occupational exposure to 'high' concentrations of titanium dioxide. The patient died of lung cancer, which was possibly associated with a 34 pack–year smoking history and not attributed to exposure to titanium dioxide. At

autopsy, about 9–10 years after the exposures to titanium dioxide, particle deposition was found to be diffuse in the lung and particles were typically found in interstitial and alveolar macrophages. Examination of lung tissue in the right upper lobe and right hilar lymph nodes showed deposits of crystalloid substances that had a high titanium content and measured 0.2–0.3 μm by 0.7 μm.

Moran *et al*. (1991) analysed lung sections from three male patients (46–57 years of age) with potential occupational exposure to titanium dioxide. Large quantities of dark granular pigment were found in macrophages in the alveolar spaces and around the bronchioles and blood vessels. X-Ray crystallography showed that the lungs of all the patients contained rutile and silica and that those of two of the patients also contained talc.

Böckmann *et al*. (2000) determined blood levels of titanium dioxide (anatase) following oral ingestion of titanium-dioxide capsules and/or powder in six adult men (24–66 years of age). Titanium dioxide was absorbed by the gastrointestinal tract in a size-dependent manner: smaller particles (0.16 μm) were more readily absorbed than larger ones (0.38 μm). Before the experiment, the background blood levels of titanium dioxide in these men ranged from ~6 to 18 μg/L. Blood levels reached up to ~50 μg/L or 100 μg/L between 4 and 12 hours after intake of 23 mg or 46 mg titanium dioxide, respectively.

In a study of 13 Caucasian skin-surgery patients (four women and nine men aged 59–82 years) who applied a microfine (10–50 nm) titanium-dioxide sunscreen for 9–31 days, Tan *et al*. (1996) found that tissue levels overlapped with those in skin samples collected post-mortem. Furthermore, there was no correlation between duration of sunscreen application and the measured concentrations of titanium dioxide. After 4 days of sunscreen application, Lademann *et al*. (1999) also reported that the deeper layers of the stratum corneum were devoid of titanium dioxide. Pflücker *et al*. (2001) performed tests with three sunscreens that contained different types of titanium dioxide (20 nm, cubic; 100 nm, needles; and 100 nm, needles composed of aggregated 10–15-nm particles). At six hours after application, punch biopsies were taken from each area. Consistent with the in-vitro study by Gamer *et al*. (2006), titanium dioxide pigments were located exclusively on the outermost layer of the stratum corneum in all cases. [The Working Group noted the lack of studies on penetration of titanium dioxide in compromised skin, and that a flex skin model was never used to address this issue.]

4.1.2 *Toxic effects*

None of the case reports provided quantitative industrial hygiene information about the exposure of workers to titanium-dioxide dust.

A small set of studies from the titanium industry where ilmenite (iron titanate) was the dust probably involved in exposure has been reviewed (IARC, 1989).

Many case studies have reported abnormalities related to exposure to titanium. In some, titanium dioxide was still identified in the lungs of workers exposed to respirable titanium dioxide years after exposure had ceased. Some case studies reported varying

degrees of fibrogenic changes to the lung associated with either brief or extended high-level exposures (Elo *et al*., 1972; Määttä & Arstila, 1975). In contrast, others that involved exposure to titanium dioxide pigment materials showed no evidence of lung inflammation or fibrosis (Schmitz-Moormann *et al*., 1964; Rode *et al*., 1981).

Elo *et al*. (1972) reported pulmonary fibrosis or fibrotic changes and alveolar macrophage responses that were identified by thoracotomy or autopsy tissue sampling in three workers who had been employed for 6–9 years in dusty work in a titanium-dioxide factory. No data on workplace exposure were reported. Two workers were 'moderate' or 'heavy' smokers but smoking information was not provided for the third worker. Small amounts of silica were present in all three lung samples and significant amounts of nickel were present in the lung tissue of the autopsied case. Exposure was confirmed using sputum samples that contained macrophages with high concentrations of titanium 2–3 years after their last exposure (Määttä & Arstila, 1975). Titanium particles were identified in the lymph nodes of the autopsied case. The lung concentrations of titanium were higher than those of control autopsy specimens from patients who had not been exposed to titanium dioxide.

A case of granulomatous lung disease was reported in a worker who had possibly been exposed to titanium dioxide at an aluminium smelting plant where he had worked near a firebrick furnace. A lymphocyte transformation test showed a proliferative response to titanium chloride but not to any other metal tested, which suggested a possible link with titanium hypersensitivity (Redline *et al*., 1986).

Yamadori *et al*. (1986) reported titanium dioxide-associated pneumoconiosis in a male titanium-dioxide packer with 13 years of potential dust exposure and a 40-year history of smoking.

In a cross-sectional study of 209 titanium metal production workers, 78 of whom were involved in the reduction process and were exposed to titanium-tetrachloride vapour, titanium oxychloride and titanium-dioxide particles had reductions in lung function (Garabrant *et al*., 1987). The authors noted that this finding could be due to exposure to titanium tetrachloride, which reacts violently with water to liberate heat and produce hydrochloric acid, titanium oxychloride and titanium dioxide. Pleural disease with plaques and pleural thickening was observed in 36 of the 209 workers, including eight of the 78 reduction-process workers. Some cases were probably caused by previous exposure to asbestos; however, among workers who were not known to have been exposed to asbestos, the risk for pleural disease after more than 10 years of employment was 3.8 times that in workers who had been employed for less than 5 years.

Oleru (1987) studied 67 workers in a small titanium oxide paint factory in Nigeria. Airway symptoms were reported by 50–54%, neurological symptoms by 20–40% and other symptoms by 10–27% of the workers. The symptoms were correlated to exposure and with pulmonary function tests. Twenty-eight cases of restrictive lung impairment were observed. Smoking prevalence was low, but several of the workers were also exposed to cotton dust.

A chest X-ray study of 336 workers at two titanium dioxide-production plants showed 19 cases of pleural abnormalities (thickening or plaques) compared with three cases among 62 unexposed workers at the same plants (Chen & Fayerweather, 1988). The odds ratio for chest X-ray abnormality associated with exposure to titanium dioxide was 1.4, although exposures at the plants included titanium tetrachloride, potassium titanate and asbestos. No lung fibrosis was observed.

Moran *et al*. (1991) reported exposure to titanium dioxide in four men and two women. Diffuse fibrosing interstitial pneumonia and bronchopneumonia were reported in three male patients (a titanium dioxide worker, a painter and a paper mill worker) with deposits of titanium dioxide (rutile) in the lung and smaller amounts of silica deposited in the tissues. Smoking information was not reported.

Keller *et al*. (1995) reported a case of pulmonary alveolar proteinosis (i.e. deposition of proteinaceous and lipid material within the airspaces of the lung) in a worker who had been employed for more than 25 years as a painter, with eight years of experience in spray painting, and who smoked two packs of cigarettes per day until he was hospitalized. Titanium was the major type of metallic particle found in his lung tissues.

4.2 Experimental systems

4.2.1 *Deposition, retention and clearance*

A considerable number of toxicological studies, both *in vivo* and *in vitro*, have characterized the disposition (deposition, absorption, distribution and elimination) of titanium dioxide particles in the respiratory tract of animals and cells. Experimental protocols and findings of many of these studies are provided in Tables 4.1–4.3.

Most animal studies on the effects of titanium dioxide on the respiratory tract have been conducted in rats. Generalizations with regard to the effects of inhaled particle size on the amount and site of deposition in the lungs and subsequent clearance are applicable to animals as well as humans and can be made but with some caution. A variety of factors other than particle parameters can influence delivered dose, distribution within the lungs and subsequent clearance. These factors complicate comparisons between studies and interspecies extrapolation of observed effects. Hence, some caution must be advised when comparing results among the various studies in Tables 4.1–4.3. For example, Bermudez *et al*. (2002) exposed rats, mice and hamsters to the same particle size, at the same particle concentration, for the same exposure time. However, despite the same study design, similar doses would not necessarily be received between the species. At a concentration of 250 mg/m^3, mice had a larger normalized pulmonary particle burden than rats and hamsters (170, 120 and 114 mg/g dry lung, respectively).

Normalized particle dose (deposited mass per body weight) delivered to the respiratory tract may decrease with increasing animal size. This was the observation of McMahon *et al*. (1975), who compared aerosol (gold particles, 0.78 μm MMAD) deposition in mice, hamsters, rats, rabbits and dogs. Ferin and Morehouse (1980) also

Table 4.1. Inhalation studies of titanium dioxide (TiO_2) disposition and responses in animal models

Species, sex (age/weight)	Aerosol characteristics	Exposure concentration	Exposure protocol	Observed effect(s)	Reference
Hooded rats of Long-Evans descent (~250 g)	TiO_2,1.48 µm MMAD (σ_g=3.26); NO_x or SO_2 + TiO_2	100 mg/m^3 for 2, 4 or 6 h; after gaseous exposure, aerosol at 15 mg/m^3 for 7 h	About 10 rats in each group killed at 1, 8, 25 and 130 days after exposure	In non-pollutant-exposed rats, about 40% of TiO_2 cleared by 25 days and 80% cleared by 130 days after inhalation; based on a linear association between TiO_2 levels in the trachea and lung burden, authors suggested that particles were removed from the alveoli via the airways by alveolar macrophages. NO_x and SO_2 stimulated clearance at low-exposure levels and suppressed clearance at higher exposure levels.	Ferin & Leach (1975)
Long-Evans and Fischer 344 rats, male (9 weeks)	TiO_2, 1.0 µm MMAD (σ_g=2.3)	14.9 mg/m^3 for 7 h	10 rats killed at 1, 8, 25 and 130 days after exposure	At days 1 and 25 after exposure, TiO_2 content in lung lobes was significantly associated with lobe weight in both rat strains. The distribution of TiO_2 between lobes was similar between strains. Normalized to lung weight, the smaller Fischer 344 rats received a slightly greater total lung burden than the Long-Evans rats (114 versus 105 µg/g lung). Lung clearance between days 1 and 8 was greater in the Long-Evans than in the Fischer 344 rats, although subsequent clearance rates were quite similar between the strains. At 25 days, 55 and 70% retention was observed in Long-Evans and Fischer 344 rats, respectively. Authors suggested that there may be strain differences affecting early alveolar clearance mechanisms.	Ferin & Morehouse (1980)

Table 4.1 (contd)

Species, sex (age/weight)	Aerosol characteristics	Exposure concentration	Exposure protocol	Observed effect(s)	Reference
Long-Evans rats, male (~300 g)	TiO_2 (anatase), 1.0 μm MMAD (σ_g=2.3), aerosol-as-generated and charge-neutralized aerosols	10.8–15.5 mg/m^3	Rats exposed for 7 h; deposition assessed in 40 rats; clearance assessed at 1, 8, and 25 days after exposure (20 rats per time-point)	On average, the deposition efficiency was 79% for charge-neutralized aerosols and 100% for aerosols-as-generated by a Wright dust feeder. The pattern of deposition within the lungs was unaffected by particle charge as indicated by a no-charge-effect on clearance.	Ferin *et al*. (1983)
Long-Evans rats, males (~300 g)	TiO_2 (anatase), 1.0 μm MMAD (σ_g=2.3); TiO_2 (rutile), 0.83 μm MMAD (σ_g=2.02)	16.5±1.7 mg/m^3, 19.3±3.1 mg/m^3	Rats exposed for 7 h; 8–10 rats killed at 1, 8, 27 and 132 days after exposure	Crystal structure had no effect on pulmonary particle clearance (half-times of 51 and 53 days for anatase and rutile, respectively).	Ferin & Oberdörster (1985)
Crl:CD rats	TiO_2 (rutile), 1.5–1.7 μm MMAD	10, 50, 250 mg/m^3	Four exposure groups of 100 male and 100 female rats exposed 6 h/day, 5 days/week up to 2 years; rats killed at 3, 6, 12 and 24 months.	No abnormal clinical signs, body weight changes or excess mortality in any group compared with controls; at 10 mg/m^3, particles were mostly phagocytosed by alveolar macrophages. At 50 mg/m^3, there was marked hyperplasia of alveolar lining cells with some alveoli adjacent to terminal bronchioles exhibiting ciliated cells; macrophages containing dust were aggregated; cellular debris, proteinosis and fibrosis were observed. Lung weights at 250 mg/m^3 were double those of the 10-mg/m^3 and control groups. Dose-related increase in particle number identified in tracheobronchial lymph nodes, cervical lymph nodes, liver and spleen was observed. Animal grooming could have been the source of the observed extrapulmonary particles.	Lee *et al*. (1985a,b, 1986)

Table 4.1 (contd)

Species, sex (age/weight)	Aerosol characteristics	Exposure concentration	Exposure protocol	Observed effect(s)	Reference
Wistar rats, female	TiO_2 (anatase), 4.8 µm MMAD, 15–40-nm primary particles	8.6 mg/m^3	6 rats exposed to each aerosol for 7 h/day for 1 year	TiO_2 particles observed mainly in interstitial macrophages of the alveolar walls; these were frequently aggregated in small granulomas. Lesions associated with TiO_2 particle accumulation distributed throughout alveolar region	Takenaka *et al*. (1986)
PVG rats, male (12 weeks)	TiO_2 (rutile); quartz	10 mg/m^3	Exposed 7 h/day, 5 days/week up to 15 weeks; 4 rats per group killed at days 2, 4, 8, 16, 32, 52, and 75; also, groups of 4 killed 62 days after exposure for 32 and 75 days	Macrophages were predominant cell type in lavages of unexposed controls. Macrophage and PMN levels in TiO_2 group remained at control levels for entire exposure period. Total lavage protein relative to TiO_2 was only slightly increased relative to controls.	Donaldson *et al*. (1988)
Long-Evans rats, male (220–260 g)	TiO_2, 1 µm MMAD (σ_g=1.4) after $CdCl_2$, 0.4–0.5 µm MMAD (σ_g=1.4–1.6) or before $CdCl_2$, 0.5 µm MMAD (σ_g=1.4)	13.3 mg/m^3 (TiO_2); 1.5, 5, 5 mg/m^3 ($CdCl_2$)	Rats (180 total) exposed nose-only to $CdCl_2$ followed 12 h after by TiO_2 for 6 h or vice versa to assess effect of Cd on particle clearance kinetics; burden of TiO_2 exposure assessed on days 1, 8, 15, 25 and 46	Relative to saline controls, the overall pulmonary clearance of TiO_2 not affected by $CdCl_2$ inhalation; however, 5-mg/m^3 exposures to $CdCl_2$ (either before or after TiO_2 inhalation) caused in increase in the amount of TiO_2 found in the lymph nodes relative to control and 1.5 mg/m^3 $CdCl_2$ which did not differ. Authors hypothesized that the cytotoxicity of $CdCl_2$ caused a decrease in macrophage clearance and an increase in transport to lymph nodes although changes in epithelial permeability could not be entirely ruled out.	Greenspan *et al*. (1988)

Table 4.1 (contd)

Species, sex (age/weight)	Aerosol characteristics	Exposure concentration	Exposure protocol	Observed effect(s)	Reference
Rats	TiO_2 (rutile),1.1 µm MMAD (σ_g=1.6), 200–700-nm primary particles; TiO_2 (anatase), 1.0 µm MMAD (σ_g=1.9), 20–40-nm primary particles; carbon black, 1 µm MMAD (σ_g=2), 14-nm primary particles	5 mg/m^3; 1, 4, 16 mg/m^3; 9.8 mg/m^3; 9 mg/m^3	30 h/week for 3 months; 30 h/week for 22.5 months; 95 h/week for 7 months; 95 h/week for 4.5 months	In general, clearance kinetics of polystyrene (3.5-µm spheres, ^{85}Sr-labelled) appeared to decrease with increasing volume burden of test materials. Despite having a lower volume burden and larger primary particle size, however, the ultrafine TiO_2 (anatase) tended to clear more slowly than carbon black (half-time 788 versus 420 days, respectively). Fine TiO_2 (rutile) caused a modest reduction in clearance relative to control (half-time 94 versus 74 days, respectively).	Muhle *et al*. (1990)
Fischer 344 rats, male and female (4 weeks)	TiO_2 (rutile), 1.0 µm MMAD (σ_g=1.6), 200–700-nm primary particles	5 mg/m^3	Rats (~12 per time-point and outcome) exposed 6 h/day, 5 days/week for up to 24 months; clearance measured from 15–100 days *in vivo* following acute inhalation of tracer aerosols: 3.5 µm ^{85}Sr-polystyrene and 0.26–0.39 µm $^{59}FeO_2$	Pulmonary clearance rates decreased in unexposed controls during study. Clearance rates in TiO_2-exposed rats were comparable with controls. Lung burden per mass of lung tissue was similar between males and females. At 15 months of TiO_2 exposure, rats had a small but significant decrease in macrophage levels and an increase in PMNs relative to controls. Epithelial permeability was not affected by TiO_2.	Muhle *et al*. (1990) [clearance kinetics, particle sizes]; Bellmann *et al*. (1991) [clearance kinetics]; Muhle *et al*. (1991) [particle sizes, cytology]

Table 4.1 (contd)

Species, sex (age/weight)	Aerosol characteristics	Exposure concentration	Exposure protocol	Observed effect(s)	Reference
Fischer 344 rats, male (180–200 g)	TiO_2 (anatase), 1.0 μm MMAD (σ_g=2.6)	50 mg/m^3	Rats exposed 6 h/day for 5 days and killed 1, 2, 4 and 9 weeks after exposure	One day after exposure, lung burden was 1.8 mg/lung for TiO_2. At 28 days after exposure, retention was 39%. Inhalation caused lesser effects, e.g. permeability and PMN influx, than instillation of similar lung burdens reported earlier (Driscoll *et al*., 1990).	Driscoll *et al*. (1991)
Fischer 344 rats (240–260 g)	TiO_2 (anatase), 0.78 μm MMAD (σ_g=1.7), 21-nm primary particles; TiO_2 (anatase), 0.71 μm MMAD (σ_g=1.9), 250-nm primary particles	23.5±3.2 mg/m^3; 23.0±4.1 mg/m^3	Chamber exposure for 6 h/day, 5 days/week, for up to 12 weeks; rats killed at 4, 8, 12, 41 and 64 weeks; 4 rats per group, except only three in 41-week group	Retention half-times were 501 and 174 days for ultrafine (21 nm) and fine (250 nm) primary particles, respectively. As a percentage of total lung burden, the unlavageable fraction of particles plus particles in the hilar lymph node were also significantly greater for ultrafine than fine primary particles at 12, 41 and 64 weeks. On average, PMN influx due to ultrafines was 43-fold and 22-fold greater than that for fine particles at weeks 8 and 12 of exposure, respectively. By week 64, PMN levels had approached control levels for both aerosols.	Ferin *et al*. (1992)

Table 4.1 (contd)

Species, sex (age/weight)	Aerosol characteristics	Exposure concentration	Exposure protocol	Observed effect(s)	Reference
Fischer 344 rats	TiO_2 (anatase), 0.78 µm MMAD (σ_g=1.7), 21-nm primary particles; TiO_2 (anatase), 0.71 µm MMAD (σ_g=1.9), 250-nm primary particles	23.5±2.9 mg/m^3; 22.3±4.2 mg/m^3; 1.3±0.3 mg/m^3	Rats exposed to aerosols (6 h/day, 5 days/week, 12 weeks); subsequently, 4 rats inhaled and 4 rats instilled with tracer aerosol (3.3 µm, ^{85}Sr-labelled polystyrene); ^{85}Sr measured *in vivo* for 180 days	Ferin *et al*. (1992) reported pulmonary retention half-times of 501 and 174 days for inhaled aerosol composed of ultrafine (21 nm TiO_2) and fine (250 nm TiO_2) primary particles, respectively. After TiO_2 exposure, inhaled/instilled polystyrene had slow-phase clearance half-times of 66/72 days (control), 117/99 days (250 nm TiO_2), 541/606 days (21 nm TiO_2). Accelerated tracheobronchial clearance was observed when pulmonary clearance was retarded. For both TiO_2 aerosols, the exposure-induced PMN influx appeared related to particle surface area. Ultrafine TiO_2 induced focal interstitial pneumonia and focal alveolitis.	Oberdörster *et al*. (1994, 1997)
Fischer 344 rats (175–225 g)	TiO_2 (anatase) fine (250 nm) and ultrafine (21 nm) particles delivered as aggregates with 1.0–1.2 µm MMAD (σ_g=1.6–2.2)	125 mg/m^3	Rats exposed for 2 h via endotracheal tube while anaesthetized and ventilated; rats (6 per time-point) killed at 0, 1, 3 and 7 days after exposure; pattern of deposition evaluated in 3 rats	Pattern of deposition (TiO_2 mass deposited per lobe) was well correlated with lung lobe size (% total lung weight). Immediately after exposure, total lavage protein significantly increased 7-fold and 3-fold in ultrafine and fine TiO_2 exposure groups, respectively, relative to unexposed controls. One day after exposure, protein levels remained increased by 3-fold in ultrafine exposure group. Significant PMN influx occurred in ultrafine exposure group relative to unexposed controls 1 day after exposure. Comparison of responses as a function of primary particle size was confounded by ~40% greater ultrafine than fine particle mass dose 1 day after exposure.	Osier & Oberdörster (1997)

Table 4.1 (contd)

Species, sex (age/weight)	Aerosol characteristics	Exposure concentration	Exposure protocol	Observed effect(s)	Reference
Crl:CDBR rats, male (7–8 weeks)	TiO_2 (rutile),1.7 µm MMAD, 0.25 µm primary particles	5, 50, 250 mg/m^3	Rats exposed 6 h/day, 5 days/week for 4 weeks; killed at 0 h, 1 week, 1, 3 and 6 months after exposure	After exposure to 5, 50, and 250 mg/m^3, lung burdens for TiO_2 were approximately 0.26, 2.7, and 12 mg, respectively. Clearance rates decreased with increasing exposure concentrations; TiO_2 half-times were 68, 110, and 330 days for 5, 50, and 250 mg/m^3, respectively. Number of particles in lymph nodes was increased in the highest exposure group relative to other exposure and control groups. The highest exposure group also had focal hypertrophy and hyperplasia which were associated with aggregates of pigmented macrophages in alveoli and at alveolar duct bifurcations. These focal lesions were evident for the entire follow-up periods. At 3 months after exposure to 250 mg/m^3 TiO_2, chemotaxis of alveolar macrophages was also reduced. Fibrosis was not observed to any significant degree in any groups.	Warheit *et al*. (1997)

Table 4.1 (contd)

Species, sex (age/weight)	Aerosol characteristics	Exposure concentration	Exposure protocol	Observed effect(s)	Reference
Syrian golden hamsters, male and female (4 weeks)	TiO_2 (rutile), 1.1 μm MMAD (σ_g=1.6), 200–700-nm primary particles	40 mg/m^3 for 5 months, then 30 mg/m^3	Animals (~9 per time-point and outcome) exposed 6 h/day, 5 days/week for up to 18 months; clearance measured 15–100 days *in vivo* following acute inhalation of tracer aerosol (3.5 μm ^{85}Sr-polystyrene)	TiO_2-exposed females tended to have slower clearance rates than similarly exposed males. On average, retention half-times in TiO_2-exposed hamsters were significantly reduced relative to controls at 3 months (control, 78 days; TiO_2, 226 days) and more so at 9 months (control, 115 days; TiO_2, 1120 days). However, at 15 months of TiO_2 exposure, clearance was more similar to controls (control, 88 days; TiO_2, 123 days). Intragroup variability in clearance rates was double that reported for rats by Bellmann *et al*. (1991). Authors suggested possible adaptation capability in hamsters.	Creutzenberg *et al*. (1998)
Wistar rats, male (12 weeks)	TiO_2 (rutile), 2.1 μm MMAD (σ_g=2.2)	25, 50 mg/m^3	Rats exposed 7 h/day, 5 days/week for up to 7 months; typically, 6 rats lavaged and 6 used to assess lung burden at each of 6 time-points	At end of exposures, lung burdens were 17 and 24 mg/g dry lung for low and high exposures to TiO_2. Macrophage levels did not change statistically during exposures. Lymph node burdens and PMN levels rapidly increased with TiO_2 exposures. Findings best associated with lung burden in terms of retained particle surface area. Lymph node burdens and PMN levels increased rapidly beyond a 'threshold' lung burden of 200–300 cm^2 particle surface area.	Cullen *et al*. (2000); Tran *et al*. (2000)

Table 4.1 (contd)

Species, sex (age/weight)	Aerosol characteristics	Exposure concentration	Exposure protocol	Observed effect(s)	Reference
Fischer 344 rats, female (6 weeks); B3C3F$_1$ mice, female (6 weeks); hamsters, female (6 weeks)	TiO_2 (rutile), 1.36–1.44 μm MMAD (σ_g=1.50–1.71), 220-nm primary particles	10, 50, 250 mg/m^3	Total of 65 rats, 65 mice and 73 hamsters exposed 6 h/day, 5 days/week, for 13 weeks; animals killed at 0, 4, 13, 26 and 52 (46 for hamsters) weeks after exposure	TiO_2 pulmonary retention half-time for the low-, mid- and high-exposure groups, respectively: 100, 324 and 838 days in rats; 50, 417 and 621 days in mice; and <110 days in hamsters. In rats and mice, PMN levels were significantly elevated in mid- and high-exposure groups and gradually decreased after exposure. However, the rate of PMN decline was far more gradual in the high-exposure groups, especially in rats. PMN levels in the high-exposure group of hamsters responded similarly to mid-exposure groups of mice and rats. In high-exposure groups of rats and mice, epithelial permeability remained elevated (>5 times low-exposure groups) up to 52 weeks, with no signs of recovery.	Bermudez *et al*. (2002)
Fischer 344 rats, female (6 weeks); B3C3F1 mice, female (6 weeks); hamsters, female (6 weeks)	TiO_2, 1.29–144 μm MMAD (σ_g=2.46–3.65), 21-nm primary particles	0.5, 2, 10 mg/m^3	Groups of 25 animals per species and time-point; animals exposed 6 h/day, 5 days/week, for 13 weeks and animals killed at 0, 4, 13, 26 and 52 (49 for hamsters) weeks after exposure	TiO_2 pulmonary retention half-times for the low-, mid- and high-exposure groups, respectively: 63, 132 and 365 days in rats; 48, 40 and 319 days in mice; and 33, 37 and 39 days in hamsters. In high-exposure groups of mice, epithelial permeability remained elevated (~twice control groups) up to 52 weeks without signs of recovery. Epithelial permeability was 3–4 times the control in high-dose rats through to 4 weeks after exposure, but approached control by 13 weeks. Epithelial permeability was unaffected in all groups of hamsters.	Bermudez *et al*. (2004)

Table 4.1 (contd)

Species, sex (age/weight)	Aerosol characteristics	Exposure concentration	Exposure protocol	Observed effect(s)	Reference
Wistar rats, adult male (250±10 g)	TiO_2, 22 nm CMD (σ_g=1.7), spark generation	0.11 mg/m^3, 7.3×10^6/cm^3	10 rats exposed 1 h via endotracheal tube while anaesthetized and ventilated at constant rate; lungs fixed at 1 or 24 h after exposure	Distributions of particles among lung compartments followed the volume distribution of compartments and did not differ significantly between 1 and 24 h post-inhalation. On average, 79.3±7.6% of particles was on the lumenal side of the airway surfaces, 4.6±2.6% was in epithelial or endothelial cells, 4.8±4.5% was in connective tissues and 11.3±3.9% was within capillaries. Particles within cells were not membrane-bound.	Geiser *et al*. (2005)

$CdCl_2$, cadmium chloride; CMD, count median diameter; MMAD, mass median aerodynamic diameter; NO_x; nitrogen oxide; PMN, polymorphonuclear neutrophils; SO_2, sulfur dioxide

Table 4.2. Instillation studies of titanium dioxide (TiO_2) disposition in animal models

Species, sex (age/weight)	Characteristics of particles and exposure	Exposure protocol	Observed effect(s)	Reference
Long-Evans rats (~300 g)	TiO_2 (rutile), 0.5, 5 mg/rat; TiO_2 (anatase), 0.5, 5 mg/rat	6 male rats per group instilled with each material in 0.2 mL saline; two control groups: non-instilled and saline-instilled; lung lavaged at 24 h after instillation.	No indication that crystal structure affected biological outcomes. High TiO_2 doses (5 mg) caused significant PMN influx relative to control and lower TiO_2 doses. Small but significant increase in macrophages after 0.5 mg rutile instillation relative to high TiO_2 doses (both rutile and anatase).	Ferin & Oberdörster (1985)
BALB/c BYJ mice, male, (7–8 weeks, ~27 g)	TiO_2 1.57 μm MMAD (σ_g=2.3), 11.8 μg/mouse	Mice (3 per group and time-point) instilled with each material in 20 μL phosphate buffered saline and killed at 6 periods between 15 min and 7 days	PMN levels in TiO_2 groups did not differ relative to saline controls at 20 h, 3 days or 7 days after instillation. Lung clearance half-time was 19 days for TiO_2.	Finch *et al*. (1987)
Fischer 344 rats, male (180–200 g)	TiO_2 (anatase, 2.1±1.5 μm), 5, 10, 50, 100 mg/kg bw	5–6 rats per exposure group killed 1, 7, 14 and 28 days after-instillation; 5-mg/kg dose not assessed at day 1	At all but the lowest instilled dose, TiO_2 caused increased PMN levels relative to saline controls at all time-points. At 5-mg/kg TiO_2, PMN levels were only increased at day 7. At 28 days following 50 mg/kg, TiO_2 was found primarily in macrophages located at the alveolar duct levels.	Driscoll *et al*. (1990)

Table 4.2 (contd)

Species, sex (age/weight)	Characteristics of particles and exposure	Exposure protocol	Observed effect(s)	Reference
Fischer 344 rats, male (240–260 g)	12-nm TiO_2 (rutile), 500 µg/rat; 21-nm TiO_2 (anatase), 65, 107, 200, 500, 1000 µg/rat; 230-nm TiO_2 (rutile), 500 µg/rat; 250-nm TiO_2 (anatase), 500, 1000 µg/rat	3–8 rats per group killed 24 h after exposure	For doses >500 µg, the unlavageable fraction appeared to correlate with instilled number of particles and decreased with increasing particle diameter.	Ferin *et al*. (1992)
Fischer 344 rats, male (~220 g)	12-nm TiO_2 (rutile), 500 µg/rat; 20-nm TiO_2 (anatase), 65, 107, 200, 500, 1000 µg/rat; 220-nm TiO_2 (rutile), 500 µg/rat; 250-nm TiO_2 (anatase), 500, 1000 µg/rat; 20-nm TiO_2 (anatase, serum-coated), 100 µg/rat; 20-nm TiO_2 (anatase, phagocytosed), 104 µg/rat	4–10 rats per group killed 24 h after exposure	Fraction of particles retained in tissues (epithelial cells or interstitium) and protein leakage correlated with surface area of retained particles. Serum coating did not affect inflammatory response or protein leakage. Phagocytosed particles did not access the tissues or induce an inflammatory response.	Oberdörster *et al*. (1992a)

Table 4.2 (contd)

Species, sex (age/weight)	Characteristics of particles and exposure	Exposure protocol	Observed effect(s)	Reference
Fischer 344 rats, male (175–225 g)	Fine (250 nm) TiO_2 (anatase), 500 µg/rat; ultrafine (21 nm) TiO_2 (anatase), 750 µg/rat	Rats instilled with particles in 0.2 mL saline (6 per time-point) and killed 0, 1, 3 and 7 days after exposure	Significantly increased PMN influx in rats exposed to ultrafine particles via instillation relative to inhalation and unexposed controls at 1, 3 and 7 days after exposure. Rats instilled with fine particles had a significant increase in PMNs relative to unexposed rats 1 day after exposure only. Significantly increased number of macrophages was present 3 and 7 days after ultrafine instillation relative to inhalation and unexposed controls. Comparison of responses as a function of primary particle size was confounded by ~40% greater ultrafine than fine particle mass dose 1 day after exposure.	Osier & Oberdörster (1997)

Table 4.2 (contd)

Species, sex (age/weight)	Characteristics of particles and exposure	Exposure protocol	Observed effect(s)	Reference
Fischer 344 rats, males (10 weeks; TiO_2 only, 211±10 g; endotoxin plus TiO_2, 235±39 g); C57BL/6J mice, male (23.3±1.6 g, TiO_2 only)	Ultrafine (20 nm) TiO_2 (anatase), 31, 125, 500 µg/rat; 6, 25, 100 µg/mouse; fine (250 nm) TiO_2 (anatase), 125, 500, 2000 µg/rat; 25, 100, 400 µg/mouse; endotoxin before ultrafine TiO_2, 70 endotoxin units, 50 µg/rat; endotoxin before fine TiO_2, 70 endotoxin units, 50 µg/rat	TiO_2 exposure only: 3 animals per group killed at 6, 24 and 48 h after instillation; endotoxin plus TiO_2: instilled with TiO_2 30 min after endotoxin inhalation and killed 24 h after instillation	On the basis of instilled particle mass, ultrafine TiO_2 caused far greater PMN influx than fine particles in both rats and mice at all time-points. Expressed as instilled particle surface area, PMN responses to fine and ultrafine TiO_2 appeared to be similar. In endotoxin-primed rats, ultrafine TiO_2 caused a significant amplification of the PMN response relative to ultrafine TiO_2 or endotoxin alone, whereas fine TiO_2 did not elicit a significant response relative to controls.	Oberdörster *et al*. (2000)
Wistar rats, male (370–470 g)	29-nm TiO_2, 125, 500 µg/rat; 250-nm TiO_2, 125, 500 µg/rat	Rats killed 24 h after exposure	Epithelial permeability, epithelial damage and inflammation were increased following 500 µg instillation of ultrafine particles (29-nm TiO_2) but not fine particles (250-nm TiO_2)	Renwick *et al*. (2004)

bw, body weight; MMAD, mass median aerodynamic diameter; PMN, polymorphonuclear neutrophils

Table 4.3. In-vitro studies of titanium dioxide (TiO_2) disposition

Cells	Characteristics of particles and exposure	Exposure protocol	Observed effect(s)	Reference
Sprague-Dawley alveolar macrophage cell line	TiO_2 [size not specified] up to 100 µg/mL	Particles untreated or opsonized with surfactant protein A, artificial bovine surfactant or rat immunoglobulin G	Opsonization with surfactant components resulted in a modest dose-dependent increase in macrophage uptake of particles compared with untreated particles. These 'inert' particles presumably phagocytosed via receptors that require neither activation nor opsonization by airway surface fluid components	Stringer & Kobzik (1996)
Sprague-Dawley rat tracheal explants	21-nm TiO_2 (anatase), 120-nm TiO_2 (anatase); 5 mg/mL suspension	Explants submerged epithelial side up; after 1 h, explants removed from suspension and placed in media for 3–7 days	Ultrafine particles appeared to enter epithelial cells more rapidly than fine particles. Results suggested that once ultrafine TiO_2 particles enter epithelial cells they are readily translocated to the interstitium, whereas fine particles tend to remain in the epithelial cells. Both fine and ultrafine particles tended to aggregate, but the aggregates of ultrafine particles were larger and encompassed a greater number of particles. The aggregate size of fine particles decreased over time, while that of ultrafine particle increased over time.	Churg *et al*. (1998)
BALB/C mouse tumour monocytic macrophages, J774.2 cell line	29-nm TiO_2, 250-nm TiO_2, 15.7–125 µg/mL (0.0975–0.78 µg/mm^2)	Cells cultured for 8 h with particles; medium subsequently changed and cells incubated for 24 h with 2-µm latex beads (5:1, bead:cell) to assess phagocytic activity	Phagocytosis was inhibited by all particle types at 125 µg/mL (0.78 µg/mm^2). However, at the lowest dose (0.0975 µg/mm^2) there was a tendency for ultrafine particles to increase phagocytic activity.	Renwick *et al*. (2001)

Table 4.3 (contd)

Cells	Characteristics of particles and exposure	Exposure protocol	Observed effect(s)	Reference
Wistar rat lung macrophages	29-nm TiO_2, 250-nm TiO_2, 125, 500 µg instilled/rat	Particles instilled in male rats 24 h before they were killed and cells collected; cells cultured 18 h with 2-µm latex beads (5:1, bead:macrophage) to assess phagocytic activity	Phagocytic activity was significantly decreased relative to control for macrophages from rats instilled with all particle types/sizes at the 500-µg dose, but was unaffected at the 125-µg dose. At the 500-µg dose, phagocytosis tended to decrease progressively from fine TiO_2 to ultrafine TiO_2. Chemotactic activity of macrophages was significantly increased by ultrafine particles at the 500-µg dose, but was similar to saline control for fine particles and the low dose.	Renwick *et al*. (2004)
Skin from domestic pigs (5 months)	Titanium formulations (T-Lite), 10% TiO_2, needle-line particles of 30–60 nm×10 nm coated with methicone or methicone and silica; 400 µg/cm^2 or 240 µg/cm^2	Test formulations applied for 24 h to 1 cm^2 exposed skin (500 µm thick)	No dermal penetration of TiO_2 wasobserved for the tested suncreen formulations. Applied TiO_2 particles were mostly aggregates up to 200 nm and occasionally up to 1 µm. Virtually all the applied TiO_2 was recovered from skin surface by washing with sponge dipped in soap solution.	Gamer *et al*. (2006)

reported that Long-Evans rats (358 g) relative to smaller Fischer 344 rats (231 g) received a lower normalized total lung dose following a 7-hour exposure to titanium dioxide (0.43 versus 0.52 μg/g bw and 105 versus 114 μg/g lung, respectively).

Bellmann *et al*. (1991) found about 40% greater lung burdens of inhaled titanium dioxide in male Fischer 344 rats compared with similarly exposed female rats. When the mass of titanium dioxide was normalized to lung weight, however, lung burdens were similar between the males and females.

The method of delivery (instillation *versus* inhalation) affects the dose rate of particles delivered to the lungs as well as the distribution of these particles within the lungs and may also potentially affect observed pulmonary responses. The lobe-to-lobe distribution of inhaled titanium dioxide is associated with lobe weight (Ferin & Morehouse, 1980; Osier & Oberdörster, 1997). Osier and Oberdörster (1997) suggested that the increased response following instillation may be due to focal areas of high particle burden, differences in dose rate or clearance kinetics. Driscoll *et al*. (1991) also reported that, for similar lung burdens of titanium dioxide, instillation induced transient increases in levels of lavage protein and polymorphonuclear neutrophils that were not observed following inhalation exposures. Following a 12-week exposure to titanium dioxide, Oberdörster *et al*. (1994, 1997) measured the clearance kinetics of both fine and ultrafine titanium dioxide as well as the clearance of subsequently administered radiolabelled particles (3.3 μm). The method of delivery of this radiolabelled particle, i.e. by inhalation or instillation, did not appear to affect the measured pulmonary clearance rates.

At 25 days after inhalation exposure to a 1.0-μm MMAD titanium-dioxide aerosol, Ferin and Morehouse (1980) reported 70% particle retention in Fischer 344 rats while Long-Evans rats retained only 55%. However, Driscoll *et al*. (1991) only observed 39% retention in Fischer 344 rats 28 days after a 5-day exposure to a 1.0-μm MMAD titanium dioxide aerosol. Pulmonary clearance in rats is also affected by age, with typical retention half-times of 45 days at 5 months versus 74 days at 23 months (Muhle *et al*., 1990).

The exposure history of animals also affects particle clearance. Exposure to the gaseous pollutants, nitrogen oxide and sulfur dioxide, stimulated particle clearance of titanium dioxide at low levels of exposure but suppressed clearance at higher levels of exposure (Ferin & Leach, 1975). The clearance might be due to macrophages and macrophage recruitment following initial exposure to gaseous pollutants would explain the stimulated clearance. Indeed, the chemotactic activity of macrophages is significantly increased following acute exposures to titanium dioxide (Renwick *et al*., 2004). However, chronic exposures to high concentrations of titanium dioxide aerosols impaired alveolar clearance to varying degrees in rats and mice (Bermudez *et al*., 2002, 2004) and possibly in hamsters (Creutzenberg *et al*., 1998). Co-exposure of rats to cytotoxic aerosols impaired macrophage clearance of titanium dioxide and increased titanium dioxide translocation to the lymph system (Greenspan *et al*., 1988).

Although differences have been observed between studies, common findings related to the behaviour of titanium dioxide particles in the respiratory tract have been reported. Following subchronic exposures to high concentrations, pulmonary clearance rates of fine

titanium-dioxide particles were decreased in both rats and mice and those of ultrafine titanium-dioxide particles were decreased to a greater extent. The evidence in hamsters is contradictory; two studies (Bermudez *et al*., 2002, 2004) showed no effect of subchronic exposure to titanium dioxide on clearance and one found impaired clearance (Creutzenberg *et al*., 1998). Rats, mice and hamsters all experienced acute inflammatory responses after exposure to fine and ultrafine titanium-dioxide particles, although the response was greater with ultrafine particles on a mass basis (Bermudez *et al*., 2002, 2004). Following exposures to titanium dioxide, rats and mice (but not hamsters) also demonstrated increased epithelial permeability which can affect the transport of titanium dioxide and other materials from the lumenal surfaces into the tissues and even the circulation.

Both in-vitro and in-vivo studies have demonstrated the rapid (~1 hour) translocation of free ultrafine-titanium dioxide particles across pulmonary cell membranes (Ferin *et al*., 1992; Churg *et al*., 1998; Geiser *et al*., 2005). Agglomerates of titanium dioxide particles may disassociate once deposited in the lungs; thus, inhaled agglomerate size is the determinant of the amount and site of deposition, but subsequent clearance is influenced by the properties of the agglomerates and the primary particles (Takenaka *et al*., 1986; Ferin *et al*., 1992; Bermudez *et al*., 2002). Following dissociation, ultrafine titanium dioxide particles are cleared more slowly and cause a greater inflammatory response (influx of polymorphonuclear neutrophils) than fine titanium dioxide particles (Ferin *et al*., 1992; Oberdörster *et al*., 1994, 2000; Bermudez *et al*., 2002, 2004). An increase in the transport by macrophages of titanium dioxide to lymph nodes has been reported following inhalation of a cytotoxin (Greenspan *et al*., 1988). However, Geiser *et al*. (2005) reported that ~80% of 22-nm titanium dioxide particles remained on the lumenal alveolar surface of rats 24 hours after inhalation. Both ultrafine and fine (0.078 and 0.2 μm in diameter) particles cross cellular membranes by non-endocytic (i.e. those that involve vesicle formation) mechanisms such as adhesive interactions and diffusion, whereas the phagocytosis of larger 1-μm particles is ligand receptor-mediated (Geiser *et al*., 2005). The differences in inflammatory effects and possibly lymph node burdens between fine and ultrafine titanium dioxide appear to be related to lung burden in terms of particle surface area and not particle mass or number (Oberdörster *et al*., 1992a; Oberdörster 1996; Oberdörster *et al*., 2000; Tran *et al*., 2000). The surface properties of titanium dioxide (e.g. roughness) may affect protein binding, and smoother titanium dioxide surfaces are more hydrophobic (Sousa *et al*., 2004).

The apparent dysfunction in pulmonary clearance as measured by lung burden of titanium dioxide following long-term exposure might not be representative for clearance of subsequently inhaled fine particles (ILSI Risk Science Institute Workshop Participants, 2000). When titanium-dioxide particles are sequestered, they may not necessarily influence nor would their clearance kinetics be reflective of macrophage-mediated removal of subsequently inhaled materials. For example, following a subchronic 12-week exposure, lung burdens of both silica and ultrafine titanium dioxide suggested impaired macrophage clearance (Oberdörster *et al*., 1994). The prolonged lung burdens were

presumed to be due to the cytotoxicity of silica dioxide and sequestration of ultrafine titanium dioxide in the interstitium. However, exposure to radiolabelled polystyrene (3.3 μm) particles also revealed a delay in clearance in animals exposed to both silica and ultrafine titanium dioxide. These large polystyrene particles were probably not sequestered even in the presence of increased epithelial permeability, and thus demonstrated impaired alveolar macrophage-mediated clearance.

4.2.2 *Toxic effects*

(a) In vivo

As reported previously (IARC, 1989), administration of high doses of titanium dioxide to experimental animals by intraperitoneal or intrapleural injection or by intratracheal instillation into the lung resulted in varying degrees of inflammation with minimal associated pathology (lung damage or fibrosis). Some studies demonstrated the fibrotic potential of titanium dioxide in rats (Muhle *et al*., 1991) in contrast to a wide range of studies that failed to demonstrate any fibrotic potential of fine titanium dioxide in rats or rabbits (IARC, 1989; Ferin & Oberdörster, 1985). However, one study showed that intratracheal instillation of 3 mg titanium dioxide to hamsters once a week for 15 weeks resulted in slight pulmonary inflammation and, subsequently, pathological evidence of interstitial fibrosis (Stenbäck *et al*., 1976). Normal clearance pathways from the lung were impaired in rats that had been exposed to 250 mg/m^3 rutile for six hours per day on five days per week for two years, and massive accumulation of dust-laden macrophages was observed. In addition, free particles and cellular debris were found in the alveoli, and alveolar proteinosis and cholesterol granulomas developed. Lung weights were increased and white patches of accumulated material were seen in the lungs at necropsy (Lee *et al*., 1985a,b, 1986). The collective results from these studies are consistent with a breakdown of normal clearance functions and altered lung structure due to the massive amount of titanium dioxide retained. The lowest exposure concentration of 10 mg/m^3 showed minimal effects whereas the 50-mg/m^3 dose also showed evidence of overload. Most of the pathology and related changes were considered by the authors to be overload-dependent.

Several studies have expanded the understanding of the toxicity of titanium dioxide, especially under conditions of lower exposure. Moreover, studies that used ultrafine or nanosize titanium dioxide showed enhanced toxicity relative to the fine particles used in earlier studies (IARC, 1989).

Baggs *et al*. (1997) compared the inhalation toxicity of fine (250 nm) versus ultrafine (20 nm) titanium dioxide (~23 mg/m^3 for six hours per day on five days per week for three months) in male Fischer 344 rats. After six months in clean air following exposure, fine titanium dioxide induced a minor degree of fibrous changes at three months, as shown by trichrome collagen staining, which was less than that in the ultrafine-treated group. The fibrous deposits (indicated by staining) decreased after six months in clean air and then became not significantly greater than those in controls at 12 months. Ultrafine

particles were more fibroproliferative in rats than fine titanium dioxide, but the fibrotic lesions (generally thought to be permanent) appeared to be reversible. Earlier work by this group (Ferin & Oberdörster, 1992) showed that inhalation of fine (250 nm) or ultrafine (20 nm) titanium dioxide (~23 mg/m^3 for 6 hours per day on 5 days per week) for 12 weeks induced differential tissue uptake of the particles (most notably at 12 weeks of exposure) and that ultrafine titanium dioxide induced much more and increasing inflammation throughout the 12-week period of exposure. It was concluded that the ultrafine particles had probably passed into the lung epithelium after having escaped phagocytosis. Intratracheal instillation with 500 μg of each type of particle yielded largely analogous findings 1 and 29 days after treatment but inflammation returned to normal at 59 days in both treatment groups.

In related studies that used the same exposures, Oberdörster *et al*. (1992a,b) provided further evidence for heightened inflammation and associated pro-inflammatory mediators in the lungs of rats exposed to ultrafine titanium dioxide as well as for reduced clearance detected by a radiotracer. The impact of the particles on inflammation correlated better with surface area than with dose mass. Osier and Oberdörster (1997) also investigated fine (250 nm) and ultrafine (20 nm) titanium dioxide in a comparative study of intratracheal instillation versus inhalation that allowed an approximation of similar acute (single exposure) lung burdens (500 and 750 μg, respectively). Acute effects (e.g. inflammation) were quantitatively similar until 7 days after exposure and the differential potency was consistent with that previously noted for ultrafine and fine particles. Instillation elicited a greater intensity of response possibly due to differences in dose rate and a less dispersed distribution of particles in the distal lung. Similar results were reported by Renwick *et al*. (2004) but the differences between the size modes that were apparent in male Wistar rats instilled with 500 μg were not evident in those administered 125 μg.

Inhalation studies with fine titanium dioxide have generally been consistent with earlier findings that suggested that its toxicity is similar to that of other poorly soluble particles (ILSI Risk Science Institute Workshop Participants, 2000). Male HAN rats exposed for 3–30 days (on 5 days per week) to 50 mg/m^3 fine titanium dioxide and followed up to 75 days showed little or no evidence of toxicity (Brown *et al*., 1992). Tests for macrophage chemotaxis with the Boyden chamber at any time after exposure showed no stimulatory effect of titanium dioxide, which was consistent with the general lack of inflammation. A similar 5-day exposure (for 6 hours per day) to titanium dioxide (1 μm) was assessed for profibrotic inflammatory end-points 7–63 days after exposure (Driscoll *et al*., 1991). Lung burden was 1.8 mg at 5 days and retention was 38.6% 28 days after exposure. Bronchoalveolar lavage indices showed no evidence (cellular, enzyme or cytokine) of damage or inflammation, nor was there evidence of macrophage activation that might lead to fibrosis under the conditions of this study.

Inhalation of 5, 50 and 250 mg/m^3 pigment-grade (fine) titanium dioxide (~1.7 μm) for 6 hours per day on 5 days per week for 4 weeks by male Crl:CDBR rats was evaluated for various inflammation-related end-points at 1 week and 1, 3 and 6 months after

exposure (Warheit *et al*., 1997). Effects consistent with prolonged (but slowly decreasing) inflammation and macrophage impairment were generally limited to the 250-mg/m^3 exposure group (12 mg retained dose). The lower-exposure groups recovered in an inverse dose-dependent manner. Pathology reflected the retained titanium dioxide in aggregated particle-laden macrophages and foamy cells, with no evidence of significant fibrosis.

Instillation of titanium dioxide (200 μg) [size of the dust particles not specified] into female C3H/He mice did not alter the clonal activity of macrophages harvested 40 days after exposure, which would be consistent with the unimpaired health of macrophages and the lack of evidence of profibrotic activity (Oghiso *et al*., 1992). A much broader array of pulmonary and systemic immunological end-points were evaluated in Fischer 344 rats exposed for 8 consecutive days (~40 mg/m^3; 2.2 μm; σ_g=1.4; 5 hours per day) (Huang *et al*., 2001). Assays up to 5 months after exposure showed minimal if any impact on associated immune function and cell mediators.

Several studies of subchronic to chronic duration have compared particle sizes and species responses to relatively low levels of titanium dioxide. Henderson *et al*. (1995) exposed female Fischer 344 rats to 0, 0.1, 1.0 and 10 mg/m^3 fine commercial-grade titanium dioxide by inhalation for 4 weeks (6 hours per day on 5 days per week). Lung burdens ranged from 4.4 to 440 μg after 1 week. Other groups of rats received instillations of 50, 200 and 750 μg to parallel these groups; higher doses were used due to the lack of apparent effects of the titanium dioxide. Measurement of cells, enzyme and cytokine markers and pathological lesions showed no effect of titanium throughout the study (1, 8 and 24 weeks after exposure) for either inhalation or instillation exposures.

Bermudez *et al*. (2002, 2004) exposed female rats, mice and hamsters by inhalation to fine (rutile; 250-nm primary particles) and ultrafine (21-nm primary particles) titanium dioxide (see Table 4.1 for details of exposure). In the study of fine titanium dioxide (Bermudez *et al*. 2002), particles accumulated in all species at 10 mg/m^3 and all species cleared the particles substantially during the period after exposure (rats>mice>hamsters), although hamsters cleared particles more completely than mice and mice more completely than rats by 1 year after exposure. At 50 and 250 mg/m^3, mice and rats accumulated more particles than hamsters and both were in overload within a minimal period after exposure in contrast to the nearly complete clearance in hamsters. Bronchoalveolar lavage indices of lung injury and inflammation at the high concentrations showed high neutrophil responses in all species and reversal in rats and mice was retarded (rat > mouse) in comparison with hamsters. Significant inflammation (but to a much much lesser extent than that with the high-level exposures) occurred in rats at 10 mg/m^3. Inflammation markers generally followed this pattern. Rats exposed to concentrations of 50 and 250 mg/m^3 developed a dose-dependent accumulation of dust in the cells, hyperplasia and alveolar lipoproteinosis. Minute collagenized fibrosis occurred in the alveolar walls that enclosed large dust–cell aggregates. The nature of the lesions in rats appeared to be actively fibroproliferative compared with those in mice and hamsters.

Indices of epithelial cell proliferation in the end airways and alveoli were seen primarily in rats and were persistent.

In the study of ultrafine titanium dioxide, Bermudez *et al*. (2004) reported that mice and rats had similar normalized lung burdens but that mice appeared to clear the particles faster than rats, except at 10 mg/m^3 when they were almost identical and appeared to have arrested clearance. In contrast, hamsters exhibited rapid clearance regardless of the exposure level. Bronchoalveolar lavage indices of lung injury and inflammation showed a greater neutrophil response in rats across the ranges of concentrations and mice showed an early high macrophagic response that decreased to below that of rats over time. Pathology (septal thickening and fibrosis) generally followed these patterns (hamsters had virtually none) and the nature of the lesions in rats appeared to be actively fibroproliferative compared with those of mice and hamsters. Indices of epithelial cell proliferation in the end airways were consistent with these observations; the reversal after exposure was most rapid in hamsters.

The impact of surface treatment on the acute lung toxicity of titanium dioxide particles was assessed in a short-term pulmonary assay with Crl:CD(SD)/GS BR rats. The particles used were R-100 titanium dioxide (1 wt% alumina; average size, 300 nm; average surface area, 6 m^2/g) and Pigment A titanium dioxide (1 wt% alumina, 3 wt% amorphous silica encapsulating the particle; average size, 290 nm; average surface area, 7.9 m^2/g), both of which were in the rutile form. Rats received a single dose of 1 or 5 mg/kg bw of the particles dispersed in phosphate buffered saline. Bronchoalveolar lavages were conducted 24 hours, 1 week, 1 month and 3 months after instillation. The inflammatory response to titanium dioxide particles was transient; this may have been the result of the instillation process itself as it was also seen in the vehicle-control group. Similar responses were observed with the lavage fluid parameters (lactate dehydrogenase, microprotein and alkaline phosphatase) and similar results were seen in the rate of lung parenchymal cell proliferation. Histopathological analyses of lung tissues showed no significant adverse effects of titanium dioxide (both types) (Warheit *et al*., 2005).

A 2-year chronic inhalation study with commercial-grade titanium dioxide (~1.6 μm; 0, 10, 50 and 250 mg/m^3 for 6 hours per day on 5 days per week) demonstrated the transmigration of particles to systemic tissues, notably the liver and spleen (Lee *et al*., 1985a,b). The authors surmised from the minimal presence of particles not associated with immune or phagocytic cells that the dose-dependent systemic evidence of particles was indicative of transmigration through the lymphatic system into the blood. There was evidence of mild focal fibrosis with few apparent interstitial particles.

Muhle *et al*. (1990, 1991) reported a series of studies that involved exposure of rats and hamsters to rutile and anatase titanium dioxide (5–30 mg/m^3) and described overload and mild inflammation in both species, although the condition appeared to be more severe (based on pathology) in rats. The anatase form was somewhat more potent in rats than the rutile form, which may reflect the 10-fold smaller size of the anatase (0.02–0.04 μm versus 0.2–0.7 μm). Pathological evidence of fibrogenesis was reported in rats.

(b) In vitro

Iyer *et al*. (1996) found that primary human macrophages cultured for up to 24 hours with 60 μg/mL commercial-grade titanium dioxide (0.45 μm) did not show apoptosis or any other evidence of DNA damage that might initiate profibrotic inflammation.

Pro-inflammatory pathways that involve IκBα degradation were assessed by examining its linkage to interleukin (IL)-8 expression (Schins *et al*., 2000) in A549 epithelial cell cultures treated with commercial fine titanium dioxide (40 μg/cm^2). Degradation of IκBα correlated with a brief induction of IL-8 (a pro-inflammatory cytokine) that rapidly decreased; this led the authors to conclude that titanium dioxide has transient but probably minimal inflammatory potential.

In a rat nasal epithelial model that predicts upper respiratory tract toxicity *in vivo* and *in vitro* (Kilgour *et al*., 2000), nasal turbinates from mice were incubated with titanium dioxide, and adenosine triphosphate was evaluated in the nasal olfactory epithelium or respiratory epithelium. Titanium dioxide caused little or no loss of adenosine triphosphate in either.

The in-vitro toxicity of ultrafine titanium dioxide particles (40 nm) was assessed by cell morphology, mitochondrial function, membrane leakage of lactate dehydrogenase and reduced glutathione levels as well as the release of reactive oxygen species in mitochondrial membrane potential (Hussain *et al*., 2005). Titanium dioxide was used as a negative control based on published data that ultrafine particles of titanium dioxide show no toxicity to these cells. Titanium dioxide appeared to have the lowest level of toxicity to cells for any of these parameters.

Donaldson and Brown (1988) compared the rutile form of titanium dioxide (medium volume diameter, 2.4 μm) with crocidolite asbestos and quartz. Rat alveolar macrophages released ^{51}Cr (indicative of cell damage) in significantly lower quantities after exposure to titanium dioxide than after exposure to either crocidolite asbestos or quartz.

Yamamoto *et al*. (2004) tested the cytotoxicity of ceramic particles of different sizes and shapes and found that dendritic particles of titanium dioxide had significantly greater toxicity than those that were spherical or spindle shaped.

Human skin fibroblasts preincubated for 18 hours with 10 μg/cm^2 titanium dioxide (anatase, 450 nm) and then irradiated with UVA showed dose-dependent photocytoxicity, which suggested that nucleic acids are a potential target for photo-oxidative damage that has been sensitized with titanium dioxide (Wamer *et al*., 1997).

Stringer and Kobzik (1998) evaluated the effect of titanium dioxide in increasing IL-8 production in primed A549 human lung epithelial cells and found that it caused significantly less tumour necrosis factor -α and IL-8 release than residual oil fly ash or pathogenic α-quartz dust. Using a mouse macrophage cell line, Thibodeau *et al*. (2003) found that exposure to α-quartz silica elicited activation of caspase 3 and caspase 9, whereas exposure to titanium dioxide did not.

Exposure of mouse peritoneal macrophages *in vitro* to 100 μg/mL titanium dioxide in the culture medium was found to inhibit the phagocytic activity of cells compared with controls (Nuuja & Arstila, 1982). The phagocytosis of alveolar macrophages was

impaired following exposure to ultrafine particles including titanium dioxide (Renwick *et al*., 2001). Oberdörster *et al*. (1992a) found that alveolar macrophages exposed to ultrafine titanium dioxide (12 and 20 nm diameter) have a greater potential to induce cytokines than those exposed to larger-sized particles.

Li (1986) and Li and Myers (1988) found that titanium dioxide caused significantly less damage than chrysotile or calcite fibres in airway epithelial cells using an in-vitro lung epithelial cell system for evaluating the potential toxicity of inhalable material. Titanium dioxide was far less toxic than calcium sulfate, chrysotile crocidolite and phosphate fibres.

4.2.3 *Genetic and related effects*

Investigations on the genetic and related effects of titanium dioxide have been performed using isolated DNA and cell culture-based test systems, as well as animals. In several of these studies titanium dioxide was used as a negative control. Other studies have evaluated the toxic properties of titanium dioxide in relation to its size (e.g. fine versus ultrafine) and/or chemistry (e.g. anatase versus rutile). Several studies have also addressed the photosensitization effects of titanium dioxide. In view of the contrasting photocatalytic and biological activities of titanium dioxide in relation to size and chemical composition (Oberdörster *et al*., 2005), specifications of each sample tested are provided whenever available.

(a) Isolated DNA

Unwinding and breakage of plasmid DNA *in vitro* has been used to investigate the generation of reactive oxygen species by various mineral dusts including titanium dioxide (Donaldson *et al*., 1996). [The Working Group noted the limited relevance of this assay for assessing particle-induced genetic damage]. A comparison of fine (500 nm) *versus* ultrafine (20 nm) titanium dioxide using ΦX174 RF plasmid DNA showed markedly stronger strand breakage for the ultrafine sample. DNA damage by the ultrafine titanium dioxide was prevented by the presence of mannitol, which suggests that the damaging effects were due to hydroxyl radicals (Donaldson *et al*., 1996). In contrast, in a more recent study, ultrafine titanium dioxide (20 nm; 49.8 m^2/g) failed to damage ΦX174 RF DNA unlike various other particles of similar size (Dick *et al*., 2003). [Different incubation times as well as different relative amounts of plasmid DNA and titanium dioxide were used in the two studies.]

The effects of UV light-irradiated titanium dioxide on isolated DNA have been investigated. Upon co-exposure with simulated sunlight (300–400 nm), both the anatase and rutile forms [particle size not specified] of titanium dioxide induced damage in pBluescript II SK^+ plasmid DNA; anatase showed stronger effects than rutile (Dunford *et al*., 1997). Photo-irradiated (365 nm; UVA) anatase and rutile (size range, 50–300 nm) also caused the formation of 8-hydroxy-2′-deoxyguanosine in calf thymus DNA in the presence of copper chloride (Hirakawa *et al*., 2004). Again, anatase showed stronger effects than rutile. In the absence of irradiation, no DNA damage was found. Following

irradiation, both samples also showed enhanced formamidopyrimidine glycosylase-mediated cleavage of DNA fragments that contained human tumour-suppressor genes *P53* and *P16* and the c-Ha-*RAS*-1 oncogene (Hirakawa *et al*., 2004). Oxidative damage in calf thymus DNA was also reported after combined treatment with UVA (320–400 nm) and titanium dioxide (average size, 450 nm) (Wamer *et al*., 1997).

(b) Cellular effects (for details and references, see Table 4.4)

Anatase titanium dioxide (21 nm) was not mutagenic to *Salmonella typhimurium* TA100, TA98 or TA102. Titanium dioxide [unspecified] did not induce somatic mutation or recombination in *Drosophila melanogaster*.

Anatase (255 nm) but not 21-nm anatase or rutile (255 or 420 nm) titanium dioxide caused DNA strand breaks in mouse lymphoma L5178Y cells. Induction of oxidative DNA damage (8-hydroxy-2′-deoxyguanosine formation) was seen in rat lung epithelial cells treated with 180-nm anatase titanium dioxide. Anatase titanium dioxide did not enhance unscheduled DNA synthesis in rat pleural mesothelial cells or induce mutation in mouse lymphoma L5178Y/$tk^{+/-}$ or RLE-6TN rat lung epithelial cells.

Titanium dioxide caused a dose-dependent increase in sister chromatid exchange in Chinese hamster CHO-K1 cells at non-toxic concentrations but not in rat pleural mesothelial or Chinese hamster ovary CHO cells. No micronucleus formation was found in Chinese hamster ovary CHO cells incubated with titanium dioxide either in the presence or absence of metabolic activation. In contrast, titanium dioxide did induce micronuclei in Chinese hamster CHO-K1 cells. Titanium dioxide samples of different size or chemistry did not cause micronucleus formation in RLE rat liver epithelial cells, but a sample of ultrafine (≤20 nm) titanium dioxide did induce micronuclei in Syrian hamster fibroblasts, while >200-nm titanium dioxide was inactive. The ultrafine sample also elicited apoptosis in these cells. Titanium dioxide did not include chromosomal aberrations in Chinese hamster CHU/IU cells (21-nm anatase), Chinese hamster CHO cells or rat pleural mesothelial cells (anatase [size unspecified]). Titanium dioxide did not cause cell transformation of Syrian hamster embryo or mouse BALB/3T3/31–1-1 cells.

Enhanced oxidative DNA damage was observed in BEAS-2B human bronchial epithelial cells with 10-nm and 20-nm anatase and 200-nm rutile. A 1:1 mixture of 200-nm anatase and 200-nm rutile caused stronger oxidative DNA damage than either of these alone. No oxidative DNA damage was observed in CRL human skin fibroblasts [unspecified titanium dioxide]. DNA strand breakage assays (alkaline unwinding) in WI-26 human embryonal lung cells showed negligible effects of titanium dioxide [unspecified]. The compound did not induce mitochondrial dysfunction (i.e. membrane potential change) in A549 human lung epithelial cells. In BEAS-2B human bronchial epithelial cells, micronucleus formation was induced with 10-nm and 200-nm anatase titanium dioxide (not with >200-nm anatase or 200-nm rutile). Increased multinucleation was found in Met-5A human mesothelial cells treated with titanium dioxide [unspecified], but no such effect was observed in primary human mesothelial cells.

Table 4.4. Genetic and related effects of titanium dioxide

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Salmonella typhimurium TA100, TA102, TA98, reverse mutation	–	NT	40 000 µg/mL (21-nm anatase)	Nakagawa *et al.* (1997)
Drosophila melanogaster, wing mosaic assay	–		300 mM[c]	Tripathy *et al.* (1990)
DNA strand breaks (comet asssay), mouse lymphoma L5178Y/tk$^{+/-}$ cells *in vitro*	+	NT	800 µg/mL (255-nm anatase)	Nakagawa *et al.* (1997)
DNA strand breaks (comet assay), mouse lymphoma L5178Y/tk$^{+/-}$ cells *in vitro*	–	NT	3200 µg/mL (255-nm rutile; 420-nm rutile); 800 µg/mL (21-nm anatase)	Nakagawa *et al.* (1997)
Oxidative DNA damage, RLE rat lung epithelial cells *in vitro*	+	NT	1700 µg/mL	van Maanen *et al.* (1999)
Unscheduled DNA synthesis, rat pleural mesothelial cells *in vitro*	–	NT	50 µg/mL (anatase) (10 µg/cm^2)[d]	Endo-Capron *et al.* (1993)
Gene mutation, L5178Y/tk$^{+/-}$ mouse lymphoma cells *in vitro*	–	NT	2000 µg/mL (21-nm anatase)	Nakagawa *et al.* (1997)
Gene mutation, *Hprt* locus, RLE-6TN rat lung epithelial cells *in vitro*	–	NT	100 µg/cm^2 (180-nm anatase)	Driscoll *et al.* (1997)
Sister chromatid exchange, Chinese hamster CHO-K1 cells *in vitro*	+	NT	1 µM[c]	Lu *et al.* (1998)
Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	–	–	25 µg/mL	Ivett *et al*. (1989)
Sister chromatid exchange, RLE rat pleural mesothelial cells *in vitro*	–	NT	37.5 µg/mL (5 µg/cm^2)	Endo-Capron *et al.* (1993)
Micronucleus formation, Chinese hamster CHO-K1 cells *in vitro*	+	NT	2 µM[c]	Lu *et al.* (1998)

Table 4.4 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Micronucleus formation, Chinese hamster CHO cells *in vitro*	–	–	10 μg/mL	Miller *et al.* (1995)
Micronucleus formation, RLE rat liver epithelial cells *in vitro*	–	NT	20 μg/cm^2 (20-nm anatase; 170-nm rutile)	Linnainmaa *et al.* (1997)
Micronucleus formation, Syrian hamster embryo cells *in vitro*	+	NT	1 μg/cm^2 (ultrafine ≤20 nm)	Rahman *et al.* (2002)
Micronucleus formation, Syrian hamster embryo cells *in vitro*	–	NT	10 μg/cm^2 (fine[e] >200 nm)	Rahman *et al.* (2002)
Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	–	–	25 μg/mL	Ivett *et al*. (1989)
Chromosomal aberrations, Chinese hamster CHU/IU cells *in vitro*	–	NT	800 μg/mL (21-nm anatase)	Nakagawa *et al.* (1997)
Chromosomal aberrations, rat pleural mesothelial cells *in vitro*	–	NT	10 μg/cm^2 (anatase)	Yegles *et al.* (1993)
Cell transformation, BALB/3T3/A31-1-1 mouse cells	–	NT	100 μg/cm^2 (anatase; rutile)	Saffiotti & Ahmed (1995–1996)
Cell transformation, Syrian hamster embryo cells	–	NT	75 μg/mL	LeBoeuf *et al.* (1996)
DNA strand breaks (alkaline unwinding), WI-26 human embryonal lung cells *in vitro*	–	NT	500 μg/mL	Kamp *et al.* (1995)
Oxidative DNA damage, CRL1634 human skin fibroblasts *in vitro*	–	NT	71.4 μg/mL (10 μg/cm^2)	Wamer *et al.* (1997)
Oxidative DNA damage (FPg-comet assay), human BEAS-2B bronchial epithelial cells *in vitro*	+	NT	10 μg/mL (1.77 μg/cm^2) 10-nm anatase, 20-nm anatase, 200-nm rutile	Gurr *et al.* (2005)

Table 4.4 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Micronucleus formation, Met-5A human mesothelial cell line *in vitro*	+	NT	2 µg/cm^2 [unspecified]	Pelin *et al.* (1995)
Micronucleus formation, primary human mesothelial cells *in vitro*	–	NT	5 µg/cm^2 [unspecified]	Pelin *et al.* (1995)
Micronucleus formation, human BEAS-2B bronchial epithelial cells *in vitro*	–	NT	10 µg/mL (1.77 µg/cm^2) 200-nm rutile, >200-nm anatase	Gurr *et al.* (2005)
Micronucleus formation, human BEAS-2B bronchial epithelial cells *in vitro*	+	NT	10 µg/mL (1.77 µg/cm^2) 10-nm anatase, 200-nm anatase,	Gurr *et al.* (2005)
Oxidative DNA damage, rat lung *in vivo*	–		2×50 mg/kg it (180 nm anatase)	Driscoll *et al.* (1997)
Gene mutation, *Hprt* locus, rat alveolar epithelial cells *in vivo*	+		100 mg/kg it (180 nm anatase)	Driscoll *et al.* (1997)
Micronucleus formation, mouse bone-marrow cells, peripheral blood lymphocytes *in vivo*	+		1000 mg/kg, ip×3	Shelby *et al.* (1993)

[a] +, positive; –, negative; NT, not tested;
[b] HID, higher inhibitory dose; LED, lower efficient dose, in-vitro tests, µg/mL; in-vivo tests, mg/kg bw; ip, intraperitoneal; it, intratracheal
[c] Dose expressed in molarity
[d] One of three experiments was positive.
[e] Dose for fine sample not specified in detail [‘similar’ concentrations were used for fine and ultrafine]

(c) Cellular effects in combination with UV irradiation

In relation to its application in sunscreens or its photocatalytic activity, several studies have addressed the effects photo-irradiated titanium dioxide. Micronucleus formation was not enhanced in rat liver epithelial cells after treatment with 170-nm anatase, 20-nm anatase or 20-nm aluminium hydroxide/stearic acid-coated rutile in combination with irradiation with UVA (at 365 nm wavelength) (Linnainmaa *et al*., 1997). In contrast, irradiated (300–400 nm wavelength) MRC-5-fibroblasts showed increased DNA strand breakage in the presence of anatase or rutile [sizes not specified] compared with cells irradiated in the absence of titanium dioxide (Dunford *et al*. 1997). In human skin fibroblasts (CRL1634, ATCC), enhanced oxidation of RNA was observed following combined titanium dioxide (particle size, 450 nm) plus UVA (320–400 nm). Treatment with titanium dioxide plus UVA did not cause increased oxidative damage to DNA (Wamer *et al*., 1997). Four titanium dioxide samples, i.e. a 21-nm anatase, a 255-nm anatase, a 255-nm rutile and a 420-nm rutile, were tested in an assay that measured DNA strand breakage in L5178Y/tk$^{+/-}$ mouse lymphoma cells. In the presence of UV light, all samples induced enhanced DNA strand breakage as determined by the alkaline comet assay at concentrations that also caused cell death (Nakagawa *et al*., 1997). In the same study, the 21-nm anatase sample induced chromosomal aberrations in the Chinese hamster CHL/IU cell line in the presence but not in the absence of UV/visible light. Besides polyploidy, the principal structural aberrations that occurred after treatment with 21-nm titanium dioxide plus UV light were chromatid breaks and chromatid exchanges, which occurred at cytotoxic concentrations (Nakagawa *et al*., 1997). The same sample was not mutagenic in *S. typhimurium* strains TA100, TAS98 or TA102, or when tested in an L5178Y/tk$^{+/-}$ colony formation assay when irradiated with UV light (Nakagawa *et al*., 1997).

(d) Studies in rodents (see also Table 4.4)

The induction of oxidative DNA damage in rat lungs was investigated after intratracheal instillation with two different samples of titanium dioxide, i.e. an untreated titanium dioxide (P-25, hydrophilic surface) and a trimethoxyoctylsilane-treated titanium dioxide (T-805, silanised/hydrophobic surface; particle size, ~20 nm). Transmission electron microscopy demonstrated a highly aggregated state of both titanium dioxide samples. Oxidative damage, as determined at 90 days in lung sections using 8-oxoguanine antibody, was not enhanced by untreated or silanised titanium dioxide (Rehn *et al*., 2003).

In-vivo mutagenesis of titanium dioxide (anatase; 180 nm median diameter; 8.8 m^2/g) was studied by *Hprt* analysis of epithelial cells isolated from the lungs of female SPF F344 Fischer rats 15 months after intratracheal instillation. Enhanced *Hprt* mutagenesis was observed with 100 mg/kg bw, a dose that also elicited persistent lung inflammation. The authors suggested that the in-vivo mutagenesis was driven by inflammation (Driscoll *et al*., 1997).

Intraperitoneal injection of titanium dioxide into mice resulted in enhanced micronucleus formation in bone-marrow cells and peripheral blood lymphocytes. No dose-dependent effect was observed over the range of 200–1000 mg/kg bw (Shelby *et al*., 1993).

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5. Summary of Data Reported

5.1 Exposure data

Titanium dioxide was first produced commercially in 1923, primarily for pigment production. Relatively small quantities of titanium dioxide are used for non-pigmentary purposes. In 2004, worldwide production of titanium dioxide was 4.4 million tonnes.

Titanium dioxide is obtained from a variety of ores that contain ilmenite, rutile, anatase and leucoxene, which are mined from deposits located throughout the world. Most titanium dioxide pigment is produced from titanium mineral concentrates by the chloride or sulfate process, either as the rutile or the anatase form. The primary particles are typically between 0.2 and 0.3 μm in diameter, although larger aggregates and agglomerates are formed. Ultrafine grades of titanium dioxide have a primary particle size of 10–50 nm and are used predominantly as ultraviolet blockers in sunscreens and plastics, and in catalysts. Most commercial titanium dioxide products are coated with inorganic (e.g. alumina, zirconia, silica) and organic (e.g. polyols, esters, siloxanes, silanes) compounds to control and improve surface properties.

Levels of occupational exposure to titanium dioxide during its manufacture have been reported from the USA and Europe between 1970 and 2000. The highest levels of exposure were observed during packing and milling, although high peak exposure also occurred in occupations such as site cleaning and maintenance. Average levels of exposure to respirable dust in these occupations up to 6 mg/m^3 (geometric mean) were reported, but have declined over time. No data were available that would allow the characterization or quantification of exposure to ultrafine primary particles. Workers in the titanium dioxide manufacturing industry may also be exposed to ore and other dusts, strong acids and asbestos.

Exposure to titanium dioxide in user industries is difficult to estimate and characterize due to the paucity of data. Exposure levels are assumed to be low in the user industries, with the possible exception of workers who handle large quantities of titanium dioxide. No significant exposure to titanium dioxide is thought to occur during the use of products in which titanium dioxide is bound to other materials, such as in paints.

5.2 Human carcinogenicity data

Three epidemiological cohort studies and one population-based case–control study from North America and western Europe were available for evaluation.

The largest of the cohort studies was among white male production workers in the titanium dioxide industry in six European countries. The study indicated a slightly increased risk for lung cancer compared with the general population. However, there was no evidence of an exposure–response relationship within the cohort. No increase in the

mortality rates for kidney cancer was found when the cohort was compared with the general population, but there was a suggestion of an exposure–response relationship in internal analyses. The other cohort studies, both of which were conducted in the USA, did not report an increased risk for lung cancer or cancer at any other site; no results for kidney cancer were reported, presumably because there were few cases.

One population-based case–control study conducted in Montréal did not indicate an increased risk for lung or kidney cancer.

In summary, the studies do not suggest an association between occupational exposure to titanium dioxide as it occurred in recent decades in western Europe and North America and risk for cancer.

All the studies had methodological limitations; misclassification of exposure could not be ruled out. None of the studies was designed to assess the impact of particle size (fine or ultrafine) or the potential effect of the coating compounds on the risk for lung cancer.

5.3 Animal carcinogenicity data

Pigmentary and ultrafine titanium dioxide were tested for carcinogenicity by oral administration in mice and rats, by inhalation exposure in rats and female mice, by intratracheal administration in hamsters and female rats and mice, by subcutaneous injection in rats and by intraperitoneal administration in male mice and female rats.

In one inhalation study, the incidence of benign and malignant lung tumours was increased in female rats. In another inhalation study, the incidence of benign lung tumours was increased in the high-dose groups of male and female rats. Cystic keratinizing lesions that were diagnosed as squamous-cell carcinomas but re-evaluated as non-neoplastic pulmonary keratinizing cysts were also observed in the high-dose groups of female rats. Two inhalation studies in rats and one in female mice gave negative results.

Intratracheally instilled female rats showed an increased incidence of both benign and malignant lung tumours following treatment with two types of titanium dioxide. Tumour incidence was not increased in intratracheally instilled hamsters and female mice.

Oral, subcutaneous and intraperitoneal administration did not produce a significant increase in the frequency of any type of tumour in mice or rats.

5.4 Mechanistic considerations and other relevant data

Humans can be exposed to titanium dioxide via inhalation, ingestion or dermal contact. In human lungs, the clearance kinetics of titanium dioxide is poorly characterized relative to that in experimental animals. (General particle characteristics and host factors that are considered to affect deposition and retention patterns of inhaled, poorly soluble particles such as titanium dioxide are summarized in the monograph on carbon black.) With regard to inhaled titanium dioxide, human data are mainly available from case reports that showed deposits of titanium dioxide in lung tissue as well as in lymph nodes.

A single clinical study of oral ingestion of fine titanium dioxide showed particle size-dependent absorption by the gastrointestinal tract and large interindividual variations in blood levels of titanium dioxide. Studies on the application of sunscreens containing ultrafine titanium dioxide to the healthy skin of human volunteers revealed that titanium dioxide particles only penetrate into the outermost layers of the stratum corneum, suggesting that healthy skin is an effective barrier to titanium dioxide. No studies on the penetration of titanium dioxide in compromised skin were available.

Respiratory effects that have been observed among groups of titanium dioxide-exposed workers include a decline in lung function, pleural disease with plaques and pleural thickening, and mild fibrotic changes. However, the workers in these studies were also exposed to asbestos and/or silica.

No data were available on the genotoxic effects in titanium dioxide-exposed humans.

Many data on deposition, retention and clearance of titanium dioxide in experimental animals are available for the inhalation route. Titanium dioxide inhalation studies showed differences—both for normalized pulmonary burden (deposited mass per dry lung, mass per body weight) and clearance kinetics—among rodent species including rats of different size, age and strain. Clearance of titanium dioxide is also affected by pre-exposure to gaseous pollutants or co-exposure to cytotoxic aerosols. Differences in dose rate or clearance kinetics and the appearance of focal areas of high particle burden have been implicated in the higher toxic and inflammatory lung responses to intratracheally instilled versus inhaled titanium dioxide particles. Experimental studies with titanium dioxide have demonstrated that rodents experience dose-dependent impairment of alveolar macrophage-mediated clearance. Ultrafine primary particles of titanium dioxide are cleared more slowly than their fine counterparts.

Titanium dioxide causes varying degrees of inflammation and associated pulmonary effects including lung epithelial cell injury, cholesterol granulomas and fibrosis. Rodents experience stronger pulmonary effects after exposure to ultrafine titanium dioxide particles compared with fine particles on a mass basis. These differences are related to lung burden in terms of particle surface area, and are considered to result from impaired phagocytosis and sequestration of ultrafine particles into the interstitium.

Fine titanium dioxide particles show minimal cytotoxicity and inflammatory/profibrotic mediator release from primary human alveolar macrophages *in vitro* compared with other particles. Ultrafine titanium dioxide particles inhibit phagocytosis of alveolar macrophages *in vitro* at mass dose concentrations at which this effect does not occur with fine titanium dioxide.

In-vitro studies with fine and ultrafine titanium dioxide and purified DNA show induction of DNA damage that is suggestive of the generation of reactive oxygen species by both particle types. This effect is stronger for ultrafine than for fine titanium dioxide, and is markedly enhanced by exposure to simulated sunlight/ultraviolet light.

In-vivo studies have shown enhanced micronucleus formation in bone marrow and peripheral blood lymphocytes of intraperitoneally instilled mice. Increased *Hprt* mutations were seen in lung epithelial cells isolated from titanium dioxide-instilled rats. In

another study, no enhanced oxidative DNA damage was observed in lung tissues of rats that were intratracheally instilled with titanium dioxide.

Most in-vitro genotoxicity studies with titanium dioxide gave negative results.

6. Evaluation and Rationale

6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of titanium dioxide.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of titanium dioxide.

6.3 Overall evaluation

Titanium dioxide is *possibly carcinogenic to humans (Group 2B)*.

6.4 Rationale

In making this evaluation the Working Group considered the human and animal evidence as well as the evidence regarding potential mechanisms through which titanium dioxide might cause cancer in humans.

The Working Group found little evidence of an increased risk for cancer among humans based on epidemiological data, although relatively few studies were available. The single most informative study was a multicountry study of titanium dioxide production workers that found a slightly increased risk for lung cancer compared with the general population and a suggestive dose–response, but no overall excess risk for kidney cancer. The two other cohort studies reported no increased risks and evidence from the case–control study did not indicate an increased risk for either lung or kidney cancer. Overall, these results led the Working Group to conclude that there was *inadequate evidence* from epidemiological studies to assess whether titanium dioxide causes cancer in humans.

In two studies of rats that inhaled titanium dioxide, one observed an excess incidence of lung tumours in both sexes and another in females only. Studies of rats exposed intratracheally found increases in the incidence of lung tumours. No increases were observed among mice and hamsters exposed intratracheally. Other studies that used different routes of administration did not observe excesses in tumour incidence. On the basis of the results of an increased incidence of lung tumours in rats, the Working Group

concluded that there was *sufficient evidence* that titanium dioxide is carcinogenic in experimental animals.

The Working Group considered the body of evidence regarding the pathways and mechanisms by which titanium dioxide or other poorly soluble particles may cause cancer. Following the same line of reasoning as that for the other particles reviewed in this volume, the Working Group considered that the available mechanistic evidence for titanium dioxide was not strong enough to warrant a classification other than Group 2B.