WCSR Advice 2016-08

SCIENTIFIC COMMITTEE REACH (WCSR)

ADVICE ON THE PROPOSED HARMONIZED CLASSIFICATION AND LABELLING OF TITANIUM DIOXIDE (CARCINOGENICITY ENDPOINT)

WCSR Advice 2016-08

CONTENTS

CONTEXT

The classification and labelling of certain hazardous chemicals must be harmonised to ensure adequate risk management throughout the European Union.

Member States, manufacturers, importers and downstream users may propose a harmonised classification and labelling of a substance. Member States can also propose a revision of an existing harmonisation.

The harmonised classification and labelling process (CLH) includes a period of public consultation that lasts 45 days.

Anyone can comment on a proposed harmonisation. Those most likely to be interested are companies, organisations representing industry or civil society, as well as individual experts.

A CLH dossier proposing a harmonized classification and labelling for Titanium dioxide (EC n° 236-675-5/CAS n° 13463-67-7) is published (31/05/2016) for commenting on the ECHA website. Titanium dioxide particles range from non-nano (bulk) to nanosizes that can aggregate or agglomerate. The CLH dossier is submitted by France

The outcome of this assessment can be used by the Belgian Competent Authority for commenting the public consultation.

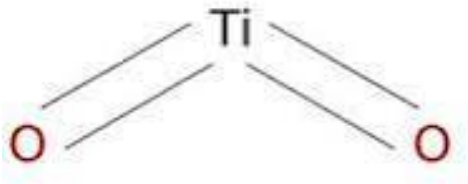
SUBSTANCE IDENTITY

Public Name: Titanium dioxide

EC Number(s): 236-675-5

CAS Number(s): 13463-67-7

Structural formula:



CONCERN

A CLH dossier is published on the ECHA website (31/5/2016) for public consultation proposing a harmonized classification and labelling for Titanium dioxide in the hazard class Carcinogenicity:

CARC 1B, H350i: May cause cancer by inhalation

ANALYSIS OF AVAILABLE INFORMATION

Physicochemical properties of TiO2 nanoparticles

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Titanium dioxide EC no.: 236-675-5	98.0 % (w/w)	>= 87.0 — <= 100.0 % (w/w)	For purity, materials were tested as uncoated and untreated material.

*These data are taken from the REACH registration dossier for EC no 236-675-5. See also, for information, public data of FAO and IARC below

Table 7: Impurities

Impurity	Typical concentration	Concentration range	Remarks
Confidential			

See also, for information public, data of FAO and IARC below

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
Aluminium oxide EC no.: 215-691-6	Stabiliser	1.0 % (w/w)	>= 0.0 — < 2.0 % (w/w)	

*These data are taken from the REACH registration dossier. See also, for information, public data of FAO and IARC below

Figure 1: *Overview of the required physicochemical properties mentioned to be considered as TiO_2 nanoparticles.*

The CLH report states the typical constituents, impurities and additives and the range in which they should be considered. When formulations are used that fall outside of this window, they should be treated with great care or be omitted from this report.

It is stated that the proposed classification and labeling for the Annex VI entry is applicable for Titanium dioxide in all phases and phase combinations; particles in all sizes/morphologies. The role of physicochemical properties (size, crystalline phase, coating, shape) of TiO_2 on carcinogenicity was discussed thoroughly in the CLH dossier prepared by France. Based on all the available data, it was concluded that all entities considered as TiO_2 are hazard equivalent, can be registered as one substance and have the same classification. We can agree on this for the proposed C&L as CARC 1B, H350i.

Carcinogenicity studies

Route of exposure

Oral exposure of animals to TiO_2 nanoparticles:

Table 4.1-01: Summary table of relevant carcinogenicity studies

Method	Results	Remarks	Reference Reliability
Oral route			
Fischer 344 rats and B6C3F1 mice (males and females) 0, 25 000, 50 000 ppm 103 weeks in diet (corresp. to 1250-2500 mg/kg bw/day in rats and 3750-7500 mg/kg bw/day with OECD conversion factors) Before guideline, no GLP status	Not carcinogenic by oral route. No firm conclusion in rats after reviewing by the Data Evaluation/Risk Assessment Subgroup of the Clearinghouse on Environmental Carcinogens.	TiO_2 anatase, purity ≥ 98%, size unspecified Tested material not fully characterized (at least size lacking) and very high tested doses	NCI, 1979 R2
Fischer 344 rats (males and females) 0, 1.0, 2.0, 5.0 % up to 130 weeks in diet (corresp. to 500, 1000, 2500 mg/kg bw/day with OECD conversion factors) Similar to guideline, no GLP status	Not carcinogenic by oral route.	TiO_2-coated mica (flat platelets, longest dimension, 10-35 µm; 28% titanium dioxide; 72% mica) Tested material not fully characterized (at least crystallinity and purity lacking) and high tested doses	Bernard, 1990 R2

Figure 2: *Overview of the two studies reported on animal studies focusing on the carcinogenicity of TiO_2 nanoparticles delivered through the oral route.*

The second study reported by Bernard et al. 1990, concerns the use of TiO_2 coated mica, where the particles were long (several micrometers) and consisted mainly out of mica. Therefore, although TiO_2 was present, it does not reach the required purity (minimum 87%) to make any analysis. The first study by NCI, 1979 was performed before any guidelines existed on Fischer 344 rats and B6C3F1 mice, both female and male for doses corresponding to 1250-2500 mg/kg bw/day for rats and 3750-7500 mg/kg bw/day for mice provided as an addition to their diet for a total duration of 103 weeks. Any conclusions drawn from this study, being the lack of any carcinogenicity should be treated with care, given the lack of material characterization and the study design not being compliant with GLP guidelines. For oral exposure studies, the data available in the CLH report does not allow one to draw any conclusions.

Other reports are available that are not mentioned, for example the study by Trouiller et al., where 50 C57Bl/6J^{pun}/^{pun} mice were given 21 nm diameter TiO_2 nanoparticles (75% anatase, 25% rutile) at 500 mg/kg bw in drinking water for 1 week which led to clear genotoxic effects as evaluated by both the in vivo Comet assay and micronucleus assay. The clear genotoxic effects at the dose lower than those described above indicates that there is a potential carcinogenic effect for TiO2 nanoparticles through oral exposure. Scarcity of the data does prevent any final conclusions on the matter.

Inhalation exposure of animals to TiO_2:

Inhalation route			
Crl:CD rats (males and females) Exposure by inhalation whole body: 0, 10, 50 or 250 mg/m^3, 6 h/day, 5 days/week for 2 years Similar to guideline, no GLP status	↑ bronchioalveolar adenoma in ♀ and ♂ and squamous lesions (mostly keratin cysts) in ♀ at 250 mg/m^3. Impairment of clearance function, pulmonary inflammation and cell proliferative responses from 50 mg/m^3.	TiO_2 (purity 99.0%), rutile particles; MMD = 1.5-1.7 µm	Lee, 1985 R2
Female Wistar rats [Crl:(WI)BR] and NMRI mice Whole body exposure by inhalation: 18h/d, 5 days/week: 7.2 mg/m^3 for the first 4 months, then 14.8 mg/m^3 for 4 months followed by 9.4 mg/m^3 for 16 months for rats and 5.5 months for mice. Not guideline, no GLP status	↑ benign keratinizing cystic squamous cell tumours, squamous-cell carcinomas, bronchioalveolar adenomas and adenocarcinomas in rats. Not carcinogenic in mice. ↑ mortality and ↓ body weight in both species. Impairment of clearance function, bronchioalveolar hyperplasia and interstitial fibrosis in rats.	TiO_2, 15-40 nm, P25 (≈ 80% anatase and ≈ 20% rutile) Purity lacking. One concentration varying during the experiment, only females tested.	Heinrich, 1995 R3
F-344 rats (males and females) Whole body exposure by inhalation, 6h/day, 5 days/week to 5 mg/m^3 TiO_2 (respirable concentration of 3.87 mg/m^3) for 24 months Not guideline, no GLP status	Not carcinogenic by inhalation. Inflammatory reaction with bronchoalveolar hyperplasia.	TiO_2, type Bayertitan T, 99.5 % rutile, MMAD = 1.1 µm Purity lacking. One low concentration tested.	Muhle, 1989 R3
SD rats (males and females) 0 or 15.95 mg/m^3 by inhalation	Not carcinogenic by inhalation. Inflammatory reaction.	TiO_2, "standard size" with 99.9% < 0.5 µm	Thyssen, 1978

Figure 3: *Overview of the four studies reported on animal studies focusing on the carcinogenicity of TiO_2 nanoparticles delivered through inhalation.*

For carcinogenic effects upon exposure through inhalation, 4 references (see Figure 3) are provided that all find inflammatory responses. Only 2 (Lee et al, 1985 and Heinrich et al, 1995) report on carcinogenicity, while the others do not find any carcinogenicity. The ones that did not report on any carcinogenicity have only a single low dose exposure (5 mg/m³; study by Muhle et al;, 1989) or short exposure times (12 weeks, Tyssen et al, 1978) as well compared to the other two studies. Of the particles tested, 2 are 99% rutile (1 for carcinogenicity positive, 1 for negative) and larger than 1 micrometer diameter. The other positive study used mixed (80% anatase, 20% rutile) of only 25 nm diameter. Though the

crystal structure is different, the size of the particles is also highly different, making it impossible to directly compare the effect of the crystal structure.

Exposure through instillation of animals to TiO_2:

Instillation route			
Hras 128 transgenic female rats DHPN (initiation) for 2 weeks. Then, 250 µg/ml or 500 µg/ml TiO_2 once every 2 weeks from the end of the week 4 to week 16 by instillation. Not guideline, no GLP status	Promotor effect observed: ↑ multiplicity of DHPN-induced alveolar cell hyperplasias and adenomas in the lung at all doses, and the multiplicity of mammary adenocarcinomas at 500 µg/ml. No carcinogenic without pre-treatment with DHPN.	TiO_2 non coated, rutile, 20 nm Purity lacking. Little experience with this model. No positive control included. Only females tested.	Xu, 2010 R3
F344/DuCrl Crj male rats DHPN (initiation) for 2 weeks, then 0.5 mg/rat TiO_2 once in week 4 by instillation. Not guideline, no GLP status	Not promotor potential by instillation. No lung lesion without pretreatment with DHPN.	Micro-TiO_2, rutile form, < 5 µm Nano-TiO_2, 80 nm (no clear crystalline identification) Many parameters did not match with standard protocol for carcinogenesis assessment; no valid positive control; only males tested.	Yokohira, 2009 R3
SPF Wistar female rats TiO_2 P25: 5x3mg, 5x6 mg or 10x6 mg by instillations TiO_2 P805: 15x0.5 mg or 30 x0.5 mg by instillation Micro TiO_2: 10x6 mg or 20x6 mg by instillation Animals sacrificed after 30 months. Not guideline, no GLP status	↑ benign tumours (adenomas and epitheliomas) and malignant tumours (adenocarcinomas and squamous cell carcinomas) with nano and micro TiO_2 at all tested doses. Higher number of tumours with nano-TiO_2 compared to fine TiO_2.	Nano-TiO_2 P25, majority anatase, 25 nm Nano-TiO_2 P805 (P25 coated with trimethoxyoctyl-silane), 21 nm Micro-TiO_2 anatase, 0.2 µm Purity lacking. Only females tested.	Pott, 2005 R2

Figure 4: *Overview of the four studies reported on animal studies focusing on the carcinogenicity of TiO_2 nanoparticles delivered through instillation.*

For instillation exposure, only 3 studies are reported, of which 1 (Yokohira et al, 2009) where it is mentioned that "Many parameters did not match with standard protocol for carcinogenesis assessment; no valid positive control; only males tested." rendering the findings of this study difficult to interpret and should therefore not be considered. The remaining two studies (Pott et al, 2005; Xu et al 2010) give different findings, where 20 nm diameter rutile TiO2 was found only to be carcinogenic in animals that had been pretreated

with N-bis(2-hydroxypropyl)nitrosamine (DHPN); while 25 nm diameter mixed TiO_2 (80% anatase, 20% rutile) gave rise to both benign and malignant tumours (adenomas and adenocarcinomas). The high similarity in size but differences in outcome of the studies performed suggests that anatase might be more carcinogenic than rutile TiO2, but as this is based on single reports and no direct comparison. Differences may also lie in the study conditions (dosage, exposure regime, as detailed in Figure 4, left column).

Other studies are available that have not been reported in the CLH report, for example the study by Park et al, 2009, who also studied 25 nm diameter mixed (80% anatase, 20% rutile) nanoparticles. Mice were exposed to TiO2 nanoparticles at 5, 20 or 50 mg/kg, by intratracheal instillation after which the animals were sacrificed at 1, 7, 14 days after treatment for a total of 12 per condition tested (dose and time point). This study, showing a dose-dependent increase in granuloma frequency in ICR mice, confirms that small-sized anatase TiO2 indeed appears to possess a carcinogenic effect.

Dermal exposure of animals to TiO_2 nanoparticles:

Dermal route			
CD1(ICR) female mice DMBA (initiation) one time. One week after: 5, 10 and 20 mg/animal TiO_2 twice weekly for 19 weeks by dermal route. Two-stage skin carcinogenesis Japanese guideline 3.2; GLP compliant	No promotor potential by dermal route.	TiO_2 coated: 79.2%, spindle shape, long axis of 50-100 nm, short axis of 10-20 nm TiO_2 non coated: 96.0%, spindle shape, long axis of 50-100 nm, short axis of 10-20 nm No information on crystallinity. Positive control valid; only females tested.	Furukawa, 2011 R2
Male transgenic Hras 128 rats and wild-type SD rats DMBA (initiation) one time. Two weeks later: 50 or 100 mg TiO_2 twice a week until week 40 by dermal route. Two-stage skin carcinogenesis Not guideline, no GLP status	No promotor potential by dermal route.	TiO_2 non coated, rutile, 20 nm. Little experience with this model. No positive control; only males tested. High tumour activity with DMBA alone in Has 128 rats.	Sagawa, 2012 R3
Female CD1 mice DMBA (initiation) one time. Two weeks later: 10 or 20 mg TiO_2 twice a week until week 52 by dermal route. Two-stage skin carcinogenesis Not guideline, no GLP status	No promotor potential by dermal route.	TiO_2 non coated, rutile, 20 nm Positive control valid; only females tested.	Sagawa, 2012 R3
Female transgenic rasH2 mice and wild type CB6F1 mice DMBA (initiation) one time. Two weeks later: 10 or 20 mg TiO_2, 5 times per week until week 8 for transgenic mice and week 40 for wild-type mice by dermal route. Two-stage skin carcinogenesis Not guideline, no GLP status	No promotor potential by dermal route.	TiO_2 coated with silicone, 35 nm No positive control; only females tested. High tumour activity in the initiated rasH2 mice.	Sagawa, 2012 R3
Hras 128 rats and wild-type rats (males and females) UVB (initiation) twice weekly for 10 weeks, then 50 mg TiO_2 twice weekly until week 52 by dermal route. Two-stage skin carcinogenesis Not guideline, no GLP status	No promotor potential by dermal route.	TiO_2 non coated, rutile, 20 nm No positive control. Little experience with this model	Xu, 2011 R3

Figure 5: *Overview of the four studies reported on animal studies focusing on the carcinogenicity of TiO_2 nanoparticles delivered through the dermal route.*

For dermal exposure, 5 reports are given, all of which indicate no carcinogenic effect. The particles to be tested are mainly 20 nm diameter rutile (3 out of 5 reports), coated or of unclear crystallinity. These findings do not allow one to make any conclusions on the impact of the crystal structure on the carcinogenic effect of TiO2 as no anatase particles of similar size have been evaluated.

The CLH report also gives a few examples of studies using other modes of administration, being subcutaneous or intraperitoneal injection, and find no carcinogenicity of the injected TiO2 nanoparticles (Bisschoff, 1982; Maltoni, 1982). Other studies that have not been mentioned do exist, such as the study by Moon et al, 2011, where chronic exposure (daily intraperitoneal administration during 28 days) was found to increase the growth of subcutaneously implanted B16F10 melanoma cells in a murine tumour model. The main difference between this study and the ones reported by CLH is likely that here a repeated exposure was evaluated, whereas the other reports used only a single administration. However, repeated exposure through intraperitoneal administration of 25 nm diameter TiO2 does have a possible carcinogenic effect. However, the report by Moon et al. only used a single dose and the study should be repeated using a larger number of dosages to confirm the initial results and to ascertain whether a true dose-dependent effects can be noticed.

Only a low absorption of TiO_2 is reported in oral and dermal toxicity studies. No carcinogenic effects could be identified after oral and dermal exposure. However, as TiO_2 nanoparticles can enter hair follicles and sweat glands, it cannot be excluded that some forms of TiO_2 could be better absorbed.

Interspecies comparison

Repeated dose toxicity studies were performed in rats, mice, hamsters. In the rat pulmonary lesions were more severe and occurred at a lower concentration and only in the rat progressive fibro-proliferative lesions and alveolar epithelial metaplasia was developed. We can agree with the discussion of the FR CA that mice are less sensitive to oxidative damage and hamsters have antioxidant protection mechanisms different form rats and humans. We agree that the rat is the most sensitive species for testing the carcinogenic potential of TiO_2.

The lung tumours observed in rats occurred in an overload context: This is also relevant for humans: e.g. workers exposed to high dust exposure.

Biological significance of cystic keratinizing squamous cell tumours in the rat and their relevance to humans: we agree not to take these tumours into account in the discussion.

Human studies

Human data from case reports, case-control and cohort studies were available, but the epidemiological data was considered inadequate/inconclusive.The various studies give confusing data. While in most cases no clear carcinogenic effects is noticed, some studies indicate increased lung tumour burden. TiO2 nanoparticles have also been found not to have a direct influence of all types of lung cancer combined, but did have a significant effect on squamous cell lung cancer. Other factors (environmental, conditional) render any analysis rather difficult, but the results do indicate a possible carcinogenicity of TiO2 nanoparticles through inhalation.

Other data

In the CLH report, 3 additional publications (Bonner et al, 2013; Warheit et al, 2007; Chen et al, 2006) are considered focusing on acute toxicity of TiO2 upon intratracheal instillation in order to derive more information from the possible impact of the physicochemical properties of the nanoparticles. The study by Bonner et al, 2013 reports on 25 nm diameter mixed (80% anatase, 20% rutile) nanoparticles, 100% anatase nanoparticles and 100% anatasenanobelts where all particles were found to induce inflammation. The study by Warheit et al, 2007 reports on 25 nm diameter mixed (80% anatase, 20% rutile) nanoparticles, 98% rutile nanoparticles of 100 nm diameter with 2% Al, 100 nm diameter rutile nanoparticles 88% TiO2 core with SiO2 (7%) and aluminium (5%) coating and 300 nm diameter rutile nanoparticles 99% TiO2 and 1% alumina and particles were delivered via intratracheal instillation. All particles were found to induce inflammation, which was mainly transient, apart from the mixed nanoparticles, which had the biggest effect. The study by Chen et al, 2006, study reports on 19-21 nm diameter TiO2 and 180-250 nm TiO2, where the crystallinity of the particles is not reported. The study observes clear inflammation induced by the smallest nanoparticles, while no such effects were observed for the larger ones. To some extent is not surprising that acute toxicity studies give different results.

The authors also give 3 repeated dose studies (Everitt et al, 2000 - Bermudez et al, 2002, 2004 - Hext et al, 2005; Baggs et al, 1997; Warheit et al, 2005), where inter-species differences were studied for hamsters, rats and mice exposed to 25 nm diameter mixed (80% anatase, 20% rutile) nanoparticles and “fine” (no diameter or crystallinity given) particles. Clear species-dependent effects were observed, where loss in body weight and

recovery was more pronounced for hamsters than for mice and rats. It is reported that rats were unique in the development of a progressive fibroproliferative lesion and alveolar epithelial metaplasia in response to a subchronic exposure to a high concentration. In a second study, smaller sized TiO2 nanoparticles were found to be more fibrogenic than larger ones. In a third study, various types of TiO2 with different levels of Al and amorphous silica surface treatments were used for inhalation and instillation studies in rats. It was observed that TiO2 with the highest levels of Al and amorphous silica resulted in mild pulmonary effects, suggesting that surface treatment can influence the toxicity of the TiO_2 nanoparticles.

In the rat pulmonary lesions were more severe and occurred at a lower concentration of 10 mg/m³ and only in the rat progressive fibro-proliferative lesions and alveolar epithelial metaplasia was developed. We can agree with the discussion of the FR CA that mice are less sensitive to oxidative damage and hamsters have antioxidant protection mechanisms different from rats and humans. We agree that the rat is the most sensitive species for testing the carcinogenic potential of TiO2. In addition, the lung tumours observed in rats occurred in an overload context, which is also relevant for humans: e.g. workers exposed to high dust exposure. On the other hand, we do agree not to take cystic keratinizing squamous cell tumours in the rat into account in the discussion as biological significance and relevance to humans is limited.

In the current REACH registration database there is one registration for "titanium dioxide" with 130 members in April 2016. This registration stated that it intends to cover "all crystal phases and hydrates of titanium dioxide including rutile, anatase, monohydrate and dihydrate". However, the types and number of compositions considered to be covered in terms of crystalline phase, morphology and surface chemistry are not transparently (and exhaustively) reported. Due to this lack of transparency, the impact on the hazard profile when the parameters vary cannot be established from the information included in the registration dossier. However, it is clearly stated in the registration dossier that all possible variations are considered equivalent in terms of hazard profile. Taking these statements into account, the approach applied in the REACH dossier was used to support the scope of the proposed entry in Annex VI of CLP.

Mode of action

Inflammation and oxidative stress

Secondary genotoxicity as the major mechanism underlying tumour formation of TiO_2: indirect oxidative stress and chronic inflammation processes: agreed.

Genotoxicity

The genotoxicity of TiO_2 is rather due to oxidative lesions as shown in the considered reliable studies (*in vitro/in vivo*): We agree that primary genotoxic mechanism by direct particle interaction with DNA cannot be ruled out, as TiO2 particles were observed in the nucleus in several genotoxicity studies.

Mechanism of toxicity of biodurable granular particles

According to NIOSH, the adverse effects produced by TiO2 exposure in the lungs are likely not substance-specific but may be due to a nonchemical-specific effect of poorly soluble low toxicity particles in the lungs at sufficiently high particle surface area exposures. We agree that a not material-specific mechanism is also involved.

CONCLUSIONS

Titanium dioxide in all phases and phase combinations, particles in all sizes/morphologies as **CARC 1B, H350i: May cause cancer by inhalation**

Non-human information

Carcinogenicity: oral

The carcinogenicity of TiO_2 through oral route requires further information as only 2 quite different studies have been reported, though both studies state that the particles tested were not carcinogenic. Other studies, not mentioned in the CLH report, did indicate clear genotoxic effects of TiO_2 nanoparticles upon oral administration, which suggests that possible carcinogenesis may exist.

Carcinogenicity: inhalation

Various reports are mentioned for inhalation and intratracheal instillation exposure. For both procedures, both positive and negative carcinogenicity has been observed. The apparent discrepancy in results may occur from the study conditions, where studies reporting a lack of carcinogenicity used lower dosages and shorter exposure times. Based on the data obtained, the possible carcinogenicity of TiO_2 nanoparticles seems to be the correct classification. The impact of the crystal structure is however more difficult to assess using the data provided and more data is needed. Based on the little data available, it seems that small sized anatase nanoparticles are more potent carcinogens than rutile ones or larger sized anatase. On the other hand, taking into account the long biopersistency and overload it is to be expected that they are all carcinogenic.

Carcinogenicity: other routes

Only minimal data are reported here that do not find any clear carcinogenicity upon subcutaneous or intraperitoneal administration of TiO_2. However, repeated exposure studies do indicate possible carcinogenicity of TiO..

Carcinogenicity: dermal

For dermal exposure, 5 reports are given, all of which find no carcinogenicity. Any impact of the crystal structure of the nanoparticles cannot be evaluated as noanatase particles were used.

Human information

Human data from case reports, case-control and cohort studies were available, but the epidemiological data was considered inadequate/inconclusive.Case reports and cohort studies are complex due to the high incidence of lung cancer and the high number of environmental factors that may contribute to it (asbestos exposure, smoking) which will influence any outcome of these studies. Some studies have reported small, but significant increases in tumouroccurence, in particular for squamous cell lung cancers, indicating thatTiO2 nanoparticles do appear to be possibly carcinogenic upon inhalation.

Summary and discussion of carcinogenicity

The authors report a lack of carcinogenic concern for TiO_2 nanoparticles after dermal or oral exposure, mainly basing their findings on limited absorption of TiO_2 and low levels of accumulation, combined with the earlier reports which did not reveal clear carcinogenicity. The authors do state that "it cannot be excluded that some forms of TiO_2 could be better absorbed, in particular with specific coating and/or size". As clear genotoxic effects have been observed after oral exposure to anatase TiO_2, and no anatase nanoparticles were studied for dermal carcinogenicity, these conclusions might be slightly premature at this stage. The authors state that high levels of nanoparticles would be required to induce carcinogenicity, but the lack of degradation and slow clearance might result in higher accumulation levels in humans upon continuous exposure. Upon inhalation, the carcinogenicity of TiO_2 has indeed been adequately shown. The authors also discuss the physicochemical properties of TiO_2 nanoparticles and their possible impact on carcinogenicity. While several studies report that smaller sized TiO_2 is more toxic than larger sized particles, the authors state that "no clear correlation has been made. In addition, carcinogenic effects were reported for nano and micro-forms. Classifying all the titanium dioxide particle sizes for carcinogenicity is therefore justified." Though carcinogenic effects have been found indeed, the influence of size and coating cannot be ignored as this will determine the final penetration of the nanoparticles and

the level of agglomeration. The lack in correlation between the hazard and size of TiO_2 is more driven by the scarcity of available data than can be used for comparison.

The authors make similar statements on the impact of the crystal structure, stating that "In conclusion, although some in vitro or in vivo acute exposure to TiO_2 suggests an impact of the crystallinity on inflammation responses, the available data on rutile and anatase do not allow drawing strong conclusion on which crystallinity is the most toxic and to which extent. In contrast, in chronic studies, no difference between crystalline forms was found in term of carcinogenic potential. Classifying all the crystalline forms for carcinogenicity is therefore justified." In various studies *in vivo*, as also indicated here for oral exposure genotoxicity studies, anatase particles appear to be more reactive and cause higher levels of inflammation than rutile ones. At this point, it is again rather difficult to draw any final conclusions on the impact of the crystal structure due to the scarcity of the data. Regarding the coating, the authors state "The data presented above show that coating can impact the toxicity of TiO_2 and that the inflammation response can differ between different forms although a clear pattern cannot be drawn from the existing data. Carcinogenicity was observed with both anatase and rutile titanium dioxide. Between these two crystal phases, Reactive oxygen species (ROS) generation and pulmonary inflammation response differs. Indeed, the quantitative aspects of the inflammatory response that are sufficient to cause a high probability of lung tumor development are not known. Therefore, it is impossible to identify a threshold of inflammation below which carcinogenicity would not occur. It is also impossible to distinguish which coating, if any, will induce inflammation below this threshold.

Moreover, based on the data generated/collected in the registration dossier and in compliance with the Annex VII-XI information requirements, that all entities they consider as "titanium dioxide" are hazard equivalent, can be registered as one substance and have the same classification. They also considered that the impact of surface treatment on titanium dioxide particles irrespective of the specific surface area or the type of chemical treatment undertaken does not impact the properties relevant for hazard. Again taking this statement at face value, it implies that they have concluded that the hazard profile of titanium dioxide in any phase or phase combination, non-surface treated and surface treated for all specific surface areas are equivalent." These statements are somewhat strange, as here, the authors do indicate differences in ROS and inflammatory responses based on the crystal structure of TiO_2, which would suggest that grouping them as a single item could be misleading. The impact of the coating is difficult to assess given the data provided. In principle, a huge number of different coating agents can be provided. Additionally, TiO_2 can be doped with other agents that may be carcinogenic in nature. Combining all these particles as a single entity again seems rather dangerous as at this stage it is not possible yet to draw any general conclusions.

The impact of the morphology is also not considered to play a major impact, though several studies have reported a higher level of inflammation for long-shaped nanobelts compared to spheres as also indicated in the CLH report.

Comparison with criteria

The authors state that "For this CLH report, data on TiO_2, whatever its morphologies, crystal phase and surface treatment, were taken into account. Based on the analysed dataset, it is concluded that criteria for classification as Carc. 1B – H350i for TiO_2 by inhalation are fulfilled." At this point, classification as Carc. 1B-H350i appears to be correct. In addition, the authors state that "Even if only some compositions without treatment of titanium dioxide have been tested for carcinogenicity, a classification as Carcinogen Category 2 for the other crystal forms, morphologies and surface treatment might underestimate the hazard since the proposed mode of action is mediated by inflammation is also considered relevant to all the forms including in the scope of the dossier.".

Conclusions on classification and labelling

The authors state that "TiO_2 should be considered as being potentially carcinogenic to humans when inhaled and thus be classified Carc. Cat 1B – H350i. This classification applied for both fine particles and nanomaterials of TiO_2 without being able of any distinction in terms of morphology, crystal phase, and surface treatment."

ADVICE

Agreement on the French CA to classify Titanium dioxide in all phases and phase combinations, particles in all sizes/morphologies as **CARC 1B, H350i: May cause cancer by inhalation**

The proposed classification appears to be appropriate given the data available, given the generation of both benign and malignant tumors in rat models upon inhalation and the increase in lung tumour occurrence in human cohort studies.

There is sufficient evidence in reliable animal studies: inhalation studies and by intratracheal instillation in the rat. There was also supportive evidence in less reliable studies. Malignant tumours were reported in 2 adequate reliable studies: one inhalation study and one intratracheal instillation study. The rat is found the most sensitive species and the benign and malignant lung tumours found in the rat studies with the fine / nano TiO2 in an overload context are relevant for human exposure. No carcinogenic effects were reported in oral or

dermal toxicity studies. Human data is considered inadequate/inconclusive and therefore insufficient to classify TiO_2 as Carc. 1A.

The suggested carcinogenic mode of action is relevant to humans: Secondary genotoxicity based on inflammation and induction of oxidative lesions (repeated dose toxicity studies, genotoxicity studies). The biopersistence and poor solubility of TiO2 is rather more relevant than the other physico-chemical parameters to explain the carcinogenic potential of tiO2. In addition, a direct genotoxic mechanism cannot be excluded as particles were found to accumulate in cell nuclei

However, at this point, we feel that a uniform classification of all TiO_2 particles, regardless of size, morphology or surface treatment is premature. The authors state that the proposed mechanism of biopersistence of the particles combined with oxidative stress and inflammation, can result in carcinogenicity. However, as the authors stated themselves, clearance of the particles is influenced by the size of the agglomerates, which can be affected by their coating and shape. The level of oxidative stress has been found to be influenced by the nature of the crystal structure. Together, these data suggest that the physicochemical properties of TiO_2 may play a role in their carcinogenicity. The scarcity of data available does not allow a direct comparison, where the particles used often differ in multiple physicochemical properties rather than only in 1 single property and the conditions used for exposure also vary widely. More data should be available to enable a more in-depth understanding of the impact of the physicochemical properties of TiO_2 on their carcinogenic potential prior to any grouping in their classification.

OTHER REFERENCES

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MEMBERS OF THE SCIENTIFIC COMMITTEE

The members are :

Willy Baeyens; Johan Bierkens; Marie-Noëlle Blaude; Steven Broekx; Peter Dubruel; Lieve Geerts; Lode Godderis; Walter Hecq; Birgit Mertens; Guy Schroyen; Stefaan Soenen; An Van Nieuwenhuyse; Jeroen Vanoirbeek; Reinhilde Weltens.

CONFLICT OF INTEREST

No member has declared any conflict of interest.

RAPPORTEURS

The Scientific Committee REACH thanks the rapporteurs Lode Godderis, Stefaan Soenen and Jeroen Vanoirbeek.

ADOPTION OF THE ADVICE

The Scientific Committee REACH advice was adopted by consensus by written procedure on 15/7/2016.

LEGAL FRAMEWORK OF THE ADVICE

Cooperation agreement of 17 October 2011 between the Federal State, the Flemish Region, the Walloon Region and the Brussels Capital Region concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)

Ministerial decree of 8 July 2014 appointing the members of the Scientific Committee REACH established under Article 3, § 3 of the Cooperation Agreement of 17 October 2011 between the Federal State, the Flemish Region, the Walloon Region and the Brussels Capital Region concerning the Registration, Evaluation, Authorisation and restriction of Chemicals (REACH)

Ministerial decree of 2 June 2016 on dismissal and appointment of members of the Scientific Committee REACH

DISCLAIMER

The Scientific Committee REACH reserves, at any time, the right to change this advice when new information and data become available after the publication of this version.

President

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