ECHA
EUROPEAN CHEMICALS AGENCY

Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

Titanium dioxide

EC Number: 236-675-5
CAS Number: 13463-67-7

CLH-O-0000001412-86-163/F

Adopted

14 September 2017

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CLH-O-0000001412-86-163/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **Titanium dioxide**

EC Number: **236-675-5**

CAS Number: **13463-67-7**

The proposal was submitted by **France** and received by RAC on **27 May 2016.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

France has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **31 May 2016**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **15 July 2016**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Normunds Kadikis**

Co-Rapporteur, appointed by RAC: **Norbert Rupprich**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **14 September 2017** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	Titanium dioxide	236-675-5	13463-67-7	Carc. 1B	H350i	GHS08 Dgr	H350i			
RAC opinion	TBD	Titanium dioxide	236-675-5	13463-67-7	Carc. 2	H351 (inhalation)	GHS08 Dgr	H351 (inhalation)			#
Resulting Annex VI entry if agreed by COM	TBD	Titanium dioxide	236-675-5	13463-67-7	Carc. 2	H351 (inhalation)	GHS08 Dgr	H351 (inhalation)			#

RAC recommends a note be included, as described in the text of the opinion

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

The EC/CAS inventory listing for Titanium dioxide (236-675-5/13463-67-7) covers any chemical that has "TiO2" as its molecular formula and is therefore the broadest possible identifier for TiO2 chemicals. This EC/CAS inventory listing has been used in the name and numerical identifiers for registration for the REACH registration, as the scope of the registration is all TiO2 chemicals. EC/CAS inventory listings for two specific TiO2crystal structures are available: Anatase (215-280-1/1317-70-0) and Rutile (215-282-2/1317-80-2). There is also a CAS inventory listing available for Brookite (12188-41-9). These EC/CAS inventory listings are not included in the Annex VI entry as they are covered by the EC/CAS inventory listing for Titanium dioxide.

Carcinogenicity was the only endpoint proposed for harmonised classification and labelling (CLH) by the dossier submitter (DS).

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

In the CLH report submitted by France, the carcinogenicity of TiO2 was assessed using studies available in the public literature and in the registration dossier. The dossier submitter (DS) indicated that all the publications used in the assessment were based on a bibliographic search carried out on all forms of TiO2 and that in addition, the information from the registration dossier (on EC 236-675-5) published on the ECHA website was considered. Only one study (Furukawa, 2011), a dermal tumour promoter study, was described as GLP and guideline compliant. One study via the oral route (Bernard, 1990) and one study via the inhalation route (Lee *et al*., 1985) were described by the DS as being "similar to guideline". Tumour promoting potential was investigated in two of the studies conducted via the instillation route (following pre-treatment with N-bis(2-hydroxypropyl)nitrosamine (DHPN)) and in all the studies conducted via the dermal route (following pre-treatment with 7,12-dimethylbenz[a]anthracene (DMBA) or UVB). In addition, the DS has drawn on data published by IARC, in particular, the monographs addressing titanium dioxide from 2006 and 2010.

In one study conducted via the oral route (NCI, 1979) both rats and mice were used and the other (Bernard, 1990) was conducted only with rats. In both studies the highest dose was 50 000 ppm in the diet, equivalent to 2500 mg/kg bw/d in rats and 7500 mg/kg bw/day in mice. None of the tumours seen were considered by the DS to be treatment-related.

In the multiple promoter studies conducted via the dermal route, no evidence of tumour promotion was seen.

In the studies conducted via inhalation or intra-tracheal administration of TiO2 in rats and/or mice, lung tumours were reported in rats in an overload context. Overload was defined by an impairment of normal pulmonary clearance due to high accumulation of particles.

Benign lung tumours (bronchioalveolar adenomas) were observed in both sexes in rats in the inhalation study by Lee *et al*. (1985) in which micro-sized rutile TiO2 was tested. In the inhalation study conducted in female rats and mice by Heinrich *et al*. (1995), nano-sized anatase/rutile TiO2 induced malignant tumours (squamous cell carcinomas and bronchioalveolar adenocarcinomas) in female rats only. Lesions described as "cystic keratinizing tumours" were also observed in female rats in Heinrich *et al*. (1995), but the relevance to humans was considered unclear. The Heinrich *et al*. (1995) study was of lower quality since it was performed in females only and the concentration level varied during the experiment. However, since the effects were consistent with those of the other studies, they were considered relevant.

After intra-tracheal administration of nano-TiO2 P25 (majority anatase), nano-TiO2 P805 (P25 coated with trimethoxyoctyl-silane, 21 nm) or micro-TiO2 anatase, an increased incidence of benign tumours (adenomas and epitheliomas) and malignant tumours (adenocarcinomas and squamous cell carcinomas) were reported by Pott and Roller (2005). A further study (Xu, 2010) reported that TiO2 treatment significantly increased the multiplicity of DHPN-induced alveolar cell hyperplasias and adenomas in the lung, and the multiplicity of mammary adenocarcinomas. Alveolar proliferative lesions were not observed in rats receiving TiO2 treatment without prior DHPN treatment, although slight inflammatory lesions were noted. As there was only little experience with this model, the DS considered that this effect needed to beconsidered with caution.

Concerning the mode of action, the DS considered that the main mechanism to explain the effects induced by TiO2, in common with effects seen with other substances, was inflammation and an indirect genotoxic effect through ROS production arising from the biopersistence and insolubility of all forms of TiO2 particles. However, the DS could also not exclude a direct interaction with DNA, since TiO2 was found in the cell nucleus in various *in vitro* and *in vivo* studies. The DS also noted that some substance characteristics (in particular shape and coating) might also lead to more potent carcinogenicity or to other specific lesions via a specific mode of action.

The DS noted that data from humans do not suggest an association between occupational exposure to TiO2 and risk for cancer. However, all these studies have methodological limitations and the reported levels of exposure are debatable.

The DS justified classification as Carc. Cat 1B – H350i for TiO2 on the basis that there was an increase in the incidence in both malignant and benign lung tumours in one species and these were reported in two studies by inhalation and two studies by instillation after exposure to TiO2. Although malignant tumours were observed only in single sex, it was noted that only females were tested in the studies reporting malignant tumours (Heinrich *et al*., 1995; Pott and Roller, 2005 and Xu, 2010). In Lee *et al*. (1985), both sexes were tested but only benign tumours (bronchioalveolar adenomas) were found (in both sexes). However, considering the type of lung tumours reported and the hypothesized mode of action, sex-specificity was not expected by the DS. Regarding the observed species difference in Heinrich *et al*. (1995), the DS considered that there may be a species difference in sensitivity to oxidative damage and/or in clearance efficiency, but there was only one study assessing carcinogenic effect of TiO2 (nano anatase/rutile P25) in mice and the high background tumour response in the control group might have limited the ability to detect any carcinogenic effects in this study. Regarding the human relevance of the observed tumours, the DS noted that although in studies with other (non-TiO2) inhaled particles inter-species variability was found in particle retention, lung overload can also occur in humans, in particular in workers exposed to high dust concentrations. Furthermore, the DS concluded that lung retention and chronic pulmonary inflammation occurring in humans were consistent with the findings in rats. According to the DS, a direct genotoxic mechanism was also possible.

Altogether, the DS concluded that there was not sufficient justification to consider the carcinogenic effects as not relevant to humans.

The DS concluded that since the data provided cannot distinguish whether a specific form of TiO2 is linked to its toxic effect, this classification should be applied to "titanium dioxide in all phases and phase combinations; particles in all sizes/morphologies".

Classification was proposed only for the inhalation route, because only local tumours were found after respiratory exposure. Insufficient evidence of carcinogenicity was identified by the oral and dermal routes, allowing them to be excluded. The DS noted that the negative results in different carcinogenicity studies might be explained by limited absorption, since the hypothesized mode of action requires sufficient accumulation of particles to induce inflammation and proliferative lesions.

Comments received during public consultation

514 comments were received during the public consultation on the CLH report. Comments were from 5 MSCA, 38 individuals and the remainder from organisations. The analysis by the DS of the issues addressed in the comments is contained in the annex to the RCOM.

Similar comments appeared more than once under different headings/endpoints in the RCOM and the DS noted that 132 comments referred to comments from Titanium Dioxide Manufacturers Association (TDMA), Titanium Dioxide Industry Consortium (TDIC) or Verbrand Der Chemischen Industrie (VCI).

Comments from three MSCA addressed substance identity. Two MSCA expressed the view that classification of the different TiO2s should be considered separately. One of these MSCA acknowledged that the lack of information on particle characteristics (size, shape, crystallinity, surface area, coating, purity, etc) used in the different studies limited the value of the data and conclusions. One MSCA pointed out that titanium dioxide may also appear in fibrous form and that such fibres would be assumed to have an asbestos-like action and therefore the carcinogenicity of rigid biodurable WHO fibres is orders of magnitude higher than for granular material. This MSCA also noted that the classification should apply to respirable particles and that use of different coatings or dopants could affect the properties of the material.

All MSCA agreed that no classification was warranted for the dermal and oral routes. Four MSCA broadly agreed with the classification proposed. Two of the MSCA suggested further discussion on the arguments addressing differences in potency between the different forms of TiO2 and the implications of tumours being secondary to chronic inflammation and oxidative stress and overload conditions.

One MSCA considered that due to uncertainties arising from the experimental conditions (test material characterisation, high concentrations, relevance to humans arising from species differences) the criteria for classification as Carc 1B; H350i were not met, but Carc. 2 should be considered.

Extensive comment on the proposal was provided by industry, which were unanimously in favour of no classification and addressed specific points made in the CLH report individually. In particular, industry questioned the need to classify for hazards arising from the physical state of the substance, and did not consider that there was concern for humans based on toxicokinetic reasons (lung burden and overload) and toxicodynamic reasons (overload concept, species differences, negative epidemiology).

Extensive commentary was also provided on the specific studies used to support the proposal. In particular, the high concentrations at which findings of lung tumours were described in the study by Lee *et al*. (250 mg/m³) which "clearly exceeded the maximum tolerable dose (MTD) and therefore it would be inappropriate to be considered as a positive tumour response". Lung tissue data, a NIOSH document and applicability of relevant OECD guidelines/ guidance and ECHA guidance were provided as support. Industry argued that particle-overload related lung tumours in rats were not relevant for human risk assessment following chronic inhalation exposures. Discussion of the identification of the lesions (whether some of these were "proliferative keratin cysts") by various groups of pathologists was also provided.

Industry also considered that intratracheal instillation studies utilised excessive doses or non-standard routes of administration "and should therefore not be considered for classification purposes". Fibrosis and bronchioalveolar hyperplasia were also not considered to be precursors of a carcinogenic response.

A number of MSCA as well as industry provided references to additional publications considered relevant to the proposal. Some of these were addressed in detail in the DS response to the comments. In responding to the comments received during public consultation, in addition to specific responses to some comments, an Annex to the RCOM was prepared in which general comments were provided. The DS also presented a revised proposal for the substance identity in their response.

Specific comments received during public consultation have been considered in detail by RAC under "Assessment and comparison with the classification criteria" (below).

Assessment and comparison with the classification criteria

Introduction

Titanium dioxide refers to a group of inorganic chemicals that have the molecular formula TiO2 and whose structural formula depends on the orientation and ordering of the bonds between the titanium and oxygen atoms. The structural formula includes highly ordered arrangements (crystalline) or highly disordered arrangements (amorphous). For the crystalline TiO2, anatase, rutile, brokelite and monoclinic TiO2(B) are the most common structures available.

In addition to its crystal structure, TiO2 can also be characterised by additional physico-chemical properties i.e. (a) size of primary particles, (b) shape/morphology of primary particles and (c) surface chemistry modified by coating of TiO2 particles. Surface coating is the intentional modification of the particle surface chemistry with a different chemical.

The substance identity reported in the REACH registration dossier covers all TiO2 in all crystal structures, morphologies and surface chemistries. The substance identity included as part of the TiO2 registration dossier under REACH and the identity of TiO2 used to generate experimental (eco-)toxicological data may not be fully equivalent in the absence of specific information.

A harmonised classification for mutagenicity was not proposed because according to the dossier submitter "*the existing data show too many discrepancies that cannot be explained with the current state of the science*" (CLH report). The dossier submitter nevertheless proposed to use the genotoxicity data as supporting evidence for the assessment of carcinogenicity. The CLH report therefore proposes to classify TiO2 with regard to its potential carcinogenicity properties only.

In addition to assessing the reliability, relevance and adequacy of the available experimental data, an additional complexity in the assessment relates to the TiO2 material that has been tested. In particular, granular or spherical nano- and micro-sized particles of TiO2 are considered to share their toxicological properties with substances commonly described as "poorly soluble low toxicity particles" (PSLT particles).

The CLH report specifically refers to the description and evaluation of TiO2-related carcinogenicity data. There are additional references to carcinogenicity data for PSLT particles. However, the PSLT data referred to in the CLH report are selective and are mainly used as supportive evidence for mode-of-action considerations. Because the scope of the CLH proposal is limited to TiO2, there might be relevant PSLT particle data which are not covered in this opinion document. RACs opinion concerns the carcinogenicity of TiO2, and it is not the intention to provide an opinion on the classification of PSLT particles in general, and in particular on their potential carcinogenic properties by inhalation.

Toxicological testing was mainly performed on TiO2 with the rutile or anatase crystal structures, or combinations thereof. However, the description of tested TiO2 is not explicit concerning the shape of the primary particles; based on the available evidence RAC assumes that the tested TiO2 materials are more or less spherical or granular, but do not show a fibrous morphology. The key experimental studies available (repeated dose toxicity and carcinogenicity studies) were performed with nano-sized anatase/rutile particles (15 to 40 nm; Heinrich *et al*., 1995) and micro-sized rutile particles (200 to 300 nm; Lee *et al.,* 1985; Hext *et al*. 2005).

The size distribution of primary particles and the resulting aerosols need to be distinguished: notably nano-sized primary particles tend to agglomerate, thus resulting in aerosol size distributions similar to micro-sized primary particles. Therefore, RAC assumes that aerosols with similar MMADs (mass median aerodynamic diameters), irrespective of the size distribution of primary particles, will result in similar distribution and deposition in the relevant regions of the respiratory tract in the rat when experimentally tested according to OECD test guidelines.

There is no detailed reporting of the type of coating modifying the surface chemistry of tested TiO2 material. According to Warheit *et al*. (2015), virtually all of the key TiO2 inhalation studies have been conducted with "standard reference" particle types with few if any surface treatments.

In brief summary, the available information indicates that: the key carcinogenicity inhalation studies were performed with respirable, non-fibrous, nano- or micro-sized primary anatase/rutile or rutile particles with few if any surface treatments.

Description and assessment of key toxicity data for TiO2

Oral carcinogenicity studies

Fischer 344 rats and B6C3F1 mice (50/sex/group) were administered TiO2 (anatase, size unspecified) in the diet for 103 weeks (before guideline, no GLP status). The top dose corresponded to 2500 mg/kg/d in rats, and 7500 mg/kg/d in mice. The low dose tested in both species was 50% of the high dose level.

Surviving animals were killed at 104 weeks. There was no appreciable effect on the mean body weights of rats and mice. There were no other clinical signs that were considered treatment-related. Survival of rats and male mice were not affected; in female mice a dose-related trend in decreased survival was noted. No treatment-related tumours were reported in male or female mice.

In female rats, the incidence of C-cell adenomas or carcinomas of the thyroid was noted (controls 1/48, low dose 0/47, high dose 6/44). Also in female rats, endometrial stromal polyps of the uterus were seen (controls 7/50, low dose 15/50, high dose 10/50). There was no statistical significance for the endometrial stromal polyps; for the thyroid tumours a trend test was considered positive, while a statistical test for pairwise-comparison was considered negative (statistical tests not specified). The CLH report did not report on tumour rates in male rats. NTP concluded that: "… *under the conditions of this bioassay, TiO2 was not carcinogenic by the oral route of exposure for B6C3F1 mice, but that no firm conclusion can be reached about the possible carcinogenicity of the compound to Fischer 344 rats, at this time*".

There was a second oral carcinogenicity study with a substance mainly composed of 72% mica (a mineral silicate) coated with TiO2 (28%), forming flat platelets with the longest dimension measuring 10 to 35 µm. Fischer 344 rats were tested for up to 130 weeks (diet study, corresponding to a maximum dose of 2500 mg/kg bw/d). Low survival rates (not dose-dependent) were noted; a transient reduction in body weight was observed; the only treatment related clinical sign was silver-coloured faeces. In high dose males, a marginally elevated overall incidence of mononuclear cell leukaemia was reported, but this was judged by the authors to be of no biological significance. The CLH report notes that the authors of the study concluded that there was no evidence of a carcinogenic effect.

RAC recognises that the characterisation of the test substances was limited: in the first study, the size of primary particles was not given (the crystal structure was anatase). The second substance tested was not pure TiO2: the particles consisted of a core of silicates coated with TiO2.

The CLH report referred to various oral absorption studies with nano- and micro-sized TiO2. Reporting of the extent of oral uptake was mainly semi-quantitative; specific percentages of oral uptake generally were not reported. Oral uptake was described as "rather low", "only low to no accumulation", "rather low uptake", "extremely low", "very little". The same conclusion was reported by EFSA (2016): (1) "The absorption of orally administered TiO2 particles (micro- and nanosized) in the gastrointestinal tract is negligible, estimated at most as 0.02-0.1% of the administered dose"; and (2) "No difference is observed in the absorption, distribution and excretion of orally administered micro- and nanosized TiO2 particles."

The dossier submitter concluded that a carcinogenicity concern after oral exposure to TiO2 had not been identified.

Dermal carcinogenicity studies

Guideline-compliant dermal carcinogenicity studies with TiO2 were not available. The dossier submitter reported on the results of five studies with a two-stage skin carcinogenesis testing protocol. Both wild-type and transgenic strains of rats and mice were tested. In all the studies, the substance DMBA was used as a carcinogenic initiator.

In all study reports it was concluded that TiO2 showed no promotor potential following dermal exposure. In some of the two-stage carcinogenesis studies, TiO2 was tested without applying the initiating agent. In these study groups there was no indication of dermal carcinogenicity potential of TiO2.

The dossier submitter concluded that there is no concern as to dermal carcinogenicity of TiO2.

Inhalation carcinogenicity studies

The CLH report summarised two publications reporting an increased incidence of lung tumours in rats following chronic inhalation of TiO2 (Lee *et al*., 1985 and Heinrich *et al*., 1995). In addition, 2 negative rat inhalation studies were summarised (Muhle, 1989 and Thyssen, 1978).

Diagnostic terms for cystic keratinizing lesions in the rat lung

Exposure to high air-borne concentrations of TiO2 particles and to other PSLT particles partly resulted in lung lesions generally described as keratinizing cysts of different morphological stages. Over the last three decades, a confusing variety of diagnostic terms have been applied to these lesions, mainly due to evolving diagnostic methods and scientific understanding of the aetiology, biological relevance and development of these lesions. Two international workshops gathering toxicological pathologists (Boorman *et al*. 1996) addressed the morphology of these lesions to reach a consensus on suitable descriptive diagnostic terms and possibly the biological relevance of these specific rat lesions for humans. At these workshops it was agreed that these cystic keratinizing lesions can be placed in 4 groups, based on their stage of development, as follows (Boorman *et al*. 1996):

1) squamous metaplasia with marked keratinization (non-neoplastic lesion)
2) pulmonary keratinizing cysts (not yet considered as neoplasms)
3) cystic keratinizing epithelioma (benign tumours)
4) cystic keratinizing pulmonary squamous cell carcinoma (malignant tumours).

The cystic keratinizing lesions tend to occur late in these experimental rat studies, rarely before 20 months of exposure, and are generally found in female rats exposed to PSLT particles by inhalation; these lesions are uncommon in male rats.

Lung tumours diagnosed in Lee *et al*. (1985) were re-evaluated based on the revised diagnostic terms by Warheit and Frame (2006). In comments received during public consultation it was claimed that evaluation of cystic keratinizing lung lesions in the Heinrich *et al*. (1995) study did not consider the revised diagnostic terms. RAC notes that the pathological diagnosis of lung tumours in the study of Heinrich *et al*. (1995) was also adapted according to the revised diagnostic terms (Rittinghausen *et al*. 1997). Thus for both rat inhalation studies, the reporting of the cystic keratinizing lung lesions complies with the revised classification scheme and terminology.

Inhalation studies reporting no tumours

- *Muhle (1989) (TiO2)*

In the Muhle (1989) study, rutile TiO2 (MMAD about 1.1 µm) was tested. Male and female F344 rats (50/sex/group) were exposed for 6h/day, 5 days/week to 5 mg TiO2/m³ for 24 months. The animals were kept without further exposure for an additional 1.5-month observation period. Minimal bronchoalveolar hyperplasia and fibrotic reactions, but no TiO2-related carcinogenic lesions were reported for this relatively low TiO2 exposure level.

- *Thyssen (1978) (TiO2)*

In the rat inhalation study of Thyssen (1978), an unspecified form of TiO2 was tested for the relatively short exposure duration of 12 weeks (the total study duration was 140 weeks). Animals were exposed to 16 mg/m³ (6h/day, 5 days/week). No treatment-related carcinogenic lesions were observed.

Assessment of studies reporting tumours

- *Lee et al. 1985 (TiO2)*

In Lee *et al*. (1985) male and female CD rats were exposed to rutile TiO2 (purity 99.0%) with an MMAD of 1.5-1.7 µm. The test material had a spherical configuration. Although the size of the primary particles was not reported, it can be however assumed that primary particles of the rutile TiO2 were in the range of 200 to 300 nm (Hext *et al*. 2005). Animals were exposed for 6 hours/day and 5 days/week for up to 2 years. Animals were killed and examined at the end of the 2-year exposure period. Three concentrations were tested: control, 10, 50 and 250 mg/m³. The test protocol of Lee *et al*. (1985) was considered comparable to current OECD guidelines by the dossier submitter (reliability 2).

No abnormal clinical signs, body weight changes or excess mortality in any exposed group were reported. Pathological lesions were restricted to the respiratory tract including the thoracic lymph nodes. Starting at the low dose level of 10 mg/m³ there was a concentration-dependent observation of white foci at the lung surface. Lung weights at 10 mg/m³ were comparable to those of the control group; at 50 mg/m³ there was a significant increase in lung weights; at 250 mg/m³ the lung weights were more than twice the weight of control lungs (male rats: 3.25 vs 7.84 g, female rats: 2.35 vs 7.21 g). The TiO2 lung burden at the end of the study was 26.5 mg/lung at 10 mg/m³, 124 mg/lung at 50 mg/m³ and 665 mg/lung at 250 mg/m³ (Lee *et al*. 1986).

The main non-neoplastic and neoplastic lesions in the lung are summarized as follows: at 10 mg/m³ the alveoli showed slight cell hyperplasia, at 50 mg/m³ there was the additional observation of alveolar proteinosis, bronchiolarization of alveoli and fibrosis. No increase in lung tumours was observed at 10 or 50 mg/m³. At 250 mg/m³ the incidence and severity of non-neoplastic lesions increased.

Table: Neoplastic lesions in CD rats with revised diagnostic criteria for cystic keratinizing lesions (Lee et al. 1985, Warheit and Frame, 2006)

	Control	**10 mg/m³**	**50 mg/m³**	**250 mg/m³**
Bronchio-alveolar adenoma (no adenocarcinoma)	2/79 (m) 0/77 (f)	1/71 (m) 0/75 (f)	1/75 (m) 0/74 (f)	12/77 (m) 13/74 (f)
Squamous metaplasia (non-neoplastic)	0/79 (m) 0/77 (f)			2/74 (f)
Pulmonary keratin cyst (non-neoplastic)	0/79 (m) 0/77 (f)	1/75 (f)		1/77 (m) 11/74 (f)
Squamous cell carcinoma (poorly keratinizing)	0/79 (m) 0/77 (f)			1/74 (f)

At the highest concentration, an increased incidence of bronchio-alveolar adenoma but no adenocarcinoma was observed. Essentially all of the cystic keratinizing lung lesions at the high dose level were re-diagnosed as non-neoplastic pulmonary keratin cysts (Warheit and Frame, 2006). Important criteria for the diagnosis of cystic keratinizing epithelioma (a benign neoplasm) were consistently not met according to the authors. In one female rat, a poorly keratinizing squamous cell carcinoma was diagnosed. Before revision and harmonisation of the pathological

diagnostic terms had occurred, the non-neoplastic pulmonary keratin cysts were reported as cystic keratinizing squamous cell carcinoma (Lee *et al*. 1985).

Inhalation of high concentrations of TiO2 (four-week exposure, pigment-grade rutile type) resulted in impaired pulmonary clearance mechanisms. Particle retention half-times of 68, 110 and 330 days were reported for the 5, 50 and 250 mg/m³ groups respectively (Warheit *et al*. 1997). Based on subchronic inhalation of pigmentary TiO2 lung retention half-times of 324 and 838 days (exposure levels of 50 and 250 mg/m³) were calculated (Bermudez *et al*. 2002).

- *Heinrich et al. 1995 (TiO2)*

In Heinrich *et al*. (1995), female Wistar rats and female NMRI mice were tested.

Ultrafine TiO2 (P25, Degussa, classical nano-sized TiO2 test material; here without further characterisation of purity) with a primary particle size of 15-40 nm and a composition of ~80% anatase and ~20% rutile was used to generate a test atmosphere with an MMAD of 0.8 µm (GSD of 1.80).

Some exposure conditions in this study were more stringent than in Lee *et al* (1985): the exposure schedule was 18 hours/day and 5 days/week. Exposure was 24 months for rats and 13.5 months for mice; surviving animals were only killed at the end of an additional recovery period of 6 months (rats) or 9.5 months (mice). Only female Wistar rats were exposed both to the control atmosphere and to the single exposure level of 10 mg/m³ (between 7.2 and 14.8 mg/m³).

The test protocol of Heinrich *et al* (1985) was considered by the DS to be of lower reliability (Klimisch reliability score of 3); according to the DS, this low reliability score was specifically based on the lack of a full characterisation of the substance tested and based on the exposure protocol (only one concentration, varying during the experiment, only females treated). In the opinion of RAC, the published results of the Heinrich *et al*. (1995) study are considered to be sufficiently reliable, relevant and adequate for the assessment of the carcinogenic potential of TiO2: the main characteristics of substance identity were reported (P_{25} from Degussa, ~80% anatase and ~20% rutile, primary particle size 15-40 nm) and the variations in the exposure concentrations (7.2 mg/m³ for the first 4 months, followed by 14.8 mg/m³ for 4 months and 9.4 mg/m³ for an additional 16 months) are not considered to compromise the basic results in female rats tested. In an external validation of the study (based on OHAT methodology) the authors acknowledged a "high external and internal validity lending an overall high level of confidence in the available evidence" (Thompson *et al*. 2016).

  - Rats

After 24 months of exposure, there was 60% mortality compared to 42% mortality in controls. At termination the mean body weight of the exposed animals was 365 g compared to a body weight of 417 g of the control rats. TiO2 exposure led to an about 5-fold increase in lung wet weight (from about 1 g in controls to about 5 g in the test group after 24 months of exposure; no relevant lung wet weight changes in controls). The retained particle lung burden was ~ 40 mg TiO2/lung. The overall lung clearance (measured by radioactively labeled test aerosols) was significantly reduced in the test group:

Table: Half-times of TiO2 lung clearance in Heinrich et al. (1995)

Duration of exposure	**Control**	**Test group**
3 months of exposure	61 days	208 days
12 months of exposure	72 days	403 days
18 months of exposure	93 days	357 days
18 months and 3 month of recovery	93 days	368 days

Nearly all test group rats showed broncho-alveolar hyperplasia and developed slight to moderate interstitial fibrosis in the lungs. These non-neoplastic lesions were already reported after 6 month of exposure. There were no lung tumours in 20 satellite rats exposed to TiO2 after 6 and 12 months of exposure.

Two histological types of rat lung tumours were observed: there was a 13/100 incidence of adenocarcinoma in test animals (versus 1/217 in controls). The original findings of cystic keratinizing lesions were re-evaluated based on the already mentioned revised diagnostic criteria and classification: following this reevaluation, the incidence in female rats with cystic keratinizing epitheliomas (considered to be benign tumours) was 16/100, the incidence of squamous cell carcinomas was 4/100 (including 3/100 cystic keratinizing squamous cell carcinoma; Rittinghausen *et al* 1997).

Table: Key lung neoplastic lesions in female Wistar rats with revised diagnostic criteria for cystic keratinizing lesions (Heinrich et al. 1995, Rittinghausen et al. 1997)

Neoplastic lesions	**Control**	**Female Wistar rats** **10 mg/m³**
Adenoma	0/217	4/100
Adenocarcinoma	1/217	**13**/100 ($p<0.05$)
Cystic keratinizing epitheliomas (benign tumours)	no corresponding lesions reported	16/100
Squamous cell carcinomas	no corresponding lesions reported	4/100 (including 3 animals with cystic keratinizing squamous cell carcinoma)

- Mice

In female NMRI mice lung tumour rates in the test group (13.8%, adenomas and adenocarcinomas) were not significantly different from the tumour rate of the control animals (30%). Treatment was however for a much shorter period (13.5 months) than for the female rats.

Comparison of key carcinogenicity studies by inhalation (TiO2)

Because of the specific importance of these two key carcinogenicity studies (Lee *et al*, 1985 and Heinrich *et al*, 1995) for classification of TiO2, their main results are further contrasted in tabular form (see the table below). The following points were noted by RAC:

Both fine and ultrafine TiO2 were tested for carcinogenicity. The ultrafine (or nano-scale) TiO2 had a primary particle size of 15-40 nm. The primary particle size of fine rutile particles tested was 200 to 300 nm. Irrespective of the different sizes of the primary particles tested, the MMADs were rather similar: fine rutile particles had an MMAD of 1.5-1.7 µm; ultrafine TiO2 had an MMAD of 0.8 µm.

Although there was a 2-year exposure period in both studies, the animals exposed to fine rutile particles were examined directly following this exposure period, while the animals exposed to ultrafine TiO2 particles were examined following the additional exposure-free period of 6 months.

Exposure levels associated with neoplastic findings are rather high: lung clearance half-times in both studies estimated to be about 1 year (Warheit et al. 1997) indicate a rather substantial slowing down of physiological lung clearance rates. Under these overload conditions, two different histological types of lung tumours developed. In the Lee *et al*. (1985) study, benign tumours (bronchio-alveolar adenoma and one poorly keratinizing squamous cell carcinoma) were observed. In the Heinrich *et al*. (1995) study both adenocarcinomas and (cystic keratinizing) squamous cell carcinomas (in addition to cystic keratinizing epitheliomas) were observed.

Table: Comparison of results of the two key inhalation carcinogenicity studies

	Heinrich *et al.* (1995)	**Lee *et al.* (1985)**
Species tested	Female Wistar rat	Male and female CD rat
Substance identity	80% anatase and 20% rutile	Rutile
Primary particles	15 to 40 nm	200 to 300 nm
MMAD	0.8 µm	1.5 -1.7 µm
Exposure schedule	18 h/day, 5 days/week	6h/day, 5 days/week
Duration of exposure	24 months	24 months
Time-point of final section	30 months	24 months
Concentration	~10 mg/m³	10, 50 and 250 mg/m³
TiO2 lung burden after 24 months	~ 40 mg/lung	26.5 mg/lung (10 mg/m³) 124 mg/lung (50 mg/m³) 665 mg/lung (250 mg/m³)
Lung clearance half-times	61-93 days in controls 368 days after recovery period	330 days at 250 mg/m³ (Warheit *et al*. 1997)
Neoplastic and non-neoplastic findings:	**At 10 mg/m³:**	**At 250 mg/m³:**
Bronchio-alveolar adenoma	4/100 (0/217) f	12/77 (2/79) m 13/74 (0/77) f
Adenocarcinoma	13/100 (1/217) f	-
Squamous metaplasia	-	2/74 (0/79) f

Pulmonary keratinizing cysts (non-neoplastic)		11/74 (0/77) f 1/77 (0/79) m
Cystic keratinizing epitheliomas (benign)	16/100 f (no lesions in controls)	-
Squamous cell carcinomas	4/100 f (no lesions in controls) (including 3 animals with cystic keratinizing squamous cell carcinoma)	1/74 (0/79) f

Note: control incidences in brackets; "-" = none reported

Although it is known that PSLT-induced tumours appear late in the rat (Gebel, 2012), malignant tumours in the Heinrich *et al*. (1995) study were already found after 18 months and at termination of exposure (24 months) (see Table below).

Table: Lung tumors (18 and 24 months of study) in Heinrich et al. (1995)

Study month	**control**	**Incidence at 10 mg/m³ TiO2**
18 (interim sacrifice)	0/18	2/20 adenocarcinoma
		3/20 squamous cell carcinoma
24	0/10	1/9 adenocarcinoma
		2/9 squamous cell carcinoma

Rat lung tumours caused by other PSLT particles

The range of tumour phenotypes observed with TiO2 was similar to that reported for other PSLT substances (Nikula, 2000).

Intra-tracheal instillation of TiO2 (Pott and Roller, 2005)

The dossier submitter presented TiO2 instillation studies as supportive evidence for the carcinogenic effects of inhaled TiO2. Intratracheal instillation is not a realistic route of exposure; this exposure regime bypasses the upper respiratory tract and may produce non-physiological patterns of deposition and clearance. Intratracheal instillation delivers a bolus dose into the lung in a relatively short period of time.

In the study by Pott and Roller (2005), female Wistar rats received up to 30 instillations of suspended particles of different types of TiO2 at weekly intervals. The study was terminated after 30 months. In the controls no primary lung tumours were diagnosed. The dose levels tested with nano-sized (20 to 30 nm) and micro-sized (200 nm) anatase particles resulted in lung tumour incidences from about 50 to 70% for the nano-sized anatase particles, and from about 30 to 60% for the micro-sized anatase particles. The dossier submitter stated that treatment with both types of TiO2 resulted in an increase of benign tumours (adenomas and epitheliomas) and an increase in malignant tumours (adenocarcinomas and squamous cell carcinomas).

Intratracheal instillation did not result in respiratory tract tumours in Syrian golden hamsters (the reference in the CLH report was to IARC monograph 93). The chronic dose schedule was similar to the dosing schedule in the Pott and Roller (2005) rat studies. However, the treated

hamster group had a markedly decreased lifespan of 70-80 weeks, as a result of which the detection of late lung tumours was impaired.

Exposure conditions in key TiO2 inhalation studies

Neoplastic lung lesions in the Lee *et al* (1985) study with micro-sized rutile particles of TiO2 were only observed at the high air-borne concentration of 250 mg/m³. No increased tumour incidences were observed at 50 mg/m³. The TiO2 lung burden after 24 months was ~ 665 mg/lung at 250 mg/m³, 124 mg/lung at 50 mg/m³ and 26.5 mg/lung at 10 mg/m³. Corresponding lung clearance rates were not reported, but these are available from other studies. Deposited TiO2 (four-week exposure, pigment-grade rutile type, size of primary particles: 200 to 300 nm) was found to be cleared from the lungs with half-times of 68, 110 and 330 days for the 5, 50 and 250 mg/m³ groups respectively (Warheit *et al*. 1997). Furthermore, pigmentary TiO2 was administered to rats for 13 weeks (0, 10, 50 and 250 mg/m³). Lung clearance half-times reported were 100 days at 10 mg/m³, 324 days at 50 mg/m³ and 838 days at 250 mg/m³ (Bermudez *et al*. 2002).

Neoplastic lung lesions in the Heinrich *et al*. (1995) study (nano-sized TiO2) were reported at the tested dose level of 10 mg/m³. The TiO2 lung burden after 24 months was ~ 40 mg/lung. The lung clearance half-time at this exposure level was 368 days compared to ~ 60 to 90 days in controls.

Comments received during public consultation emphasised the view that the experimental exposure level of 250 mg/m³ in Lee *et al*. (1985) clearly exceeded the MTD and therefore the corresponding tumour data would be inappropriate to be considered as a positive tumour response.

RAC specifically refers to the Guidance on the Application of the CLP Criteria (2015) and the OECD Guidance Document (GD) 116 (2012). The CLP Guidance proposes that testing protocols should maximise the sensitivity of the test without significantly altering the accuracy and interpretability of the biological data observed. Generally the MTD is defined as the highest dose to produce toxic effects without causing death and at which decreases in body weight gain of no more than 10% relative to controls are observed. The CLP Guidance refers to overload conditions in general terms, stating that the "relevance of lung overload in animals to humans is currently not clear and is subject to continued scientific debate" (section 3.9.2.5.3., page 470 of the CLP Guidance). In OECD GD 116 it is noted that inhalation of doses that overwhelm pulmonary clearance may lead to tissue responses that are specific to the tested species. It is proposed that for substances with poor solubility the degree of lung overload and the delay in clearance needs to be estimated. There is the recommendation that the "use of concentrations exceeding an elimination half-time of approximately 1 year due to lung overload at the end of the study is discouraged." It needs to be emphasized however, that there is no specific justification for this concrete definition. More information on the conduct of acute and repeated dose inhalation studies is given in OECD GD 39 (2009); in this guidance no recommendation is given on a maximum lung clearance half-time. It is however expressed that (1) "the study design of repeated inhalation studies … need to reveal and quantify the clearance of test articles" and (2) "exposure concentrations should be selected to cover the entire range of lung burdens, i.e. those which do not delay clearance to those which do delay clearance." (OECD GD 39, page 44).

The Draft ECHA Guidance on Nanomaterials (2016) gives specific advice on testing PSLT particles for repeated dose toxicity. For PSLT particles the rat lung burden is considered to be an important issue when assessing the toxicological outcome of an inhalation study. It was concluded that "lung effects observed in animals exposed to PSP by inhalation should be considered relevant for humans unless it can be clearly substantiated otherwise" (page 15). There is no specific

recommendation as to the level of overload that might compromise the relevance of the corresponding toxicological outcome for humans.

RAC acknowledges that rat tumours only developed under conditions of marked reduction of lung clearance. Corresponding lung clearance half-times reached and exceeded 1 year.

RAC notes the overload concept proposed by Morrow (1988 and 1992). Whereas it might not be a generally accepted concept, it might assist in a constructive discussion of the term "overload": Long-term exposure to increasing exposure levels of PSLT particles leads to increasing pulmonary dust burdens whereby the clearance of deposited particles by alveolar macrophages becomes progressively reduced to the point that pulmonary clearance essentially ceases. Parallel to these changes alveolar macrophages change their response pattern within the lung. In the rat lung, chronic inflammation is a key consequence of increased particle lung burdens. Morrow (1988 and 1992) developed an overload concept relating the extent of lung dust burden and reduction of alveolar clearance with the extent of the volumetric loading of alveolar macrophages. According to Morrow (1988 and 1992) increasing inhalation exposure to particles "brings about a significant prolongation of particle clearance" if 6% of the volume of alveolar macrophages is occupied by particles. When volumetric loading of alveolar macrophages reaches about 60%, "pulmonary dust clearance appeared to cease almost completely" (Morrow 1992).

Morrow roughly estimated the levels of lung burdens related to the 6% and 60% particle volume loading of alveolar macrophages (AM): he calculated a total AM pool volume of 25 mm^3 in a rat lung of about 1.5 g (about 2.5 x 10E+7 alveolar macrophages with an AM volume of about 1000 μm^3). A volume of 6% of the AM pool then corresponds to 1.5 mm^3 or 1.5 mg of particles with unit density (AM volume loading of 60% corresponds to 15 mm^3 or 15 mg of particles with unit density).

Table: Substance-specific lung burdens and degree of AM particle volume loading

Density of substance	**Substance-specific lung burden in the rat lung corresponding to a 6% volume loading of alveolar macrophages**	**Substance-specific lung burden in the rat lung corresponding to a 60% volume loading of alveolar macrophages**
1	1.5 mg/rat lung	15 mg / rat lung
1.5 (e.g. coal dust)	2.3 mg / rat lung	23 mg / rat lung
2.7 (talc)	4.1 mg / rat lung	41 mg / rat lung
4.3 (TiO2)	6.5 mg / rat lung	65 mg / rat lung

If one follows this concept for TiO2 with a density of 4.3, a TiO2 lung burden range between 6.5 mg and 65 mg per rat lung would cover a range from significant prolongation to an almost complete cessation of alveolar clearance. As a rule of thumb, for TiO2 with a density of 4.3, the numerical value of the TiO2 rat lung burden is identical to the numerical value of the percentage of AM particle volume loading.

Table: Lung burden and particle volume loading in key inhalation studies

	Heinrich *et al*. 1995	**Lee *et al*. 1985**
Exposure levels	~10 mg/m³	10, 50 and 250 mg/m³
TiO2 lung burden after 24 months	~ 40 mg/lung	26.5 mg/lung (10 mg/m³) 124 mg/lung (50 mg/m³) 665 mg/lung (250 mg/m³)
Particle volume loading in alveolar macrophages	~ 40%	A 60% volume loading is already reached between 10 and 50 mg/m³

Based on this overload concept of Morrow (1988 and 1992) the TiO2 lung burden in Heinrich *et al*. (1995) (40 mg/lung after 24 months) corresponds to an AM particle volume loading of 40% (ie. below the 60% level for almost complete cessation of alveolar clearance). In the Lee *et al*. (1985 study) the corresponding lung burdens at 50 mg/m³ and 250 mg/m³ (with 124 and 665 mg/lung) both exceeded the 60% level for the almost complete cessation of alveolar clearance.

Criteria to agree on a specific guidance level for the maximum reduction of lung clearance in experimental testing are complex in nature. Evidence indicates that highly exposed coal miners experience particle lung burdens that can be reached in the rat lung only under conditions of a marked degree of overloading (Kuempel *et al*. 2009 and 2014): historically lung burdens in coal miners in the range of 10-20 mg/g lung were reported (it was not reported whether coal miners with these lung burdens developed pneumoconiosis). Referring to the Morrow overload concept (as described above) the 6 to 60% range for AM particle volume loading in the rat corresponds to a lung burden of 2.3 to 23 mg coal dust per rat lung (or ~1.5 to 15 mg coal dust per g rat lung). With reference to these considerations, the high lung burden of 20 mg/g lung in coal miners can be reached in rats only under conditions of an almost complete inhibition of lung clearance (15 mg/ g rat lung corresponds to a 60% particle volume loading). Part of an explanation for these relationships may be that lung clearance of particles in humans is considerably slower than in rats (Gregoratto et al. 2010). If particle lung burden can be considered a relevant dose metric, these considerations indicate that experimental testing of PSLT particles in the rat under exposure conditions strictly avoiding a significant degree of overload is not justified. In view of this, RAC acknowledges that high particle loading in rats is a condition that can not be dismissed in the classification of TiO2.

Genotoxicity of TiO2

The dossier submitter did not propose a harmonised classification for the hazard class "germ cell mutagenicity" because in their opinion the existing mutagenicity data showed too many discrepancies which could not be adequately explained. Nevertheless, the dossier submitter decided to use the genotoxicity data as one of the parameters relevant for a mode-of-action discussion on carcinogenicity.

The dossier submitter noted that particle-related inflammatory reactions resulting in oxidative stress can be considered the main pathway explaining positive genotoxic results obtained with TiO2. Although they considered primarily this indirect mode of action as the mechanism of genotoxicity, the dossier submitter did not exclude a direct genotoxic effect. The DS indicated that TiO2 was found in the cell nucleus in some *in vitro* and *in vivo* studies and thus it might directly interact with DNA. However, the dossier submitter also noted that accumulation of TiO2

in the nucleus had not been systematically investigated and when reported, it was not quantified. Comments received during public consultation questioned the dossier submitter's statement that TiO2 might reach the nucleus and cause primary genotoxicity.

Reporting of the study-specific results in the CLH report was generally limited; there was no detailed study-specific information on parameters related to a possible mechanism of genotoxicity. The way the genotoxicity data were presented in the CLH report did not enable RAC to form an independent opinion on the dossier submitter's conclusion on genotoxicity.

The opinion of the dossier submitter, which considered oxidative stress as the main pathway explaining positive genotoxic results obtained with TiO2, is supported by a recent review on the genotoxicity of titanium dioxide nanoparticles: "Current data indicate that the genotoxicity of TiO2 NPs is mediated mainly through the generation of oxidative stress in cells" (Chen *et al*. 2014).

RAC is expected to appropriately take into account scientific opinions of other European committees/agencies. In 2016 EFSA published their "Reevaluation of titanium dioxide (E 171) as a food additive" The EFSA document contains a thorough and detailed summary and discussion of TiO2 genotoxicity data. Much emphasis was placed on a study-specific discussion of the reliability and relevance of the data, including the possible mechanism of genotoxic action. The Panel concluded that "orally ingested TiO2 particles (micro- and nanosized) are unlikely to represent a genotoxic hazard *in vivo*."

Based on the reviews referred to (CLH report, Chen *et al*. 2014, EFSA 2016) RAC considers it justified to assume that oxidative stress plays a central role in TiO2 genotoxicity.

For the purposes of this TiO2 carcinogenicity assessment, RAC considered possible genotoxicity (and carcinogenicity) in the rat lung as a direct consequence of inflammatory reactions resulting in an increased tissue level of reactive oxygen species. This approach is specifically supported by the findings of Driscoll *et al*. (1997) (see next chapter).

Mode of action in rats

TiO2 particles (without WHO fibre characteristics or surface coating resulting in specific toxicity) are considered to be "poorly soluble low toxicity particles" (PSLT). Inhaled respirable TiO2 particles are phagocytosed by alveolar macrophages. Morrow (1988) proposed that when the macrophage intracellular volume of ingested particles becomes greater than 6% of the cell volume, macrophage mobility slows; when particle content in the macrophages reaches 60% of the macrophage volume , movement of macrophages ceases. High particle volume loading of macrophages is considered responsible for the elicitation of alveolar chronic inflammatory reactions with oxidative stress, formation of reactive oxygen species (ROS) and cytotoxicity. As a consequence, secondary mutagenicity is likely to occur.

Driscoll *et al*. (1997) published a corresponding key study: for rats exposed to poorly soluble particles (quartz, carbon black and micro-sized titanium dioxide) by intratracheal instillation the relationships between particle exposure, inflammation and mutagenesis in rat alveolar type II cells were characterised. The studies demonstrated that *in vivo* exposure to particle doses eliciting a neutrophilic inflammatory response (verified by bronchoalveolar lavage) resulted in increased mutation in rat alveolar type II cells. The mutagenic effect was observed for all three materials and was dose-related.

The relative potency of these materials in causing *in vivo* mutation in lung cells varied (the lowest mutation frequency was seen with TiO2) and corresponded to their relative *in vivo* neutrophilic

inflammatory activity. The potential contribution of lung inflammatory cells to *in vivo* mutagenic responses was evaluated by co-culturing *in vivo* particle-elicited BAL cells with a rat alveolar epithelial cell line *in vitro*. Particle-elicited BAL cells were shown to exert a mutagenic effect on these epithelial lung cells *in vitro*. The addition of catalase inhibited the BAL cell-related increase in mutations which the authors considered as an indication that reactive oxygen species are required for the mutagenic activity of particle-elicited rat lung inflammatory cells.

Histological investigations in rats showed the occurrence of alveolar hypertrophy and hyperplasia of type II alveolar cells surrounding clusters of particle-laden macrophages. Hyperplastic reactions seem to be more prominent in alveoli compared to terminal bronchiolar cells. There was evidence of lipoproteinosis in the alveolar lumen. Alveolar metaplasia will eventually result in tumour development in the rat (adenocarcinoma and cystic keratinizing squamous cell carcinoma). With increasing doses, translocation of particle-laden macrophages to the lung interstitium increased and interstitial fibrosis developed as well.

Tumour development is not considered to be triggered by direct contact of TiO2 with epithelial lung cells, but by high particle loading of macrophages with TiO2 particles and subsequent modification of macrophage activity, essentially resulting in marked and sustained inflammatory responses in the lung. RAC considers it plausible to assume a practical threshold for this mode of action.

The information provided to RAC on this mode of action comes partly from studies with TiO2, but also from studies with other PSLT particles (e.g. Lee *et al*. 1985, Heinrich *et al*. 1995, Bermudez *et al*. 2002 and 2004, Warheit *et al*. 1997, Warheit *et al*. 2016).

Interspecies differences and relevance to humans

90-day TiO2 toxicity studies in rats, mice and hamsters (Bermudez et al. 2002 and 2004)

The TiO2 studies by Bermudez *et al*. (2002 and 2004) are directly relevant because they contribute to the understanding of mode of action and enable the assessment of interspecies differences for lung responses in three laboratory rodent species (hamsters, mice and rats).

The main features of the study protocol were as follows: exposure to TiO2 was for 13 weeks (6 h/days, 5 days/week) followed by a recovery period without TiO2 exposure for up to 52 weeks. The main parameters investigated were TiO2 lung burden at different time points, pulmonary inflammation reactions and cytotoxicity via broncho-alveolar lavage, lung cell proliferation (BrdU) and histopathology. TiO2 of both micro- (pigmentary/fine) and nano-(sub-pigmentary/ultra-fine) primary particle sizes were tested.

Pigmentary TiO2 was tested at concentrations up to 250 mg/m³ (0, 10, 50 and 250 mg/m³). Dose-dependent lung retention half-times of TiO2 were 100 days for rats in the low concentration group and 324 days to 838 days in the higher concentration groups. The number of macrophages and the proportion of neutrophils as markers of chronic inflammation along with parameters of cytotoxicity (LDH, total protein) significantly increased in bronchoalveolar lavage (BAL) fluid. The BrdU labelling index in alveolar cells (but not in terminal bronchiolar cells) was significantly increased at the highest concentration. Immediately post-exposure, both mid- and high-dose rats had alveolar hypertrophy and hyperplasia of type II epithelial cells surrounding aggregations of particle-laden macrophages. Infiltration of neutrophils indicated histological evidence of chronic active inflammation. By 52 weeks, post-exposure mid-dose rats additionally showed alveolar metaplasia; high-dose rats developed more severe alveolar type II hypertrophy, hyperplasia and metaplasia and additionally significant alveolar septal fibrosis and

interstitialization of particle-laden macrophages. The alveolar lumens in lesion areas were characterised by lipoproteinosis.

Bermudez *et al*. (2002) compared these responses in rats with pulmonary responses in hamsters and mice: the particle lung retention pattern in hamsters was different to the pattern in mice and rats: the lung retention half-time in hamsters at the high-dose level was lower compared to the other species (110 days compared to 621 days in mice and 838 days in rats). Correspondingly, parameters of chronic inflammation rapidly declined in hamsters compared to mice and rats. Significant lung cell replication (BrdU) was only measured in high-dose rats, not in mice and hamsters. There was minimal type II alveolar hyperplasia in hamsters, but not in mice. The most marked difference between hamsters and mice compared to rats was that alveolar metaplasia and fibro-proliferative changes were exclusively observed in high-dose rats with a retention half-time of 838 days.

Similar investigations were performed with nano-sized TiO2 (Bermudez *et al*. 2004). The primary particle size was approximately 20 nm. The aerosol generated was made up of particle agglomerates with an MMAD of 1.37 µm (similar to the micro-sized TiO2 tested). The maximum concentration tested was 10 mg/m³ (compared to 250 mg/m³ of the micro-sized TiO2). The high dose level of the nanomaterial indicated substantial retardation of alveolar clearance, with lung clearance half-times around 50% of the lung clearance half-times for the high dose micro-sized material (in rats, mice and hamsters; according to the summary of lung clearance rates in Hext *et al*. 2005). According to the authors (Bermudez *et al*. 2004), the toxicity profile of the nanoparticles was consistent with the profile obtained with the micro-sized particles (Bermudez *et al*. 2002).

In comparing these subchronic studies, it is evident that for both nano- and micro-sized TiO2 there is a higher sensitivity of rats compared to mice and hamsters. At high concentrations of nano- and micro-sized TiO2, alveolar metaplasia and fibro-proliferative changes were only observed in rats, not in mice and hamsters. Particle retention patterns and pulmonary responses were different in these rodent species (for mice and hamsters robust carcinogenicity data are not available).

Supporting evidence from PSLT particle data: rats versus monkeys (Nikula et al. 1997)

In comments received during public consultation it was additionally proposed that reference should be made to publications on interspecies differences related to other PSLT particles. The publication by Nikula *et al*. 1997 is one of the corresponding key studies. Male cynomolgus monkeys and F344 rats were exposed to 2 mg/m³ of respirable particles of coal dust (and to diesel exhaust soot as well) for 24 months (7h/day, 5 days/week; for the monkeys an exposure duration of 24 months does not correspond to a lifetime study).

At these identical air-borne concentrations, relatively more particulate material was retained in the lungs of monkeys than in rat lungs. The volume percentage of lung occupied by particulate material was about 0.3% in the rat and about 1% in the monkey. However, the compartmental coal dust retention pattern in the lung was different in rats and monkeys: Rats retained a greater portion of coal dust in lumens of alveolar ducts and alveoli than monkeys. In contrast, monkeys retained a greater portion of the particulate material in the interstitium than rats. The retention percentages for the alveolar versus interstitial compartments was 73% versus 27% in rats and 47% versus 53% in monkeys. The different retention sites are assumed to be linked to differences in sensitivity for tumour development; see chapters below.

The corresponding histopathological findings in rats and monkeys are summarised in the following table (for a complete picture see tables 2 and 3 in Nikula *et al*. 1997). The differences

observed in pulmonary responses between rats and monkeys should be interpreted with caution in view of the weak responses observed in the rats. Indeed, less than minimal inflammation was observed in 7/15 animals and less than minimal septal fibrotic reactions was observed in 4/15 animals (see Table below).

Table: Pulmonary responses to identical low concentrations of coal dust in rats and cynomolgus monkeys (Nikula et al. 1997)

	Rat Control	**Rat 2mg/m³**	**Monkey Control**	**Monkey 2 mg/m³**
Type of lung pathology and average grading scores				
Number of animals examined	15	15	14	14
Alveolar macrophage hyperplasia	1(1)	15(1.5)	2(1.0)	14(1.4)
Alveolar epithelial hyperplasia	1(1)	14(1.5)	2(1.5)	3(1.0)
Particle-associated inflammation	0(-)	7(1.0)	1(1.0)	4(1.0)
Septal fibrotic reaction	0(-)	4(1.0)	3(1.3)	0(-)
Alveolar proteinosis	0(-)	2(1.0)	0(-)	0(-)

Footnote: The first number is the number of animals with the findings. The number in parentheses is the average severity score which ranges from 1 (slight) to 5 (marked). Score 2 means minimal.

Overall, RAC recognises that in the monkeys there was still a uniform distribution of particles in alveolar spaces and interstitium of the lung. As compared to the rather weak effect profile in rats (at the dose level tested), a weaker response in monkeys is not considered by RAC sufficient reasoning for claiming alveolar findings to be unique to the rat.

Supporting evidence from PSLT particle data: particle retention pattern in rats and humans (Nikula et al. 2001)

Nikula *et al*. (2001) extended their morphometric lung examinations of particle retention patterns to humans. The authors compared the retention pattern within the compartments of the lung (parenchymal lumen versus interstitium) of Diesel exhaust soot in rats and of coal dust in humans. In this study, lungs from 5 control persons and from 11 low-dose miners and 5 high-dose coal miners were examined for compartmental particle location in the lung.

The major compartment both in the human and rat lung was the alveolar parenchyma with roughly about 85% and the interstitium with roughly about 15% of the total lung volume. About 80% of diesel soot particles were retained in the rat parenchymal lumen (compared to about 20% in the interstitium). In low-dose miners, nearly 70% of coal dust particles were retained in the interstitium (compared to about 30% in the parenchymal lumen). The few data available indicate an even higher percentage in the human lung interstitium for the high-dose miners.

The authors finally calculated a "relative compartmental retention", a lung burden parameter, indicating the density of packaging of particulates in a specific compartment. The relative compartmental retention (figure 7 in Nikula *et al*. 2001) of Diesel soot particles was slightly higher in rat parenchyma than in the rat interstitium (about 1.2 versus 0.8 at the high-dose level). In coal dust miners the particulate material was more densely packed in the interstitium

(relative compartmental retention of about 2 in the interstitium versus about 0.5 in the parenchymal lumens; low-dose miners; figure 8 in Nikula *et al*. 2001).

The authors discuss that these different retention patterns for Diesel exhaust soot in rats and coal dust in humans could mean that different lung cells are predominantly in contact with the particles in humans and rats, which suggests that PSLT particle responses may differ between the two species.

- *Dosimetric modelling of human retention patterns (Gregoratto et al. 2010)*

Gregoratto *et al*. (2010) refined the Kuempel *et al*. (2001) lung retention model based on coal miner data including further data from human lung retention studies after the inhalation of radioactive particles. The Gregoratto dosimetry model predicts a clearance from the alveolar space to the mucociliary escalator for 60% of alveolar deposit (T1/2 = 400 days) and a clearance from the alveolar space to the interstitium for 40% of alveolar deposits (with T1/2 of 700 days).

- *Supporting evidence from PSLT particle data: Review on the overload concept by Warheit et al. 2015*

The differences in lung distribution patterns between species is considered to be one reason for the differing pulmonary responses among the several species studied. While inhaled particles are predominantly retained in the alveolar duct compartments of rats, resulting in the rat lung tumours observed, in humans there is a greater tendency of transmigration of alveolar deposits to interstitial sites of the lung. A relatively lower particle load in human alveolar spaces is considered to result in a lower extent of alveolar hyperinflammatory responses. Some authors (e.g. Warheit *et al*. 2015) propose to assume that the interstitialisation of particles appear to serve as a repository in humans which is deemed to be less reactive as to tumour development. According to the authors coal workers exposed to high concentrations may develop interstitial-based progressive fibrosis, but possibly less or no risk of developing pulmonary tumours.

- *Human non-neoplastic lung responses due to PSLT particle exposure by inhalation (Schultz 1996, Green 2007, NIOSH 2011 and ECETOC 2013)*

Schultz (1996) compared the pathology of dust-induced pulmonary lesions in rats and humans. He stated that "lung particle burdens equivalent to those producing overload in rats have occurred in coal workers". Furthermore, he concluded that "lesions commonly seen in overload studies in rats, such as marked accumulation of alveolar macrophages, inflammation, necrosis of pneumocytes, alveolar proteinosis, and cholesterol granulomas, were not present in humans with coal worker's pneumoconiosis."

Significantly, Green *et al* (2007, quoted in NIOSH, 2011) compared the tissue responses in human and rat lungs to a range of poorly soluble particles (coal dust, talc and silica). Criteria for selection of human pathology materials was based on known exposure to dust aerosols, a history of not smoking cigarettes, and a lack of major confounding diseases. Similar criteria were used for selection of rodent pathology material. It is obvious that documentation of exposure to dust aerosols was good in studies with rats and poor in human populations. The cellular responses in the lungs were graded for severity (scores 1 to 4) using a standardized grading system. Key morphological changes were documented. Based on the corresponding bar charts in the original publication the following scores were estimated for the various morphological changes documented for coal dust and talc. Both similarities and differences in response to the same agent were shown. Specifically, acute intra-alveolar inflammation, alveolar epithelia hyperplasia and alveolar lipoproteinosis were all greater in rats than in humans; relevant fibrosis was observed both in rats and humans. The authors indicate that these differences may account for

differences in carcinogenic responses as well. RAC notes that human pathology material still showed low scores for centriacinar alveolar hyperplasia.

Table: tissue responses in human and rat lungs to a range of poorly soluble particles

Scores 1-4		**Coal Low Dose**		**Coal High Dose**		**Talc**	
		Humans	**Rats**	**Humans**	**Rats**	**Humans**	**Rats**
Inflammation, acute,	centriacinar intra-alveolar	< 0.2	0.4	< 0.2	1.1	< 0.2	2.4
Inflammation,	granulomatous nodular	< 0.2	< 0.2	< 0.2	0.3	1.6	1.7
Lipoproteinosis,	alveolar	< 0.2	< 0.2	< 0.2	0.8	< 0.2	3.8
Hyperplasia,	centriacinar, alveolar	0.5	0.6	0.4	2.5	1.1	2.3
Fibrosis,	centriacinar	1.0	< 0.2	1.6	1.4	1.8	2.3

NIOSH (2011) concluded that "collectively, these studies indicate that, while there are uncertainties about the rat as a model for particle-related lung cancer in humans, and specifically for TiO2, there is insufficient evidence for concluding that the rat is not a valid model" (chapter 3.6 in NIOSH 2011). Specifically with respect to non-cancer responses, NIOSH stated that "the rat and human lung responses to poorly soluble particles of low or high toxicity (e.g. coal dust and silica) are qualitatively similar in many of the key steps for which there are data, including pulmonary inflammation, oxidative stress, and alveolar epithelial cell hyperplasia" (again chapter 3.6 in NIOSH 2011).

ECETOC (2013) stated that "that the abundance of available clinical and epidemiological data in occupationally-exposed workers is consistently negative for lung cancer as well as non-neoplastic lung diseases." RAC notes some contradiction in the ECETOC assessment of interspecies non-neoplastic lung responses: stating first that there are no non-neoplastic lung diseases, then describing the basic mechanisms in the etiology of Coal Workers Pneumoconiosis (with direct cytotoxicity, oxidant production by pulmonary phagocytes, mediator release from alveolar macrophages and secretion of growth factors from alveolar macrophages). ECETOC (2013, page 73) concluded: "Chronic particle exposure may cause adverse health effects other than lung cancer in humans, such as systemic inflammation and pneumoconiosis. However, further analysis of these data including toxicokinetic modelling to evaluate hypotheses about relationships between lifetime exposures, retained lung dust, overloading of lung clearance and disease development are recommended."

ECETOC (2013) stated that there is a low severity of pulmonary inflammation in humans. This statement is supported by Morfeld *et al*. (2015): "The PMNs however, play a unique role in rat experiments, findings that do not appear to occur in high dust exposed workers, such as coal miners." A comprehensive review on coal dust toxicity takes a totally different view on the coal dust related inflammation in humans (Attfield in Patty 2012): "Taken together, these data support the hypothesis that initially disease is prevented by the upregulation of protective antioxidants and the downregulation of inflammatory cytokines. Induction and progression of the

disease process are associated with an increase in oxidant generation, loss of antioxidant protection, and progressive pulmonary inflammation and damage."

Overall, RAC notes that a sufficiently detailed and transparent presentation of TiO2- or PSLT-related non-neoplastic lesions in humans is neither provided in the CLH report nor in the references referred to in the comments received during public consultation. The data available to RAC does not provide a sufficiently consistent view. The main concern of RAC is that reporting of dose response relationships for non-neoplastic lesions in humans is essentially lacking. Reporting of human data on non-neoplastic consequences of inhalation exposure to TiO2 and PSLT particles is however considered necessary in order to judge whether the mode-of-action in rats is at least partly operative in humans.

Considerations on human relevance of rat lung tumours

The American Cancer Society considers non-small cell lung cancer the most common type of human lung cancer (percentage of about 85%). Squamous cell carcinoma, adenocarcinoma and large cell carcinoma are all subtypes of non-small cell lung cancer (https://www.cancer.org/cancer/non-small-cell-lung-cancer/about/what-is-non-small-cell-lung-cancer.html).

Maronpot *et al*. (2004) compared the major morphological categories of pulmonary neoplasia in rodents and humans. They concluded that without the lung cancer consequences of smoking the major subtypes in humans would then be "adenocarcinomas and bronchioalveolar carcinomas, which would correspond very closely to the types of lung tumours generally occurring in rodents".

In Lee *et al*. (1985) and Heinrich *et al*. (1995) and Rittinghausen *et al*. (1997) two different tumour types were observed: (1) cystic keratinizing epitheliomas (benign) or cystic keratinizing squamous cell carcinomas and (2) bronchiolo-alveolar adenoma or adenocarcinoma.

The discussion of interspecies differences preferentially focuses on the cystic keratinizing lung tumours. The WHO list of classification of human lung tumours does not contain cystic keratinizing lung tumours (Travis *et al*. 2015); the absence of this lung tumour type in the WHO list is considered by RAC as an indication that this type of cystic keratinizing lesions so far might not have occurred in humans. Different from the cystic keratinizing lung tumours seen in rats, other types of lung tumours, such as adenoma and adenocarcinoma do occur in humans (Travis *et al*. 2015).

Schultz (1996) stated that "responses such as keratinizing squamous cysts and adenomatous proliferation within areas of bronchiolarization, which are common in rats in lung overload, have not been observed in humans". RAC does not know whether the author considers "adenomatous proliferations within areas of bronchiolarization" identical to the adenomas and adenocarcinomas observed in the rat lung following exposure to TiO2 and other PSLT particles. In the literature provided to RAC, no convincing evidence is reported which allows considering the adenoma and adenocarcinoma observed in the TiO2 rat inhalation studies as unique to the rat.

RAC acknowledges that TiO2 epidemiology studies provide no consistent evidence of an association of exposure to TiO2 and excess lung cancer risks. But in the opinion of RAC this human TiO2 epidemiology alone does not exclude a carcinogenic potential of TiO2 in humans and does not necessarily question the positive evidence for rat lung tumours, bearing also in mind the methodological limitations of the epidemiology studies available. Epidemiology describes risks: TiO2 lung cancer potency in rats is already comparatively low. If in addition there is low exposure to TiO2, then TiO2 lung cancer potential in humans would not be expected to be shown in the available epidemiology studies.

Available chronic rat inhalation studies with nanosized PSLT particles were used to model and estimate ranges of concentration-risk relationships (www.baua.de/en/Topics-from-A-to-Z/Hazardous-Substances/TRGS/pdf/910/nanoscaled-GBP.pdf).

Where dosimetry adjustments of the rat lung cancer data are included, the human work-life adjusted BMD10 values (10% excess risk of lung cancer) for nanosized PSLT particles cover a range of about 5 to 25 mg/m³ respirable PSLT particles (TWA, daily work life-time exposure). In the studies evaluated, cystic keratinizing lesions could not be differentiated as to their specific subtypes and were counted as lung tumours. A slightly lower carcinogenic potency (slightly higher BMD10) should be assumed for microsized PSLT particles. In this estimate of BMD10 values it is conservatively assumed that there is potential for carcinogenicity in humans and that humans possess an identical sensitivity based on identical PSLT particle lung burdens. For the reasons given above, it can be assumed that human BMD10 values are higher than calculated.

The maximum average exposures to the respirable fraction of TiO2 at workplaces covered in the study by Boffetta *et al*. 2004 (mainly microscale TiO2) were reported to be around 0.7 mg/m³(summary of data in Hext *et al*. 2005; corresponding data on the respirable fraction of TiO2 are not available for the other epidemiology studies). If (over-conservatively) a human BMD10 is assumed for workers of 5 to 25 mg/m³ for TiO2 and linearity in the dose range of interest, then a presumed chronic exposure level of 0.7 mg/m³ implies an excess worker lifetime lung cancer risk of 0.3 to 1.4%. Against the background of 5 to 7% lung cancer incidence in the general population, an excess risk of probably less than 0.3 to 1.4% cannot easily be recognized under conditions of current epidemiology studies.

Influence of specifications of TiO2 on lung toxicity

The following chapter reports on selected key studies illustrating the influence of specifications of TiO2 on lung toxicity. Reference is made to these studies to assist RAC in considering whether to relate the TiO2 classification proposal to a narrow or broad definition of chemical identity.

Shape/morphology

The TiO2 materials tested for carcinogenicity by inhalation are known to have a granular shape. RAC is not aware of inhalation studies or studies with other routes of administration where fibrous shapes of TiO2 have been tested. The chronic inhalation toxicity observed is considered to be related to high concentrations of respirable TiO2 particulates of non-fibrous morphology.

Crystal structure and primary particle size

Micro-sized rutile and nano-sized anatase/rutile primary particles were tested for lung carcinogenicity (Lee *et al*. (1985), Heinrich *et al*. (1995)). Particle size distribution (MMAD) in both chronic inhalation studies was similar and within the range of respirability. Based on these studies the specific influence of the crystal structure or the size of primary particles on experimental lung carcinogenicity in rats cannot be demonstrated. RAC is not aware of toxicity studies with TiO2 with brookite crystal structure.

There is some evidence from other PSLT substances regarding the influence of the size of primary particles. When comparing the findings of different carcinogenicity studies it needs to be recognized that the particle-related tumour rates increase with age and these lung tumours in the rat are known to appear late, mainly after study durations longer than 24 months. Taking the different study durations into account, a comparative analysis indicates that nano-sized particles are somewhat more potent than micro-sized particles. But it was concluded by the author that the difference in carcinogenic potency between PSLT nano- and micromaterials is relatively low in general and can differ by a factor of about 2 to 2.5 if related to the cumulative

mass concentration as the dose metric (Gebel, 2012). RAC takes this conclusion cautiously, as the underlying database was limited and because the CLH report and opinion document does not contain a comprehensive analysis of the quantitative differences in the carcinogenic potential of nano- and microsized PSLTs.

Coating of particles (particle surface chemistry)

The key inhalation studies (Lee *et al*. 1985, Heinrich *et al*. 1995) used TiO2 materials without or at most with marginal surface coating (Warheit *et al*. 2016).

The impact of surface treatment on TiO2 particle toxicity was tested in a subacute rat inhalation study (Warheit *et al* 2005): Several TiO2 materials were tested in a 4-week inhalation study in male Sprague Dawley rats at the very high concentration of 1130 to 1300 mg/m³ (6h/day, a total of 20 exposures). Primary particle sizes ranged from 290 to 440 nm; the particle size distribution in the aerosol is characterised by MMADs between 1.3 and 1.8 µm. Lung tissues were evaluated by histopathology immediately after exposure as well as up to 12 months post-exposure. In summary, particle-laden macrophage accumulation and light alveolar cell hyperplasia were observed in all TiO2-exposed rats; there was a marked reduction of these lesions towards the end of the post-exposure period. Slight collagen deposition was only observed in the TiO2 formulation with the highest percentage of coating materials (7% alumina, 8% amorphous silica, thus 85% TiO2). While the airborne concentrations of the different aerosols were similar (between 1130 and 1300 mg/m³), the surface area per particle mass was significantly higher in the formulation resulting in fibrogenic responses (27.8 m²/g vs 6 to 12.2 m²/g). The authors of the study noted that surface treatments can influence the toxicity of TiO2 particles in the lung, but noted that they viewed these impacts as minor when compared to other types of dusts (without specifying which other dusts were meant).

The CLH report refers to further data on the impact of coating. The dossier submitter concluded that coating can impact the toxicity of TiO2 and that the inflammation response can differ between different forms of TiO2, although a clear pattern cannot be drawn from the existing data.

Reference to IARC TiO2 assessment

The CLH report contains a summary of the IARC assessment. In the opinion of RAC, this IARC summary of results of the carcinogenic studies is consistent with the toxicological TiO2 data presented in the CLH report.

The IARC working group concluded that there was inadequate evidence from epidemiological studies to assess whether TiO2 causes cancer in humans. The IARC working group did not conclude that there was human evidence suggesting lack of carcinogenicity. On the basis of results of increased incidence of lung tumours in rats, the IARC working group concluded that there was sufficient evidence that TiO2 is carcinogenic in experimental animals. The IARC working group considered the available mechanistic evidence for TiO2 not strong enough to conclude that TiO2 is "probably carcinogenic to humans" (group 2A). The IARC working group therefore decided to categorize TiO2 as "possibly carcinogenic to humans" (group 2B).

Although IARC extensively describes the variability of TiO2 (crystal type, size of primary particles, coating), there is no explicit IARC discussion on possible differences in carcinogenic potential or potency.

Human epidemiological data

One case report study, three case-control studies as well as five cohort studies were summarised in the REACH registration dossier or mentioned during public consultation; the majority of them

have already been analysed in the IARC monograph (volume 93). Apart from the detailed analysis of the strength of the studies in question given below, RAC concludes that the single case report investigation observed TiO2 in lung tissue of a lung cancer patient, but did not allow any conclusion on a causal relationship. In addition, one case-control study carried out by Siemiatycki (1991) showed some indication (statistically non-significant) of an association between TiO2 exposure in the occupational environment and lung cancer. The other two case-control studies and five cohort studies did not indicate an association between TiO2 exposure and lung cancer. Detailed description and analysis of the epidemiological studies mentioned is given in the summary table (see Annex 3 at the end of the document).

RAC underlines that no information is given on TiO2 particle characteristics, including surface area and size distribution in the occupational environment to which the workers are exposed. Only the cohort study carried out by Boffetta *et al.* (2004) in 11 European companies producing TiO2 specifically considered the respirable TiO2 dust. Three out of five cohort studies assessed the exposure quantitatively by direct long-term stationary and personal sample measurements. In other cases, details of exposure are unclear or they are derived from typical workplace conditions based on self-reported occupational histories. The total exposure time was different starting with 6 month or 1 year employment at TiO2 production industries. Only in some case-control studies was frequency of exposure during a normal work-week clearly considered, however, the cohort studies applied derivation of average intensity, duration and cumulative exposure used for the assessment. Generally, adjustment for different confounders (for example, presence of asbestos, smoking, etc.) was done with few exceptions concerning smoking in cohort studies.

Case reports

Deposits of TiO2 in lung tissue were reported for a 53-year old man engaged in packing of TiO2 for 13 years and suffering from pneumoconiosis accompanied by a papillary adenocarcinoma of the lung (Yamadori *et al*., 1986). However, as the person had also a smoking history of ~40 years, it is not possible to conclude on the role of TiO2 deposits in causing cancer and the subsequent death of the person.

Case-control studies

The case-control study based on interviews with more than 4000 persons in Montreal (Canada) including patients with different types of cancer as well as population controls indicated a statistically non-significant association between substantial exposure to TiO2 defined as ≥ 10 years in the industry or occupation up to 5 years before onset of a disease and lung cancer (odds ratio or OR 2.0, 95% CI 0.6 – 7.4, based on 5 exposed cases) (Siemiatycki, 1991). However, when considering any exposure to TiO2, only the risk of squamous-cell lung cancer was increased but without statistical significance (OR 1.6, 95% CI 0.9 – 3.0, based on 20 exposed cases). In addition, the risk of urinary bladder cancer was increased when considering any TiO2 exposure (OR 1.7, 95% CI 1.1 – 2.6, based on 28 exposed cases). No information on magnitude or frequency of exposure was given, making it impossible to do dose – response relationship analysis.

Two other related case – control studies carried out in Montreal involved persons with histopathologically confirmed cases of lung cancer (up to 2093 persons) and control groups of randomly selected healthy people (up to 2045 persons) as well as persons with cancer in organs other than the lung (up to 1349 persons) (Boffetta *et al*., 2001 based on Siemiatycki, 1991 and Ramanakumar *et al*., 2008). Low, medium and high exposure as well as frequency of exposure to TiO2 was reconstructed based on self-reported occupational histories and typical workplace conditions being in place. Neither an increased risk of lung cancer caused by TiO2 exposure, nor

a correlation between occurrence of lung cancer and the frequency, level or duration of exposure was reported (OR ~1.0).

Cohort studies

Chen and Fayerweather (1988) compared observed numbers of incident cases of lung cancer and associated mortality within a population of 1576 workers exposed to TiO2 in USA with expected numbers based on both company rates and USA national rates. Duration and time-weighted exposure average was used, but it is not clear whether it is based on direct measurements. Mortality from lung cancer was profoundly lower than expected both on the basis of national rates (standardized mortality ratio or SMR 0.52) and company rates (SMR 0.59). Neither was there an increase in the incident cases of lung cancer (SMR 1.04). However, it was indicated that only cancer cases in active employees of the company were included, This applies both to calculation of the cases observed in exposed persons and in calculation of the company reference rates. Nevertheless, RAC considers that no association between TiO2 exposure and lung cancer is indicated.

Another retrospective mortality cohort study included 4241 workers employed at four TiO2 production plants in the USA (Fryzek *et al*. 2003). Workers started their career in 1960 and afterwards, and have been followed-up until the end of 2000. A pool of 1472 participants who had worked exclusively in administration or in jobs not associated with TiO2 exposure formed the control group. Low, medium and high categories of exposure were derived based on historical exposure reconstruction involving long-term stationary as well as full-shift or near full-shift personal samples for total TiO2 exposure estimation. Average intensity, duration and cumulative exposure were adjusted by Cox proportional hazard models. Workers with the highest exposure to TiO2 had significantly smaller number of deaths than that expected for all causes (SMR 0.7) with no excess for lung cancer (SMR 1.0). RAC therefore considers that no association between TiO2 exposure and lung cancer is indicated.

A large cohort study in 11 European companies covering TiO2 production plants in Finland, France, Germany, Italy, Norway and UK was conducted by Boffetta *et al*. (2004). In this mortality follow-up study, 15017 workers who started their employment from 1927 to 1969 and ended in 1995-2001 were involved. Yearly average cumulative exposure was derived based on exposure reconstruction which in its turn was based on personal sample measurements that were mainly collected during the 1990s. The estimated cumulative exposure to respirable TiO2 dust (recalculated from total dust) was from 0 to ≥ 13.20 mg/m^3 years. When considering any TiO2 exposures mortality from lung cancer was slightly higher than in the general national population (SMR 1.19 - 1.23 depending on the statistical model used) with large variations among countries (from SMR 0.76 in Finland to 1.51 in Germany). Nevertheless, it must be stressed that 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. Besides, adjustment for smoking was lacking. The analysis with respect to respirable fraction of the TiO2 dust showed no dose – response relationship: cumulative exposure 0 – 0.73 mg/m^3 year – Relative Risk (RR) 1, 0.73–3.43 mg/m^3 year – RR 1.19, 3.44–13.19 mg/m^3 year– RR 1.03, ≥13.20 mg/m^3 year – RR 0.89. In addition, there was no relationship with exposure to TiO2 considering duration of employment and concentration. Irrespective of some possible methodological problems (possible exposure misclassification mentioned, some of the factories were relatively new and therefore follow-up periods were short), RAC considers that no clear association between TiO2 exposure and lung cancer is indicated.

RAC notes however, in the above three cases the issue of level of exposure; this is further discussed at the end of this chapter.

Two cohort studies on individuals (5054 participants for study in 2010 and 3607 participants for study in 2013) employed in three DuPont titanium dioxide production facilities in the US and followed from 1935 through 2006 were conducted by Ellis *et al*. (2010 and 2013). The first study was a general follow-up without an attempt to analyse dose-response, while the latter study used exposure reconstruction taking into account work history and static as well as personal monitoring data for TiO2 and TiCl4 (in total, 3488 industrial hygiene monitoring records from 1971 to 2002 with differing measurement durations). A number of cumulative exposure categories from <5 to >80 mg/m^{3}year were established. No increase in causes of death compared to the US population (all causes of death: SMR 0.81 (95% CI 0.77-0.85); all malignant neoplasms: SMR 0.90 (95% CI 0.82- 0.99); lung cancer: SMR 0.90 (95% CI 0.75-1.05)) was revealed in the 2010 study.

In the 2013 study, the same conclusion was drawn with respect to overall SMRs. However, compared to the DuPont workers not involved in TiO2 production, SMR for lung cancer was 1.35 (95 % CI 1.07–1.66). Comparing increasing exposure groups to the lowest group, disease risk (lung cancer mortality assessed without lag) did not increase with exposure: cumulative exposure 5–15 mg/m^3 year – Relative Risk (RR) 1.68, 15–35 mg/m^3 year – RR 1.65, 35–80 mg/m^3 year– RR 1.20, >80 mg/m^3 year – RR 1.38. Although the RR was greater than "1" at every exposure level, the CIs were wide and overlapped across all levels (Figure below). RAC considers that no clear association between TiO2 exposure and lung cancer was demonstrated.

Additionally, two publications dealing with systematic review of the literature on both experimental and epidemiological data on TiO2 analysed above were mentioned during public consultation (Hext *et al*., 2005 and Thompson *et al*., 2016). Both came to the overall conclusion that there was no suggestion of any carcinogenic effect associated with workplace exposure to TiO2.

Hext *et al*. stressed that regarding the large cohort study in 11 European companies covering TiO2 production plants (Boffetta *et al*., 2004), the average estimated respirable TiO2 dust concentration fell at most factories over the study period to current typical levels of 0.2–0.4 mg/m^3.

Thompson *et al*. (2016) which is the most recent study, gave a summary of lung cancer risk estimates from epidemiological studies of TiO2 (Figure below) and assessed the internal and external validity of these investigations (the other Figure below)[1].

[1] Study quality and relevance were evaluated by Thompson *et al* (2016) as follows: Internal validity using the National Toxicology Program Office of Health Assessment and Translation (NTP OHAT); Risk of Bias tool, external validity and other quality and relevance elements (e.g., indirectness) using direction from the OHAT handbook and grading of recommendation, assessment, development and evaluation (GRADE) approach (Guyatt *et al*., 2011; NTP OHAT, 2015). Summary characterizations of hazard were generated based on validity assessments, including considerations for strengths and weaknesses, risk of bias, magnitude of effect, dose-response, and consistency. Based on such, a confidence in the body of evidence was assessed and candidate datasets identified.

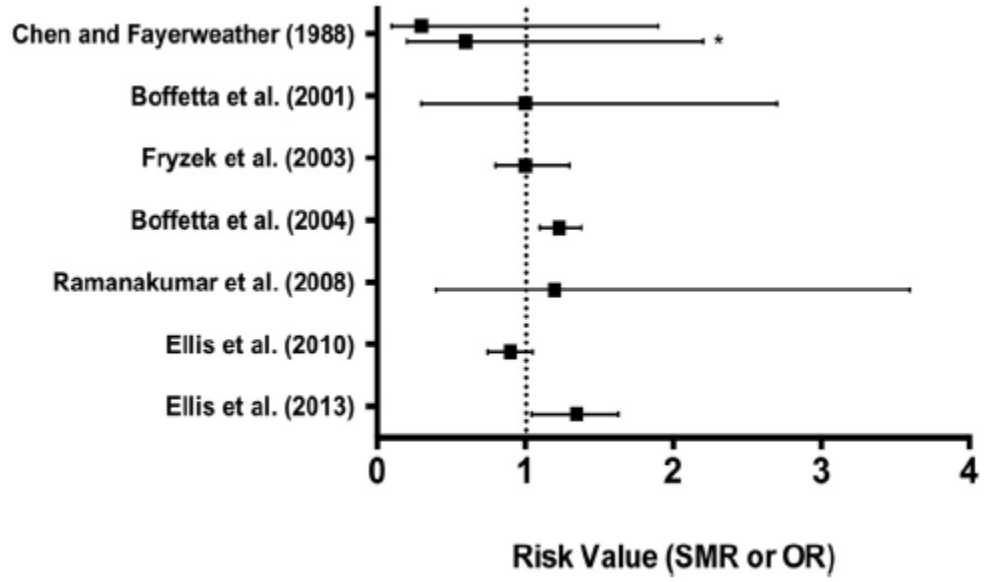

Figure. A summary of lung cancer risk estimates from epidemiologic studies of TiO2 pigment production workers showing standardised mortality ratio (SMR) or odds ratio (OR) for lung cancer. The 90% or 95% confidence interval for each risk estimate is also presented. In most cases, risk estimates were extracted for the highest exposure groups as identified by the study authors. All studies except Chen and Fayerweather (1998) reported SMRs for lung cancer or ORs based on lung cancer cases. Chen and Fayerweather (1998) reported ORs for lung cancer mortality and lung cancer incidence (indicated with an asterisk) (extracted from Thompson *et al*., 2016).

With respect to the Boffetta *et al*. (2004) study reflected in the Figure above, many of the regions where the factories were located had a higher death rate from lung cancer than the national rate for their country. The SMR for lung cancer would have been lower if regional reference mortality had been used. In addition, the Ellis *et al*. (2013) study mentioned here reflects the SMR compared to workers of the same plants not involved in TiO2 production. The SMR compared to the general populations was below "1".

Study	External Validity (Indirectness)	Internal Validity (Risk of Bias)					
		Study participants (Q3)	*Confounding (Q4)*	*Data completeness (Q7)*	*Exposure characterization (Q8)*	*Outcome assessment (Q9)*	*Reporting (Q10)*
Boffetta et al (2001)	Low	++	++	++	-	++	++
Boffetta et al (2004)	Low	--	-	-	+	++	++
Chen et al (1988)	Low	+	+	+	+	++	+
Ellis et al (2010)	Low	--	-	+	-	++	++
Ellis et al (2013)	Low	++	-	+	+	++	+
Fryzek et al (2003)	Low	++	--	++	++	++	++
Ramanakumar et al (2008)	Low	++	++	++	-	++	++

Figure (extracted from Thompson *et al*., 2016). Internal and external validity assessment results of human TiO2 data. External validity based on the level (very low [dark green] to very high [dark red]) of indirectness. Low indirectness indicates high external validity and vice versa. Internal validity based on risk of bias (definitively low risk of bias [dark green; ++] to definitely high risk of bias [dark red–]). Low risk of bias indicates high internal validity and vice versa.

Thompson *et al*. (2016) concludes that similar to other observational epidemiological studies evaluating risk from chemical exposures, the findings of TiO2 epidemiologic studies are likely to be impacted by exposure misclassification (exposure characterization in the Figure above) and

confounding factors. However, when considering all quality elements, Thompson *et al*. (2016) concluded that the data support a moderate level of confidence for the human evidence.
Summarising all the epidemiological data, taking into account the assessment of the internal and external validity of these investigations performed by Thompson *et al*. (2016) and acknowledging that all these studies have their methodological limitations, confounding factors and the level of exposure, type/size of particles as well as dose-metrics reported is debatable, applying a weight of evidence analysis, RAC considers that human data do not consistently suggest an association between occupational exposure to TiO2 and risk for lung cancer. Currently there are no epidemiological data to distinguish any potential carcinogenic effect of specific TiO2 micro and nano particle sizes and/or specific physical forms. However, one cohort study by Boffetta *et al*. (2004) deals specifically with the respirable fraction of TiO2 dust and did not observe a clear dose – response relationship between estimated exposure level and RR for lung cancer. Taking into account the general lack of epidemiological investigations on respirable fraction of TiO2 dust and indications made by Boffetta *et al*. (2004) and repeated by Hext *et al*. (2005) that the investigated TiO2 concentrations in the occupational environment are rather low (for example, in the Boffetta *et al*., 2004 study the median cumulative exposure of workers was 1.98 mg/m^3 years with interquartile range 0.26-6.88 mg/m^3 years) to cause lung cancer, RAC concluded that the epidemiological data was not sufficient to conclude on a carcinogenicity classification as the exposure data was inconclusive and that the epidemiological data could not overrule the outcome of the animal studies.

Comparison of carcinogenicity data with classification criteria

Oral and dermal carcinogenicity

Considerations on classification of TiO2 for the oral and dermal route rely on experimental animal data; for these routes of exposure human evidence is not available.

TiO2 was tested in two oral carcinogenicity studies. TiO2 forms tested were anatase with an unspecified size of primary particles and a mineral silicate covered with TiO2. Experimental animal species tested were rats and mice. The dossier submitter concluded that a carcinogenic concern for the oral route was not identified. This conclusion was not questioned during public consultation. Based on the negative oral carcinogenicity data reported in the dossier and the low oral absorption of TiO2, **RAC concludes that a classification for TiO2 for oral carcinogenicity is not warranted.**

Standard dermal carcinogenicity studies with TiO2 are not available. The dossier submitter reported on the results of five studies with a two-stage skin carcinogenesis testing protocol. The dossier submitter concluded that there is no concern for dermal carcinogenicity of TiO2. Comments during public consultation did not question this conclusion. Based on the negative dermal carcinogenicity data and the low dermal absorption of TiO2, **RAC confirms that available data do not support a classification of TiO2 for dermal carcinogenicity.**

Carcinogenicity by inhalation

Introduction

The dossier submitter proposed a Category 1B classification for TiO2 carcinogenicity by inhalation. As noted in the CLH report, the dossier submitter proposed the following CLP Annex VI entry: "Titanium dioxide in all phases and phase combinations; particles in all sizes/morphologies". Following public consultation the dossier submitter provided the following revised proposal: "Particles of titanium dioxide in all phases, phase combinations and morphologies with at least one dimension below 10 µm." Parallel to this textual definition the CLH report (Part A, chapter 1.1) referred to 3 different substance identifiers: titanium dioxide, anatase and rutile each with

specific EC and CAS numbers. This classification proposal was substantially challenged in many comments received during public consultation.

The dossier submitter responded to the comments received during public consultation in the RCOM annex, in which they concluded to retain the proposal for a category 1B classification for TiO2 carcinogenicity by inhalation.

Epidemiological studies

A substance is classified into category 1A if it is known to have a carcinogenic potential in humans. Category 1A is largely based on human evidence. Category 1A requires that human studies establish a causal relationship between human exposure to a substance and the development of cancer.

Taking into account the available information and acknowledging that all these studies have their methodological limitations, RAC is of the opinion that the human data do not consistently suggest an association between occupational exposure to TiO2 and risk for lung cancer. RAC however emphasises that average respirable TiO2 dust concentrations at workplaces (Boffetta *et al*. 2004, Hext *et al*. 2005) are estimated to be at levels below 1 mg/m³ (TWA). Hence, RAC concludes that the animal carcinogenicity studies cannot be overruled by these epidemiological studies (see corresponding chapter above).

The dossier submitter considered the human data insufficient for a category 1A classification. The proposal not to classify TiO2 based on human data was not questioned in comments received during public consultation. It is the opinion of RAC that the human studies were not adequate to establish a causal relationship between exposure to TiO2 and the development of cancer. **RAC concludes that a carcinogenicity category 1A for TiO2 is not warranted.**

Experimental animal studies

RAC considered whether a carcinogenicity classification for TiO2 can be justified based on the experimental animal data available. Carcinogenicity in experimental animals can be evaluated using conventional bioassays, and other *in-vivo* bioassays that focus on one or more of the critical stages of carcinogenicity. For carcinogenicity classification, reference is made to chapter 3.6 of the Guidance on the Application of the CLP Criteria (version 4.1 / June 2015).

The tested TiO2 particles are considered to be "poorly soluble low toxicity" particles. This "grouping" is intended to set these TiO2 particles and other PSLT particles apart from other particle types, such as poorly soluble fibrous particles or poorly soluble particles with specific toxicity.

Based on the available experimental evidence for TiO2, additionally referring to selected carcinogenicity data for poorly soluble low toxicity particles as supporting evidence, RAC takes the view that the tested TiO2 particles experimentally induced lung tumours in rats under conditions of marked particle loading in the lung.

- *Carcinogenic effects in two or more animal species?*

A causal relationship between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in two or more species of animals is indicative of a category 1B classification. It is the conclusion of RAC that exposure to respirable TiO2 particles resulted in treatment-related lung tumours in rats. There was no increased lung tumour incidence in a female NMRI mice study (Heinrich *et al*. 1995, but the duration of exposure of 13.5 months may have been to short. Other species have not been tested for carcinogenicity. The specific condition addressed (an increased incidence of malignant

neoplasms or of an appropriate combination of benign and malignant neoplasms in two or more animal species) is not considered to be fulfilled for TiO2. This TiO2 carcinogenicity profile corresponds to the carcinogenicity profile of other PSLT substances.

- *Neoplasms in two or more independent studies in one species?*

In case of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in two or more independent studies in one species, Category 1B is indicated. The Lee *et al*. (1985) study revealed an increased incidence of benign neoplasms (bronchio-alveolar adenoma) in male and female rats at the rather high exposure level of 250 mg/m³. This exposure level was linked to cessation of alveolar clearance. RAC takes the view that this marked condition of overload should not have a determining influence on classification of TiO2.

The only TiO2 inhalation study with malignant neoplasms was the Heinrich *et al*. (1995) study. In this study nano-scaled TiO2 was tested at a single exposure level of 10 mg/m³ in female rats. The experimental exposure schedule resulted in a particle volume loading of alveolar macrophages in the region of 40% resulting in a marked, but not total cessation of alveolar clearance. Two types of malignant lung tumours were observed: adenocarcinoma and cystic keratinizing squamous cell carcinoma. Benign cystic keratinizing epitheliomas were also reported in this study. Intratracheal instillation of TiO2 resulted in increased rat lung tumour rates as well; these results are consistent with the results of the chronic rat inhalation studies.

This TiO2 carcinogenicity profile corresponds to the carcinogenicity profile of other PSLT substances (Nikula *et al*. 2000; Gebel, 2012). RAC refers to these PSLT particle carcinogenicity data as supporting evidence. Adding data from other PSLT particles, RAC considers this condition of classification (malignant neoplasms or an appropriate combination of benign and malignant neoplasms in two or more independent studies in one species) to be fulfilled. For its final recommendation however, RAC used a weight-of-evidence approach integrating other modifying conditions and criteria.

- *Tumours in both sexes of a single species?*

Category 1B might also be indicated if there is an increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practice (GLP). In the context of the formal conditions for "sufficient evidence of carcinogenicity", it is the interpretation of RAC that the wording "increased incidence of tumours" is a short form for an "increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms". The carcinogenicity study with TiO2 nanoparticles was only performed with female rats (Heinrich *et al*. 1995); the only rat study in which both sexes were tested (Lee *et al*. 1985) did report an increased incidence of bronchio-alveolar adenoma in both sexes at the rather high exposure level of 250 mg/m³, but did not report an increased incidence of treatment-related malignant tumours. Data available for other PSLT substances (Nikula *et al*. 2000 and Gebel, 2012) generally indicate a lower sensitivity of the male rat. RAC does not in this case consider this condition for category 1B ("tumours in both sexes of a single species") to be sufficiently fulfilled.

- *Unusual degree of malignant tumours in a single study in one species and sex?*

A single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites. The results in the Heinrich *et al*. (1995) rat study with an increased incidence of both adenocarcinoma and cystic keratinizing squamous cell carcinoma in the order of magnitude of

10% are not considered to fulfil this category 1B condition of an "unusual degree of malignant tumours in a single study".

- *Weight-of-evidence approach*

In the context of a preferred weight-of-evidence approach RAC discussed additional considerations for classification (chapter 3.6.2.3.2 of the Guidance on the Application of the CLP criteria). RAC considers it essential to additionally take the following factors into consideration: the overload concept, specifically the related mode of action for genotoxicity and carcinogenicity and species differences, including consideration of human relevance of experimental animal data.

- *Particle clearance and lung dust burden*

In the OECD Guidance Document 116 it is recommended not to use experimental exposure levels for particles "exceeding an elimination half-time of approximately 1 year due to lung overload at the end of the study". This recommendation however lacks a specific justification for the duration of 1 year. The draft ECHA Guidance on Nanomaterials (2016) refers to this issue as well but does not give a specific recommendation as to the level of overload that might compromise the relevance of the corresponding toxicological outcome for humans.

The justification for any specific guidance level for the maximum reduction of lung clearance in experimental testing are considered to be complex: evidence from coal miners indicate that highly exposed coal miners experience particle lung burdens that can be reached in the rat lung only under conditions of a significant degree of overloading (Kuempel *et al*. 2009 and 2014). If particle lung burden can be considered a relevant dose metric, then the experimental testing of PSLT particles in the rat under exposure conditions which strictly avoid a significant degree of overload may not be sufficient.

RAC is of the opinion that it is generally justified that TiO2 or other PSPs are tested under overload conditions. The maximum degree of overloading of alveolar macrophages necessary remains undecided. RAC does not set aside the available rat carcinogenicity findings because rat lung tumours were only observed under exposure conditions resulting in marked overload. However, RAC acknowledges that overload conditions resulting in a complete cessation of alveolar clearance (beyond a 60% particle volume loading of alveolar macrophages) can be considered "excessive exposure" with questionable relevance for humans. This extreme degree of overload was observed in the Lee *et al*. (1985) study. However, with a particle volume loading of about 40%, there was a marked, but not complete cessation of alveolar clearance in the Heinrich *et al*. (1995) study.

Because of the complete cessation of alveolar clearance, RAC takes the view that the results of the Lee *et al*. (1985) rat study should not have a determining influence on classification of TiO2. In the context of a weight-of-evidence approach RAC recognises that the described experimental conditions for rat lung tumour development indicate that TiO2 can be considered a relatively weak rat lung carcinogen.

- *Mode of action in rats*

In rats, chronic TiO2 exposure levels associated with marked overload resulted in increased incidences of lung tumours. High TiO2 lung burdens cause a functional impairment of rat alveolar macrophages with associated impaired pulmonary clearance and provocation of chronic pulmonary inflammatory responses. The potential contribution of lung inflammatory cells to *in vivo* mutagenic responses was ascertained by co-culturing *in vivo* particle-elicited BAL cells with a rat alveolar epithelial cell line (Driscoll *et al*. 1997). Sustained alveolar inflammation can be considered the causative link to indirect genotoxicity and tumour development in the rat lung.

Based on the overall evidence available, RAC considers it plausible to assume that inflammatory reactions and reactive oxygen species play a central role in TiO2 genotoxicity and carcinogenicity.

In the opinion of RAC, the mode-of-action proposed for the rat is consistent with the assumption a practical threshold. Based on the experimental data available, rat lung tumours develop if exposure levels are associated with marked overloading of macrophages and chronic alveolar inflammation. The Guidance on the Application of the CLP Criteria (chapter 3.6.2.2.4) refers to such considerations and indicates that the assumption of a practical threshold can be viewed as decreasing the level of concern for human carcinogenicity.

- *Are different conclusions necessary for specific TiO2 materials?*

The evidence outlined in the CLH report and in this opinion do not indicate substantial differences in the toxicity profile of the tested TiO2 materials. Rat lung carcinogenicity of the two tested TiO2 materials is characterised as "particle carcinogenicity". Regarding the specific influence of the size of primary particles, nanoscale particles are generally considered to be somewhat more potent than microscale particles, although there are some indications that the difference is not very large. The integrating approach, i.e. to not split up classification for carcinogenicity by inhalation for micro- and nano-sized TiO2, is supported by RAC, the dossier submitter and by many of the comments during public consultation.

RAC is not aware of experimental data on TiO2 materials that may not be considered as PSLT particles (e.g. TiO2 particles fulfilling the WHO fibre criteria or coated TiO2 particles with specific surface toxicity) for which their toxicity profiles must be separately assessed and compared with the CLP criteria.

- *Interspecies differences and relevance to humans*

Substances which have induced benign and malignant tumours in well-performed experimental studies on animals are considered also to be presumed or suspected human carcinogens unless there is strong evidence that the mechanism of tumour formation is not relevant for humans (CLP 3.6.1.1). The Guidance defines a rather strict corresponding condition: "Only if a mode of action of tumour development is conclusively determined not to be operative in humans may the carcinogenic evidence for that tumour be discounted" (Guidance on the Application of the CLP Criteria, page 380).

As shown in subchronic studies only (there are no adequate carcinogenicity studies in animal species other than rats available) there are distinct species differences related to early steps of lung tumour development.

In this context, RAC considered the reported data on species-specificity of lung retention patterns, of site-specific development of non-neoplastic lung lesions and of lung tumour development. Specific reference is made to TiO2 data; however, the CLH report and especially comments during public consultation extensively referred to selected data on other PSLT particles as well.

Based on subchronic inhalation studies (both with nano- and microscale TiO2) rats were more sensitive than other small rodents. For both TiO2 specifications, it has been shown that at high identical respirable concentrations of TiO2 particles alveolar metaplasia and fibrosis was observed in the rat lung, but not in mice and hamsters. Particle retention patterns and pulmonary responses were different in these small rodent species (Bermudez *et al*. 2002 and 2004).

The discussion on species differences of TiO2 toxicity is supported by selected data for other PSLT particles. Species differences were not only observed between rats and other small rodents, but between rats and monkeys as well (Nikula *et al*. 1997).

There is further supporting evidence from PSLT particles as to the relative particle retention pattern in rats and humans based on comparative morphometric lung examinations (diesel exhaust soot in rat lungs versus coal dust in humans) and based on revised human lung retention models (Nikula *et al*. 2001; Kuempel *et al*. 2001; Gregoratto, 2010). Again, data indicate that retention of inhaled particles in the human lung interstitium was much more pronounced than in the rat lung interstitium. Again, these data do not justify a black-white conclusion: the evidence-based Gregoratto human dosimetry model predicts a clearance from the alveolar space to the mucociliary escalator for 60% of alveolar deposits (T1/2 = 400 days) and a clearance from the alveolar space to the interstitium for 40% of alveolar deposits (with T1/2 of 700 days).

Overall, RAC acknowledges that there is a quantitative difference in retention patterns of PSLT particles (no data for TiO2) in lung compartments of rats versus monkeys and humans. The data available document that relative retention in alveolar spaces (compared to lung interstitium) is higher in rats than in monkeys or humans. The data however do not indicate that retention of particles in human alveolar spaces can be disregarded.

Available data indicate toxicodynamic differences as well. There are various reviews (Schultz, 1996; Green, 2007; NIOSH, 2011; ECETOC, 2013) which report on similarities and dissimilarities of non-neoplastic lung responses in experimental animals and humans.

Overall, these reviews all lack a dose-related description of human non-neoplastic lung responses. Specifically, for important aspects such as chronic inflammation in humans following coal dust exposure, contradictory statements have been published (see summary in preceding chapters).

This gap of analysis is not filled by the CLH report or the comments received during public consultation. Because of this missing link in relating the human non-neoplastic responses to human exposure levels, RAC is not in a position to finally judge the comparability of the adverse outcome pathway for non-neoplastic lesions in rats and humans.

Experimental animal testing of TiO2 resulted in lung carcinogenicity in male rats (tested only in the Lee *et al.* 1985 study) and female rats. TiO2 was not adequately tested for lung carcinogenicity in hamsters and monkeys; nor in mice. Other PSLT particles mainly resulted in lung carcinogenicity in the female rat.

The human relevance of observed types of rat lung tumours is the subject of on-going discussion. TiO2 (and other PSLT particles) essentially resulted in two types of lung tumours in the rat: (1) cystic keratinizing epitheliomas (benign) or cystic keratinizing squamous cell carcinomas and (2) bronchiolo-alveolar adenoma or adenocarcinoma.

The cystic keratinizing lesions tend to occur late in these studies, rarely before 20 months of exposure. These lesions are generally found in female rats under overload conditions; these lesions are uncommon in male rats. RAC acknowledges that the cystic keratinizing lesions can be considered unique to the rat; a corresponding type of lesion is not known in humans. However, bronchio-alveolar adenoma or adenocarcinoma are well-known in humans; based on the scarce data available, RAC does not see the evidence to judge this type of tumour observed in the rat as irrelevant to humans.

RAC acknowledges that TiO2 epidemiology does not consistently provide evidence of an association of workers' exposure to TiO2 and increased incidences of lung cancer. However, average long-term exposure to workers in the study of Boffetta *et al. (*2004) was relatively low (below 0.7 mg/m³; with reference to Hext *et al*. 2005). In association with the given rat lung cancer potency of TiO2 and the species differences already known, RAC is of the opinion that a

possible carcinogenic potential in humans cannot easily be recognised by the usual epidemiology studies.

Some authors (e.g. Nikula *et al*. 2001; ECETOC, 2013; Warheit *et al*. 2015) propose to assume different adverse outcome pathways for lung tumour development in rats and humans:

In the rat, inhaled particles are predominantly retained in the alveolar duct compartments. Rat macrophages are considered to be more sensitive to overload conditions than macrophages in other species, resulting in a more pronounced degree of inflammation in alveolar spaces. Inflammation in the alveolar lumen is considered to be the cause for indirect mutagenicity in epithelial lung cells. Lung tumours (at least the keratinizing cysts and corresponding squamous cell carcinoma) are considered to originate in the alveolar lung compartment.

For humans, the same authors propose a greater tendency for transmigration of alveolar particle deposits to interstitial sites of the lung. A relatively lower particle load in the human alveolar spaces is assumed to result in a lower extent of alveolar inflammatory responses. Warheit *et al*. (2016) propose to assume that interstitialization of particles could be considered to serve as a repository in humans which is deemed to be less reactive as to tumour development. They presume that human cells in closest contact with the particles and macrophages in the interstitium are mesenchymal cells rather than epithelial cells. In this context, the authors refer to coal workers who may develop interstitial progressive fibrosis, but are possibly at less risk of developing pulmonary tumours.

These complex data on interspecies differences need to be thoroughly taken into account in an overall assessment on the possible human potential of TiO2-related lung tumour development (see "overall conclusion" below).

- *Intrinsic properties*

Comments during public consultation generally questioned the adequacy of any classification of TiO2. These comments noted that TiO2 toxicity is particle toxicity, that the adverse effects observed do occur irrespective of the chemical composition of the substance and thus are not to be considered intrinsic properties. The comments emphasised that classification of TiO2 would imply that any insoluble solid matter thus would require a classification for carcinogenicity.

RAC acknowledges that the TiO2 inhalation toxicity observed in rats is particle toxicity and accepts the general understanding that the development of rat lung tumours is mediated by the pathological consequences of a higher loading of macrophages with particles of rather low solubility. The deposited particles, but not solutes of TiO2 molecules, can be assumed to be responsible for the observed toxicity. RAC acknowledges as well that the carcinogenicity profile described for TiO2 is not exclusively characteristic for TiO2 but applies to the whole group of chemicals referred to as "poorly soluble low toxicity particles".

The CLP regulation requires a classification to be based on the intrinsic properties of substances. The CLP Guidance defines the intrinsic property of a substance as the basic properties of a substance as determined in standard tests or by other means designed to identify hazards. RAC considers the toxicity profile observed as a basic property of inhaled and respirable particles of TiO2. With reference to the CLP definition of intrinsic properties, RAC considers that the CLP regulation regards the properties of TiO2 or other substances which are PSLT particles as relevant for classification.

Overall conclusion

Following a weight-of-evidence approach,

- taking note that TiO2 was not shown to be a multisite carcinogen,
- being aware that TiO2 is a lung carcinogen especially in female rats,
- recognising that there are no robust carcinogenicity studies in species other than rats,
- recognising that the majority of rat lung tumours occurred late in life,
- recognising that rat lung tumours only developed under inhalation exposure conditions associated with marked particle loading of macrophages,
- presuming a practical threshold for lung tumour development (mutagenicity in lung cells is considered to depend on chronic inflammation and oxidative stress),
- taking note of experimental, mainly repeated dose toxicity data indicating a lower sensitivity of other small rodents, monkeys and humans compared to rats,
- being aware of TiO2 epidemiology studies which do not consistently suggest an association between occupational exposure to TiO2 and lung cancer mortality

RAC takes the view that the experimental and human evidence does not support titanium dioxide to be classified as Carc. 1A or 1B.

RAC also considered whether TiO2 fulfills the classification criteria for category 2 for carcinogenicity or whether no classification for carcinogenicity is more appropriate. Balancing the reasons for category 2 or no classification, RAC looked closely at the experimental conditions in the rat inhalation studies and at interspecies differences.

The experimental schedule of the Lee *et al*. (1985) study resulted in a complete cessation of alveolar clearance already at the non-carcinogenic exposure level of 50 mg/m³. Alveolar clearance half-times measured in different studies at the exposure level of 250 mg/m³ reached and exceeded 1 year. RAC takes the view, that these exposure conditions represent excessive exposure which invalidates the results of the Lee *et al*. (1985) study on their own for classification purposes. The exposure schedule of the Heinrich *et al*. (1995) study deviated from standard protocols (18 hours exposure/day), but because of the relatively low exposure level tested (10 mg/m³) the degree of particle loading was substantially lower compared to the Lee *et al*. (1985) study (particle volume loading in the Heinrich *et al*. (1995) study did not yet result in a complete cessation of alveolar clearance). The Heinrich *et al*. (1995) study resulted in an excess incidence of lung adenocarcinomas (and benign and malignant cystic keratinizing lesions). Although not performed according to standard testing guidelines, the results of this study are considered reliable and relevant and consistent with rat inhalation carcinogenicity findings of other PSLT substances (Gebel, 2012). Evidence from coal miners indicates that highly exposed workers experience particle lung burdens that can be reached in the rat lung only under conditions of a marked degree of overloading (Kuempel *et al*. 2009 and 2014). These considerations moved RAC to consider TiO2 as a rat lung carcinogen under marked, not yet excessive conditions of particle loading of lung macrophages.

Arriving at a conclusion also involves making a judgement on whether the available information on interspecies differences is already sufficient reasoning for considering TiO2 particles not being operative in humans at all. In this respect RAC specifically points out that:

- the dosimetry-related data and models available document a higher particle sequestration to the human lung interstitium, but do not exclude and in fact still reveal a significant degree of particle retention in human alveolar spaces as well,

- the evidence presented indicates a lower sensitivity of non-human primates and humans to PSLT induced lung inflammation (including alveolar inflammation), but does not sufficiently document a quantitative dose response relationship of alveolar inflammation in humans,
- there is no convincing scientific evidence to question the human relevance of observed rat lung adenocarcinomas,
- the epidemiological studies, although not consistently suggesting associations between occupational exposure to TiO2 and lung cancer mortality, do not allow this to be interpreted as the absence of a human hazard.

According to the CLH guidance, carcinogenic evidence can only be discounted if the mode of action of tumour development is conclusively determined not to be operative in humans. RAC holds the view that a sufficiently detailed and specific adverse outcome pathway for humans is not yet available. In the opinion of RAC the experimental and human evidence currently available supports a lower human sensitivity but does not conclusively exclude a carcinogenic potential or hazard of TiO2 in humans.

Based on the lines of evidence outlined in this opinion document and summarised in this overall conclusion, RAC concludes that TiO2 warrants a Category 2 classification for carcinogenicity. In the context of drafting the Annex VI entry for TiO2 RAC considers it essential to take note of the following:

RAC acknowledges that the mode of action for the rat lung carcinogenicity in rats can not be considered "intrinsic toxicity" in a classical sense: the deposited particles, but not solutes of TiO2 molecules can be assumed to be responsible for the observed toxicity. Nevertheless, this mode of action results in relevant toxicity and carcinogenicity which in principle merits consideration in classification and labelling. The CLP regulation does not exclude a health hazard classification triggered by physico-chemical characteristics of a chemical.

Generally, classification for carcinogenicity does not specify a route of exposure. However, the profile of lung carcinogenicity described for TiO2 is specifically linked to the inhalation route of application. Currently, there is no experimental evidence for TiO2 carcinogenicity for the oral or dermal route of application. TiO2 lung carcinogenicity is associated with inhalation of respirable TiO2 particles. Based on the data available today RAC considers it conclusively proven that no other route of exposure causes the carcinogenicity hazard. Correspondingly, RAC proposes to classify TiO2 as a Category 2 carcinogen, with the hazard statement H351 (inhalation).

Titanium dioxide tested for carcinogenicity by inhalation comprised samples of non-fibrous shape, differing crystal structures and particle sizes (including both nano- and microscale primary particles) and no or minor toxicologically relevant surface treatment. The toxicity profile determined designates the titanium dioxide tested as a "poorly soluble low toxicity" particle. The toxicity profile of fibrous titanium dioxide with WHO fibre characteristics (which has not been tested) is considered substantially different in terms of specific mode of action and cancer potency. Specifications of titanium dioxide with surface coatings resulting in a mode of action which is not any longer defined by the basic "granular particle toxicity" but by additional specific chemical toxicity, are also not covered by the toxicity profile of the tested titanium dioxide substances.

The carcinogenicity profile observed thus is specifically related to exposure to respirable TiO2 particles with different crystal structures and different primary particle sizes, but which do not possess WHO fibre characteristics or additional specific surface toxicity because of coating of the TiO2 particles.

RAC considered various options for the Annex VI entry of TiO2. The dossier submitter proposed a TiO2 Annex VI entry specifying the CAS number 13463-67-7 combined with the supplementary physico-chemical description "Titanium dioxide in all phases and phase combinations; particles in all sizes/morphologies". In the "attachment to the responses to comments" the DS refined the proposed scope as "particles of titanium dioxide in all phases, phase combinations and morphologies with at least one dimension below 10 µm". These proposed substance identity descriptions include TiO2 with WHO fibre characteristics in the definition of the TiO2 entry.

With such a supplementary physico-chemical description the Annex VI entry would not be adequately based on the hazard assessment of the specific TiO2 materials referred to in this dossier; furthermore the corresponding Annex VI entry runs the risk of incorrect classification for forms of TiO2 with WHO fibre characteristics (and possibly of TiO2 with surface coatings introducing specific chemical toxicity as well).

To ensure that all relevant scientific and regulatory aspects are taken into account RAC proposes the following scope of an entry in Annex VI of CLP: "Titanium dioxide" (without a further physico-chemical description) is proposed to be used as chemical name (international chemical identification). The CAS number to be used is 13463-67-7.

In addition to the classification (category 2 carcinogen including the hazard statement H351 (inhalation)) RAC proposes the following "Note": "***If the substance is placed on the market as particles of the substance fulfilling the WHO fibre criteria or as particles with surface coating their hazardous properties must be evaluated in accordance with CLP Title II to assess whether a higher category (Carc. 1B or 1A) and/or additional routes of exposure (oral or dermal) should be applied.***" The classification is based solely on the hazardous properties of the substance. It does not take into account the likelihood of exposure to the substance and therefore does not address the risks of exposure.

RAC acknowledges that the carcinogenicity profile described for TiO2 is not exclusively characteristic for TiO2 but applies to a group of chemicals with similar toxicity profile addressed as "poorly soluble low toxicity particles". The CLH report and this RAC Opinion concentrates on TiO2 data and do not fully consider the data for other PSLT substances.

Additional references

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ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).

Annex 3 Summary of human epidemiology investigations concerning carcinogenicity of TiO2 (next page)

ANNEX 3

Table 1. Summary of human epidemiology investigations concerning carcinogenicity of TiO2

Type of study	**Study description**	**Size of TiO2 particles and exposure value**	**Total exposure time and frequency**	**Results**	**Remarks**	**Authors**	**Conclusion by RAC on the study outcome with respect to possible association of TiO2 to lung cancer**
Case report	Health effects of a 53-year old man engaged in packing of TiO2	No information	13 years. No information on frequency.	Pneumoconiosis accompanied by a papillary adenocarcinoma of the lung. Deposits of titanium dioxide in lung tissue. Slight fibrosis of the interstitium around bronchioles and vessels.	Smoker for 40 years	Yamadori *et al*., 1986 (IARC monograph volume 93)	This case report does not allow to conclude on clear causality between the Tio2 exposure and lung cancer
Case – control study	**857** histopathologically confirmed cases of lung cancer in the male population (aged 35–70 years) of Montreal from 1979 to 1985 and control groups of **533** randomly selected healthy people and **533** people with cancer in organs other than the lung. Questionnaire on	No information on size. Low, medium and high exposure estimated to be 0.05-1 mg/m^3, 1-10 mg/m^3 and >10 mg/m^3 acc. to typical workplace conditions.	Different time. Frequency of exposure during a normal work-week: <5%, 5–30% or >30% of the time.	**33** cases and **43** controls identified as being ever exposed to TiO2. No indication of a correlation between lung cancer development and the frequency, level or duration of TiO2 exposure (**OR** about "1" - statistically significant at 95 % CI for different exposure scenarios).	No measurement of exposure, based on self-reported occupational histories. Results adjusted to other covariates.	Boffetta *et al*., 2001 (IARC monograph volume 93)	No indication of an increased risk of lung cancer

	previous work experience regarding TiO2 production, manufacture and use of TiO2 containing products.						
	Combination of results from two studies in Montreal (1979-1986 and 1996-2001) involving **857** + **1236** lung cancer cases, **533** + **1512** population controls, **1349** people with cancer in organs other than the lung. Males and females aged 35-75 years. Questionnaire on previous work experience.	No information on size. Low, medium and high exposure acc. to typical workplace conditions determined.	Different time. Frequency of exposure during a normal work-week: <5%, 5–30% or >30% of the time.	~4 % of participants with lifetime exposure to TiO2. No detectable excess risk of lung cancer determined. **OR** about "1" - statistically significant at 95 % CI for different exposure scenarios.	No measurement of exposure, based on self-reported occupational histories. Results adjusted to other covariates.	Ramanakumar *et al*., 2008	No indication of an increased risk of lung cancer
	More than **4000** subjects were interviewed in Montreal (males aged 35–70 years) including patients with 20 different types of cancer diagnosed from 1979 to 1985 and a series of population controls. A panel of industrial hygienists reviewed each job history reported by study subjects and	No information on size and exposure magnitude.	Substantial exposure divided and defined as ≥10 years in the industry or occupation up to 5 years before onset of a disease. No information on frequency.	For substantial exposure excess risk were found in relation to urinary bladder cancer (**OR 4.5**; 90% CI 0.9–22.0) and lung cancer (**OR 2.0**; 90% CI 0.6–7.4). For any exposure no excesses were observed in relation to all lung cancers (**OR 1.0**; 90% CI 0.7–1.5) but to some extent with respect to squamous-cell lung cancer (**OR, 1.6**; 90% CI 0.9–3.0) and urinary bladder	No measurement of exposure, based on self-reported occupational histories. Results adjusted to other covariates.	Siemiatycki, 1991 (IARC monograph volume 93)	Some indication of an increased risk, but without statistical significance

	assessed exposure to 293 substances.			cancer (**OR 1.7**; 90% CI 1.1–2.6).			
Cohort study	**1576** male workers exposed to TiO2 and employed for more than one year in two USA factories were observed between 1956 and 1985 for cancer and chronic respiratory disease incidence and from 1935 to 1983 for mortality. 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.	No information on size. Exposure from 0 to >20 mg/m^3. Duration and time-weighted exposure average was derived.	Different time (>1 year). Duration and time-weighted exposure average was derived.	Mortality from all cancers was lower than expected. For lung cancer 9 deaths were observed, with 17.3 expected on the basis of national rates (**SMR 0.52**; 95% CI 0.24–0.99) and 15.3 expected on the basis of companies rates (**SMR 0.59**; 95% CI 0.27–1.12). Incident cases of lung cancer approximately the same as expected (8 cases of lung cancer observed, 7.7 cases expected (**SMR 1.04**; 95% CI 0.45– 2.05)). Some tumours of the genitourinary system recorded (**SMR 1.59**; 95% CI 0.76–2.92).	Details of exposure to TiO2 were not described - unclear if quantitative exposure results were used. Incident cases of cancer only in actively employed persons were used for both observed and company reference rates. Adjustment for some confounders (presence of asbestos, etc.) was done.	Chen and Fayerweather (1988) (IARC monograph volume 93)	No indication of an increased risk of lung cancer
	Retrospective mortality cohort study of **3832** male and **409** female workers employed for ≥6 months at 4 titanium dioxide production industries in the USA on or after 1 January 1960; follow-up until December 2000. As control group, **1472** persons worked exclusively in	No information on size. Low, medium and high categories of exposure were derived based on historical exposure reconstruction .	Different time. Average intensity, duration and cumulative exposure adjusted by Cox proportional hazard models.	35 % 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. Workers with the highest exposure to titanium dioxide had a similar pattern of mortality, i.e. significantly smaller number of	Some long-term area samples as well as 914 full-shift or near full-shift personal samples for total titanium dioxide exposure estimation were used. Cox proportional hazard models that adjusted for the effects of age, sex,	Fryzek *et al*., 2003 (IARC monograph volume 93)	No indication of an increased risk of lung cancer

	administration or in other jobs that did not involve exposure to titanium dioxide.			deaths than that expected for all causes (**SMR 0.7**; 95 % CI 0.6–0.9) with no excess for lung cancer (**SMR 1.0**; 95 % CI 0.5–1.7). No trend of increasing SMRs for malignant or non-malignant lung disease with increasing duration of employment was evident.	geographical area and date of first employment were used. No individual adjustments for smoking were done.		

	A mortality follow-up study of **15017** workers (~95 % males) for at least 1 month in production (employment started from 1927-1969 and ended in 1995-2001) was carried out in **11** European companies (in Finland, France, Germany, Italy, Norway and UK) manufacturing TiO2	No information on yearly average exposure to total TiO2 dust based on historical exposure reconstruction . The estimated cumulative exposure to respirable TiO2 dust from 0 to ≥13.20 mg/m^3 year.	Different time (>1 year). No information on frequency, but yearly average cumulative exposure derived.	Mortality from lung cancers due to all exposures was higher than expected death cases in the general national population (**SMR 1.23**; 95% CI 1.10-1.38 (a fixed-effects statistical model) or **SMR 1.19**; 95% CI 0.96–1.48) (a random-effects 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. This conclusion is not supported in relation to exposure to respirable TiO2 dust: <u>0–0.73 mg/m^3 year</u> - **RR 1** (reference) <u>0.73–3.43 mg/m^3 year</u> – **RR 1.19**; 95 % CI 0.80–1.77 <u>3.44–13.19 mg/m^3 year</u>– **RR 1.03**; 95 % CI 0.69–1.55 <u>≥13.20 mg/m^3 year</u> – **RR 0.89**; 95 % CI 0.58–1.35. Generally, there was no relationship with exposure to TiO2 considering duration	Exposure reconstruction was based on personal sample measurements that were mainly collected during the 1990s. Not clear whether the respirable dust fraction directly measured or recalculated from total dust. It is important to note that 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. Some relatively new factories provided short follow-up periods. Adjustment for smoking was lacking. Possible exposure misclassification	Boffetta *et al*., 2004 (IARC monograph volume 93)	No indication of an increased risk of lung cancer

				of employment and concentration.	mentioned. Exclusion of part of the early experience of the cohort from the analysis.		

	A cohort of **5054** individuals (of them ~10 % women) employed in three DuPont titanium dioxide production facilities in the US was followed from 1935 through 2006. SMRs for TiO2 process workers (combined and plant specific) was compared with that of the population of the United States. Poisson regression was used to estimate SMRs.	No information on particle size. No exposure values were estimated.	Different time (>6 months). No exposure frequency was assessed.	1475 deaths observed in the cohort. No statistically significant increase in causes of death compared to the US population (all causes of death: **SMR 0.81** (95% CI 0.77-0.85); all malignant neoplasms: **SMR 0.90** (95% CI 0.82-0.99); lung cancer: **SMR 0.90** (95% CI 0.75- 1.05). Only exception for cancers of other respiratory organs – **SMR 2.49** (95% CI 0.62- 6.46) (based on 3 deaths in the cohort, each at a different respiratory site).	Smoking history data were not available.	Ellis *et al*., 2010	No indication of an increased risk of lung cancer
	A cohort of **3607** workers (of them ~12 % women) employed in three DuPont titanium dioxide production facilities in the US was followed from 1935 through 2006. Combined and plant-specific cohort mortality was compared with the overall US population and other DuPont employees based on SMR. The relationships between	No information on particle size. The estimated 8-h TWA concentrations ranged from 31.5 mg/m3 in 1971-1975 to 1.75 mg/m3 in 2001-2005.	Different time (>6 months). Cumulative exposure categories (<5, 5 to 15, 15 to 35, 35 to 80, and >80 mg/m3 year) estimated.	Among the 833 deaths (all causes, all cancers, lung cancers, non-malignant respiratory disease, or all heart disease), no causes of deaths were statistically significantly elevated either overall or plant-specific when compared to the US population (SMRs below "1"). However, comparing to the DuPont workers not involved in the TiO2 production, **SMR** for	Exposure reconstruction was based on work history and monitoring data for TiO2 as well as titanium chloride (TiCl4) (3488 industrial hygiene monitoring records, both static and personal measurements) were collected from 1971 to 2002. The 8-h TWA TiO2 concentrations by	Ellis *et al*., 2013	No indication of an increased risk of lung cancer

	selected causes of death and annual cumulative exposures to titanium dioxide and chloride were investigated using Poisson regression methods to examine trends with increasing exposure.			lung cancer is **1.35** (95 % CI 1.07–1.66). Comparing increasing exposure groups to the lowest group, disease risk did not increase with exposure, example of lung cancer (with not lagged exposure): 5–15 mg/m^3 year - **RR 1.68**; 95 % CI 0.83–3.43 15–35 mg/m^3 year – **RR 1.65**; 95 % CI 0.82–3.36 35–80 mg/m^3 year– **RR 1.20**; 95 % CI 0.54–2.59 ≥80 mg/m^3 year – **RR 1.38**; 95 % CI 0.62–3.03	5-year calendar intervals and by job types were grouped. By job type, the 8-h TWA concentrations ranged from 0.15 mg/m^3 (purification) to 14.70 mg/m^3 (utility). Smoking history data were not available but potential for asbestos exposure was taken into account.		

OR – odds ratio; RR – relative risk; SMR - standardized mortality ratio; CI – confidence interval; TWA - time weighted average