Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

silicon carbide fibres (with diameter < 3 µm, length > 5 µm and aspect ratio ≥ 3:1)

EC Number: 206-991-8
CAS Number: 409-21-2; 308076-74-6

CLH-O-0000001412-86-200/F

Adopted

9 March 2018

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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: **silicon carbide fibres (with diameter < 3 µm, length > 5 µm and aspect ratio ≥ 3:1)**

EC Number: **206-991-8**

CAS Number: **409-21-2; 308076-74-6**

The proposal was submitted by the **Netherlands** and received by RAC on **31 January 2017.**

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

PROCESS FOR ADOPTION OF THE OPINION

The Netherlands 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 **14 March 2017**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **28 April 2017**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Agnes Schulte**

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 **9 March 2018** 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 and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	014-RST-VW-Y	silicon carbide (fibres fulfilling the WHO definition: diameter < 3 µm, length > 5 µm and aspect ratio ≥ 3:1)	-	-	Carc. 1B	H350i	GHS08 Dgr	H350i			
RAC opinion	014-RST-VW-Y	silicon carbide fibres (with diameter < 3 µm, length > 5 µm and aspect ratio ≥ 3:1)	206-991-8	409-21-2 308076-74-6	Carc. 1B	H350i	GHS08 Dgr	H350i			
Resulting Annex VI entry if agreed by COM	014-RST-VW-Y	silicon carbide fibres (with diameter < 3 µm, length > 5 µm and aspect ratio ≥ 3:1)	206-991-8	409-21-2 308076-74-6	Carc. 1B	H350i	GHS08 Dgr	H350i			

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Silicon carbide (SiC) fibres currently have no entry in Annex VI to the CLP Regulation.

The inhalation route is the only exposure route of concern.

During public consultation, a comment was received about the possibility to include a CAS number for this entry. The dossier submitter (DS) responded that no specific CAS number has been assigned to SiC fibres with this specific definition (diameter < 3 µm, length > 5 µm and aspect ratio ⩾ 3:1). However, they also stated that two CAS number exist for SiC: 308076-74-6, which is specific for fibres and could contain whiskers and certain cleavage fragments, and 409-21-2, which covers all forms of SiC. RAC concluded that both CAS numbers should be included in Annex VI because in combination with the international chemical identification information, the scope would be adequately defined (by limiting the scope of the broader CAS No. (409-21-2) to the fibrous forms included under this CAS No.) while at the same time ensuring that the classification is more readily identified by CAS No. RAC agrees with the DS.

RAC notes that the phrase ”fulfilling the WHO definition”, which is not included in the CLP Regulation, can be modified outside the context of the CLP Regulation, and it is therefore proposed not to include this in the entry in Annex VI, while maintaining the defining technical text, i.e. “diameter < 3 µm, length > 5 µm and aspect ratio ⩾ 3:1”.

RAC evaluation of germ cell mutagenicity

The SiC fibres were not assessed for classification for germ cell mutagenicity either by the DS or by RAC. The DS stated that basic *in vivo* and *in vitro* studies are available in the literature but provided no information on their results. Only the overall negative conclusion of an Ames test (Bioservice, 2008) was reported. Genotoxicity data for SiC fibres were, however, also presented in Section 4.1.7.6 of the CLH report as supportive information, to provide relevant data for the assessment of carcinogenicity of SiC fibres.

No proposal on the classification of SiC fibres regarding the endpoint genotoxicity was included in the CLH dossier.

Comments received during public consultation

No comments were received during the public consultation as this hazard class was included for information only in the CLH report.

Assessment and comparison with the classification criteria

RAC did not assess this hazard class as no classification was proposed.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The DS proposed classification of all forms of SiC fibres (including fibres, whiskers and cleavage fragments) fulfilling the WHO fibre definition (WHO, 1985) as Carc. 1B; H350i. SiC whiskers and SiC cleavage fragments of certain size and form fall within the scope of this definition.

The DS concluded that SiC fibres induce tumours based on several carcinogenicity studies with SiC dust and fibres via inhalation, intraperitoneal and intrapleural injections in rats, and a review of the available epidemiology studies in humans. They also included a summary of the IARC evaluation and a description of the possible toxicological mechanisms involved.

The classification proposal was mainly based on one non-guideline inhalation carcinogenicity study and one meta-study reanalysing the results of this non-guideline carcinogenicity study, as well as other studies on fibres in general (see table below). The classification proposal was further supported by a low-dose repeated dose inhalation toxicity study and several other studies using other routes of administration (intraperitoneal or intrapleural injection; a list of these studies can be found in the Table 36 of the CLH report).

Animal data

Table: Summary table of relevant non-human carcinogenicity studies (inhalation route)

Species, exposure route	Test material	Method	Results	Remarks	Reference
Rats Inhalation Intraperitoneal injection	SiC whiskers (single crystal) mean diameter of 0.45 µm and > 5 µm in length	For the long-term studies, 2 groups of 40 specific-pathogen-free (SPF) rats of the AF/HAN strain (the number of rats per sex is not specified; no controls were used) were exposed to SiC dust cloud (984 fibres > 5 µm/ml) for 238 days during a period of approximately 1 y. Dusting was for 7 h each day, 5 days each week. After 1 y, groups of 4 rats from each experimental study were killed for the examination. The remaining animals were left for their full life span except that the study was terminated when the number of survivors in each group had dropped to six. To assess the ability to produce mesotheliomas, a dose of $1x10^9$ fibres (length > 5 µm) was suspended in 2 ml of PBS and was injected intraperitoneally into groups of 24 rats For studies of whisker durability in lung tissue, intratracheal injection was undertaken. Doses of 1 mg of SiC whiskers were suspended in 1 mo PBS and injected as a single dose into groups of 16 rats . SiC fibre dissolution in vitro was tested at pH 7.0, 4.6 and 0.6.	SiC whiskers induced fibrosis and tumors (pleural mesotheliomas) in rats after inhalation and IP treatment.. Significant clearance of SiC whiskers occurred following intratracheal injection and extremely little clearance of this material in the year following a 12-month inhalation period. No dissolution was determined (0.0 – 0.2%).	Positive (KEY STUDY)	Davis J.M.G. *et al.*, 1996
Rats Inhalation	SiC whiskers 0.95 * 6 µm MMMF: (D x L: ≤ 1 x > 20 µm)	Reanalysis of existing carcinogenicity studies on fibres to determine the relevant fibre characteristics for carcinogenicity. The data used were from the studies carried out at the IOM under the Colt Fibre Research Program (CFRP) (Davis J.M.G. *et al.*, 1996), and from studies carried out in Switzerland and the USA under the program of the Thermal Insulation Manufacturers Association (TIMA).	The results suggested a primary influence of the airborne concentrations of the numbers of fibres thinner than 1 µm and longer than 20 µm, and of the measured dissolution rate of the fibres. Lung carcinogenicity of man-made fibres in rats is a function of fibre length and that the man-made fibres longer than 20 µm had the greatest potency to be carcinogenic. SiC fibres showed a clear increase in lung cancer incidence, lung tumour incidence and especially mesothelioma incidence.	Positive (same study as Davis J.M.G. et al., 1996)	Miller B.G. *et al.*, 1999a

In the key study by Davis *et al.* (1996), a clear increase in carcinomas, adenomas and mesotheliomas in lungs of rats exposed via inhalation to SiC whiskers (SiCW, single crystal, mean diameter of 0.45 µm and > 5 µm in length) was observed after 1-year (238 days of exposure) with a full-life span follow-up. In a second inhalation study (Akiyama *et al.,* 2007), rats exposed to SiCW (mean diameter of 0.5 µm and length of 2.8 µm) developed broncho-alveolar hyperplasia and advanced fibrosis of the lung parenchyma but not tumours. The DS concluded based on these two studies that the carcinogenicity observed is a function of the fibre length.

Stanton *et al.* (1981) reported an increased incidence of pleural carcinomas in rats 1 year after intrapleural administration of SiCW (metallic crystalline, highly variable in diameter and length). They concluded that the probability of pleural sarcoma correlates best with fibres that in general measure $\leqslant$ 0.25 µm x > 8 µm. In two other studies, rats were administered SiCW intrapleurally (Vasil'eva *et al.*, 1989; Johnson and Hahn, 1996). In Vasil'eva *et al.* (1989), no information on the dimensions of the fibres were given; the overall frequency of mesotheliomas was found to be comparable to that of rats injected with asbestos (positive control). Johnson and Hahn (1996) tested three types of mono crystalline whiskers: SiCW 1 (diameter 0.42 and length 4.5 µm), SiCW 2 (diameter 0.75 and length 20.1 µm) and SiCW 3 (diameter 0.32 and length 6.6 µm). Adenocarcinomas, in combination with pleural mesotheliomas, were observed for all whiskers types, although the pleural mesotheliomas were not statistically significant for SiCW 3. This study aimed also to investigate the effects of length/diameter and number of the fibres. SiCW 3 was the less carcinogenic: 23% of animals developed mesothelioma vs 0, 90% and 87% for saline control, SiCW 1 and SiCW 2 respectively. As these differences cannot be explained only by fibre number and length/diameter distribution, the authors concluded that other aspects must also be important, although in the case of SiCW, surface chemistry may have a limited influence on their carcinogenic potency.

Intraperitoneal administration of SiCW (mean diameter < 0.95 µm and length > 0.4 µm) and of unspecified SiCW led to early development of peritoneal mesotheliomas (Miller *et al*., 1999b; Adachi *et al.*, 2001) in rats. In Adachi *et al.* (2001), the frequency of mesotheliomas between rats exposed to SiCW and the positive control, asbestos, was comparable. Also Pott (1991) observed a dose-response relationship for tumour incidences in rats exposed intraperitoneally to unspecified SiCW with dimensions of 3.1 x 0.31 µm.

No increased tumour incidence was found in rats which had received an injection of non-fibrous SiC (Pott *et al.*, 1994) or granular SiC (Roller *et al.,* 1996).

Human data

Several SiC epidemiology studies were included in the CLH report. Most of them were conducted in Norway and referred to the same SiC industry source population (Bugge *et al*., 2010, 2011 and 2012; Romundstad *et al.*, 2001 and 2002). Other cohort studies were conducted in Canada and Sweden, but they have low power due to the small sample size (Infante-Rivard *et al.*, 1994; Jakobsson *et al*., 1997; Järvholm *et al*., 1982; Edling *et al.*, 1987). Characterisation of SiC fibres (and other dust components) was not reported in any of the epidemiological studies.
Overall, the epidemiological studies found exposure-response associations between increased risk of cancer (or risk of mortality from cancer) and exposure to total dust (respirable quartz, cristobalite, SiC particles and SiC fibres) in the Norwegian SiC industry (Bugge *et al.*, 2010 and 2011; Romundstad *et al.*, 2001 and 2002; Infante-Rivard *et al.,* 1994).
The DS reported that no increment in risk could be observed with increasing duration of employment. Smoking was reported not to act as a confounder.
Due to the cumulative exposure to total and respirable dust, including respirable quartz, cristobalite, SiC particles and SiC fibres, the causative agents in dust for increased risk of cancer could not be conclusively identified.

In the most recent study by Bugge *et al.* (2012), cumulative exposure to total and respirable dust, including respirable quartz, cristobalite, SiC particles and SiC fibres was assessed with respect to lung cancer in 1687 long-term workers employed during 1913 – 2003. The study cohort was based on a previously established cohort in the Norwegian SiC industry (Bugge *et al.*, 2010; Romundstad *et al.*, 2001). In order to estimate exposure to specific agents, a large comparative study was performed in 2002 - 2003, with around 700 parallel personal measurements of total dust and respirable dust, and total dust and fibres. The amounts of quartz, cristobalite and SiC dust in the respirable dust fraction were determined. Standardized incidence

ratios (SIR) for lung cancer were calculated including a follow-up period (1953 – 2008) stratified by cumulative exposure categories. Associations between exposure level and lung cancer incidence for SiC particles and SiC fibres were reported (see table below).

Table: Observed number of cases (Obs) and standardized incidence ratio (SIR), with 95% confidence intervals (CIs) of lung cancer among 1687 long-term Norwegian SiC industry workers employed during 1913 - 2003 and followed up during 1953 - 2008, by tertiles of cumulative exposure, and with exposure lagging 0 and 20 years (Bugge *et al.* 2012).

Cumulative exposure	No lag					20 years lag of exposure				
	N	Person-years	Obs	SIR	95% CI	N	Person-years	Obs	SIR	95% CI
SiC particles (mg x years/m^3)										
0 – 0.83	970	14111	14	1.3	0.7 – 2.1	1616	32293	27	1.3	0.9 – 1.9
0.83 – 3.0	941	14096	14	1.3	0.8 – 2.2	677	5865	14	1.6	0.9 – 2.7
3.0 – 60	697	14703	34	2.2	1.6 – 3.1	357	4752	21	2.6	1.7 – 3.9
SiC fibres (fibres x years/cm^3)										
0 – 0.50	925	13788	13	1.2	0.7– 2.1	1619	31648	24	1.2	0.8 – 1.8
0.50 – 2.0	1018	14897	15	1.3	0.8– 2.2	682	6466	14	1.6	0.9 – 2.6
2.0 – 93	614	14225	34	2.2	1.6– 3.0	336	4796	24	2.6	1.8 – 3.9

The relative importance of the specific exposure factors for cristobalite, SiC, and SiC fibres was studied by constructing Poisson regression models including two or more exposure variables at a time (log-transformed). The DS reported that crystalline silica in the form of cristobalite was the most important occupational exposure factor responsible for lung cancer excess in the Norwegian SiC industry, but SiC fibres seemed to have an independent additional effect (IRR 1.7; 95% CI 1.1 to 2.9). Exposure to quartz and SiC particles did not seem to influence the lung cancer incidence significantly (table below).

Table: Incidence rate ratios (IRR) and 95% CIs for lung cancer related to log-transformed cumulative exposure to cristobalite, SiC fibres and SiC particles among 1166 male ever-smoking Norwegian long-term SiC industry workers employed during 1913 - 2003 and followed up during 1953 - 2008, adjusted for age and the other exposure factors (Bugge *et al.*, 2012)

	Smokers, N=1166, 30714 PYR, 58 cases				
	IRR	**95% CI**	**LR-test***	**AIC**	**$r_{Pearson}$†**
Cristobalite	1.9	1.2 to 2.9		275.6	
Cristobalite adjusted for SiC	2.0	1.2 to 3.3	p=0.8	277.5	0.74
Cristobalite adjusted for SiC and fibres	1.6	0.8 to 3.3	p=0.4	278.8	
Cristobalite adjusted for fibres	1.5	0.8 to 2.9	p=0.4	276.9	0.76
Cristobalite adjusted for fibres and SiC	1.6	0.8 to 3.3	p=0.8	278.8	
Fibres	1.9	1.2 to 2.9		276.7	
Fibres adjusted for SiC	1.7	1.1 to 2.9	p=0.6	278.4	0.51
Fibres adjusted for SiC and cristobalite	1.3	0.7 to 2.6	p=0.2	278.8	
Fibres adjusted for cristobalite	1.3	0.7 to 2.6	p=0.2	276.9	0.76
Fibres adjusted for cristobalite and SiC	1.3	0.7 to 2.6	p=0.8	278.8	
SiC particles	1.4	1.0 to 2.1		281.4	
SiC particles adjusted for fibres	1.1	0.7 to 1.8	p=0.03	278.4	0.51
SiC particles adjusted for fibres and cristobalite	0.9	0.5 to 1.6	p=0.2	278.8	
SiC particles adjusted for cristobalite	0.9	0.5 to 1.6	p=0.02	277.5	0.74
SiC particles adjusted for cristobalite and fibres	0.9	0.5 to 1.6	p=0.4	278.8	

*LR-test: Likelihood ratio test comparing the actual model with the model containing one less exposure factor.
†$r_{Pearson}$: Pearson's correlation coefficient.
PYR, person - years; AIC, Akaike's Information Criterion; SiC, silicon carbide.

There were also two case-control studies available showing an association between pneumoconiosis and exposure to SiC dust (Dufresne *et al.,* 1993; Massé *et al.*, 1988) but the studies included a very small number of cases in total.

Overall, the DS considered that the epidemiological studies showed a positive association between exposure to total dust in the SiC industry and risk of cancer, but limited information is available about exposure-response associations between specific dust constituents and increased risk of cancer.

Only the recent study by Bugge *et al.* (2012) indicated that crystalline silica in the form of cristobalite has to be considered the most important occupational exposure factor responsible for lung cancer excess in the Norwegian SiC industry. SiC fibres seemed to have an independent additional effect, while exposure to quartz and SiC particles did not seem to influence the lung cancer incidence.

Fibres assessment

The DS included assessments of SiC fibres by several international institutions. IARC in 2014 (Grosse *et al.*, 2014) concluded that differences in the nature of SiC fibres warranted separate

evaluation and classification. Fibrous SiC was classified in Group 2B based on limited evidence in humans that it causes lung cancer, as correlations between exposures to SiC fibres and cristobalite made it difficult to disentangle their independent effects. SiC whiskers were classified in Group 2A on the basis that the physical properties of the whiskers resemble those of asbestos and erionite fibres, which are known carcinogens.

In 2012, the Health Council of The Netherlands concluded that fibrous SiC (fibres, whiskers) may cause cancer through a non-stochastic mechanism of action and should be classified as carcinogenic to humans (CLP Category 1A).

In respiratory toxicology, it is generally accepted that high aspect ratio particles (fibres) pose an additional hazard beyond that produced by conventional compact particles. A high aspect ratio is defined by the WHO as a ratio of fibre length to diameter $\geqslant$ 3 (WHO, 1988). The key factors for fibre toxicity are dose, dimensions (length and diameter) and durability. Fibres with a diameter over 3 µm cannot be inhaled into the deep part of the lung (Harrison, 2015), and the length determines whether the fibres can be engulfed and removed by the macrophages. The durability depends on the dimension and composition of the fibres and is influenced by possible dissolution and/or breaks. Transversal breaks, which decrease the fibre length, reduce the fibre durability and the toxicity, while longitudinal breaks increase the number of thin long fibres in the lungs.

Generally, it is considered that long fibres (20 µm) cannot be completely taken up by macrophages, resulting in frustrated phagocytosis, release of ROS and growth factors and secondary effects which may result in carcinogenesis. When this occurs in the lung, lung adenoma and carcinoma can be expected. Short fibres (5 µm) are normally fully engulfed by microphages and behave comparably to non-fibrous particles. Except for overload conditions, the involvement of these short fibres in carcinogenesis is considered low (Bernstein, 2007). Donaldson (2010) proposed the following mechanism of mesothelioma induction: A fraction of the inhaled fibres are transported by the draining lymphatic fluid into the pleural space. Short fibres are transported over the parietal pleura towards the lymph nodes. However, long fibres cannot pass the stomata in the parietal pleura, resulting in stoma retention. Frustrated phagocytosis of the fibres at the stomata can result in local effects including mesothelioma. A threshold of 5 µm is considered to apply for stoma retention and inflammation (Lippmann, 2014).

Another mechanism for fibre carcinogenicity is mesothelial piercing of the pleura. The available *in vitro* data show that the diameter is more important than length, with smaller diameters (50 nm) being more toxic than wider diameter (150 nm). The length of the tested nanotube fibres was shorter than 10 µm. These short fibres also induced mesotheliomas after i.p. injection (Nagai, 2011).

Lippmann (2014) reviewed the available data on fibres in general and suggested critical minimal fibre lengths of 2 µm for fibrosis, 5 µm for mesothelioma and 15 µm for lung cancer. The related predominant diameters were > 0.15 µm, > 0.15 µm and < 0.1 µm respectively. A more general observation is that fibres with a diameter above 3 µm are not considered respirable.

Conclusions

The DS considered that the criteria for classification as Carc. 1B were fulfilled for SiC fibres based on the animal studies and the limited evidence from the epidemiological studies. The available data on SiC fibres, and more generally on durable fibres, showed that the potential for carcinogenicity increases with increasing fibre length and decreases with increasing diameter. Therefore, the DS decided to adopt the WHO fibre definition (diameter < 3 µm, length $\geqslant$ 5 µm and aspect ratio $\geqslant$ 3:1) to take into account these parameters in their proposal.

The DS proposed to classify SiC fibres as a carcinogen by the inhalation route only. This was because local tumours were observed after inhalation, i.p. and intrapleural installation and the DS acknowledged the absence of dermal and oral studies. However, the DS considered that

carcinogenicity via other routes of exposure can be excluded based on the proposed mechanism of toxicity for SiC fibres and fibres in general. Overall, they proposed to classify SiC fibres as Carc. 1B; H350i, May cause cancer by inhalation.

Comments received during public consultation

Two Member State Competent Authorities (MSCAs) and 4 Industry or trade associations commented. One MSCA inquired about the fibres definition and about the physical and toxicological properties of the tested fibres (including rigidity in addition to length and diameter, toxicological differences between whiskers and fibres, the observation that biopersistence was already observed for fibres > 0.4 µm lengths). Overall, they agreed with the proposal to classify SiC whiskers (as Cat. 1B) but considered classification in Cat. 2 more appropriate for SiC fibres. The second MSCA agreed with the proposal (Carc. 1B) and provided several comments to improve and clarify the CLH dossier proposal. They also provided additional studies for consideration.

All Industry commenters disagreed with the proposed classification. The main reasons were unclear definition of fibre characteristics, scientific data not applicable to the type of fibres (e.g. data on raw SiC fibres instead of on the fibres on the marked which are mixed with other materials), and exposure considerations. In their comments it was stated that currently no evidence of carcinogenicity exists on SiC cleavage fragments.

Assessment and comparison with the classification criteria

Summary and assessment of animal data

RAC shares the conclusion of the DS that SiC fibres have been shown to induce tumours in animals when administered via the inhalation route or following intrapleural and intraperitoneal administration.

Inhalation studies

SiC whiskers (single crystal, mean diameter 0.45 µm, > 5 µm length) were carcinogenic in a rat inhalation study (Davis *et al.*, 1996) and induced increased rates of lung adenocarcinomas and mesotheliomas.

Table (extracted from Table 16 of the CLH report)

Fibre type	**No. of rats**	**No. of carcinomas (%)**	**No. of adenomas (%)**	**No. of mesotheliomas (%)**
Amosite	42	7 (17)	9 (21)	2 (5)
SiC	**42**	**5 (12)**	**5 (12)**	**10 (24)**
Microfibre	38	0	4 (11)	0

The results have to be assessed taking into account the deviations of the Davis *et al.* (1996) study from standard carcinogenicity studies with guidance-conformity. No air control group was included in this study. In comparison to a group of rats with inhalation exposure to microfibers, clear increases in lung carcinomas and mesotheliomas were observed for the SiC whiskers and (the positive fibre control) amosite asbestos. The ranges of historical incidences of lung tumours and (pleural) mesotheliomas in comparable laboratory control animals were not given in the

study report. They may be assumed to be at a very low level based on the limited information given for a previous batch of animals (with no data on the size of the batch). IARC (2017) in their evaluation referred to control data from a previous study (Davis *et al.*, 1991) with a similar design (pulmonary carcinoma 1/47, pulmonary adenoma 1/47, pleural mesothelioma 0/47).

It has to be noted that the carcinogenic effect was observed despite the number of animals being lower than required (42 rats in total compared to 50 rats/sex/group suggested in the TG) and the shortened exposure duration (1 instead of 2 years) followed by an observation period. The incidence of adenomas was at the same level as for the group that inhaled microfibre and less markedly increased compared to the amosite group. As information from an air control group is not available and the information on the laboratory historical data is limited, it is difficult to confirm the rate of adenomas as increased. A remarkable observation is the high rate of mesotheliomas observed in 10 SiC rats (24%) following the relatively short (1-year) inhalation exposure period.

No information is given in the CLH report on the (lung) effects in 9 additional rats that were killed by the end of exposure to SiC whiskers.

In summary, despite the limitations of the study that may have resulted in a lower sensitivity to assess the carcinogenic potential of SiC whiskers, it was concluded that SiC whiskers tested in the study of Davis *et al.* (1996) was carcinogenic in rats after inhalation.

The second inhalation study (Akiyama *et al.,* 2007) did not reveal a carcinogenic response of SiC whiskers in rats. The lack of tumour response was attributed by the DS to the low exposure level (2.6 ± 0.4 mg/m³, 98 fibres ± 19 fibres/mL), the shorter fibre length (mean diameter of 0.5 µm and mean length of 2.8 µm; MMAD 2.4 µm) and the small number of rats (11) examined after 2 years (unlike the full life span in the Davis study). Broncho-alveolar hyperplasia with fibrous aggregations were seen in 2 out of 11 rats at the age of 2 years (0/13 in controls). Fibre-aggregated foci in the alveoli and interstitial deposition of whiskers accompanied by collagenous material were observed in the alveolar space 6 days after cessation of treatment after 1 year of exposure. Progression to severe fibrotic changes around fibre-aggregated regions and fibrous thickening of the alveolar wall around fibre aggregations and infiltrated with inflammatory cells were found at the end of the 1-year recovery period. Fibre deposition in the pleura and slight thickening of the pleura was also noted.

Additional evidence was available from studies with single (or multiple) intrapleural or intraperitoneal administration of fibres that are commonly used in the testing of fibres as models to demonstrate their potential to induce mesotheliomas.

Intrapleural studies

Stanton *et al.* (1981) reported an increased incidence (17/26 (65.4%) vs. 29/1518 (1.9%) in combined controls, including also groups of sham-treated controls and controls that received non-fibrous material) in pleural sarcomas (sarcomatoid mesotheliomas) in rats 1 year after intrapleural administration of 40 mg SiCW (metallic crystalline, strongly variable in diameters and lengths) after thoracotomy.

47.7% of rats injected intrapleurally three times with 20 mg SiW at intervals of one month developed pleural mesotheliomas (Vasil'eva *et al.*, 1989). Even higher percentages of pleural mesotheliomas (90%, resp. 87% vs. none in the control group) corresponding to a significantly shortened survival time were observed in groups of 30 rats that received 20 mg of two different SiC fibres (SiCW 1, SiCW 2), while a third sample (SiW 3) caused a tumour response of 23% in a lifetime study of Johnson and Hahn (1996). The difference in tumour response could not be explained by the fraction of fibres > 20 µm in length or the fibre numbers.

Intraperitoneal studies

A high rate of mesotheliomas (22/24, 90%) and shortened mean survival time were seen in rats that received a single dose of $1x10^9$ fibres (length >5 µm) intraperitoneally (Davis *et al*., 1996). No information on the timing of the intraperitoneal injection of fibres to groups of 24 rats was given, but based on the Fig. 1 of the study of Davis *et al*. (1996) an application at the beginning of the study appears likely. The same data on study design and outcome was reported in Miller *et al.* (1996b) which was conducted at the same institute (and both published in 1996) as in the study of Davis *et al*. (1996).

In the Adachi *et al.* study (2001), the frequency of mesotheliomas was 70% in rats exposed to 5 mg SiCW one year after a single intraperitoneal administration (and 100% at 10 mg SiCW, no data on size distributions). Mesotheliomas started to appear as early as 200 days after injection.

A dose response relation (based on mg/rat and total no. of fibres) of the tumour rates and mean survival time was observed in rats exposed intraperitoneally to unspecified SiCW with dimensions of 3.1 µm x 0.31 µm (Pott, 1991).

Summary and assessment of the human data

RAC agreed with the overall conclusion of the DS that the epidemiological studies (Infante-Rivard *et al*., 1994, Bugge *et al*., 2012) showed a positive association between exposure to total dust in the SiC industry and risk of lung cancer. However, there is only limited information about exposure-response associations between specific dust constituents and increased risk of cancer. RAC shared the view of the DS that the analysis by Bugge *et al.* (2012) indicates that SiC fibres may have an independent additional lung cancer effect in workers. The unadjusted incidence rate ratio was more strongly associated with lung cancer incidence for cristobalite exposure than for SiC fibres (2 vs. 1.9, see Table on Incidence rate ratios (IRR) and 95% CIs for lung cancer, above).

The DS reported that crystalline silica in the form of cristobalite was the most important occupational exposure factor responsible for lung cancer excess in the study by Bugge *et al*. (2012), but SiC fibres seemed to have an independent additional effect (IRR 1.7; 95 % CI 1.1 to 2.9). Exposure to quartz and SiC particles did not seem to influence the lung cancer incidence significantly. This is generally true, as stated in the CLH report "*when two or more exposure factors were included in a Poisson model, lung cancer risk was most strongly associated with cristobalite exposure. An association with exposure to SiC fibres was also demonstrated, but this association was less marked than the cristobalite association*". However, the study authors also pointed out that "*although fibres had a stronger association with lung cancer than quartz and SiC, this effect was somewhat reduced when cristobalite was included in the multivariate model*". The study authors thus put this finding into perspective: "*However, the effect estimate (IRR) of SiC fibres after inclusion of cristobalite and SiC particles in a multivariate model was still 1.3, and we cannot from this study exclude an effect of SiC fibre exposure on lung cancer incidence".*

RAC noted that the association was weaker after adjustment for cristobalite and non-fibrous SiC and did not reach significance (IRR 1.3, CI 0.7-2.6). In this study the IRR for SiC particles is reported to be 1.4 (adjusted for fibres still 1.1) and thus similar to the IRR value of SiC fibres. Nevertheless, the study authors and the DS reported that *"exposure to quartz and SiC particles did not seem to influence the lung cancer incidence significantly*".

RAC noted that characterisation of SiC fibres (and other dust components) was not reported in any of the epidemiological studies. Thus the data do not allow a conclusion to be drawn on a specific association between SiC fibres of specific ranges of diameters and lengths and cancer risk.

It was also noted that no control population was included in epidemiological studies, and comparisons were made to calculated "expected incidence" numbers based on 5-year national incidence rates for various age groups. One exception is the study by Jakobsson *et al.* (1997) (controls = fishermen and other industrial workers, respectively). However, in this study participants were not only exposed to various forms of SiC, but rather to metal dust (stainless steel; 18% nickel (Ni), 8% chromium (Cr)) and dust from the abrasives, including SiC, aluminium oxide, amorphous silicon dioxide, clay, and phenol-formaldehyde resins at the same time.

Similarly, in the study by Järvholm *et al.* (1982), industry workers were exposed to a mixture of tallow, beeswax, petroleum jelly, carnauba wax, alundum (Al_20_3) or carborundum (SiC), ferric oxide, and chalk within a metal polish paste. In the study by Edling *et al.* (1987), where no significant increase was found in mortality or in cancer morbidity among the workers, they were exposed to aluminium oxide, SiC, and formaldehyde when manufacturing abrasive materials.

With respect to the studies by Romundstad *et al.* (2001a,b) and Bugge *et al.* (2010, 2011 and 2012), the DS reported that total dust was composed of respirable quartz, cristobalite, SiC particles and SiC fibres. The DS also stated (only in the tables) that carbon monoxide and sulphur dioxide gases were released with the SiC dust, together with small amounts of volatile polycyclic aromatic hydrocarbons (PAH); such impurities might impact the study outcome. In the study by Bugge *et al.* (2012), a few historical measurements of PAH were mentioned (ca. 1 µg/m^3), showing low exposure levels compared with current occupational exposure limits. PAH was therefore neither included in the measurement programme for the comparative measurement study nor in the subsequent modelling in this study. The authors concluded that "*other cancers associated with PAH exposure, such as bladder cancer, was not increased in the SiC industry indicates that other factors than PAH were the more important carcinogenic agents*."

Moreover it is noteworthy that Bugge *et al.* (2011) reported that in the earlier periods, parallel exposure of workers to asbestos could not be excluded, although the use of asbestos has been moderate in this industry, mainly restricted to maintenance work between 1940 and 1980.
Generally, estimation of exposure was based mainly on industrial hygiene measurements and on descriptions of changes in the process technology and work practices over time. The proportion of crystalline silica, SiC fibres, and SiC particles in total dust was assumed to be constant over time.

Lagging of exposure by 10 and 20 years implies that each person-year of follow-up is assigned a cumulative level of exposure corresponding to the cumulative level 10 or 20 years earlier. Bugge *et al.* (2012) demonstrated that the 10 year lag gave no different results than the non-lagged analyses, whereas with a 20 year lag in exposure, more significant exposure-response associations were seen, indicating a longer induction and latency period for lung cancer development than after 10 years. However, in contrast to this finding, a Jahr model analysis did not find any time-weighted exposure-response associations. RAC notes that this finding was not specifically addressed in the CLH report.

Bugge *et al*. (2012) pointed out that the exposure assessment study does not take into account the use of personal protective equipment (PPE) due to limited information about historical use of respirators and that "*not adjusting for the use of respirators might thus lead to an overestimation of the inhaled dose, especially for the recent years*". This was not referred to in the CLH dossier but might have led to an underestimation of the exposure-response relationship in the epidemiological studies.

Comparison with the classification criteria

RAC agrees with the DS' conclusion that according to Annex I, CLP Regulation (Chapter 3.6.2) classification in Category 1A is not warranted as the available epidemiology data show limited evidence of carcinogenicity. Positive associations between exposure to SiC fibres and lung cancer were identified, but confounding factors as exposure to other lung carcinogens could not be ruled out.

Limited evidence as defined by the criteria a)-d) in Annex I, 3.6.2.2.3(b) of the CLP Regulation could justify classification in Category 2. RAC found that there is neither doubt about the causal relationship between SiC fibres and the increase in lung carcinomas and mesotheliomas, nor are there unresolved questions about the interpretation of the observed tumours. There may be unresolved questions about the carcinogenic responses of SiC fibres with mean lengths shorter than 5 µm; however these are outside the scope of the DS' classification proposal.

According to the CLP criteria (Annex I, 3.6.2.2.3 (b)) classification in Category 1B for carcinogenicity is warranted if there is sufficient evidence of carcinogenicity, i.e. when a causal relationship has been established between the agent and an increased incidence of malignant neoplasms, or of an appropriate combination of benign and malignant neoplasms from either two or more species, or from two or more independent studies experiments in one species. The available studies on SiC fibres do not fulfil these specific criteria since no other species besides rats were tested and only one positive inhalation study is available.

However, despite the limitations of the dataset, RAC considered that a clear causal relationship was demonstrated in the inhalation study of Davis *et al*. (1996). Taking the supporting evidence from intrapleural/intraperitoneal studies into consideration, there is sufficient evidence to fulfil the criteria that "*a single study in one species and sex might be considered to provide sufficient evidence of carcinogenic 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*."

Silicon carbide fibres were carcinogenic in rats only in the absence of long-term studies in other species. Information on the sex affected was not available for the study of Davis *et al*. (1996). Female rats were treated in most of the studies with intrapleural/intraperitoneal administration. The only study in male and female rats (Vasil'eva *et al.*, 1989) did not provide information about sex-specific responses.

Taking also into account the general knowledge on fibre carcinogenicity (e.g. from asbestos) there are no reasons to assume a sex-specific carcinogenic effect.

The DS added information on the rate of spontaneous tumours in their responses to comments received during PC (in the RCOM document): *Pleural mesotheliomas occur rarely in rats and in humans (Blackshear, 2014). According to Table 3.3 of the IARC monograph on asbestos (Volume 100c), no pleural mesotheliomas were observed in a range of rat strains*.

The DS proposed that the criteria for classification in Carc. Cat. 1B was fulfilled for SiC fibres based on the animal studies and the limited evidence from the epidemiological studies. RAC considered the evidence from human data to be limited to the indication from the SiC workplace studies (Bugge *et al*. 2010, 2011 and 2012; Romundstad *et al*., 2001 and 2002) that SiC fibres may have an additional independent effect on the risk of lung cancer. The overall evidence (taking also into account other studies with co-exposure to lung carcinogens) may be considered weak and uncertainty exists about the level of its significance towards the overall strength of evidence in the absence of epidemiologic studies with information on SiC fibres.

Based on the studies of Davis *et al*. (1996) and Akiyama *et al.* (2007), the DS postulated that the carcinogenicity of SiC fibres is a function of the fibre length which can be considered as one of several parameters that contributes to fibre carcinogenicity. Fibres with mean lengths > 20 µm were assumed to significantly contribute to the carcinogenic responses. In the Akiyama study SiC whiskers with mean lengths < 5 µm persisted in the lung tissue (with a half-life of 16 months), translocated to the alveolar interstitial sites and to pleural regions and induced interstitial (alveolar) fibrosis, bronchiolar-alveolar hyperplasia and fibrotic foci in the pleura at the sites of fibre deposition. The latter two findings could optionally be interpreted as early pre-neoplastic findings (bronchiolar – alveolar hyperplasia – adenoma – carcinoma) or precursor lesions (pleura fibrosis – plaque formation – mesothelioma). Uncertainty remains whether the findings from this study on SiC whiskers should be considered as evidence that only fibres of mean lengths > 5 µm have carcinogenic potential as the treatment duration was too short and the number of tested animals too small to allow any conclusion to be reached on the carcinogenic potential.

The dose-related tumour formation after single intraperitoneal injection of fibres with 3.1x0.31 µm dimensions (Pott, 1991) indicated that SiC fibres with mean lengths < 5 µm may also be carcinogenic.

Studies with intrapleural and intraperitoneal administration supported the conclusion that SiC whiskers with different dimensions, but all with mean diameter < 3 µm and lengths > 5 µm are carcinogenic (Davis *et al.*, 1996; Stanton *et al.*, 1981; Johnson and Hahn, 1996). Other studies with intrapleural or intraperitoneal administration of SiC did also induce mesotheliomas, however no detailed information on the fibre size were given (Vasil'eva *et al.*, 1989; Adachi *et al.*, 2001),

Nevertheless, at this state of knowledge and based on the available studies, RAC recognised that a carcinogenic response was demonstrated for SiC whiskers with mean diameters of 0.45 µm and > 5 µm as used in the inhalation study of Davis *et al.* (1996). Evidence for SiC fibres with shorter mean lengths (< 5 µm) to induce lung cancer and mesotheliomas was at present considered by RAC to be insufficient.

The inhalation study of Davis *et al.* (1996) and the studies with intrapleural/intraperitoneal administration (at least all those with size characterisation) provided evidence of carcinogenicity of SiC whiskers with mean dimensions of < 3 µm diameter and lengths > 5 µm. The DS suggested to define the entry (in Annex VI of CLP Regulation) as for SiC fibres (in general) with these dimensions due to the comparable dimensions of whiskers and fibres and similarities in their dissolution and surface active properties. RAC agreed with this proposal, recognising that fibrous SiC contains fibres of variable diameters and lengths. According to the information provided by the DS, polycrystalline SiC fibres with a diameter < 3 µm and lengths > 5 µm may contain fibres indistinguishable from monocrystalline whiskers. Moreover, cleavage fragments are polycrystals that may split into monocrystals of smaller diameters. Knowing that there are no long-term inhalation data and only limited data from intracavial testing on mesothelioma production, the DS suggested to include SiC cleavage products in the classification proposal. SiC fibres, whiskers and cleavage fragments are SiC fibres which, if they fulfil the WHO fibre definition, should be considered to be carcinogenic and should be covered by the entry. Although the evidence is only strong for SiC whiskers, RAC considered it justified that all three fibre types should be considered as carcinogens based on the present understanding of the pathological mechanism of fibre carcinogenicity (Lippmann, 2014) and based on the fact that these SiC forms are not clearly defined due to their highly variable composition, but these SiC forms may contain fibres with a diameter < 3 µm and lengths > 5 µm. Observations (inflammation/fibrosis and tumour sites/types) correspond to the fibre carcinogenicity paradigm which is known for other carcinogenic fibres (e.g. asbestos, e-glass microfibres, refractory ceramic fibres).

Differences in the fractions of insoluble fibres and differences in the size distribution of SiC fibres occur, but do not support the lack of carcinogenicity for a certain fibre type. Rödelsberger and

Brückel (2006) in their study concluded that the carcinogenic potency of SiC cleavage products (based on the i.p. data from Pott and Roller, 1996) could be lower than that of whiskers, which may be attributed to the lower concentration of fibres/mg sample (58 000 fibres/mg granular sample vs. 48 000 000 fibres/mg whiskers (in the study of Pott and Roller, 1996) or 107 000 000 fibres/mg whiskers and/or to the low fraction (10%) of fragments with diameters < 1 µm or no fibres at lengths > 10 µm. Limitations of the Pott and Roller study are noted and in the end no firm conclusion on their relative potency can be drawn.

As for the SiC fibres, RAC decided to follow the DS' proposal to include the SiC cleavage products. Although only limited data was available, it was shown (e.g. in Bruch *et al.* 2014) that SiC cleavage products contain fibres of the critical dimensions, and it was not demonstrated that all SiC cleavage products are free from fibres or fibre-like structures and/or that polycrystalline structures do not split into fibres with relevant sizes.

RAC recommends that the Annex VI entry should not refer to the full text of the WHO fibre definition (1997) that includes fibres with a diameter < 3 µm and lengths > 5 µm with an aspect ratio of ⩾ 3:1. One reason was that the CLP Regulation does not refer to fibres as 'fibres with WHO definition'. Instead, it is mainly the fibre dimensions and their biopersistence which determine the carcinogenic potential of SiC fibres and for which the evidence was provided. The specific dimensions should be considered by the entry (SiC fibres (with diameter < 3 µm and lengths > 5 µm with an aspect ratio of ⩾ 3:1).

<u>Classification for the inhalation route only</u>

RAC agreed with the proposed classification for the inhalation route (H350i, May cause cancer by inhalation). The DS argued that the proposed fibre pathological mechanism acts only after inhalation and that only local tumours were seen after inhalation, intraperitoneal or intrapleural instillation. The latter two administration routes were accepted as sensitive to demonstrate the carcinogenic potential of fibres with WHO dimensions, but do not represent relevant routes for normal use and exposure.

RAC noted that the available evidence on carcinogenicity is based on inhalation studies and supporting evidence from intraperitoneal and intrapleural administration. Studies on other routes such as oral and dermal are not available, but chronic exposures via these routes were considered as unlikely to cause carcinogenic effects. This view is in line with RAC's previous decision on E-glass microfibers. It should be noted that the existing classification on asbestos as a carcinogen, Category 1A was not restricted to the inhalation route, and the underlying reasons are not known (it is not clear whether an indication of the route was possible at that time). According to present knowledge, there is no evidence that other carcinogenic fibres meeting the WHO definition have carcinogenic properties after oral or dermal exposure. However, uncertainties remain since the absence of evidence is based on the absence of dermal and oral studies on SiC fibres (and E-glass microfibers). For asbestos fibres, some data exist after long term oral exposure. No increase in gastrointestinal tumours were observed in rats and hamsters after lifetime administration of chrysotile, crocidolite and amosite (asbestos) fibres with the diet (IARC, 2012). IARC found positive associations between asbestos exposure and tumours along the gastrointestinal tract, however interpretation of the findings need careful consideration of the exposure assessment (swallowing of a fraction following inhalation may be considered) and strength of evidence based on the available epidemiological studies. A more recently published prospective cohort study (Offermans *et al.*, 2014) showed an association between several gastrointestinal cancer types and prolonged occupationally highly exposed subjects.

RAC concluded that based on the present knowledge the inhalation route is the only relevant route and the SiC fibres should be classified for this route only.

Comparison with criteria for applying notes specific to fibres

Note A:
Without prejudice to Article 17(2), the name of the substance must appear on the label in the form of one of the designations given in Part 3 of Annex VI. In Part 3, use is sometimes made of a general description such as '... compounds' or '... salts'. In this case, the supplier is required to state on the label the correct name, due account being taken of section 1.1.1.4.

RAC agreed with the DS' view not to propose Note A as the proposed international chemical identifier is not for a group entry but for a specific substance with defined physical properties.

Note Q:
The classification as a carcinogen need not apply if it can be shown that the substance fulfils one of the following conditions:

- *a short term biopersistence test by inhalation has shown that the fibres longer than 20 µm have a weighted half-life less than 10 days; or*
- *a short term biopersistence test by intratracheal instillation has shown that the fibres longer than 20 µm have a weighted half- life less than 40 days; or*
- *an appropriate intra-peritoneal test has shown no evidence of excess carcinogenicity; or*
- *absence of relevant pathogenicity or neoplastic changes in a suitable long term inhalation test.*

RAC agreed with the DS that Note Q is not appropriate as data show high biopersistence of SiC fibres and excessive carcinogenicity in line with the RAC opinion on E-glass microfibres and glass microfibres. Moreover, the dimensions of SiC fibres are defined in the entry (with length > 5 µm) and exemptions for types of fibres > 20 µm are not needed.

Note R:
The classification as a carcinogen need not apply to fibres with a length weighted geometric mean diameter less two standard geometric errors greater than 6 µm.

RAC followed the DS' proposal not to apply Note R. SiC fibres shown to be carcinogenic in the study of Davis *et al*. (1996) had a mean length of 5 µm, but do also contain fractions of fibres with much larger fibres. Also SiC fibres within the proposed definition (mean diameter < 3 µm and length > 5 µm and aspect ratio $\geqslant$ 3:1) may contain variable fractions of longer and shorter fibre lengths (with different diameters). RAC noted that Note R is a measure for the diameter (not length). SiC fibres may be polycrystalline with a potential to split into shorter and thinner fibres than the original ones. No SiC fibre type is known with thick fibres only and fibres with > 6 µm were outside the scope of the classification proposal.

(The RAC opinion on glass microfibers provides further information about the history and intention of Note R).

Conclusion on classification

RAC agreed with the DS that silicon carbide fibres with diameter < 3 µm, length > 5 µm and aspect ratio $\geqslant$ 3:1) should be classified as **Carc. 1B; H350i, "May cause cancer by inhalation"**.

RAC comments of specific target organ toxicity – repeated exposure (STOT RE)

The SiC fibres were not assessed for classification for STOT RE either by the DS or by RAC. However, the DS included in the CLH report repeated dose toxicity data from studies in animals as well as from *in vitro* testing in cell cultures on SiC particles and fibrous forms of the substance as additional information relevant to the proposal. Please note that RAC has added comments and additional information from the references.

NOTE: RAC's comments are added to the DS' summary and are indicated as *italicised* text below.

Silicon carbide dust

In a repeated dose inhalation study in rats, two sets of independent tests conducted with respirable dust particles with a (average) grain diameter of < 3 µm revealed a slight (non-significant) increase in mediastinal lymph node weight after a second series of a 5-d inhalation of 20 mg SiC/m³ (5 h/d, after a first series of 5 days of exposure followed by 2 days rest). A high number of total cells and of alveolar macrophages in the broncho-alveolar lavage (BAL) fluid (without stimulation of granulocytes) was observed three days after end of the inhalation in the first set of testing only (Bruch *et al.*, 1993a). No information was given on the fibre concentration and/or fibre distribution in the dust samples. Only in Bruch and Rehn (1996) was it was clearly stated that the SiC dust samples were free of fibrous SiC varieties.

> *RAC also notes that no information is given on the particle size distribution or histology. No effect on lung weight or maximum flow values for respiratory function was observed.*

A single intratracheal injection of SiC dust (50 mg/rat) with a (average) grain diameter of < 3 µm led to increased lymph node weight after 8 months (first series of testing) and 3 and 12 months (second series of testing) of inhalation exposure. According to the CLH report, at 3 and 8 months after exposure, the dust deposited in the lungs was *compactly located and was not accompanied by any cellular response* and was considered by the authors of the study to be a "*completely inert deposition*" (because it was not accompanied by any (granulocytic) cellular response or collagen deposition) of SiC dust in the lungs (Bruch *et al.*, 1993b). Based on these studies, the DS concluded that SiC particles were practically "inert", i.e. that they produced no tissue damage (Bruch *et al.*, 1993b) nor increased number of granulocytes (following repeated inhalation) (Bruch *et al.*, 1993a).

> *RAC notes that no BAL parameters were examined in this study* and that *no data* are available for *the first 90 days after intratracheal application. No information* was *given on the particle size distribution.*

In a later study, Bruch and Rehn (1996) observed that a single intratracheal instillation of 20 mg/animal SiC-B dust (mean diameter 1.14 µm) and SiC-A (mean diameter 2.26 µm) elicited increased numbers of total cells and a granulocytic response in the BAL. While SiC-B causes a significant drop of the granulocytic response at day 14 followed by a new elevation of the granulocytic percentage up to primary levels which persisted until day 90, the granulocytic percentage of SiC-A decreased continuously during the 90-d follow-up period.

The DS concluded that relevant differences in bio-pathogenicity do exist for the tested varieties of SiC (Bruch and Rehn, 1996). The authors suggested that SiC dusts are biologically inert when in particulate form (with grain diameter of < 3 µm), but have biological activity when they are in fibrous form (Begin *et al*., 1989; Bruch and Rehn, 1996).

> *RAC notes that the Bruch and Rehn study intratracheally applied a low dose of particles < 3 µm (20 mg/animal) which caused increased total and granulocytic cell numbers in*

BAL. The organ weights were not assessed in this study. The higher dose (50 mg/animal) in the earlier study (Bruch et al., 1993b) (without BAL and without time-course data for the period up to day 90) was found to induce higher lymph node weights that persisted up to 12 months without any other signs of lung lesions. The conclusion of "complete inert deposition of SiC dust particles < 3 µm average diameter" appeared in conflict with the Bruch and Rehn study (which indicated an inflammatory response) and was regarded as uncertain due to the lack of an investigations during the first 90 days and the lack of BAL parameters, and due to the limitations of the chosen test model (single intratracheal administration). The persistently increased weights of lymph nodes indicated that at the very least dust particles were translocated - most likely within histiocytic cells from the alveolar space through intercellular/vascular pathways - into the alveolar wall and to the local lymph nodes. It was stated that any granulocytic response in the lymph node was absent and this was considered not unlikely at the late phases of examination, as it is an expected finding in the early phase of inflammation after single exposure. Demonstration of the presence of abnormal lympho-histiocytic cell responses in the lymph nodes at the late phase in recovery may need appropriate methods (e.g. using immunohistopathology) during the course of the recovery period. Overall, the interpretation is that the inertness of SiC dust particles cannot be concluded based on the limited studies available, with different parameters examined in the studies and the conflicting results from different studies on the dust.

Moreover it is necessary to define 'inertness': Following (sub-)chronic inhalation exposure, (dust) particles deposited in alveolar macrophages (synonymous with findings described as particle-laden 'alveolar histiocytosis' or 'alveolar macrophages') and interstitial macrophages without being accompanied by (microscopically visible in standard haematoxylin and eosin sections) inflammatory cells (granulocytic or lympho-histiocytic inflammatory cells, depending on the duration of exposure and nature of the agent) and interstitial (alveolar/perbronchiolar) fibrosis are considered as 'inert dusts'. Depending on the doses and the time course, biomarkers of inflammatory responses and of broncho-alveolar lesions may be affected in BAL parameters. Although no overt abnormal tissue lesions or fibrosis may have been seen, the alterations in BAL parameters could be more sensitive and could indicate that at the dose tested there was no 'inert' deposition of dust particles.

In contrast to repeated dose inhalation studies, instillation studies are of limited value for identifying the dose-responsiveness of dust exposure. The available repeated dose inhalation study (Bruch et al., 1993a) indicated increases in weights of regional lymph nodes and inflammatory cell responses in the BAL (at least from one experimental series), but examined only a short (subacute) treatment period and only one concentration. Thus, no reliable (sub-)chronic inhalation study on SiC dust is available to indicate dose- and time-dependent responses or to enable a robust conclusion to be derawn on the "inertness' of the SiC dust. Based on the available limited information it can be stated that no evidence of fibrosis was identified for the applied doses and test regimens. The situation may be different at higher doses or after appropriate chronic inhalation testing.

Bruch and Rehn (1996) discussed that SiC-B induced a granulocytic response together with an 'epithelial stimulation' which the authors considered to be consistent with dust-related carcinogenicity based on the formation of reactive oxygen species (ROS), which might be important for silica carcinogenicity. As the epithelial stimulation was mentioned as a result of in vitro testing on cell toxicity and ROS generation in alveolar macrophages, it remains unclear which data led to the conclusion that there was epithelial stimulation. The material tested in this study was reported to be free of fibrous SiC.

Please note that the data on SiC dust was considered to be additional information to identify (dis-)similarities between SiC dust and fibres. The classification proposal does not cover the particulate non-fibrous SiC.

Silicon carbide fibres

Inhalation of 0.09-60.5 mg/m³ SiC whiskers (average diameter 0.577 µm and length 4.68 µm) for 13 weeks resulted in concentration-related increased incidences of lung lesions. These included inflammatory lesions, lymphoid hyperplasia in bronchial and mediastinal lymph node lesions and bronchiolar, alveolar and pleural wall thickening and pleural fibrosis (Lapin *et al.*, 1991). After 26 weeks of recovery, the lung inflammatory lesions had decreased and fewer rats had enlarged lymph nodes. However, the incidence of alveolar wall thickening, focal pleural wall thickening and adenomatous hyperplasia of lung had increased further.

After single intratracheal administration of 100 mg SiC fibres with diameter 0.27 ± 0.27 µm and length of 6.8 ± 11.2 µm in 100 mL saline, the results were (according to the authors) somewhat similar to other fibrous materials of comparable dimensions (such as crocidolite asbestos fibres or chrysotile) in the lung tissue (Begin *et al*., 1989). The SiC fibres (raw or ashed) are retained in the tissue (*the exposed tracheal lung lobe*), and they cause a nodular fibrosing alveolitis and sustained accumulation of inflammatory cells. These cells, mainly macrophages, are activated to produce an excessive amount of fibronectin and other fibroblast growth factors. This altered fibroblast growth regulation leads to a chronic alteration of the interstitial lung matrix which could lead to the SiC pneumoconiosis reported in humans and in the sheep model (Begin *et al*., 1989).

The DS conclusion (*based on their interpretation of Bruch et al., 1993b*) was that SiC dust had no effect, while SiC whiskers in *in vitro* studies (Svensson *et al*., 1997) showed (*that some, but not all of the tested SiC whiskers*) generate ROS and DNA breakage, which was considered to be in line with the results of the *in vitro* tests. A high capacity of SiC whiskers to activate neutrophils (*to generate reactive oxygen metabolites*) was observed and it was higher than for crocidolite. These observations suggested that SiC whiskers exert their activity via the induction of oxidative stress and possibly via a subsequent inflammatory response, and both processes were considered by the DS to have a threshold.

Furthermore, SiC whiskers were observed to be cytotoxic (Vaughan *et al*., 1991; Svensson *et al*. 1997), to disrupt cell membranes, and to be cytostatic (Vaughan *et al*., 1991). Within 24h of being added to BALB/3t3 embryonic mouse cell cultures, SiC whiskers were found associated with the cells, attached to the cell surface, internalised or found penetrating cell surfaces. Additionally, significant alterations in the genome were observed by Vaughan *et al*. (1991). In this study, SiC whiskers induced increased DNA synthesis and total cellular DNA content in embryonic mouse cells. The authors concluded that the amount of damage appears to be more a function of the number of whiskers present than of their size.

However, Brown *et al.* (1998) did not find a significant difference in free radical activity compared to the controls in plasmid DNA assays. The authors concluded that free radicals are either not involved in SiC fibres carcinogenicity, or that the assay conditions were not sensitive enough to detect free radical generation in this case (while it was positive for amosite asbestos with a similar length distribution). The SiC fibres used in the study had a length distribution of 60.86% > 10 µm and 27.6% > 20 µm. The diameter or aspect ratio of the fibres were not given in this study.

Comments received during public consultation

No comments were received during the public consultation as this hazard class was included for information only in the CLH report.

Assessment and comparison with the classification criteria

Not relevant as no proposal for classification of SiC fibres for STOT RE is included in the CLH dossier.

Additional references

IARC (2012) IARC Monographs on the evaluation of carcinogenic risks to humans. Volume 100C, Arsenic, Metals, Fibres and Dusts. http://monographs.iarc.fr/ENG/Monographs/vol100C/

IARC (2017). IARC Monographs on the evaluation of carcinogenic risks to humans. Volume 111. Some Nanomaterials and some fibres. http://monographs.iarc.fr/ENG/Monographs/vol111/index.php

Offermans, Vermeulen, Burdorf, Goldbohm, Keszei, Peters, Kauppinen, Kromhoug, Van den Brandt (2014) Occupational asbestos exposure and risk of esophgeal, gastric and colorectal cancer in the prospective Netherlands Cohort Study. Int J Cancer 135: 1970-1977.

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).