CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

Substance Name: Silicon Carbide (fibres fulfilling the WHO definition: diameter <3 µm, length > 5 µm and aspect ratio ≥ 3:1)

EC Number:

CAS Number:

Index Number:

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Version number: 1.2 **Date: January 2017**

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	***Silicon Carbide, (fibres fulfilling the WHO definition: diameter <3 µm, length > 5 µm and aspect ratio ≥ 3:1)***
EC number:	
CAS number:	
Annex VI Index number:	***none***
Degree of purity:	***unknown***
Impurities:	***unknown***

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation
Current entry in Annex VI, CLP Regulation	-
Current proposal for consideration by RAC	Carc. 1B (H350i)
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Carc. 1B (H350i)

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	None		None	Not evaluated
2.2.	Flammable gases	None		None	Not evaluated
2.3.	Flammable aerosols	None		None	Not evaluated
2.4.	Oxidising gases	None		None	Not evaluated
2.5.	Gases under pressure	None		None	Not evaluated
2.6.	Flammable liquids	None		None	Not evaluated
2.7.	Flammable solids	None		None	Not evaluated
2.8.	Self-reactive substances and mixtures	None		None	Not evaluated
2.9.	Pyrophoric liquids	None		None	Not evaluated
2.10.	Pyrophoric solids	None		None	Not evaluated
2.11.	Self-heating substances and mixtures	None		None	Not evaluated
2.12.	Substances and mixtures which in contact with water emit flammable gases	None		None	Not evaluated
2.13.	Oxidising liquids	None		None	Not evaluated
2.14.	Oxidising solids	None		None	Not evaluated
2.15.	Organic peroxides	None		None	Not evaluated
2.16.	Substance and mixtures corrosive to metals	None		None	Not evaluated
3.1.	Acute toxicity - oral	None		None	Not evaluated
	Acute toxicity - dermal	None		None	Not evaluated
	Acute toxicity - inhalation	None		None	Not evaluated
3.2.	Skin corrosion / irritation	None		None	Not evaluated
3.3.	Serious eye damage / eye irritation	None		None	Not evaluated
3.4.	Respiratory sensitisation	None		None	Not evaluated
3.4.	Skin sensitisation	None		None	Not evaluated
3.5.	Germ cell mutagenicity	None		None	Not evaluated
3.6.	Carcinogenicity	Carc. 1B: H350i	None	None	
3.7.	Reproductive toxicity	None		None	Not evaluated
3.8.	Specific target organ toxicity – single exposure	None		None	Not evaluated
3.9.	Specific target organ toxicity – repeated exposure	None		None	Not evaluated

3.10.	Aspiration hazard	None		None	Not evaluated
4.1.	Hazardous to the aquatic environment	None		None	Not evaluated
5.1.	Hazardous to the ozone layer	None		None	Not evaluated

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Pictogram: GHS05

Signal word: Danger
Hazard statements: H350i: May cause cancer via inhalation
Precautionary statements: Not relevant for harmonisation.

Proposed notes assigned to an entry:

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

SiC (silicon carbide) fibres has not previously been assessed for classification by RAC or TC C&L.

2.2 Short summary of the scientific justification for the CLH proposal

This proposal is based on the information as available in the registration dossiers of SiC (crude and grain) (14 January 2014), the evaluation of the Health Council of the Netherlands (2012) and other available information.

Epidemiological evidence indicates that inhalation exposure to dust in Norwegian SiC industry is related to increased risk of lung cancer for workers (Romundstad P *et al*., 2001, 2002; Bugge MD *et al*., 2010, 2011, 2012). In all epidemiological studies concomitant exposure to several other (potentially) carcinogenic substances occurred; therefore lung cancer risk or mortality observed may not be assigned with complete certainty to a single exposure factor. In one recent epidemiological study, internal analyses indicated 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 additional effect (Bugge M.D. *et al*., 2012). Non-fibrous SiC did not seem to contribute to the cancer risk (Bugge M.D. *et al*., 2012). Case studies of lung tissue samples from SiC manufacturing workers revealed silicotic nodules and ferruginous bodies and indicated that SiC fibres are durable and can exist in high concentrations in lung parenchyma (Massé S. *et al*., 1988).

Available animal studies demonstrate that SiC whiskers (SiCW) and fibres can induce the development of various tumours, including mesotheliomas, after inhalation, intrapleural or intraperitoneal administration. Upon inhalation of SiCW (mean diameter of 0.45 µm and > 5 µm in length) Davis *et al.* (1996) reported the development of carcinomas, adenomas and mesotheliomas in lungs of rats exposed to SiC whiskers. In addition, no tumor induction was found in the limited inhalation study of Akiyama I. *et al.* (2007) when rats were exposed to SiCW (mean diameter of 0.5 µm and length of 2.8 µm) although broncho-alveolar hyperplasia and advanced fibrosis of the lung parenchyma were found. This result supported the results in previous studies that the carcinogenicity is a function of the fibre length. Stanton *et al.* (1981) reported the increased incidence of pleural carcinomas, resembling mesenchymal mesotheliomas in man, 1 year after intrapleural administration of SiCW (range of diameters 0.05 to > 1.5 µm and length of >1.5-2.5 µm to > 8 µm) in rats. The probability of pleural sarcoma correlates best with fibres that measure ≤ 0.25 µm x > 8 µm. The overall frequency of mesotheliomas in rats injected with SiCW was found to be comparable to that of rats injected with asbestos, used as a positive control in some studies (Vasil'eva L.A. *et al.*, 1989; Adachi S. *et al.*, 2001). The development of adenocarcinomas in combination with mesotheliomas, and development of peritoneal mesotheliomas upon intrapleural administration of SiCW (SiCW 1: diameter of 0.42 and length of 4.5 µm; SiCW 2: diameter of 0.75 and length of 20.1 µm; SiCW 3: diameter of 0.32 and length of 6.6 µm) to rats were also reported (Johnson N.F. and Hahn F.F., 1996). This study also showed that other aspects of a fibre must also be important although fibre dimensions are a critical factor for carcinogenesis. In the case of SiCW, surface chemistry may have a limited influence on their carcinogenic potency. No animal data on intrapleural administration of non-fibrous SiC were retrieved.

Intraperitoneal administration of SiCW (mean diameter of < 0.95 µm and length of > 0.4 µm) and unspecified SiCW to rats could lead to early development of peritoneal mesotheliomas (Miller B.G.

et al., 1999b, Adachi S. *et al.*, 2001). No increased tumour incidence was found in rats which had received an injection of non-fibrous SiC (Pott F. *et al.*, 1994) or granular SiC (Roller M. *et al.,* 1996).

In conclusion, classification with Carc. 1B –H350i is warranted for all forms of SiC fibres (table 4) fulfilling the WHO fibre definition[1] (WHO, 1985). SiC whiskers and SiC cleavage fragments of certain size and form fall within the scope of this definition.

Table 4. Forms of SiC fibres for which classification is proposed.

Form	Definition	Classification
SiC fibres (WHO definition)	polycrystalline fibres	Carc. 1B
SiC whiskers (WHO definition)	monocrystalline fibres	Carc. 1B
SiC cleavage fragments (WHO definition)	Elongated particles produced by the splintering of larger crystals	Carc. 1B

2.3 Current harmonised classification and labelling

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

SiC a fibre has currently no harmonised classification (Annex VI, CLP Regulation).

2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

The self-classification as available from the C&L Inventory Database includes self-classification of a total of 732 notifiers. The majority (600) proposed for no classification. 132 notifiers self-classified for skin irritant, eye irritant, specific target organ toxicity (single exposure and repeated exposure) and carcinogenicity.

From these 732 notifiers, 52 proposed a self-classification Carc 1B. None of the notifiers proposed a self-classification Carc. 1A. One notifier proposed a self-classification of H351 Suspected of causing cancer. The large difference in classification for carcinogenicity between the notifiers maybe due to the form (fibrous or non-fibrous).

SiC nano particles and fibres (i. e. whiskers) are not in the scope of REACH registration dossier. The registrants use no classification. It is concluded that there is currently no registration of SiC fibres. However, at least one producer of SiC fibres has pre-registered and intents to register before the 2018 deadline (http://acm-usa.com/faq/?#13).

[1] WHO definition: diameter <3 µm, length >5 µm and aspect ratio ≥3:1

2.4.2 Current self-classification and labelling based on DSD criteria

This paragraph is considered irrelevant seen the repeal of Directive 67/548/EEC with effect from 1 June 2015.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

A substance with the classification of Carc. 1B; H350i is normally subject to harmonised classification (CLP article 36.1.b). SiC is currently not classified according to Annex VI of CLP. However, based on the experimental animal data and epidemiological human data, a classification as Carc. 1B; H350i for the endpoint carcinogenicity is warranted to SiC whiskers and fibres but not to non-fibrous SiC.

Repeated-dose toxicity and genotoxicity data of SiC are also presented in this report as supportive information, as they may provide relevant data for the assessment of carcinogenicity of SiC. However, the classification of SiC regarding repeated-dose toxicity and genotoxicity endpoints is not discussed in this report.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	
EC name:	Silicon Carbide (fibres fulfilling the WHO definition: diameter <3 µm, length > 5 µm and aspect ratio ≥ 3:1)
CAS number (EC inventory):	
CAS number:	
CAS name:	
IUPAC name:	
CLP Annex VI Index number:	
Molecular formula:	SiC
Molecular weight range:	40.0 g/mol

In addition, SiC appears in several crystal modifications based on how the different silicon and carbon layers are stacked. Also the form of SiC varies depending on the production. SiC crude and grains are produced using an Archeson graphite electric resistance furnace at high temperatures (1600 – 2500 °C). The crude SiC is crushed/milled and sieved depending on the required grades.

The crude and grains contain some fibres and cleavage fragments but normally no whiskers. SiC whiskers are produced using a different processes of which pyrolysis of agricultural waste is the main process. Also the uses differ between SiC whiskers (reinforcement of ceramics) and SiC crude and grains (ceramic, refractory and foundry industry) (source: Silicon carbide manufacturers (SiCMa), personal communication).

- non-fibrous SiC (SiC crude and grains), consisting of amorphous angular/globular particles.

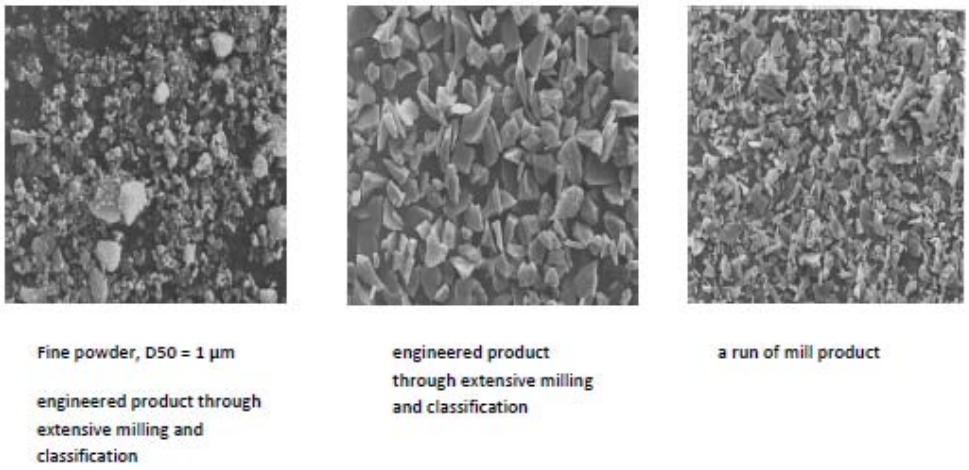

Figure 1: SEM pictures of SiC crude and grains materials (source: SiCMa)

- SiC fibres: polycrystalline fibres; particles longer than 5 µm with a width of less than 3 µm and an aspect ratio of more than 3 are defined as WHO fibres (Health Council of the Netherlands, 2012). According to IARC, SiC fibres are generally poly-crystalline; of variable length and diameter, and may include fibres that are indistinguishable from whiskers (Grosse et al, 2014). However, also monocrystalline fibres are marketed such as:

SI-TUFF™ SF-7 7-Series SiC Fiber

Chemical Composition	High Purity, β-Silicon Carbide (β-SiC)
Crystal Structure	Diamond Cubic
Geometry	Discontinuous Fibre
Mean Diameter, µm	7
Mean Length, µm	65-70 (D50)
Modulus, GPa	350 (estimated)
True Density, g/cm3	~ 3.04
Hardness (Mohs)	9.5

(http://acm-usa.com/site/user/files/1/Datasheet_SF_7.pdf)

- SiCW: monocrystalline whiskers (i.e. threadlike SiC fibres). Whiskers are single crystal structures possessing a fine fibrous morphology similar to that of amphibole asbestos. Several different definitions exist for whiskers. They are approximately cylindrical in shape with an aspect ratio equal to or greater than 3 and a diameter less than 5 µm (Health Council of the Netherlands, 2012). Whiskers are short, discontinuous, rod- or needle-shaped single crystals in the size range <1µm in diameter and >10 µm in length, with aspect ratio equal to or greater than 10 (Rodelsperger K and Bruckel B, 2006). Rod- or needle-shaped single crystals with a diameter < 3 µm and an aspect ratio lentgh/diameter > 10 (SiCMa).

Examples of marketed SiC whiskers are:

Silar® SC-9M Deagglomerated Silicon Carbide Whiskers (Advanced Composite Materials)

Crystal type	Beta (Polytype)
Geometry	Long, rigid rod nanotube
Diameter, µm	0.65
Length, µm	10-12 (D50)
Modulus, GPa	450
Density, g/cm3	3.21
Free carbon, wt%	0.05-0.30
Silica, wt%	0.35-0.75

(http://acm-usa.com/site/user/files/1/Datasheet_SC_9M.pdf)

Other available forms are available here:

http://en.sinetam.com/products/whiskers.html

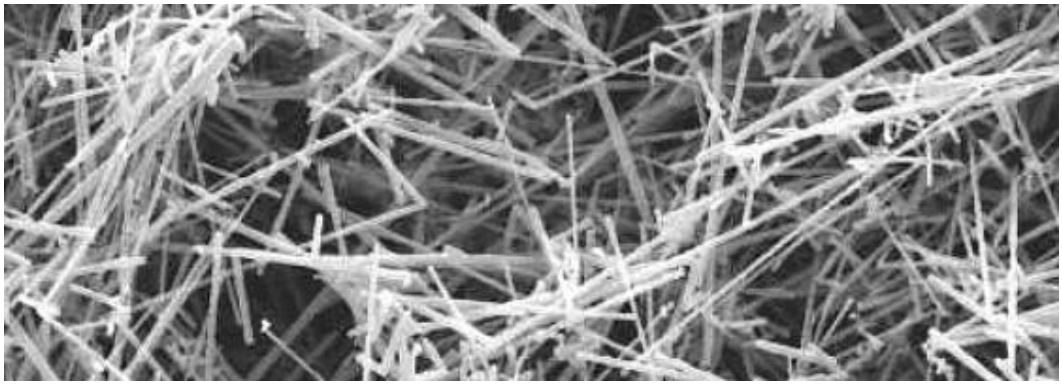

Figure 2: Silar® SC-9M silicon carbide whisker (Source: http://www.acm-usa.com/silar-sc-9m/)

- SiC cleavage fragments; elongated particles produced by the splintering of larger crystals during preparation (grinding and classifying) of SiC. By their irregular shape, they can mostly be distinguished from fibrous particles (asbestos, glass fibres, whiskers). Typically they fulfil the definition of WHO criteria for fibres in term of size and shape (Bruch J. et al., 2014; Rodelsperger K and Bruckel B, 2006). However, the cleavage fragments, unlike fibres, do not split into a large number of fibrils (Rodelsperger K and Bruckel B, 2006). Pictures of cleavage fragments are available in Bruch et al (2014).

Structural formula:

SiC exists in about 250 crystalline forms. The most common polytypes of SiC are 3C-SiC (β), the hexagonal 4H-SiC and 6H-SiC (α), and the rhombohedral 15R-SiC.

1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Silicon Carbide	unknown	unknown	No registration information on SiC fibres

Current Annex VI entry: no harmonized classification

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
unknown	unknown	unknown	No registration information on SiC fibres

Current Annex VI entry:

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
unknown	unknown	unknown	unknown	It is known that some forms of SiC fibres are coated with other substances (example: SI-TUFF™ SC-210 http://acm-usa.com/default.aspx)

Current Annex VI entry:

1.2.1 Composition of test material

1.3 Physico-chemical properties

Table 9: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	Solid: fibres	Electron microscopy images	
Melting/freezing point	Not applicable SiC does not melt congruently, instead it dissociates into graphite and silicon vapour above 2700 °C.	Ruff (1935) as summarised in the ECHA registration dossier.	
Boiling point	The substance decomposes before boiling.	Not available	
Relative density	The relative density of SiC is 3120 kg/m^3 ar 20°C.	Bmelin Hand book of Inorganic Chemistry Silicon Supplement Volume B2. Springer-Verlag Berlin	
Vapour pressure	The substance does not melt at temperatures below 300 °C.	Not available	
Surface tension	Water solubility is below 1 mg/L at 20 °C.	Not available	
Water solubility	Silicon Carbide is practically insoluble in water. Water solubility is below 1 mg/L at 20 °C.	Confidential	
Partition coefficient n-octanol/water	The substance is inorganic.	Not relevant	
Flash point	The substance is inorganic.	Not relevant	
Flammability	Based on the structure and experience in handling and use of the substance, it can be reasoned that flammability is not a concern.	German BG-Institute for Occupational Safety and Health (BGIA) GESTIS-DUST-EX	
Explosive properties	Max. Ex-Overpressure:1.5 bar K_{St} Value: 6 bar m/s Explosibility: St 1 (K_{St} < 200 bar m/s)	German BG-Institute for Occupational Safety and Health (BGIA) GESTIS-DUST-EX	
Self-ignition temperature	Self-heating of the substance can be excluded up to 400 °C.	German BG-Institute for Occupational Safety and Health (BGIA) GESTIS-DUST-EX	
Oxidising properties	The substance is incapable of reacting exothermically with combustible materials. Value used for CSA: Oxidising: no	Not relevant	
Granulometry	mass median diameter 70-90 µm	Confidential	
Stability in organic solvents and identity of relevant degradation	The substance is inorganic.	Not relevant.	

products			
Dissociation constant	Not relevant.		
Viscosity	Not relevant.		

The described physico – chemical properties are based on the registered non-fibres form of SiC.

2 MANUFACTURE AND USES

2.1 Manufacture

Information on production of SiC particles and whiskers are provided in Part B Section 1.1.

2.2 Identified uses

Information on the use of SiC particles and whiskers are provided in Part B Section 1.1.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Not evaluated in this report.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

Inhalation is the only exposure route that is of concern in relation to the direct effects of SiC particles on human health (ECHA registration dossier). Absorption in the lung represents the main route of uptake for dust particles. Transport and deposition of the fibres in the airways are determined by their aerodynamic behaviour. The fibre size, their chemical composition and the deposited dose in the lung define their retention kinetics. The fate of deposited fibres within the respiratory system depends on both the site of deposition and the characteristics of the fibre. Once deposited in the lung, most particles are removed by various clearance mechanisms. Insoluble particles deposited on ciliated airways are generally cleared from the respiratory tract by mucociliary activity in 24-48 hours and will be swallowed in the mouth (ingestion) (ECHA registration dossier). Clearance from the pulmonary region may occur through the action of alveolar macrophages or by alternative mechanisms. Migration through the intercellular spaces of the alveolar membrane to the lymphatic system of the lungs may occur. Clearance of insoluble particles deposited in the pulmonary region of the lung has half-times that are measured in months to years (ECHA registration dossier).

The specific clearance patterns of fibrous and non-fibrous forms of SiC have been examined in the sheep model (Dufresne A. et al., 1992). All particles in the non-fibrous sample were angular in shape. Analysis by X-ray diffraction indicated that this sample contained essentially a particular polymorph of SiC carborundum but also $A_{12}O_3$ corundum. The 'fibrous' sample contained fibres of several morphological types (at least isolated fibrils, aggregated fibrils, rectilinear fibres, corrugated fibres), but also angular particles (27% by weight) and graphite (5% by weight). Because of their complicated morphological structures, no attempt was made to get the size distribution of the fibres. On the basis of some characteristics revealed by TEM examination (special image features at high magnification, electron diffraction patterns) the numerous constitutive fine fibrils could be positively identified as SiC. The larger fibres were too thick to be studied by electron diffraction. The larger fibres were around 30 µm long and 0.5 µm thick. Although their energy dispersive spectrometer of X-rays (EDS) all exhibited a single Si peak, their SiC nature could not by affirmed. They could eventually correspond to vitreous siliceous material. The same is true for the angular particles in the 'fibrous sample'.

Sheep weighing between 25 and 45 kg were used in this study. The flock was divided into groups of eight sheep. The tracheal lobe was exposed to 100 ml of saline containing 100 mg of particulates from either the non-fibrous sample (group G, for angular) or the fibrous sample (group F). Exposure of the tracheal lobe was carried out via bronchoscopic catheterization of the tracheal bronchus and slow infusion of the suspension in the lobe. The animals were studied by broncho-alveolar lavage (BAL) prior to exposure and post-exposure at months 2, 4, 6 and 8. At month 8 of the study, all sheep were sacrificed and the lungs removed from the chest cavity. The tracheal lobe was identified and nine tissue samples were taken for analysis. The study showed that 8 months after intra-tracheal

injection of angular and fibrous SiC, the overall retention rate was 30 times less for fibrous than for angular SiC particles. The retention rates of angular particles were similar in the experimental groups G and F. In each of the two groups, the extent of individual variation was much greater for fibrous than for angular particles. Some of the fibres had been transformed into ferruginous bodies, more in the broncho-alveolar lavage fluids (BAL) than in the lung samples (Dufrense A. et al. 1992). Assuming a one component exponential decrease, the half-life of decrease would be 5.8 months for angular particles and 1.7 months for fibres. Although it was speculated that a higher pulmonary retention of the fibrous type could be responsible for its higher toxicity in the sheep model, in this study, there was a lower retention of the toxic agent (fibrous SiC) than the inert one (angular SiC) (Dufrense A. et al. 1992).

Similar observations were made by Bruch J. et al. (1993-1; see chapter 4.7) who compared pulmonary early retention and subsequent clearance of SiC dust (Wacker GmbH batch No D Mikro-F1200 M678) and crystalline silica (quartz) after inhalation of 20 mg/m3 on five consecutive days during two weeks. The inhalation schedules, including two sets of independent inhalation series. Seven rats per group were killed with an overdose of pentobarbital 3, 11, 21, and 90 days after exposure in the first inhalation series, and 3, 21, and 90 days after exposure in the second series. The lungs were removed and stored in acetone for measuring the dust content in the tissue. Dust content was determined gravimetrically with a formic acid digestion method. Dust deposits at day 3 after inhalation were higher in groups exposed to SiC than to quartz. Subsequently SiC was eliminated more effectively. The lowest initial retention of quartz was attributed to higher activity.

Pulmonary deposition and the clearance of deposited fibre particles from lungs were assessed by Akiyama I. et al. (2003). Forty-two Wistar male rats (9 weeks old) were exposed to SiCW (SiC whiskers), by inhalation for 6 hours/day, 5 days/week for 4 weeks. The SiC (CAS nr. 409-21-2) used during this experiment was commercially purchased by Tokai Carbon Co (Japan). The type of SiCW was TWS-100. The mass median aerodynamic diameter was 2.5 μm and the geometric mean fibre diameter and length were 0.4 and 2.2 μm, respectively. No information was available whether these particles were mono or polycrystalline. The daily average exposure concentrations were 10.4 ± 0.5 mg/m3 (214 ± 31 fibres/ml) during the exposure period. The rats were sacrificed after 3 days, 2 weeks, 1, 2, 3, 6 and 12 months after 4-weeks exposure. At each sacrifice time, 5 or 12 rats in the exposure groups and 5 rats in the controls were used. The body and wet organ weights (lungs, livers, kidneys and spleens) were measured. There were no significant differences between the exposed rats and the controls in the body weights and wet organ weights. The weighted lungs were ashed with acid solution (HNO_3 and H_2O_2) by a microwave ashing method. The lung burden after the exposure and the clearance of deposited SiCW from rat lungs were measured. The estimated amount of total inhaled whiskers was 12.5 ± 0.2 (mg). The maximum SiCW content in rat lung was 0.60 ± 0.09 mg in the exposed rat group. The apparent deposition fraction was 4.8 ± 0.7 %. The SiCW deposited in the rat lungs decreased exponentially with the increase in length of the clearance period after the 4 weeks exposure. Based on the one-compartment model a biological half time of 4.0 months was calculated.

In the study of Davis and co-workers (Davis J.M.G. et al., 1996) SiC fibre durability was examined both in vivo and in vitro. It was found that compared to microfibres more longest SiC fibres were present in the lung tissues. While significant clearance of SiC occurred following intratracheal injection, there was extremely little clearance of this material in the year following a 12-month inhalation period. This lack of clearance was probably due to the very significant lung damage caused by the heavy dose of SiC, which was already well marked by the end of dusting. Practically no in vitro dissolution occurred at pH 7.0, 4.6 and 0.6 in a period of up to 56 days (0.0 – 0.2%).

4.1.2 Human information

No relevant human information.

4.1.3 Summary and discussion on toxicokinetics

There was a lower retention of fibrous SiC or quartz compared to higher retention of angular (non-fibrous) SiC in sheep model of pneumoconiosis (Dufrense A. *et al*., 1992) and Female Wistar rats (Bruch J. *et al,*. 1993-1). After exposure of female Wistar rats to SiC aerosols by inhalation for two periods of 5 consecutive days, SiC was deposited practically inert in the lung (Bruch J. *et al*, 1993-1). SiC showed practically no lymphatic penetration.

Assuming a one component exponential decrease, the half-life of decrease would be 5.8 months for angular particles and fibres (Dufrense A. *et al*. 1992). Based on the one-compartment model a biological half time of 4.0 months was calculated for SiCW (Akiyama I. *et al*., 2003). The apparent deposition fraction was 4.8 ± 0.7 %. The SiCW deposited in the rat lungs decreased exponentially with the increase in length of the clearance period after the 4 weeks exposure (Akiyama I. *et al*., 2003).

Compared to microfibres more long SiC fibres were presented in the lung tissues after administration (Davis J.M.G. et al., 1996). It has been noted that there was extremely little clearance of SiC fibres in the year following a 12-month inhalation period while significant clearance of SiC occurred following intratracheal injection. The in vitro dissolution of SiCW was practically absent.

4.2 Acute toxicity

Not evaluated in this report

4.3 Specific target organ toxicity – single exposure (STOT SE)

Not evaluated in this report

4.4 Irritation

Not evaluated in this report

4.5 Corrosivity

Not evaluated in this report

4.6 Sensitisation

Not evaluated in this report

4.7 Repeated dose toxicity

Repeated dose toxicity data in animals and in vitro testing in cell cultures are presented for information as they may provide relevant data for assessment of carcinogenicity. No classification is discussed and proposed for this endpoint.

Table 10: Summary table of relevant repeated dose toxicity studies

Method	Test material	test concentration	Results	Remarks	Reference
Inhalation Four groups of rats were exposed to air only or to one of three concentrations of SiC for 6 hours/day, 5 days/week for 13 weeks	SiC whiskers (average diameter x Length was 0.577 µm x 4.68 µm)	0.09, 3.93, 10.7 and 60.5 mg/m^3 (0, 630, 1746 and 7276 SiC whiskers/ml)	Increased lung weight; inflammatory lesions; bronchiolar, alveolar, and pleural wall thickening; local pleural fibrosis in lung; reactive lymphoid hyperplasia in bronchial and mediastinal lymph nodes.		Lapin C.A et al., 1991
Female SPS Wistar rats injected intra-tracheally with a single dose (50 mg SiC) followed by observation period of 3, 8 and 12 months.	Dust samples: - SiC (F 1200 grün, Elektroschmelzwerk, Kemptem, Germany) - untreated clay, ground clay, and tempered clay, kaolinite (DMT, Essen, Germany), - Dorentruper quartz (DQ12, DMT, Essen, Germany). The dust samples were separated to give an equal respirable particle size distribution. The mean grain diameter was <3 µm. No information was available regarding the presence of fibres in the samples.	First series (30 rats per group): 1. 50 mg SiC suspended in 0.5 ml physiological saline 2. 0.5 ml physiological saline served as control. Second series (30 rats per group): 1. 50 mg SiC suspended in 0.5 ml physiological saline 2. 2 mg quartz DQ12 suspended in 0.5 ml of physiological saline. 3. 0.5 ml physiological saline served as control.	Lymph node weights: First series: slight increase (after 8 months) Second series: slight increase (after 3 and 12 months); no alterations over the period from 3 to 12 months. Histology of the lungs and lymph nodes: Completely inert deposition of SiC dust without accompanying cellular responses (no granulocytes, no collagen development).		Bruch J. *et al.* (1993-2)
Female Wistar rats were exposed oronasally in a modified Kimmerle inhalation chamber. 5 hours/day on five consecutive days, followed by a rest period of 2 days and a re-exposure period of five consecutive days. Observation periods: - investigation of bronchoalveolar lavage (BAL fluid): three and 90 days (first series), and three. 21, and 90 days (second inhalation series). - Lung function test: 90 days (first series) - Elimination of dust	Dust samples: - SiC (Wacker GmbH batch No D Mikro-F1200 M678), - corundum (fused alumina; Wacker GmbH batch No D F1200/3 M74375), - kaolinite, - tempered and ground clay, - quartz (DQ12, DMT, Essen). dust samples with an average grain size below 3 µm. No information was available regarding the presence of fibres in the samples.	First series: 1. 20 mg SiC/m^3 2. 20 mg quartz/m^3 for one hour followed by an exposure of 20 mg SiC/m^3 for four hours. 3. 20 mg kaolinite/m^3 for one hour followed by 20 mg SiC/m^3 for four hours. 4. 20 mg corundum/m^3. Second series: 1. 20 mg SiC/mi^3 2. 20 mg quartz/m^3 3. 20 mg tempered, ground clay dust/m^3 4. Sham exposure (to air).	Organ weight: First series; No increase lymph node weight in SiC group; increased weight lymph node weight in SiC + quartz group; no changes in lung weight. Second series: increased in all groups; highest in quartz group as early as day 3 after ending inhalation; no changes in lung weight. BAL fluid : first series: no changes in cells in SiC group; increased numbers of granulocytes in quartz group; no change in total phospholipids in SiC group; increased at 90 days in quartz group; ratio of LSF (PG:PI) subfractions at day 90		Bruch J. *et al.* (1993-1)

Method	Test material	test concentration	Results	Remarks	Reference
from the lung: 3, 11, 21, and 90 days (first inhalation series) and 3, 21, and 90 days (second series).			after exposure is roughly 2:1 for control and SiC groups and 1:2 for SiC + quartz and SiC + kaolinite groups. second series: no changes in SiC group; increased numbers of granulocytes in quartz and clay group; total phospholipids increased at 90 days in the quartz group, no changes in SiC group, ratio of LSF subfractions at day 90 after exposure were all above 1.5 except for quartz group at day 90 (< 0.5). Lung function: Maximum peak flow values at 90 days after exposure: for SiC and control >8.5 ml/s; for SiC + quartz and SiC + kaolinite groups <8.0 ml/s. Elimination: Dust deposits at day 3 after inhalation were higher in SiC group. Subsequently SiC was eliminated more effectively.		
72 sheep, exposure to single tracheal lobe via bronchoscopic catheterization of the tracheal bronchus and slow infusion in the lobe, followed by 8 month observation period.		100 ml saline (Sa group-control) 100 mg latex beads in 100 ml saline (latex group) 100 mg SiC raw particles in 100 ml saline (SiCp group); 100 mg SiC ashed particles in 100 ml saline (SiCpa group); 100 mg Minusil-5 in 100 ml saline (quartz group) 100 mg crocidolite fibres in 100 ml saline (Cro group) (diameter of 0.24 ± 0.13 µm, length of 2.60 ± 3.05 µm) 100 mg SiC raw fibres in 100 ml saline (SiCf group) (average diameter 0.27 ± 0.27 µm, average length of 6.8 ± 11.2 µm). 100 mg SiC ashed fibres in 100 ml saline	Pathologic scores of disease in the sheep groups were 0±0 for Sa, latex, graphite, SiCp and SiCpa group, 2.9±1.0 for the quartz group, 1.9±0.25 for crocidolite, 1.2±0.21 for SiCf, 1.6 ±0.20 for SiCfa groups. Quartz groups: 500% increase in cellularity which decreased to 250% at month 4 and remained elevated; other groups slight and transient early increase. Quartz groups had significant increased glycosaminoglycan, fibronectin production and fibroblast growth at month 8 of study. SiCf and SiCfa were producing a nodular fibrosing alveolitis.		Begin R. *et al.* (1989)

Method	Test material	test concentration	Results	Remarks	Reference
		(SiCfa group) (average diameter 0.27 ± 0.27 µm, average length of 6.8 ± 11.2 µm).			
In vivo test: dust samples were instilled into rat lungs, 20 mg per animal. The animals were sacrificed after 2, 14, 21 and 90 days. The animals were evaluated by broncho-alveolar lavage (BAL),in the BAL-fluid (BALF), protein, cells, and lung surfactant lipids (LSL) were determined.	SiC-A (Wacker Chemie, Germany, labelled as F1200 according to FEPA; SiC-B (Wacker-Chemie under the label NF2). Mean diameter: FI200 2.26 µm, NF2 1.14 µm: the probes were free of fibrous SiC varieties.	*In vivo*: Dust samples were instilled into rat lungs, 20 mg per animal.	*In vivo*: Total cells were increased 5 days (d) post exposure but decreased continuously during the observation time of 90 d to values close to control animals. The granulocytic response was high in both groups immediately following the exposure whereas the following terms show considerable differences. Si-B causes a significant drop at d 14 followed by a new elevation of granulocytic percentage up to the primary level which persisted unchanged until d 90. In contrast to this, the granulocyctic percentage in the group SiC-A decreased continuously and was statistically different to SiC-B at d 21 and 90 ($p < 0.001$). SiC-A produces an elevation of LSL in the BALF until d 14 post exposure whereas SiC-B effects a sharp drop in LSL even to subnormal level at d 14 post exposure followed by strong and persistent increase.		Bruch and Rehn,1996
In vitro testing on alveolar macrophages (male guinea pig) by a set of toxicity parameters (Lactate dehydrogenase (LDH), Fluorescein diacetate (FDA)) and through determination of inducible H_20_2 release.		*In vitro*: Dusts were tested in doses ranging from 20 to 180 mg/l 0^6cells. Positive controls were quartz (DQI2), the negative ones corundum.	*In vitro*: SiC-B elicits a lasting granulocytic response together with an epithelial stimulation. SiC-A and corundum lack particular bio-pathogenic effectively.		

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

No oral data on repeated dose toxicity have been reported for SiC.

4.7.1.2 Repeated dose toxicity: inhalation

A toxicity study was undertaken to examine whether the inhaled SiC whiskers of specific dimensions (average diameter x Length was 0.577 μm x 4.68 μm) by a natural inhalation route cause lung damage in rats (Lapin C.A et al., 1991). In this study four groups (50 males/50 females each) of rats were exposed to air or to one of three concentration of SiC (0.09, 3.93, 10.7 and 60.5 mg/m3) for 6 hours per day, 5 days per week for 13 weeks. Half of the rats were euthanized at the end of exposure, the remainders were examined 26 weeks later. No concentration-related changes in body weight, clinical chemistry and haematological data attributable so SiC have been observed. Lung weights were increased in the high concentration exposure group at both euthanization times. In all SiC exposure groups, the incidence of the following lung and lymph node lesions was higher than in the controls: inflammatory lesions; bronchiolar, alveolar, and pleural wall thickening; focal pleural fibrosis in lung; and reactive lymphoid hyperplasia in bronchial and mediastinal lymph nodes. After 26 weeks of recovery, lung inflammatory lesions had decreased and fewer rats had enlarged lymph nodes, but the incidence of alveolar wall thickening, focal pleural wall thickening, and adenomatous hyperplasia of lung had increase further. Incidence and severity of these observations were dose-related.

Begin R. *et al.* (1989) conducted an experiment in their sheep model of pneumoconiosis in order to study the biological mechanisms of SiC pneumoconiosis and identify the active component(s) of the particulates. Sheep weighing between 25 and 45 kg were used by Begin R. et al. (1989). The flock was divided into groups of eight sheep. The tracheal lobe was exposed to 100 ml of saline containing 100 mg of particulates from either the non-fibrous sample or the fibrous sample. Samples of graphite, quartz, angular SiC (particulate SiC raw or ashed were 99.5 percent <5μm) , fibrous SiC (raw or ashed were of average diameter 0.27 ± 0.27 μm, average length of 6.8 ± 11.2 μm) and crocidolite asbestos were tested comparatively. These SiC materials were collected from the production sites in the Acheson furnaces of two Quebec SiC plants. The non-fibrous SiC was collected from the centre of large lumps of produced materials. The SiC fibres were collected mainly from the outside part of the main cylindrical lump produced by the process.

Exposure of the tracheal lobe was carried out via bronchoscopic catheterization of the tracheal bronchus and slow infusion of the suspension in the lobe. The animals were studied by bronchoalveolar lavage (BAL) prior to exposure and post-exposure at months 2, 4, 6 and 8. At month 8 of the study, all sheep were sacrificed and the lungs removed from the chest cavity. The tracheal lobe was identified and nine tissue samples were taken for analysis. The BAL analyses of cellularity, cytotoxicity and fibrogenicity, in association to necropsy histopathology, documented that granular SiC was inert. The SiC fibres (with an average diameter 0.27 ± 0.27 μm, an average length of 6.8 ± 11.2 μm) produced a sustained nodular fibrosing alveolitis comparable to that induced by crocidolite asbestos fibres or chrysotile. This fibrosing activity of SiC fibres was accentuated by ashing of the fibres which combusts the graphite on surface of the fibres (Begin R. et al. 1989).

This experiment clearly identified SiC fibres as bioactive with fibrogenic activities comparable to crocidolite asbestos fibres of similar size. Angular SiC was inert. This study therefore documents that when a mineral with the same chemical composition is in a fibrous form, it behaves somewhat as other fibrous materials of comparable dimension in the lung tissue. The long fibres are retained in the tissue, and they cause a 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, the SiC pneumoconiosis as reported in this animal model (Begin R. *et al.*, 1989).

Female Wistar rats, initial body weight, 180-220 g, were exposed oronasally in a modified Kimmerle inhalation chamber to dust samples (Bruch J. *et al*., 1993-1). The dust samples under study were SiC, corundum, kaolinite, tempered and ground clay, and quartz. The average grain size was below 3 µm. The animals were exposed for five hours a day on five consecutive days, followed by a rest period of two days and a re-exposure period of five consecutive days. Total exposure time was 50 hours. There were two sets of independent inhalation series. The first inhalation series (50 animals each group) included:

1) exposure to a constant concentration of 20 mg SiC/m^3 respirable air for five hours a day.
2) Exposure to a constant concentration of 20 mg quartz/m^3 respirable air for one hour a day followed by an exposure of 20 mg SiC/m^3 respirable air for four hours a day.
3) Exposure to a constant concentration of 20 mg kaolinite/m^3 respirable air for one hour a day followed by an exposure of 20 mg SiC/m^3 respirable air for four hours a day.
4) Exposure to a constant concentration of 20 mg corundum/m^3 respirable air for five hours a day (15 animals).

Second inhalation series (42 animals each group), included:

1) Exposure to a constant concentration of 20 mg SiC/mi^3 respirable air for five hours a day.
2) Exposure to a constant concentration of 20 mg quartz/m^3 respirable air for five hours a day.
3) Exposure to a constant concentration of 20 mg tempered, ground clay dust/m^3 respirable air for five hours a day.
4) Sham exposure (to air).

Three and 90 days after exposure in the first series, and three, 21, and 90 days after exposure in the second inhalation series, seven rats per group and seven control rats exposed to air were killed and the lungs were degassed and lavaged in situ five times through the trachea, each time with 5 ml physiological saline. The lavage fluid was centrifuged to sediment the cells and to extract lung surfactant factor (LSF) phospholipids from supernatant lavage fluid. Phospholipid composition was calculated by comparison with standard phospholipid mixtures (phosphatidyl glycerol (PG), phosphatidyl inositol (PI)).

In both inhalation series, the rats showed normal behaviour and normal development after the inhalation and during the observation period of 90 days. First inhalation series showed no increase lymph node weight in SiC, corundum and SiC + kaolinite group. A significant increase in lymph node weight until the end of the investigation occurred only in SiC + quartz group. In second inhalation series all animals exposed to dust showed increased lymph node weights; however, the weights for the quartz group were clearly greater than those for the SiC and clay dust groups as early as day 3 after ending inhalation. There were no changes in lung weight for both inhalation series.

The first inhalation series showed high total cell numbers as well as alveolar macrophages three days after the end of inhalation in the SiC group. These conditions were reversed after 90 days. In the quartz treated group increased cell numbers were found after 90 days. These values must be regarded as the result of an adaptive adjustment to dust exposure in association with the toxic effects of the individual samples. The number of granulocytes reflects the pathological inflammatory stimulus. SiC and kaolinite showed no specific stimulation of granulocytes. Quartz on the other hand produced a strong inflammatory stimulation. Similar results were found in the

second series of studies. The number of cells in lavage fluid was similar in the sham control and SiC group over the entire experimental period. More cells were found in the BAL fluid of groups exposed to clay and quartz. The numbers in animals exposed to clay mineral were normal by day 90.

The relation between sub-fractions of LSF (PG:PI) is a particularly sensitive response to the inflammatory or fibrogenic effects of dusts. On exposure to quartz and kaolinite abnormal ratios of < 1:1 were obtained. By contrast, SiC induced no such alterations in the LSF subfractions, with values corresponding to the normal ratios range from 2:1 to 3:1 of the untreated control.

A lung function test was carried on eight animals of the first inhalation series, which included exposure to SiC, kaolinite, quartz, and corundum. Eight lungs of each exposure group were excised 90 days after finishing the inhalation and tested for peak flow. Control animals as well as animals exposed to SiC showed similar maximum flow values (>8.5 ml/s), whereas exposure to quartz and to a lesser degree kaolinite gave lower flow rates (<8.0 ml/s).

Seven rats per group were killed with an overdose of pentobarbital 3, 11, 21, and 90 days after exposure in the first inhalation series, and 3, 21, and 90 days after exposure in the second series for determination of dust deposits and their retention. Dust deposits (expressed as mg per gram lung tissue?) at day 3 after inhalation were higher in SiC group. Subsequently SiC was eliminated more effectively. The lowest initial retention of quartz was attributed to higher activity.

Bruch and Rehn (1996) conducted *in vivo* and *in vitro* studies (see 4.7.1.6 other relevant information for in vitro studies) with two dust samples SiC-A and SiC-B. The mean diameter of SiC-A was 2.26 µm and of SiC-B was 1.14 µm. Both probes were free of fibrous SiC varieties. For the *in vivo* testing, the dust samples were instilled into rat lungs, with single dose of 20 mg per animal, under controlled conditions providing exact doses to each animal. The animals were sacrificed after 2, 14, 21 and 90 days. The animals were evaluated by broncho-alveolar lavage (BAL), in the BAL-fluid (BALF), protein, cells, and lung surfactant lipids (LSL) were determined. Total cells were increased 5 days post exposure but decreased continuously during the observation time of 90 days to values close to control animals; no substantial difference could be observed between the samples tested when compared to overall change during the study (Bruch and Rehn, 1996). The granulocytic response was high in both groups immediately following the exposure whereas the following terms show considerable differences (Bruch and Rehn, 1996). SiC-B causes a significant drop at day 14 followed by a new elevation of granulocytic percentage up to the primary level which persisted unchanged until day 90. In contrast to this, the granulocyctic percentage in the group SiC-A decreased continuously and was statistically different to SiC-B at day 21 and 90 ($p < 0.001$). Both samples induced distinct and qualitatively different reactions of the epithelial system. SiC-A produces an elevation of LSL in the BALF until day 14 post exposure whereas SiC-B effects a sharp drop in LSL even to subnormal level at day 14 post exposure followed by strong and persistent increase.

4.7.1.3 Repeated dose toxicity: dermal

No dermal data on repeated dose toxicity have been reported for SiC.

4.7.1.4 Repeated dose toxicity: other routes

Bruch J. *et al* (1993-2) investigated the tissue response of the lung itself as well as lymph nodes associated of SiC dust with long term injection tests. Female SPS Wistar rats were injected

intratracheally with a single dose (50 mg SiC dust with main grain diameter < 3µm (no further information) followed by observation period of 3, 8 and 12 months. SiC led to a slight increase in average lymph node weights after eight months (first series) and three and 12 months (second series). No alterations in lymph node weight over the period from three to 12 months (second series). Completely inert deposition of SiC dust in the lung and the lymph nodes. The dust was compactly located without accompanying cellular responses so that in particular no granulocytes were found. No collagen development could be identified. There was a slight increase in lymph node weights. These nodes accumulate all dusts drained lymphatically from the lung and concentrate these particles in lymph node tissue. This slight increase in lymph node weights could be attributed to the natural response to an inert dust deposit.

4.7.1.5 Human information

No data.

4.7.1.6 Other relevant information

A broad range of in vitro studies are provided which demonstrated that there are reactions started in cells which could lead to fibrosis or cancer.

Inhalation of man-made vitreous fibres (MMVF) leads to both inflammatory and fibrotic processes, as well as expression of genes linked to cell proliferation and antioxidant defence in a dose-related fashion. These processes are associated with the activation of alveolar macrophages, lymphocytes, polymorphonuclear cells, mast cells, and fibroblasts and the release of a number of cellular mediators, e.g. tumour necrosis factor α (TNFα), interleukin-1α (IL-1α), interleukin-6 (IL-6), and basic fibroblast growth factor (bFGF) and the upregulation of proto-oncogenes (SCOEL/SUM/88, 2012). Injury to alveolar epithelial cells is followed by hyperplasia and hypertrophy and occasionally by neoplastic transformation resulting in tumourigenesis. Fibre activated macrophages and other inflammatory cells generate reactive oxygen species (ROS), e.g. O^{2-}•, H_2O_2, and NO. The hydroxyl radical (OH •), peroxynitrite, and nitronium ions may also be formed. ROS can also originate from redox reactions occurring at the fibre surface, e.g. by fibre iron catalysis, leading finally to generation of OH •. These oxidants induce oxidative stress in the target cells (SCOEL/SUM/88, 2012).

These processes, being the underlying mechanism of fibre carcinogenicity, are considered to have a threshold (SCOEL/SUM/88, 2012). Cellular antioxidative systems including superoxide dismutase (SOD), catalase, and glutathione-S-transferase-dependent systems, protect against cellular injury and DNA damage as long as the release of ROS is not sufficient to overwhelm this defence. Consequently, the lung is able to deal with a considerable number of fibres without detectable molecular or pathogenic events, which has been shown in epidemiologic and experimental studies.

The parameters analysed in the in vitro studies as descripted in this section relates to pathological stimulus for inflammatory processes and toxic effects on cell metabolism and membrane integrity. Such changes could lead to malignant tumors and as a concomitant of carcinogen induced cellular transformation.

Table 11: Summary table of supporting *in vitro* studies

Test material	Method	Results	Remarks	Reference
SiC cleavage fragments fulfilling the WHO criteria for fibres (length >5 µm, width <3 µm, aspect ratio (length/width) >3). Five test samples of SiC commercial products with different concentrations of CFs prepared for the respirable fraction according to aerodynamic diameter ≤ 4 µm with the precision cascade impactor. Numerical concentrations of CFs and size characteristics are given in 11. The following reference materials were used: (1) Quartz DQ12, respirable fraction. (2) Cristobalite, respirable fraction, a CS isomorph, obtained by heating quartz. (3) SiC whiskers, commercially available, respirable in size. (4) UICC crocidolite. (5) Electrocorundum (fused aluminum oxide; Al_2O_3; ground and sieved for particles ≤ 1.5 µm aerodynamic diameter) used as an inactive sample.	*In vitro* vector model: 1. Cytotoxicity: H_2O_2 release test with guinea pigs alveolar macrophages 2. Glucuronidase test with guinea pigs alveolar macrophages 3. The release of TNF-α with rat alveolar macrophages 4. Assessment of dust induced oxidative burst (ROS) with alveolar macrophages from guinea pigs. Doses: 0, 15, 30, 60 and 120 µg/10^6 alveolar macrophages. The in vitro testing concept was developed, based on the primary reactions of alveolar macrophages activated by phagocytosis of dust particles. Certain reactions (vitality, membrane damage) or secretory products (enzymes, mediators, radical molecules) can be regarded as independent "vectors" of the alveolar macrophages dust reactions. Analysis and combination of the vectors ("vector model") give multidimensional reaction patterns. The vector model has been validated with different dust types of known in vivo reactions (inert, fibrotic, carcinogenic).	All SiC commercial samples showed a very low response in all parameters at all doses tested The results for the doses 15 and 30 mg/10^6 alveolar macrophages are not different statistically from the electrocorundum and the blank (untreated cells). Sample 5 evoked a low, borderline increase of TNF at the higher doses. A dose-dependent increased secretion of ROS for the test samples of SiC beginning at the dose 60 mg/10^6 alveolar macrophages, without any sign of adverse effects at the other parameters. Absence of relationship between WHO fibres (Cleavage Fragments) in SiC test samples and in vitro increase of ROS. Electrocorundum at all doses exerted minimal reaction, not statistically different from blank. Quartz DQ12 prompted a strong dose-dependent toxicity for all parameters but ROS. Cristabolite differ from quartz by a lower TNF response, but very high ROS response, clearly dose-dependent. Crocidolite exhibited a dose-dependent toxicity for all parameters but cytotoxicity. In comparison, SiC whiskers triggered the same three parameters dose dependently.		Bruch J *et al* (2014)
Size distribution (%) of the fibres used in the study: Long-fibre amosite asbestos: length distribution 64.75 >10 µm and 35.25 > 20 µm SiC fibres: length distribution 60.86 >10 µm and 27.6 > 20 µm. Code 100/475 glass fibres: length distribution 50.00 >10 µm and 19.32 > 20 µm. TIMA fibre: mixture of MMVF10 (85.24 >10 µm and	*in vitro* ϕX174RF plasmid DNA scission assay Fibres were coated with female Wistar rats, rat lung lining fluid to determine whether the oxidant-generating ability could be modulated. Fibres were suspended in the DNA solution at 924,900 fibres/20 µl. Test conditions were fibres (coated and uncoated) in DNA/water	All fibres displayed some free radical activity as assessed by their ability to decrease the percentage of supercoiled plasmid DNA, but this was only significant in the case of long-fibre amosite asbestos, which caused 55% depletion of supercoiled DNA. The remaining fibres had free radical activity that ranged from 5 to 20%, but these values were not		Brown DM *et al* (1998)

Test material	Method	Results	Remarks	Reference
67.17 > 20 µm), RCF1 (77.36 >10 µm and 45.27 > 20 µm), RCF4 (59.35 >10 µm and 17.96 > 20 µm).	only, or fibres (uncoated) in DNA/water plus mannitol (4 mM). Each treatment was incubated at 37°C for 8 h. Four microliters of tracking dye was then added to each sample and the DNA plasmid was separated by electrophoresis for 16 h at 20 °C on 0.8% agarose gel. After staining in ethidium bromide, a photograph of the gel under ultraviolet (UV) light was taken and the bands indicating damage to the plasmid were quantified by densitometry. The results were expressed as the percentage of the treatments to the untreated plasmid.	significantly different from control.		
Five whiskers (four SiCs, SiCW-1, -2, -3, -4, and one silicon nitride, SiNW) and two powders (one SiC SiCP, and one silicon nitride, SiNP). One SiCW whisker, SiCW-3, was also ball-milled in water for two different time periods–3 hours (SiCW-3S, short-milled) and 58 hours (SiCW-3L, longmilled). The materials and their dimensions are summarized in Table 12.	Cloning efficiency Assay Survival of the V 79 Chinese hamster lung fibroblasts was determined by cloning efficiency from a single cell suspension. Concentrations used were 0.25, 0.5, 1.0, 2.0, and 4.0 µg/cm^2 (SiCW-2, SiNW, and crocidolite), 0.5, 1.0, 2.0, 4.0, and 8.0 µg/cm^2 (SiCW-1), 1.0, 2.0, 4.0, 8.0, and 16 µg/cm^2 (SiCW-3, SiCW-4, SiCW-3L, and SiCW-3S) and 10.0, 20.0, 30.0, 40.0, and 80.0 µg/cm^2 (SiNP and SiCP) Fibres were suspended in Millipore water.	The inhibition by the most toxic whiskers (EC50 0.9 to 4.2 µg/cm^2) was in the same order of magnitude as that of crocidolite (1.4 µg/cm^2). SiC powder was less toxic (EC50 31.4 µg/cm^2) than the whiskers.		Svensson I *et al.* (1997)
	Analysis of DNA Strand Breaks in V 79 Chinese hamster lung fibroblasts with Nick Translation Assay Concentrations varied from 0.3 to 15 µg/mm^3 Fibres were suspended in Millipore water.	There was a high DNA breaking potential (of the same magnitude as crocidolite asbestos).		
	Three radical formation measuring test: - Deoxyguanosine Hydroxylation Assay (concentration used 1 mg/ml) - Dimethylsulfoxide as Scavenger (concentration used 0.6 mg/ml) - Deoxyribose Assay (concentration used 0.6 mg/ml) Fibres were suspended in Millipore water.	The radical tests showed that only crocidolite and SiCW-4 could potentiate the formation of hydroxyl radicals.		
	Activation of neutrophils	SiCW had the highest		

Test material	Method	Results	Remarks	Reference
	Fibres were suspended in phosphate-buffered saline	ability to stimulate human neutrophils to generate reactive oxygen species		
The dust samples used were: SiC (F 1200 grin, Elektroschmelzwerk, Kempten, Germany), untreated clay, ground clay, and tempered clay, kaolinite (DMT, Essen, Germany), and Dorentruper quartz (DQ12, DMT, Essen, Germany). . The dust samples were separated to give an equal respirable particle size distribution. The mean grain diameter was <3 µm.	*In vitro* methods 1. H_2O_2 release test with alveolar macrophages from guinea pigs were introduced into wells of microtitre plates (300 000 per well); two hours after settlement and conditioning the dust samples were added at doses of 20 and 60 µg/300 000 cells respectively and incubated for 16 hours. (De la Harpe and Nathan) 2. The release of TNF-α Bone marrow macrophages were seeded in microtitre plates at a density of 10^6/ml (2 x 10^5/well). The mineral dust suspensions (concentration of 10 mg/ml in phosphate buffered saline) were added to the cultures together with 0.25 U α-antitrypsin/ml to a final volume of 200 µl/well. After a 24 hour incubation, the supernatants were tested for TNF-α activity.	1. No difference in H_2O_2-release with SiC and Corundum at highest concentration compared with untreated cells. Quartz at a concentration of 60 µg gave a complete inhibition of stimulation of H_20_2-release and the lower concentration of 20 µg quartz resulted in about 40% reduction. Quartz concentrations up to 10 µg/well led to a significant growth inhibition compared with the controls. When tempered and ground clay and pure clay were compared with SiC, SiC did not result in an apparent growth inhibition of L 929 cells at doses up to 50 µg/well		Bruch J. *et al.* 1993-2
- SiCW-1 with a diameter of 0.8 (SD = 0.3) µm, average length of 18.1 (SD = 14.3) µm and aspect ratio of 23.3 (SD = 18.7), - SiCW-2 with the diameter of 1.5 (SD = 0.6) µm, average length of 19.0 (SD = 11.0) µm and aspect ratio of 15.3 (SD = 11.2) - crocidolite asbestos	*In vitro* study Dye Exclusion test (moribund cells or with leaky membranes) BALB/3T3 embryonic mouse cells 5.0, 10.0, 15.0 or 20.0 µg/cm^2 Test material were suspended in phosphate buffered saline	Crocidolite asbestos and SiCW-1 exhibited similar levels of dose dependent cytotoxicity within the first 24 hr.	Dye exclusion assays do not report cells which survive the first 24 hr to be compromised and die later.	Vaughan GL. *et al*., (1991)
	In vitro study ^{51}Cr Release (membrane damage or cell death) in BALB/3T3 embryonic mouse cells At final conc. 5.0 µg/cm^2 Test material were suspended in phosphate buffered saline	Cells in cultures exposed to SiCW-1 or crocidolite at 5.0 µg/cm^2 release 20-30% of label ^{51}Cr in excess of controls, while SiO_2 induces an excess loss of approximately 8.0%.	Radiochromium release assays do not report cells which survive the first 24 hr to be compromised and die later.	
	In vitro study Colony-Forming Efficiency (proliferative ability)	On a mass per surface area basis, SiCW-1 is slightly more toxic than equal quantities of crocidolite.	However, when expressed as a function of the number of fibres	

Test material	Method	Results	Remarks	Reference
	in BALB/3T3 embryonic mouse cells Test material were suspended in phosphate buffered saline	The larger SiCW is less cytotoxic than SiCW-1 ($P < 0.01$) but not significantly different from asbestos ($P > 0.05$).	to which the cells are exposed rather than mass, no significant difference ($P<0.05$) was found between the cytotoxicities of 0.8 and 1.5 µm SiCW of similar lengths.	
	In vitro study Tritiated Thymidine Incorporation Assay (rate of DNA syntheses) in BALB/3T3 embryonic mouse cells Concentration: 0.0 to 2.0 µg/cm^2 Test material were suspended in phosphate buffered saline	DNA synthesis rates for fibre-/whisker-exposed cells were generally elevated relative to controls, often by a factor of as much as 2.5.	However, the results were inconsistent and not reported.	
	Incidence of Binuclear Cells (rate of multi-nuclearity) Concentration used: 5 µg/cm^2 Test material were suspended in phosphate buffered saline	Increase in the number of binuclear cells (six to eight fold).		
	Cellular Transformation (loss of contact inhibition) Concentration: 5 µg/cm^2 Test material were suspended in phosphate buffered saline	On a mass per surface area basis, SiCW-1 is slightly more toxic than equal quantities of crocidolite. The larger SiCW is less cytotoxic than SiCW-1 ($P < 0.01$) but not significantly different from asbestos ($P > 0.05$). When the data for SiCW-2, the larger whisker, are recalculated so that a comparison of transformation frequencies can be made on the basis of the number of particles rather than mass, there is, as with colony-forming efficiency, little difference between the effects of SiCW-1 and SiCW-2.		
	Cellular DNA Content (total DNA content) Test material were suspended in phosphate buffered saline	Significant increases in total cellular DNA content, however, were consistently observed 10-20 generations after treatment. Cells treated with SiCW and crocidolite contained an excess of DNA ranging from approximately 40% to near 70%.		

Bruch J. *et al.* (2014) studied the toxicological significance of cleavage fragments (CFs) in commercial products fulfilling the WHO criteria for fibres. The test samples were respirable fractions of five different commercial samples of SiC grains (Table 11). The CF content (scanning electron microscopy) was in the range 17–493 fibres/mg. Crystalline silica and whiskers could not be detected. Quartz DQ12, cristobalite, SiC whisker and UICC crocidolite were used as positive control reference samples, whereas electrocorundum was used as negative reference. Biological activity was assessed with the *in vitro* vector model on *ex vivo* rat and guinea pig alveolar macrophages.

The response of the positive references was clearly different from that of the SiC grains which were close to the low activity dust electrocorundum for the other vectors. Electrocorundum at all doses exerted minimal reaction, not statistically different from blank. Quartz DQ12 prompted a strong, dose-dependent toxicity for all parameters but ROS. Glucuronidase was very high, saturation starting at the 30 µg dose. Cristobalite differed from quartz by a lower TNF-α response, but a very high ROS response, clearly dose dependent. A very special effect pattern occurs after exposure to cristobalite: the ROS increases at fairly low doses accompanied by a moderately strong stimulus of TNF-α secretion. Crocidolite exhibited a dose-dependent toxicity for all parameters but cytotoxicity. In comparison, SiC whiskers triggered the same three parameters dose dependently. A particular feature of fibrous dust alveolar macrophages interaction was the dose-dependent ROS increase induced by SiC whiskers and UICC crocidolite. UICC crocidolite evoked a moderate increase in TNF-α secretion.

In general, all SiC commercial samples showed a very low response in all parameters and at all doses tested. The results for the doses 15 and 30 mg/10^6 alveolar macrophages are not different statistically from the electrocorundum and the blank (untreated cells). Sample 5 evoked a low, borderline increase of TNF-α at the higher doses. Most striking, however, was the dose-dependent increased secretion of ROS for the test samples beginning at the dose 60 mg/10^6 alveolar macrophages, without any sign of adverse effects at the other parameters. A particular feature of fibrous dust alveolar macrophages interaction was the dose-dependent ROS increase induced by SiC whiskers and UICC crocidolite. This ROS increase by the SiC samples was not related to the contents of CFs; the most pronounced level of ROS occurred with SiC grain no. 1, which contained practically no CFs. In the study of the significance of surface characteristics, the heated SiC grains showed that the original capacity to evoke ROS secretion in alveolar macrophages was drastically lowered. Moreover, the ROS stimulatory characteristic of all samples (native and heated) was negatively correlated to the oxygen content.

The study was designed as an in vitro screening investigation in order to find out whether the presence of WHO fibres (in fact CFs) in SiC commercial products was of some biological significance. The parameters analysed address pathological stimulus for inflammatory processes and toxic effects on cell metabolism and membrane integrity. The principal findings of the SiC samples compared with the reference samples in the vector model include the following: (1) the SiC commercial products demonstrated a very low level of biological activity in the vector model comparable to the external standard electrocorundum; however, in the mid and the top dose range an isolated stimulation of ROS secretion was observed in some samples. This increase was not related to the contents of CFs; the most pronounced level of ROS occurred with SiC grain no. 1, which contained practically no CFs; (2) all reference samples triggered biological responses similar to those reported in previous studies with the vector model; (3) the heating of sample nos 1, 2 and 3 changed the biological activity of the SiC grains as determined by an overall drop in the particle dependent ROS release in alveolar macrophages.

The results showed that cleavage fragment seem to have no biological relevance when present in SiC grains at the tested concentrations. The corresponding figures for the commercial SiC samples

were 0.279 x 10^9 CF/g (mean from second column Table 11); the geo-means of the CF dimensions (Table 11) were diameter 1.34, length 5.12 µm and aspect ratio 3.82. The typical fibre types from airborne samples in the Norwegian SiC Industry differ significantly from the CF of commercial grains (Skogstad *et al*., 2006). From 2263 analysed fibrous particles only 6 were assigned as CF (0.3%), a negligible proportion. Aside from the morphological appearance, the CF of the commercial samples differed in thicker diameters and lower aspects ratios from the fibre categories of the airborne samples of Norwegian SiC Industry (Skogstad et al., 2006). The morphological characteristics (aspect ratio) of the CF in this study (Table 12) show comparatively thick (0.8–1.8 µm) and short particles (3.2–6.8 µm). Typically they fulfil the definition of WHO criteria for fibres in terms of size and shape (length >5 µm, thickness < 3µm, aspect ratio >3:1). In the commercial SiC products no whiskers were present.

Table 12. Physical characteristics of respirable fractions of SiC grains; scanning electron microscopy (SEM) and phase contrast microscopy (PCOM): numerical concentrations (10^6/g) of cleavage fragments: columns 2–6. Size characteristics of cleavage fragments: columns 7–10 (Bruch J. *et al.*, 2014).

		Meeting WHO criteria; Particles longer than 5 µm			Particles longer than (SEM)		Size characteristics (µm) Of cleavage fragments (SEM) Diameter		Length	
Samples	All (SEM)	SEM	PCOM	Average	10 µm	20 µm	Range	Mean	Range	Mean
#1	11	4	30	17	0.3	nd	0.3–3.0	1.19	1.5–19.5	4.74
#2	644	519	467	493	37	nd	0.5–2.9	1.81	2.4–14.6	6.83
#3	1882	118	483	300	nd	nd	0.25–2.6	0.83	1.4–9.2	3.02
#4	250	113	137	125	12	1	0.25–3.0	1.32	1.5–25.4	5.25
#5	511	392	383	387	41	nd	0.30–3.0	1.83	1.1–16.0	6.85

Brown DM. *et al*. (1998) compared three types of synthetic vitreous fibre: glass fibres (Code 100/475 and MMVF10), refractory ceramic fibres (RCF1 and RCF4), and silicon carbide in an in vitro plasmid assay. These were compared with amosite asbestos for ability to generate free radicals in solution to determine whether this was a predictor of carcinogenicity. The ability of asbestos fibres to cause lung diseases such as fibrosis or cancer has been considered to be related to length of fibres (Davis et al., 1996) and biopersistence (Davis & Donaldson, 1993). However, in recent inhalation studies noncarcinogenic fibres were found to accumulate in the lung to similar extents as carcinogenic fibres and were of comparable length distribution and biopersistence in vivo; examples of noncarcinogenic fibres that showed this effect were Code 100/475 glass fibre (Davis et al., 1996) and MMVF10. Thus long fibres can persist in the lung and no apparent pathological alterations develop, and this suggests that another factor associated with carcinogenic fibres is important in leading to disease.

This putative additional factor could be the ability of fibres to generate free radicals at their surface, hydroxyl radical in particular. Arguments in favor of this as a factor in toxicity are the following: Asbestos causes hydroxylated adducts of DNA and DNA breakage in vitro; asbestos causes DNA breakage in cells in vitro, an event that is mediated by hydroxyl radical in vitro; Fe chelators and

antioxidants can inhibit production of cytokines stimulated by asbestos in vitro; and antioxidants can inhibit the inflammation caused by short-term in vivo exposure to asbestos.

The intrinsic hydroxyl radical activity of each fibre type was assessed by plasmid DNA scission and by high-performance liquid chromatography (HPLC) using a hydroxyl radical trap, salicylate. Fibres depositing in the lung become coated with lung lining material, which may modify the fibre surface reactivity and hence the fibre's oxidant-generating ability. Brown DM. et al. (1998) therefore used rat lung lining fluid to coat the fibres to determine whether the oxidant-generating ability could be modulated. The role of iron in mediating hydroxyl radical production was assessed by the use of the chelator desferrioxamine-B (DSF-B), and the hydroxyl radical scavenger mannitol was utilized in some assays. ϕX174RF plasmid DNA was added to ultrapure sterile distilled water at a concentration of 240 ng/20 µl. Fibres were suspended in the DNA solution at 924,900 fibres/20 µl. Test conditions were fibres (coated and uncoated) in DNA/water only, or fibres (uncoated) in DNA/water plus mannitol (4 m*M*). Each treatment was incubated at 37°C for 8 h. Four microliters of tracking dye was then added to each sample and the DNA plasmid was separated by electrophoresis for 16 h at 20 V on 0.8% agarose gel. After staining in ethidium bromide, a photograph of the gel under ultraviolet (UV) light was taken and the bands indicating damage to the plasmid were quantified by densitometry. The results were expressed as the percentage of the treatments to the untreated plasmid.

SiC fibres (length distribution 60.86 > 10 µm and 27.6 > 20 µm) displayed some free radical activity as assessed by their ability to decrease the percentage of supercoiled plasmid DNA. However, this result was not significant. Only for amosite asbestos (length distribution 64.75 > 10 µm and 35.25 > 20 µm), which caused 55% depletion of supercoiled DNA, the difference was significant. The intrinsic hydroxyl radical production by SiC fibres was additionally assessed by measuring the amount of 2,3-dihydroxybenzoic acid (2,3-DHBA) formation after incubation with salicylic acid using HPLC. The product was not detected with SiC fibres. 2,3-DHBA was detected when amosite asbestos and RCF1 (length distribution 77.36 > 10 µm and 45.27 > 20 µm) were tested. These results are important for development of screening assays for predicting the carcinogenicity of fibres, as well as for understanding the mechanism of fibre toxicity. Despite the fact that asbestos has the ability to stimulate cells and cause inflammation via free radical mechanisms, this does not appear to be generally true for other carcinogenic fibre types. Silicon carbide has proven to be one of the most carcinogenic fibres to be investigated in experimental pathology studies (Davis et al., 1996), as well as being as cytotoxic and cytostatic as asbestos (Vaughan et al., 1991). The absence of free radical activity of this fibre in the two assays used here suggests either that free radicals are not involved in silicon carbide carcinogenicity or that the conditions of the assays are not sensitive to detect free radical generation. (Brown DM. *et al* 1998).

Svensson I. *et al* (1997) investigate the toxicity of SiC and silicon nitride (SiN) whiskers and granular SiC and compare their toxicity with the toxicity of crocidolite. The materials and their dimensions are summarized in Table 13.

Tabel 13 Characteristics of the Whiskers and Powders[a] (Svensson I. et al., 1997)

Composition and sample no.[b]	Manufacturer/ type	Content of discriminated whiskers[c] $x10^{10}$/g	Content of long fibres (≥20 µm) $x10^{10}$/g	Length, µm	Diameter, µm	Length/ diameter	Specific area, m^2/g
SiCW-1	Tokai 100	1.2	0.23	14 ± 10	0.8 ± 0.4	18 ± 11	3.0
SiCW-2	Tokai 400	0.9	0.20	14 ± 9	0.9 ± 0.4	17 ± 10	1.5
SiCW-3	Tateho SCW10	4.3	0.52	12 ± 10	0.7 ± 0.4	19 ± 13	4.2
SiCW-4	Tateho SCW1S	1.1	0.14	12 ± 9	0.7 ± 0.4	21 ± 12	4.9
SiCW-3Ld	Tateho SCW10	3.9	0.12	9 ± 5	0.6 ± 0.3	16 ± 10	11.7
SiCW-3Se	Tateho SCW10	5.2	0.44	11 ± 7	0.7 ± 0.4	16 ± 9	5.2
SiNW	UBE	2.2	0.42	13 ± 8	0.9 ± 0.4	16 ± 8	2.2
SiNP	UBE E10				0.5 ± 0.4		10.9
SiCP	UF 15, Lonza				0.4 ± 0.3		14.9

[a]Further information about measurement and characterization in Nyberg et al. (1995). Data are means ± *SD.*
[b]Length/diameter ≥5, diameter ≤3 mm. Discrimination limit in image analysis.
[c]SiCW = silicon carbide whisker, SiNW = silicon nitride whisker, SiNP = silicon nitride powder, SiCP = silicon carbide powder.
[d*]L = long-milled (58 hours).
[e]S = short-milled (3 hours).

The tests used in this study were cloning efficiency of V79 cells, nick translation in V79 cells, three radical formation measuring tests, and activation of neutrophils. In forthcoming analyses the results will be correlated with physicochemical data in an attempt to establish structure-activity relationship models (SARs) whereby the toxicity of a new fibre may be predicted. Svensson I. *et al* (1997) presented information on the in vitro toxicity of nine ceramic whisker materials and their preparations and compared to UICC crocidolite,

The cytotoxic effect of asbestos and SiCW has been attributed to the disruption of cell membranes (Vaughan et al., 1991), although effects on the mitotic process by ceramic fibres have also been reported. Concentration-dependent inhibition of cloning efficiency of V79 cells have been found for all ceramic materials. However, there was a clear distinction between the whiskers, the milled whiskers, and the powders. The cytotoxic effects of the unmilled whiskers were all comparable to those for standard reference crocidotile asbestos UICC (Union Internationale Centre le Cancer). The EC50 for the whiskers ranged between 0.9 and 4.2 µg/cm^2, whereas the EC50 of crocidolite was 1.4 µg/cm^2. The milled whiskers and the powders had less toxicity than the whiskers. The most toxic whisker, SiNW, had an EC50 even lower than that of crocidolite. SiNW has a high content of long whiskers, ≥20 µm. SiCW-3 and SiCW-3S have a low cytotoxicity compared with SiNW but their content of long whiskers (≥20 µm) is of the same magnitude as that of SiNW. Therefore these data give no clear explanation as to why SiNW is the most toxic whisker. A significant structure activity relationships (SAR) was established for the cytotoxicity, involving only morphological descriptors. The present set of ceramic fibres follows the already established relationship between fibre morphology and cytotoxicity, although fibre length was found to be more important than fibre diameter or length/diameter ratio.

Anaphase abnormalities, aneuploidy and chromosome aberrations, and sister chromatid exchanges have been documented as the result of exposure to asbestos in vitro, and silicon carbide whiskers have recently been found to cause DNA transformation in maize. Although the mechanisms behind these effects are poorly understood, one mechanism involving the generation of DNA strand breaks is likely. In the nick translation assay an increased formation of DNA strand breaks was found for all fibres except SiCW-2 and SiNW. Taking into account the concentration used, there was a high DNA breaking rate for all the silicon carbide whiskers (of the same magnitude as crocidolite) and a low rate for the other materials. The highest effect was found for SiCW-3S and the lowest for SiNW and the powders (SiCP and SiNP). SiNW contains a high fraction of long whiskers, but the DNA breaking potential is of the same magnitude as that of the powders. SiCW-2 had a low effect on DNA breakage and also a low fraction of long whiskers, whereas SiCW-4 had a high effect and a low fraction. Thus, a clear relationship between the number of long whiskers and the formation of DNA strand breaks could not be found (Svensson I. *et al.* 1997).

The action of reactive oxygen metabolites is considered important for the toxic action of asbestos. It has been shown that the ability of different fibres to generate hydroxyl radicals correlates well with their ability to induce mesothelioma in rats and humans. The whiskers that significantly increased the formation of hydroxyl radicals in this study were SiCW-4 and crocidolite. In comparison with the controls, they increased the formation of 8OHdG approximately 10 times and the degradation of deoxyribose approximately 10 and 60 times, respectively. The rest of the materials tested did not differ from control. A significant SAR was also derived for hydroxyl radical production in the DMSO assay. A subset of the chemical descriptors was found important, whereas the contribution from the morphology variables was very limited. The radical production was higher for fibres with an increased trace element background.

There was a considerable variation between the different materials' ability to activate neutrophils. (Svensson I. *et al.* 1997). In this investigation the SiCWs had the highest ability to stimulate human neutrophils to generate reactive oxygen metabolites, and all fibres except SiNW (for Chemiluminescence) and SiNW and SiNP (for H_2O_2) were higher than crocidolite. Phorbol-12-myristate-13-acetate (PMA) is often used to activate neutrophils to release reactive oxygen metabolites and PMA-stimulated neutrophils have been found to induce DNA damage in neighbouring epithelial cells in vitro and malignant transformation in mice The high capacity of SiCW to activate neutrophils (SiCW-3, SiCW-4, and SiCW-3S of the same magnitude as PMA) may increase toxic effects in the lung. It is tempting to speculate that if ceramic fibres increase neutrophil-mediated transformation of epithelial cells, then they might also increase the risk of cancer.

Vaughan GL et al. (1991) used in vitro methods to make an initial determination of cytotoxicity for single-crystal SiCW. Vaughan GL et al. (1991) found that SiCW-1 (with a diameter of 0.8 (SD = 0.3) μm, average length of 18.1 (SD = 14.3) μm and aspect ratio of 23.3 (SD = 18.7)) and SiCW-2 (with the diameter of 1.5 (SD = 0.6) μm, average length of 19.0 (SD = 11.0) μm and aspect ratio of 15.3 (SD = 11.2)) proved as cytotoxic and cytostatic as crocidolite asbestos. Within 24 hr of being added to cell cultures, many, perhaps a majority, of the whiskers that can be detected by phase contrast microscopy are found associated with the cells, attached to cell surfaces, or internalized. As demonstrated by the micrographs SiCW whiskers too large to be engulfed are found penetrating cell surfaces, often entering the cell on one side, and exiting on the other as if the cell were "skewered." It seems likely that transmembrane particles, compromising membrane integrity, are responsible for much of the cytotoxicity observe to be associated with crocidolite asbestos and SiCW (Vaughan GL. *et al.,* 1991). This possibility is circumstantially supported by the results of cytotoxicity assays which are based upon evaluation of membrane selectivity. The dye, trypan blue, is excluded by healthy living cells but not by moribund or dead cells which stain blue. Based on dye exclusion,

crocidolite asbestos and SiCW-1 exhibited similar levels of dose dependent cytotoxicity within the first 24 hr. Similar results were obtained with the radiochromium release assay wherein cells were allowed to accumulate ^{51}Cr and then placed in growth medium without the label where they are exposed to the test materials. As cells die, or are damaged, plasma membranes deteriorate and lose natural selective permeability. As a consequence, ^{51}Cr leaks to the medium surrounding the cell. Cells in cultures exposed to SiCW-1 or crocidolite at 5.0 μg/cm^2 release 20-30% of label in excess of controls. Dye exclusion and radiochromium release assays do not report cells which survive the first 24 hr to be compromised and die later.

Analysis of colony-forming efficiency, however, measures cellular proliferative capacity. Cells must not only survive the first 24 hr but must also be sufficiently viable to produce individual colonies of at least 50 cells each. On a mass per surface area basis, SiCW-1 is slightly more toxic than equal quantities of crocidolite. The larger SiCW is less cytotoxic than SiCW-1 ($P < 0.01$) but not significantly different from asbestos ($P > 0.05$). This finding as to the relative toxicities of SiCW-1 and SiCW-2 seems to follow from the common understanding that fibres of smaller diameter are more toxic than larger ones. On the other hand, consider the obvious fact that a given mass of small diameter fibres will contain more particles than the same mass of larger fibres of similar length. On a numerical basis, Vaughan et al found no significant difference ($P < 0.05$) between the cytotoxicities of 0.8 and 1.5 μm SiCW of similar lengths. It seems likely that, within the narrow size range examined, cellular response probably depends more on the number of particles a cell encounters than on relative size. In addition to being immediately cytotoxic, SiCW-1, SiCW-2, and crocidolite induce, within eight generations of exposure, changes in cellular growth habits and structure generally held to be characteristic of cellular transformation. Perhaps the most significant alteration in cells exposed to SiCW and asbestos occurred at the level of the genome. Vaughan et al. (1991) found that, although DNA synthesis rates for fibre-/whisker-exposed cells were generally elevated relative to controls, often by a factor of as much as 2.5, the results were inconsistent. Significant increases in total cellular DNA content, however, were consistently observed 10-20 generations after treatment. Cells treated with SiCW and crocidolite contained an excess of DNA ranging from approximately 40% to near 70%. This observation is consistent with those in other systems where transformed cells and those cells associated with malignancies have elevated levels of DNA. Although the processes involved in cellular transformation and those in the development of malignancy are not necessarily identical, they are similar. When, therefore, a material is shown to be an agent capable of transforming cells, there is cause for concern. In light of the general hypothesis that cell transforming and carcinogenic potentials in durable whisker/fibre form materials are largely functions of particle size and aspect ratio, it was not unexpected that SiCW-I and crocidolite would be more effective than SiCW-2 in cell transformation capability. When, however, transformation frequency for SiCW is shown as a function of the relative number of whiskers rather than mass there is no difference between SiCW-1 and SiCW-2. This supports the probability that, as with cytotoxicity, the effects of these materials within this size frame depend more upon the number of fibres than on the size.

Besides the two series of studies involved an inhalative burden to the animals' lung from an intratracheal injection of high dose of 50 mg per animal (see 4.7.1.4 Repeated dose toxicity: other routes) Bruch J et al. (1993-2), also in vitro testing was performed. To substantiate and control the detectable changes in the alveolus in vitro tests were used that assess the specific interactions between dust and the alveolar macrophages with particular respect to the dose. Cytopathogenicity (H_20_2 release test) as well as stimulation of alveolar macrophages to produce TNF-α were used as criteria of in vitro effects (Bruch J et al 1993-2). Alveolar macrophages from guinea pigs were introduced into wells of microtitre plates (300 000 per well). After two hours settlement and conditioning the dust samples were added at doses of 20 and 60 μg/300 000 cells respectively and incubated for 16 hours. Quartz at a concentration of 60 μg gave a complete inhibition of stimulation

of H_20_2-release and the lower concentration of 20 µg quartz resulted in about 40% reduction. SiC dust (mean grain diameter <3 µm) at a concentration of 60 µg, showed no difference compared with untreated cells both for the temporal course of events as well as end point measurements. The authors assume that these test indices in general reflect the pathogenic effects of mineral dusts and, to a lesser extent, the specific cytotoxic effect. For example, in these cases there is a the direct correlation of cytopathogenic indices with that of dust penetrating the lymph nodes (lymphotropism) as well as the ability to evoke graded reactions in quartz typical regions of the lymph nodes.

Among several alveolar macrophage-derived fibrogenic factors now identified, tumour necrosis factor-α (TNF-α) seems to play a key part in that a single instillation of silica in mice leads to a pronounced increase in lung TNF-α production. Also, silica induced lung fibrosis is almost completely prevented by TNF- α antibodies. To better characterise the mechanism of test dust dependent alveolar macrophage activation, Bruch J et al. (1993-2) have investigated the effects of silica and SiC on the secretion of TNF- α from isolated alveolar macrophages. Bone marrow cells from CBFl-mice were seeded in microtitre plates at a density of 10^6/ml ($2x10^5$/well). The mineral dust suspensions were added to the cultures together with 0,25 U α-antitrypsin/ml to a final volume of 200 µl/well. After a 24 hour incubation, the supernatants were tested for TNF-α activity. Quartz concentrations up to 10 µg/well led to a significant growth inhibition compared with the controls. When tempered and ground clay and pure clay were compared with SiC dust (main grain diameter <3 µm), SiC dust did not result in an apparent growth inhibition of L 929 cells at doses up to 50 µg/well.

For the *in vitro* testing, the harmfulness was assessed on alveolar macrophages (male guinea pig) by a set of toxicity parameters (Lactate dehydrogenase (LDH), Fluorescein diacetate (FDA)) and through determination of inducible H_20_2 release (Bruch and Rehn, 1996). Dusts samples SiC-A (mean diameter: FI200 2.26 µm) and SiC-B (main diameter: NF2 1.14 µm) were tested in doses ranging from 20 to 180 mg/l06 cells, tests were performed in triplicate in one term at four independent terms (independent: animals, cell harvest, dust weighing, dust dosing, plate reader assessing). The samples SiC-A and SiC-B were free of fibrous SiC varieties Positive controls were quartz (DQI2), the negative ones corundum (crystalline aluminium oxide (Al_2O_3)). Using the original non-sized SiC samples, cell viability measured by FDA showed no significant differences. Taking loss of hydrogen peroxidase secretion as a measure for cell damage, macrophages burdened with quartz or SiC-B sample exert a significant and dose dependent reduction in hydrogen peroxide release. In contrast the SiC-A sample did not show this effect. In contrast to the results obtained for cell viability, marked differences between the two samples could be measured in the H_20_2 secretion (Bruch and Rehn, 1996).

In conclusion, dust samples SiC-A and SiC-B show different biological effects (Bruch and Rehn, 1996). SiC-B leads to marked pathological reactions in the animal test and in the *in vitro* testing whereas the SiC-A sample is inert in the frame of the specificity and sensitivity of the investigational procedures used here. However, the differences cannot be solely explained on the basis of different grain size distribution. The sample B contains a higher concentration of the finer particles. The fine fraction is more toxic; but when the samples were separated into fractions of distinct grain size diameter, each fraction of sample B is more toxic than sample A. In conclusion the data show that relevant differences in bio-pathogenicity do exist for the tested varieties of SiC (Bruch and Rehn, 1996).

The experiments clearly show that SiC-B elicits a lasting granulocytic response together with an epithelial stimulation (Bruch and Rehn, 1996). These cellular events fit into the concepts of dust related carcinogenicity based on the formation of Reactive Oxygen Species (ROS) which might be

important for silica carcinogenicity. SiC-A and corundum lack particular bio-pathogenic effectiveness.

4.7.1.7 Summary and discussion of repeated dose toxicity

Repeated dose toxicity data in animals and in vitro testing in cell cultures are presented for information as they may provide relevant data for assessment of carcinogenicity. No classification is discussed and proposed for this endpoint.

Several repeated dose studies have examined the histopathological changes, inflammatory responses and fibrosis formation of SiC particles and SiC in a fibrous form.

Repeated dose studies conducted with dust samples with respirable particles with a grain diameter of < 3 μm were without any positive results (Bruch J. et al., 1993-1; Bruch J. et al., 1993-2; Rehn et al., 1989). No information is given on the fibre concentration and/or fibre distribution in the dust samples. Only in Bruch and Rehn (1996) it is clearly stated that the SiC dust samples were free of fibrous SiC varieties. Based on these studies, it was assumed that SiC particles were practically "inert", i.e. that it produced no tissue damage (Bruch J. et al., 1993-2) nor increased number of granulocytes (Bruch J. et al., 1993-1).

Inhaled SiC whiskers (average diameter 0.577 μm and length 4.68 μm) resulted in higher incidence of lesions of the lung and lymph node (Lapin C.A et al., 1991). SiC fibres with diameter 0.27 ± 0.27 μm and length of 6.8 ± 11.2 μm behaves somewhat as other fibrous materials of comparable dimension in the lung tissue (Begin R. et al., 1989). The long fibres are retained in the tissue, and they cause a 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 leads to the SiC pneumoconiosis as reported in humans and in the sheep model (Begin R. et al., 1989).

Bruch and Rehn (1996) observed that SiC-B elicits a lasting granulocytic response together with an epithelial stimulation. These cellular events fit into the concepts of dust related carcinogenicity based on the formation of Reactive Oxygen Species (ROS) which might be important for silica carcinogenicity. SiC-A and corundum lack particular bio-pathogenic effectively. In conclusion the data show that relevant differences in bio-pathogenicity do exist for the tested varieties of SiC (Bruch and Rehn, 1996). These data suggests that SiC dusts is biologically inert when in particulate form (with grain diameter of < 3 μm), however have biologic activity when they occur in a fibrous form (Begin R. et al., 1989; Bruch and Rehn, 1996).

This is in line with the in vitro tests. SiC dust had no effect (Bruch J. et al 1993-2). However, SiC whiskers in vitro studies show to generate reactive oxygen species and DNA breakage (Svensson I. et al., 1997; Vaughan GL. et al., 1991). These observations suggest that SiC whiskers exert its activity by induction of oxidative stress and possibly a subsequent inflammatory response. These processes are considered to have a threshold. The extent of genotoxicity may depend on a cell's ability to adapt to oxidant stress. Further, SiC whiskers have proven to be as cytotoxic, disrupting cell membranes, and cytostatic as crocidolite (Vaughan GL. et al., 1991; Svensson I et al. 1997). SiC whiskers also exert a significant alteration in the genome. SiC whiskers demonstrated to induce increased DNA synthesis and total cellular DNA content in embryonic mouse cells (Vaughan GL. et al., 1991). Increases in DNA synthesis rate is consistent with observations in other systems where transformed cells and those cells associated with malignancies have elevated levels of DNA (Vanderlaan et al., 1983). Further, Vaughan GL. et al. (1991) concluded that the amount of damage appear to be more a function of the number of whiskers present than of their size.

However, Brown DM. et al. (1998) did not found 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 carcinogenicity, or that the assay conditions were not sensitive enough to detect free radical generation in this case (Brown DM et al 1998). The SiC fibres used by Brown DM et al (1998) had a length distribution of 60.86 >10 µm and 27.6 > 20 µm. The diameter or aspect ratio of the fibres are not given in this study.

Table 14 summarize the changes in reactive oxygen species and alterations in DNA after exposure of in vitro cells to SiCW. Taken as a whole, such changes in cells could lead to carcinogen induced cellular transformation and lead to carcinogenicity.

Table 14 Summary of vitro studies on different mechanisms related to SiC characteristics

Method	SiC characteristics	Parameter: effect	Reference
In vitro vector model: 1. Cytotoxicity: H_2O_2 release test with guinea pigs alveolar macrophages. 2. Glucuronidase test with guinea pigs alveolar macrophages. 3. The release of TNF-α with rat alveolar macrophages. 4. Assessment of dust induced oxidative burst (ROS) with alveolar macrophages from guinea pigs.	Commercial SiC dust with cleavage fragments mean diameter of 0.8–1.8 µm and 3.2–6.8 µm	1. No 2. No 3. No 4. Yes, but not related to the concentration of cleavage fragments	Bruch J. et al., 2014
In vitro ϕX174RF plasmid DNA assay (hydroxyl radical generation assay) 1. Fibre-mediated free radical damage to plasmid DNA 2. Hydroxylation of salicylic acid by fibres	SiC fibres mean length distribution 60.86 > 10 µm and 27.6 > 20 µm	1. Damage to DNA: No 2. Hydroxylation of salicylic: No	Brown DM. et al. (1998)
In vitro studies with V79 Chinese hamster lung fibroblasts 1. Cloning efficiency Assay 2. Analysis of DNA Strand Breaks 3. Three radical formation measuring test: a. Deoxyguanosine Hydroxylation Assay b. Dimethylsulfoxide as Scavenger c. Deoxyribose Assay 4. Activation of neutrophils	SiCW-1 mean length 14±10 µm and diameter 0.8±0.4 µm SiCW-2 mean length 14±9 µm and diameter 0.9±0.4 µm SiCW-3 mean length 12±10 µm and diameter 0.7±0.4 µm SiCW-3S mean length 11±7 µm and diameter 0.7±0.4 µm SiCW-3L mean length 9±5 µm and diameter 0.6±0.3 µm SiCW-4 mean length 12±9 µm and diameter 0.7±0.4 µm SiCP mean diameter 0.4±0.3 µm	1. Yes, for whiskers (except SiCW-3L); No for SiCP 2. Highest for SiCW-3S and lowest for SiCP 3. Yes for SiCW-4 No for other whiskers and powder 4. Yes for SiCW-1, SiCw-3, SiCw-4, SiCW-3L and SiCW-3S No for SiCW-2 and SiCP	Svensson I et al. (1997)
In vitro methods 1. H_2O_2 release test 2. release of TNF-α	SiC dust mean grain diameter <3 µm	1. No 2. No	Bruch J. et al. 1993-2

In vitro tests 1. Dye Exclusion test (moribund cells or with leaky membranes) 2. ^{51}Cr Release (membrane damage or cell death) 3. Colony-Forming Efficiency (proliferative ability) 4. Tritiated Thymidine Incorporation Assay (rate of DNA syntheses) 5. Incidence of Binuclear Cells (rate of multi-nuclearity) 6. Cellular Transformation (loss of contact inhibition) 7. Cellular DNA Content (total DNA content)	SiCW-1 mean diameter 0.8±0.3 µm and length 18.1±14.3 µm and aspect ratio 23.3±18.7 SiCW-2 mean diameter 1.5±0.6 µm and length 19.0±11.0 µm and aspect ratio 15.3±11.2	1. SiCW-1: yes SiCW-2: results not described 2. SiCW-1: yes SiCW-2: results not described 3. Yes 4. Yes, but inconsistent and not reported 5. Yes 6. Yes 7. Yes	Vaughan GL. et al., (1991)

4.7.1.8 Summary and discussion of repeated dose toxicity findings relevant for classification according to DSD

This paragraph is considered irrelevant seen the repeal of Directive 67/548/EEC with effect from 1 June 2015.

4.7.1.9 Comparison with criteria of repeated dose toxicity findings relevant for classification according to DSD

This paragraph is considered irrelevant seen the repeal of Directive 67/548/EEC with effect from 1 June 2015.

4.7.1.10 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification according to DSD

This paragraph is considered irrelevant seen the repeal of Directive 67/548/EEC with effect from 1 June 2015.

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

Not evaluated in this report.

4.9 Germ cell mutagenicity (Mutagenicity)

4.9.1 Non-human information

Standard genotoxicity tests in vitro or in vivo have been recovered for public available literature when available. The mutagenicity of SiC was first approached through the Ames test. The bacterial reverse mutation assay (Ames test) clearly showed SiC to be non-mutagenic (Bioservice, 2008; OECD 471) (REACH registration dossier).

4.9.2 Human information

No humans data on mutagenicity of SiC is available.

4.9.3 Other relevant information

No other relevant information on mutagenicity of SiC is available.

4.9.4 Summary and discussion of mutagenicity

Standard genotoxicity tests *in vitro* or *in vivo* were not available.

4.9.5 Comparison with criteria

No evaluated in this report.

4.9.6 Conclusions on classification and labelling

No classification is discussed and proposed for this endpoint for SiC.

4.10 Carcinogenicity

4.10.1 Non-human information

Table 15: Summary table of relevant non-human carcinogenicity studies

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	Positive (same study as Davis J.M.G. et al., 1996)	Miller B.G. *et al.*, 1999a

Species, exposure route	Test material	Method	Results	Remarks	Reference
			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.		
Rats Inhalation	SiC whiskers (mean D x L was 0.5 x 2.8 µm)	Male rats/Wistar/n=42 Inhalation; 98±18 fibres/ml, 6 hrs/d, 5 d/wk for one year	Increased lung weight; fibrotic changes in lungs. No tumor induction.	Positive for these parameters but not for tumor induction.	Akiyama I. *et al.*, 2007
Rats, intrapleural administration	SiC fibres (range of diameters 0.05 to > 1.5 µm and length of >1.5-2.5 µm to > 8 µm)	40 mg dose of different particles (including SiC fibres) uniformly dispersed in hardened gelatine was applied by open thoracotomy directly to the left pleural surface of 12- to 20-week-old outbred female rats. In each experiment, 30-50 rats were treated and followed for 2 years, at which time the survivors were killed.	A positive response was the increase of pleural sarcomas after 1 year compared to controls after exposure to SiC fibres. The probability of pleural sarcoma correlates best with fibres in general that measure ≤ 0.25 µm x > 8 µm. Relatively high correlations were also observed with fibres in other categories having a diameter up to 1.5 µm and a length greater than 4 µm.	Positive	Stanton M.F. *et al.*, 1981
Rats, intrapleural administration	SiC whiskers	In experiments with intrapleural injections of SiCW (20 mg x 3, with one month interval) to random-inbred rats.	The pleural mesotheliomas were induced in 47.7% (SiC) and 34.1% (positive control chrysotile B) of rats, respectively. Controls 0%	Positive	Vasil'eva L.A. *et al* 1989 (Article in Russian)
Rats intrapleural administration	SiC whiskers (Mean values: SiCW 1 D x L: 0.42 x 4.5 µm; SiCW 2 0.75 x 20.1 µm; SiCW 3	SiC whickers were injected (single or repeated was not specified) to 3 groups of 30 female F344/N rats, intrapleurally with 20 mg (corresponding to ca. 73 mg/kg bw2) of 3 different SiC whiskers samples (SiCW 1, SiCW 2 and SiCW 3), suspended in 0.4 ml saline. Saline was injected to the controls. The mortality changes of the rats were followed for proximally 3 years.	Rats inoculated with SiCW 1 or 2 had the shortest life spans. The life spans of the rats treated with SiCW 3 were not significantly different from those of control rats. SiCW 1 and SiCW 2 significantly developed pleural mesotheliomas in rats ($p \leq 0.05$). SiW	Positive	Johnson N. F. and Hahn F.F., 1996

Species, exposure route	Test material	Method	Results	Remarks	Reference
	0.32 x 6.6 µm)		3 also developed pleural mesotheliomas but not significant. Fibres were found in sections from all treatment groups.		

Species, exposure route	Test material	Method	Results	Remarks	Reference
Rats Intraperitoneal administration	Non-fibrous SiC	SiC was injected intraperitoneally repeatedly at intervals of two weeks into Wistar rats at two dose levels (5 times 50 mg and 20 times 50 mg). Observation period 90 weeks.	One year after the first intraperitoneal injection of SiC, lower average body weight of the rats injected with 20×50 mg SiC was observed. Six months later this difference was smaller. No serosal tumours were found.	Negative	Pott F. *et al.*, 1994
Rats Intraperitoneal administration	Granular SiC	Two groups of 48 female and 72 male rats were injected intraperitoneally either 5 or 20 times with 50 mg of granular SiC (corresponding to total doses of approximately 667 mg/kg bw and 2,666 mg/kg bw) for 30 months.	Two mesotheliomas were found in a total of 395 evaluated rats treated with saline or granular SiC.	Negative	Roller M. *et al.*, 1996
Rats Intraperitoneal administration	SiC whiskers (D x L: < 0.95 x > 0.4 µm)	Groups of about 24 rats received single intraperitoneal injections of a range of fibres in suspension, and were monitored for the rest of their lives for the development of mesothelioma.	22 out of 24 rats administered with SiCW developed mesothelioma, with median mesothelioma survival of 257 days (SD = 52)	Positive	Miller B.G. *et al.*, 1999b
Rats Intraperitoneal administration	SiC whiskers (not specified)	330 rats divided into 24 groups received single dose intraperitoneal administration of 5 to 20 mg/rat of 9 types of the JFM (Japan Fibrous Material Research Association) standard fibre samples (glass wool, rock wool, micro fibre glass, three types of refractory fibre, potassium titanate whisker, SiCW, titanium oxide whisker), wollastonite (natural fibre) and UICC chrysotile B, and were observed for two years.	All rats administered of SiCW developed peritoneal mesothelioma within a year.	Positive	Adachi S. *et al.*, 2001
Rats Intraperitoneal administration	SiC whiskers (0.31 (d) * 3.1 (l) µm)	Groups of 36 or 48 rats were intraperitoneal injected once with 0.05, 0.25, 1.25, 6.25 or 25 mg SiC. The rats were observed for an unknown period of at least 115 weeks.	There was a clear dose response relation for the induction of tumours and a reduction in life span.	Positive	Pott, 1991
Adapted from Pott F. *et al.*, 1991 intraperitoneal and intrapleural	SiC granular (< 30% with L/D > 10) and SiC whiskers (> 80% with L/D > 10 and D < 0.25 µm)	Samples were suspended in water and filtered. One half of each filter was analyzed by scanning electron microscopy (SEM, magnification ×2500), and transmission electron microscopy (TEM, ×10,000) was also performed for the SiC whiskers.	The concentration of WHO fibers was 58,000 fibers/mg for the granular sample compared to 48,000,000 (SEM) and 42,000,000 (TEM) fibers/mg for the whiskers. In addition, 0% of the fragments compared to 44% and 30% of the whiskers were more than 10 µm long.	-	Rödelsperger K and Brückel B, 2006

Species, exposure route	Test material	Method	Results	Remarks	Reference
			In total, 15 and 58 × 10^6 WHO fibers were injected with the granular SiC even though only 0.8% and 0% tumours were recorded.		

4.10.1.1 Carcinogenicity: oral

No relevant information is available.

4.10.1.2 Carcinogenicity: inhalation

The available study reports for inhalation carcinogenicity of SiC fibres mostly contain only limited information on study details because often several fibres were studied. The identification of the tested substance is also limited as in most cases no information on crystal form and presence as monocrystalline or polycrystalline is provided.

Davis and co-workers (Davis J.M.G. *et al.,* 1996) demonstrated that SiC whiskers was fibrogenic and carcinogenic in rats in a long-term inhalation study with full-life-span follow-up. In this study, the pathogenicity of mineral fibres such as amosite, SiC and microfibre were investigated. In the long term inhalation studies, 2 groups of 40 specific-pathogen-free (SPF) rats of the AF/HAN strain were exposed to amosite, SiC whiskers (single crystal; mean diameter of 0.45 μm and > 5 μm in length) or microfibre dust respectively. Controls were not used in the studies. However, in a previous batch of controls of the same rat strain and maintained in the same laboratory, one adenoma and one carcinoma was observed. The numbers of rats per sex was also not specified in the study. The period of exposure was approximately 1 year (238 days) for 7 hours per day and 5 day per week. During the exposure, some animals were removed for examination for various effects from SiC. Following the 1-y inhalation period, groups of 4 rats from each experimental study were killed for the carcinogenicity 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. In practice for pathology study, groups of 9 rats treated with amosite, 9 rats treated with SiC and 11 rats treated with microfibre were killed by the end of exposure while 42 rats treated with amosite, 42 rats treated with SiC and 38 rats treated with microfibre were examined for carcinogenicity at the end of their full life span. To assess the ability of amosite, SiC whiskers and microfibre 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. It has been found that both amosite and SiC were very carcinogenic in the present study (Table 16). SiC produced slightly fewer tumors in the lung parenchyma than amosite but produced more mesotheliomas compared to amosite. (Key study)Table 16 Summary of pathological findings from inhalation studies: minimum, maximum and mean level of advanced fibrosis (percent of lung area) and numbers of animals with tumours (percentage in italics)

Fibre type	No. of animals for fibrosis	Advanced fibrosis (%)			No. of animals for pathology	Carcinoma		Adenoma		Mesothelioma	
		Minimum	Mean	Maximum		No.	%	No.	%	No.	%
Amosite	9	3.5	7.6	14.8	42	7	17	9	21	2	5
SiC	9	6.6	8.7	20.2	42	5	12	5	12	10	24
Microfibre	11	0	0.2	0.7	38	0	0	4	11	0	0

Miller and co-workers examined the influence of fibre dimensions, persistence in the lung, and dissolution and cell toxicity in vitro, on the risks of developing lung tumours in rats from fibres (Miller B.G. *et al,* 1999a). 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). To avoid including the very large number of variables represented by a complete bivariate

set of length and diameter variables, airborne fibre concentrations were summarized not only in cumulative length categories but also in two diameter classes according to whether the fibre diameters were greater or smaller than 0.95 μm. The incidence of tumors was treated as a binomial response variable. Its relationship with characteristics of individual fibre types was investigated by multiple logistic regression. The relevant data generated from this study is summarized in Table 17 below. It confirms that fibre clouds contained mostly fibres thinner than 1 μm. It also shows that long fibres (fibres with length of >20 μm) were most persistence in the lung. Despite the small number of data points, the results were consistent with the hypothesis that, for inhalation studies, 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.

Table 17 Characteristics of fibres and pathology results from IOM and TIMA studies (Controls were not included).

Fibre label	Exposure concentration (fibre hr^{-1} $litre^{-1}$)								Biopersistence at 12 months (%)						Inhalation pathology		
	Mass conc. (mg/m^3)	Diameter (µm)	Length (µm)														
			>0.4	>5	>8	>10	>15	>20	Lengths >0.4 µm	Lengths >5 µm	Lengths >8 µm	Lengths >10 µm	Lengths >15 µm	Lengths >20 µm	Lung cancer (%)	Lung tumour (%)	Mesothelio mas (%)
100/475	5.8	<0.95	6580	1730	810	533	138	52	9.9	21.3	22.9	29.6	17.2	23.7	0	11	0
		>0.95	46	46	36	34	21	12									
SiC 1	11.4	<0.95	3268	1581	786	530	237	89	52.6	53.7	47.7	49.2	54.5	59.2	12	24	24
		>0.95	26	26	16	16	3	0									
Amosite	5.5	<0.95	6311	1551	814	530	204	127	8.9	21.9	29.4	34.4	46.2	68.8	21	38	5
		>0.95	102	83	78	77	36	21									
MMVF10	3	<0.95	27	22	16	13	8	5	13.1	9.4	6.0	4.4	2.1	0.6	0	0	0
		>0.95	73	70	59	50	34	24									
MMVF10	16	<0.95	136	110	81	63	38	24							0	1	0
		>0.95	366	347	297	253	168	120									
MMVF10	30	<0.95	218	176	129	102	61	38							1	6	0
		>0.95	586	556	475	404	269	192									
MMVF21	3	<0.95	54	44	36	32	23	18	36.9	37.8	37.7	36.3	36.6	43.0	1	4	0
		>0.95	65	63	53	49	39	33									
MMVF21	16	<0.95	238	195	160	140	102	77							1	4	0
		>0.95	286	276	236	216	172	146									
MMVF21	30	<0.95	385	316	259	228	164	125							1	4	0
		>0.95	463	447	382	350	279	236									
MMVF22	3	<0.95	58	47	38	32	23	17	16.6	10.6	6.1	3.8	2.2	1.7	1	2	0

		>0.95	49	47	42	40	31	25								
MMVF22	16	<0.95	254	205	164	141	102	73							0	0	0
		>0.95	214	204	184	172	136	110								
MMVF22	30	<0.95	414	334	266	229	165	19							1	3	0
		>0.95	348	332	299	280	222	178								
RCF1	3	<0.95	53	37	25	23	15	11	40.3	42.8	44.9	46.6	49.6	50.5	0	2	0
		>0.95	47	46	40	39	32	27								
RCF1	9	<0.95	153	106	73	65	43	31							1	4	1
		>0.95	135	131	115	111	91	77								
RCF1	16	<0.95	244	169	117	105	69	50							1	2	0
		>0.95	216	210	184	178	146	122								
RCF1	30	<0.95	398	296	222	186	135	106							6	12	2
		>0.95	300	291	265	238	189	157								
RCF2	30	<0.95	431	276	176	144	96	59	59.1	70.8	83.9	99.9	130.0	157.3	4	7	2
		>0.95	434	425	369	337	290	235								
RCF4	30	<0.95	198	58	22	9	2	0	72.2	81.2	95.7	108.4	113.1	142.7	2	3	1
		>0.95	496	444	300	220	116	59								

Akiyama and co-workers exposed 42 male Wistar rats to SiC whiskers for 6 hours/day, 5 days/week for 1 year by inhalation (Akiyama I. *et al.,* 2007). The control rats were exposed to clean air in identical, adjacent chambers under similar conditions of flow, temperature, and humidity. The mass median aerodynamic diameter, the geometric mean fibre diameter and the geometric mean fibre length were 2.4 µm (± 2.2), 0.5 µm (± 1.5) and 2.8 µ m (± 2.3), respectively. The daily average exposure concentrations were 2.6 ± 0.4 mg/m^3 (98 ± 19 fibres/mL). The rats were sacrificed at 6 days and 3, 6 and 12 months after the exposure. There was no significant difference in survival rates between the exposure and control rats (data not shown) but there were significant differences in the lung weights at the time of 6 days and 6, 12 months (Table 18). The amount of SiC whiskers deposited in rat lungs 6 days after the end of the inhalation period of 12 months was 5.3 ± 1.4 mg. This amount declined exponentially. The estimated half-time was 16 months. Long fibres (20 µm) persisted more than short fibres. Histopathological observations were made at 6 days and 12 months after 1 year of inhalation. Small fibre-aggregated foci were diffused in the alveolar space in the entire lung field shortly at 6 days after 1 year of inhalation. Some of the SiC whiskers were deposited in the interstitial tissue and some of them were accompanied by collagenous material. The infiltration of inflammatory cells around the aggregated fibres was not remarkable. There was a slight thickening of a part of the pleura due to fibre deposition. One year after the end of the 1-year inhalation exposure, fibrotic changes were remarkable around some fibre-aggregated regions. In these regions, fibrous thickening of the alveolar wall around fibre aggregations and infiltration of inflammatory cells, mainly macrophages and monocytes, were found. They were observed in the lung field as a magnified image of alveolitis at low magnification. Broncho-alveolar hyperplasia formation was observed in two animals in the exposed group. Fibrous aggregations were scattered in the broncho-alveolar hyperplasia. No neoplastic lesions were observed. However, only 11 rats were available for the 2 year necropsy due to planned interim sacrifices. No follow up more than 1 year was performed after the end of the inhalation period.

Table 18 Body and wet organ weights after 1 year of inhalation

Clearance time after inhalation	Group	Number of rats	Body weight (g)	Lung weight (g)
6 days	E	10	616.9 ± 61.1	2.13 ± 0.20[a]
	C	10	655.4 ± 47.0	1.88 ± 0.13
3 months	E	5	648.4 ± 133.6	2.00 ± 0.17
	C	5	612.6 ± 51.4	1.62 ± 0.36
6 months	E	5	602.2 ± 164.2	2.16 ± 0.16 [a]
	C	5	749.4 ± 94.9	1.81 ± 0.15
12 months	E	11	683.1 ± 173.6	2.18 ± 0.27 [a]
	C	13	643.8 ± 100.8	1.87 ± 0.18

Note: E, exposure group; C, control group. [a] Significant at $p < 0.01$

The absence of lung tumour formation may be explained by the low exposure level, the short fibre length or the small number of rats examined after 2 years.

4.10.1.3 Carcinogenicity: dermal

No relevant information is available.

4.10.1.4 Carcinogenicity: Other routes

Intrapleural administration

Stanton and co-workers reported increased incidence of pleural carcinomas, after 1 year intra-pleural administration of SiC in rats (Stanton M.F. *et al,* 1981). A total of 72 experiments were performed, by applying a standard 40 mg dose of particles (corresponding to ca. 145 mg/kg bw[2]) uniformly dispersed in hardened gelatine by open thoractomy directly to the left pleural surface of 12- to 20-week-old rats. In each experiment, 30-50 rats were treated and followed for 2 years. A positive response was the occurrence of pleural sarcomas that resembled the mesenchymal mesotheliomas of man, developing after 1 year. Three types of controls were considered: untreated rats, rats that received thoractomies but no pleural implant, and rats with pleural implants of non-fibrous material. There were two types of spontaneous tumours observed in the studies: the fibro-sarcomas of left mammary gland and the subcutaneous fibro-sarcomas induced by suture material. SiC used in the study was a single sample (metallic crystalline whiskers), which was of exceptionally fine uniform dimension. The incidence of pleural sarcomas in all 3 control groups combined was 7.7 ± 4.2% (calculated by the life table method). For SiC, actual tumour incidence was 17/26 with the fibre dimensions of range of diameters 0.05 to > 1.5 μm and length of >1.5-2.5 μm to > 8 μm (Table 19). In general, the results indicated that particles in the relatively thin- and long-dimensional categories were associated with higher tumour probabilities. The best correlation was obtained with the fibres that measure ≤ 0.25 μm × > 8 μm (diameter x length).

Table 19 Incidence of pleural sarcomas in different groups of control rats and in rats treated with SiC.

Group	Tumour incidence	Percent tumour probability ± SD
SiC treated group	17/26	100
Combined controls*	29/1518	7.7 ± 4.2

** Combined controls included untreated controls, non-carcinogenic pulmonary implants and non-carcinogenic pleural implants.*

Vasil'eva *et al.* (1988; article published in Russian) studied the carcinogenicity of SiC whiskers by injecting three times groups of 93 male and female rats into the pleural cavity with 20 mg of SiC in 1 mL of physiological solution. The interval between injections was one month. 96 rats of the second group were injected three times with the same doses of chrysotile B (positive control) and 52 rats with physiological salt solution (negative control). The animals were observed until their natural death. The tumours, as well as organs, were subjected to morphological evaluation. Pleural mesothelioma's were induced in 47.7% of the SiC treated group and in 34.1% of chrysotile B treated group, while in the negative control group no mesothelioma's were seen. [The details of this study could not be further evaluated.]

In another study, Johnson and Hahn (Johnson N. F. and Hahn F.F., 1996) investigated whether SiCW are carcinogenic in the intrapleural inoculation assay, by injecting (single or repeated are not specified) 3 groups of 30 female F344/N rats, 6 to 8 weeks old, intra-pleurally with 20 mg (corresponding to ca. 73 mg/kg bw[2]) of 3 different SiC whiskers samples (SiCW 1, SiCW 2 and

[2] The value has been calculated using the default value for average body weight of female rats of 275 gram in chronic studies.

SiCW 3), continuous ceramic filaments (CCFs), International Union Against Cancer crocidolite asbestos or saline. The mean fibre length of SiCW (mono crystalline) in three samples was determined by scanning electron microscopy and amounted to 4.5 (±0.23), 20.1 (±1.01) and 6.6 (±0.40) µm, respectively, and the diameter < 1 µm. The number of fibres in three samples was 7.6×10^6 , 1.6×10^5 and 1.1×10^7 fibres per 1 mg samples, respectively, resulting in the doses of 5.6×10^8 fibres/kg bw, 1.2×10^7 fibres/kg bw and 8×10^8 fibres/kg bw. The life span of the rats treated with SiC W 1 or 2 were significantly shorter than those of the animals treated with saline. Out of 30 animals treated with SiCW 1 and SiCW 2, 27 (90%) and 26 (87%) developed pleural mesotheliomas, with the median survival time (days after injection) of 453 (±21) and 519 (±20) days, respectively. In contrast, 7 rats (23%) of the rats treated with SiCW 3 developed pleural mesotheliomas, in comparison to 57% of those treated with the positive control (crocidolite). No tumours were identified in the animals treated with saline. The tumours identified, with one exception, were sarcomatous in appearance and, in all but one case, involved the visceral pleura. The detailed results on tumour development are presented in Table 20 below. In the present study, the differences in the biological activity of the three SiCW samples could not be explained by differences in fibre morphology. The fibre length/diameter distributions were dissimilar. The SiCW 2 contained a disproportionate number of long, thin fibres which was highly carcinogenic. However, SiCW 1 had a similar carcinogenic potency as SiCW 2 but had a lower fraction of fibres ≥ 20 µm in length than SiCW 3 which was less carcinogenic than either SiCW1 or 2. The difference in reactivity could not be explained on a fibre number basis as SiCW3 (the least reactive SiCW) contained the highest number of fibres in the inoculum. Although fibre dimensions are a critical factor for carcinogenesis these results indicate that other aspects of a fibre must also be important.

Table 20 Occurrence of pleural mesotheliomas by treatment group

Sample	No. of animals	No. of animals with mesothelioma	Animals with mesothelioma (%)	Time of first tumour (days)	Mean time to tumour (days (SEM))	Median survival time (days after injection (SEM))
Saline	50	0	0	-	-	753 (25)
CCF (PRD-166)	50	0	0	-	-	708 (18)
SiCW 1	30	27	90	320	465 (25)	453 (21)*
SiCW 2	30	26	87	273	499 (15)	519 (20)*
SiCW 3	30	7	23	349	651 (30)	635 (26)
Crocidolite	30	17	57	416	608 (23)	548 (24)*

* p≤ 0.05v controls with pairwise comparisons with either generalized Savage or Wilcoxon test statistics corrected for multiple (five) comparisons.

Pott and co-workers (Pott F. *et al.,* 1994) observed no increase in tumours in a carcinogenicity study with non-fibrous SiC (type NF2)(no information on particle dimensions), which was injected in Wistar rats (WU/Kiβlegg-Iva: WIWU, 8-10 weeks) intra-peritoneally under CO_2 anesthesia as dust suspensions in 2 ml buffered 0.9% sodium chloride solution. SiC was injected for 5 times at intervals of two weeks into 48 female and 72 male rats at two dose levels (5 times 50 mg and 20 times 50 mg, corresponding to total doses of ca. 667 mg/kg bw and 2,666 mg/kg bw[3]). One year after the first intra-peritoneal injection of SiC, the average body weight of the rats injected with

[3] This value has been calculated based on the default value for average body weight of female rats of 275 gram in chronic studies.

20×50 mg was about 5% lower in both sexes than in the control group injected 20 times with 2 ml saline. Six months later this difference was between 7 and 8% in both sexes. The mortality was less than 20% after 90 weeks in all SiC groups. No serosal tumours were found in the abdominal cavity of 35 histo-pathologically examined rats. Observations 90 weeks after the start of the experiment did not indicate any obviously acute or chronic toxic effect in male and female rats due to 1000 mg non-fibrous SiC dust administered intraperitoneally.

In the study of Davis and co-workers (1996) following the intraperitoneal injection of 1 x 10^9 fibres (length > 5 µm), the numbers of mesotheliomas developing in groups of 24 rats were 21 for amosite, 22 for SiC and 8 for microfibre. A figure with estimated cumulative survival from deaths associated with mesothelioma against time was presented in the study (Figure 3). The SiC produced mesotheliomas particularly rapidly. The median survival time (at which 50% survival was achieved) was 257 days for SiC. SiC was also found to produce rapid pulmonary inflammation as determined by the presence of significant numbers of neutrophils in pulmonary lavage fluid. Similarly, SiC inhalation was found to cause a rapid increase in the rate of proliferation of broncho-alveolar lining cells.

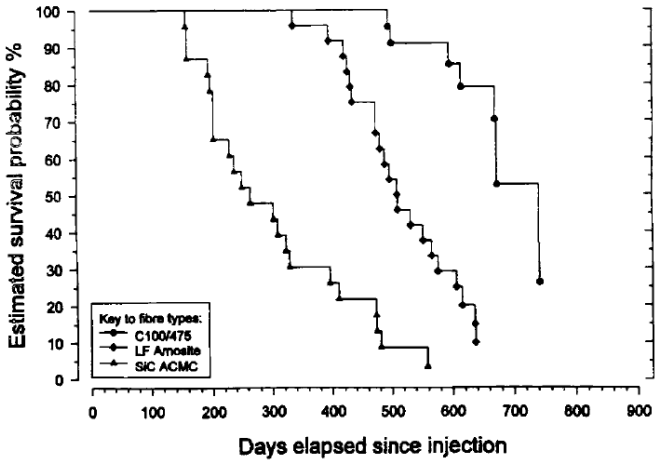

Figure 3 Survival functions for mesothelioma, adjusted for deaths from other causes

Roller and co-workers (Roller M. *et al.,* 1996) examined groups of male or female rats for 30 months for tumours in the abdominal cavity after repeated intra-peritoneal injections with dust suspensions of mineral and vitreous fibres. Two groups of 48 female and 72 male rats were injected either 5 or 20 times (interval between the injections was 2 weeks) with 50 mg of granular SiC (no further specifications) (corresponding to total doses of approximately 667 mg/kg bw and 2,666 mg/kg bw[4]). Two mesotheliomas were found in a total of 395 evaluated rats treated with saline or granular SiC (Table 21). Other tumours are listed in Table 22 below. The results do not show an increase in any type of tumors.

Table 21 Findings of mesotheliomas in granular SiC treated groups compared to controls.

Treatment	Dose injected	Sex	No. of rats	Rats with mesotheliomas	Survival time (weeks after 1st injection

[4] This value has been calculated using the default value for average body weight of male and female rats of 375 gram.

	i.p.		At start	16 weeks	evaluated	Hist. exam	No.	%	20% ≤	50% ≤	80% ≤
Untreated controls	-	F	40	40	37	7	0	0	95	115	129
0.9% NaCl sol.	20x2ml	F	96	95	93	95	0	0	93	110	130
0.9% NaCl sol.	20x2ml	M	72	72	69	72	1	1	75	103	123
Granular SiC	5 x 50mg	F	48	48	47	48	1	2	87	105	130
Granular SiC	5 x 50mg	M	72	71	71	72	0	0	86	109	128
Granular SiC	20 x 50mg	F	48	48	45	48	0	0	91	107	130
Granular SiC	20 x 50mg	M	72	71	70	72	0	0	92	104	125

Table 22 Tumours except mesothelioma in the abdominal cavity of rats

Treatment (total dose)	**NaCl 40 mL**		**SiC (granular) 250 mg**		**SiC (granular) 1000 mg**	
Sex	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**
No. of rats	**93**	**69**	**47**	**71**	**45**	**70**
Uterus	12	-	6	-	7	-
Ovary	-	-	2	-	1	-
Testicle	-	2	-	-	-	1
Liver	1	-	-	1	-	-
Pancreas	-	-	-	-	-	-
Kidney	-	-	-	-	-	-
Suprarenal gland	-	1	-	2	-	-
Mesentery	1	-	1	1	1	-
Lymph nodes	1	1	-	-	-	-
Scrotum	-	1	-	-	-	-
Intestine	-	-	-	-	-	-
Bile-duct	-	-	-	-	-	-
Abdominal cavity	1	-	-	-	-	-

Miller *et al.* (1999b) tested a range of man-made mineral fibres, including SiCW, for evidence of carcinogenicity by single injection into the peritoneal cavity of 24 male SPF Wistar rats and monitored them for the rest of their lives for the development of mesothelioma. The target dose was

designed as the estimated mass required to contain 10^9 fibres > 5 µm in length and amounted to 14.2 mg SiC (corresponding to ca. 30 mg/kg bw and 2.1×10^9 fibres/kg bw). The fibres were < 0.95 µm in diameter (Table 23). Out of 24 rats administered SiC whiskers, 22 (92% CI) developed mesothelioma, with median mesothelioma survival of 257 days (SD = 52) (Table 24).

Table 23 Distribution of injected fibre dose (diameter) and six cumulative length classes, and persistence injected fibres at 12 months

Fibre label	Fibre type	Mass dose (mg)	Diameter class	Number of injected fibres						Persistence (%) of injected fibres at 12 months					
				Length category (µm)						Length category (µm)					
				> 0.4	> 5	> 8	> 10	> 15	> 20	> 0.4	> 5	> 8	> 10	> 15	> 20
100/475	Glass microfibre	8.3	< 0.95 µm	11034	1868	680	421	186	9	9.9	21.3	22.9	29.6	17.2	23.7
			> 0.95 µm	12	12	12	12	0	0						
SiC 1	SiCW	14.2	< 0.95 µm	821	577	387	307	185	121	52.6	53.7	47.7	49.2	54.5	59.2
			> 0.95 µm	4	3	3	3	3	1						
Amosite	Amosite asbestos (long-fibre)	6.1	< 0.95 µm	1791	402	225	164	103	63	8.9	21.9	29.4	34.4	46.2	68.8
			> 0.95 µm	10	8	8	8	8	8						
MMVF 10	Glass wool	144.4	< 0.95 µm	376	314	264	236	155	119	13.1	9.4	6.0	4.4	2.1	0.6
			> 0.95 µm	665	659	598	567	506	436						
MMVF 21	Stonewool	183.1	< 0.95 µm	1349	1012	744	628	439	344	36.9	37.8	37.7	36.3	36.6	43.0
			> 0.95 µm	701	644	558	514	411	335						
MMVF 22	Slagwool	129.6	< 0.95 µm	898	671	492	402	263	142	16.6	10.6	6.1	3.8	2.2	1.7
			> 0.95 µm	570	544	466	388	291	207						
RCF 1	Refractory ceramic fibre	110.9	< 0.95 µm	713	394	280	228	129	85	40.3	42.8	44.9	46.6	49.6	50.5
			> 0.95 µm	398	374	302	260	194	140						
RCF 2	Refractory ceramic fibre	188.8	< 0.95 µm	958	619	392	320	201	111	59.1	70.8	83.9	99.9	130.0	157.3
			> 0.95 µm	565	550	480	455	340	231						
RCF 4	Heat-treated RCF 1	90.4	< 0.95 µm	648	264	134	81	15	6	72.2	81.2	95.7	108.4	113.1	142.7
			> 0.95 µm	548	466	311	230	111	36						

Table 24 Summary of mortality experience for each fibre type (controls are not included in the study)

Fibre label	Animals in group	Number with mesothelioma	%	Median all-cause survival (days)	Estimated standard error	Median mesothelioma survival (days)	Estimated standard error
100/475	24	8	33	642	*	679	24
SiC 1	24	22	92	250	45	257	52
Amosite	24	21	88	509	27	509	27
MMVF 10	22	13	59	643	87	676	43
MMVF 21	20	19	95	281	*	284	*
MMVF 22	24	13	54	658	*	695	*
RCF 1	24	21	88	337	17	337	17
RCF 2	18	13	72	376	25	391	25
RCF 4	22	0	0	725	*	#	#

* Sparse data – no reliable estimate
# No deaths – function not defined

Adachi and co-workers evaluated the carcinogenic risk of man-made fibres, including SiCW, based on mesothelioma incidence in female F344 rats after a single intra-peritoneal administration (Adachi S. *et al.,* 2001). Female F344/Jslc rats were administered intraperitoneal as suspended solution (1 mg/ml) of fibres in saline and were observed for two years after the administration. The number of fibres were counted by scanning electron microscopy, resulting in $414x10^3$ mono crystalline fibres/µg (size is not specified). All rats administered 10 mg of SiCW developed peritoneal mesothelioma within a year. In the group of rats administered 5 mg of SiCW, incidence of mesothelioma was 70% at one year after the administration. 20 mg of SiCW was not tested. No controls were used in the study. The carcinogenic potency of SiCW was estimated 2.4 times in comparison with VICC chrysotile asbestos. The fastest development of peritoneal mesothelioma was identified in the rat administered 5 mg of SiCW at 133 days of the experiment.

Pott (1991) reported the dose –dependent increase in tumour incidence and life span reduction in female Wistar rats after single intraperitoneal injection of 0.05, 0.25, 1.25, 6.25 or 25 mg per animal. The fibre size was 3.1 (L) * 0.31 (D) µm without specification whether it was mono or polycrystalline. The rats were observed for at least 115 weeks. The study was limitedly reported and many animals were killed or died of an infectious lung disease but it was tried to recover as much information as possible. The applied evaluation method (not specified) overestimated the tumour incidence. For example, the true percentage of tumour bearing animals at the highest dose of SiC was less than 97% but higher than 75%. The reported values are provided in table 25.

Table 25. Tumour incidences in female rats after intraperitoneal injection with SiC fibers.

dose injected mg per animal	fibres * 10^9	rats with tumour	mean lifespan of rats with tumour
1 * 0.05	0.005	12.5%	115 weeks
1 * 0.25	0.027	21.7%	111 weeks
1 * 1.25	0.13	61.9%	61 weeks
1 * 6.25	0.67	76.7%	54 weeks
1 * 25	2.68	97.3%	39 weeks

Rödelsperger K and Brückel B (2006) analysed the amount of WHO fibres per milligram of the granular SiC in the study by Pott (1991) as it has been realized that even granular SiC may contain cleavage fragments that fulfil the definition of WHO fibres. In addition, whether the potency per WHO fibre was different for the SiC fragments and the SiC whiskers was examined. Samples of the original granular and fibrous SiC were suspended in water and filtered. One half of each filter was analyzed by scanning electron microscopy (SEM, magnification ×2500), and transmission electron microscopy (TEM, ×10,000) was also performed for the SiC whiskers. The concentration of WHO fibres was 58,000 fibres/mg for the granular sample compared to 48,000,000 (SEM) and 42,000,000 (TEM) fibres/mg for the whiskers. The aspect ratio of the WHO fibres exceeded 10/1 for only 3.3% of the fragments but in each analysis for 96% of the whiskers. In addition, 0% of the fragments compared to 44% and 30% of the whiskers were more than 10 µm long. The injection of 250 and 1000 mg respectably of the granular SiC led to the observation of 0.8 % and 0 % tumors with upper limits of the 95% confidence interval of 4.29% and 2.84%. Since the granular SiC contained 58,000 WHO fibres/mg, in total 15 and 58 × 10^6 WHO fibres were injected with the granular SiC. However, 20.1% and 43.3% tumours would have been expected if the carcinogenic potency were the same for the fragments and for the whiskers. The study concluded that the carcinogenic potency is a function of the shapes of the WHO fibres and is much lower for SiC fragments than for whiskers. Hence carcinogenicity mainly is restricted to a subgroup of WHO fibres longer than about 10 and thinner than about 1 µm.

4.10.2 Human information

Exposure-response associations between increased risk of cancer and exposure to total dust in SiC industry have been indicated in the epidemiological studies (Bugge M.D. et al., 2010; Romundstad P. et al., 2001, Bugge M.D. et al., 2011; Romundstad P. et al., 2002; Infante-Rivard C. et al., 1994). However, limited information is available about exposure-response associations between specific dust constituents and increased risk of cancer. Most epidemiological studies in the SiC industry come from the same source population (Norwegian studies) (Bugge M.D. et al., 2012; Bugge M.D. et al., 2010; Romundstad P. et al., 2001, Bugge m.D. et al., 2011; Romundstad P. et al., 2002). This implies that there is no or only limited replication of the results in other populations in the world. The other cohort studies conducted in Canada and Sweden have low power, because of small sample size (Infante-Rivard C. et al., 1994; Jakobsson K. et al., 1997; Järvholm B. et al., 1982). Case-control studies did show an association between pneumoconiosis and exposure to SiC dust (Dufresne A. et al., 1993; Massé S. et al., 1988) but the studies included a very small number (4) cases in total.

Table 26: Summary table of human cohort studies on cancer

Study type	Test material	End point	Population	Exposure assessment	Observations and Remarks	Reference
Cohort	Total and respirable dust, respirable quartz, cristobalite, and SiC particles and SiC fibres	Lung cancer	The study cohort based on Bugge M.D. *et al*. (2010) and Romundstad P. *et al*, (2001). 1687 men, employed in 1942 and onwards, with ≥3 years employment in the Norwegian SiC industry between 1913 and 2003 and alive after 1 Jan 1953. Control: General male population.	Historic job exposure matrix based on about 8000 measurements. Exposure categories in low, medium and high. SiC particles (mg x years/m^3): - 0-0.38 - 0.83-3.0 - 3.0-60 SiC fibres (fibres x years/cm^3): - 0-0.50 - 0.50-2.0 - 2.0-93	- Results adjusted for age and smoking and asbestos exposure inside the SiC industry - Lung cancer incidence was about twofold increased at the highest level of exposure to each of the exposure factors. SIR 1.9-2.3 for all agents in the highest exposure group. - 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.	Bugge M.D. *et al.*, 2012
Cohort	Dust in SiC industry	Lung and total cancer	Study population based on Romundstad P. *et al*, 2001. From which 121 persons refuse to participate in the follow-up and 130 new employees from period 1997-2003 were added, leading to cohort of 2631 men employed in the SiC industry for a total of ≥6 months, and first employed at one of the three plants between 1913 and 2003. Control: general population (the Cancer Registry of Norway)	Long-term employees were defined as ≥3 years of total employment in the industry and short-term workers as <3 years.	- Results adjusted for smoking and age - Short-term workers: an overall excess incidence of cancer (SIR 1.4, 95% CI 1.2–1.6) and of lung cancer (SIR 2.6, 95% CI 1.9–3.5) - Long-term workers: an excess incidence of total cancer (SIR 1.2, 95% CI 1.1–1.3) and lung cancer (SIR 1.7, 95% CI 1.3–2.2). - Dust exposure in SiC carbide industry may have contributed to the increased risk among long-term workers, whereas the increased risk among short-term workers may be due to a combination of occupational and lifestyle factors. - The causative agents in dust for increased risk of cancer could not be identified in this study	Bugge M.D. *et al.*, 2010

Cohort	Total dust: SiC fibres, SiC particles, and crystalline silica	Cancer	2,620 men employed for more than 6 months in three Norwegian SiC smelters are studied. Control: general population (the Cancer Registry of Norway)	Job exposure matrix based on more than 6,000 measurements. Four exposure categories were defines: never exposed, low, medium, high.	- Results adjusted for age and smoking. - Overall excess risk of lung cancer (74 observed versus 39.9 expected; standardised incidence ratios (SIR) 1.9; 95% CI 1.5-2.3) and elevated risk of stomach cancer (39 observed versus 26.5 expected; SIR 1.5; 95% CI 1.1-2.0). - A high correlation between exposures to the different dust constituents makes differentiation of the effects from separate exposure factors difficult.	Romundstad P. *et al,* 2001
Cohort	Total and respirable dust, respirable quartz, cristobalite, SiC particles, and SiC fibres	Mortality from obstructive lung diseases (OLD)	Based on Romundstad P. *et al.* (2002). 1687 long-term workers (≥3 years) employed in 1913-2003 in the Norwegian SiC industry. Control: national mortality rate	Cumulative exposure, by historical job exposure matrix, were characterized with respect to quartz, cristobalite, SiC particles and SiC fibres. The exposure estimation process is described in Føreland *et al* (2011). Three exposure groups were defined: low, medium, high.	- Adjusted for age, smoking and period of diagnosis (before/after 1990) - increased total mortality risk (SMR 1.1, 95% CI 1.0 to 1.2; 788 cases) and increased risks of cancer (SMR 1.2, 95% CI 1.0 to 1.4; 201 cases), respiratory diseases (SMR 1.6, 95% CI 1.3 to 2.0; 91 cases) and external causes (SMR 1.5, 95% CI 1.1 to 2.0; 44 cases). - Internal analyses indicated that SiC was the exposure factor with the highest risk estimate among workers with less than 15 years of employment, and cristobalite seemed to be the most important factor among workers with more than 15 years of employment.	Bugge M.D. *et al.*, 2011
Cohort	Total dust in SiC industry	Mortality from obstructive lung diseases (OLD)	2562 men, working in one of three SiC smelters in Norwegian SiC industry between 1962 and 1996 Control: Norwegian male population.	Job exposure matrix. Four exposure categories were defines: never exposed, low, medium, high.	- Results adjusted for age and smoking - An excess mortality from cancer, SMR 1.18 (95% CI, 1.03 to 1.35), and an excess mortality from non-malignant respiratory diseases, SMR 1.36 (95% CI 1.07 to 1.70) was found. An excess mortality from asthma, emphysema, and chronic bronchitis combined was also found, SMR=2.21(95% CI 1.6 to 2.95), increasing from 1.05 in the unexposed category to 2.64 (95% CI 1.44 to 4.43) in the upper category of exposure to total dust - High correlation between exposure to the different	Romundstad P. *et al.*, 2002

					agents made it difficult to separate potential effects posed by different types of exposures	
Cohort	Total dust, including: respirable quartz, cristobalite and polycyclic aromatic hydrocarbons concentrations	Cancer	585 Québec SiC production workers who had worked from 1950 to 1980. Follow-up was to December 31 1989.	Job exposure matrix based on 121 dust samples.	- increased mortality from lung cancer (24 observed versus 14.14 expected; standardized mortality ratio (SMR) 1.69; 95% CI 1.09-2.52; significant $p < 0.05$) and stomach cancer (7 observed versus 3.19 expected; (SMR) 2.18; 95% CI 0.88-4.51; not significant) - lung cancer is relation to cumulative exposure of total dust - study very small (=585) and the power of the study was too weak to give statistically significant results	Infante-Rivard C. *et al.,* 1994
Cohort	Metal dust (stainless steel; 18% nickel, 8% chromium) and dust from the abrasives (including SiC, aluminium oxide, amorphous carbon dioxide, clay, and phenol-formaldehyde resins).	Respiratory, stomach, and colorectal cancer.	727 Swedish males were exposed for at least one year (follow up 41 years) Control: standardised mortality or incidence ratios (SMRs, SIRs; county reference rates)	A crude categorisation of workers into exposure level was made, based on notations in the plant records of jobs held.	- The risk estimates were higher in workers with long employment time (1-14 years: four observed cases, SIR 1.7, 95% confidence interval (95% CI) 0.4 to 4.5; > 15 years: three observed cases, SIR 4.3, 95% CI 0.9 to 13) and the increased risk was especially pronounced among those first employed before 1942. - The limited size of the exposed cohort makes a detailed exposure-response analysis unstable, and the confidence limits are wide.	Jakobsson K. *et al.,* 1997
Cohort	Polishing pastes (tallow, beeswax, carnauba wax, alundum, SiC, ferric oxide	Mortality	86 males, Sweden exposed for at least 5 years. Control: Swedish male population.		- 18 died compared to 13.3 expected number of death. 7 died of cancer against 3 expected. 4 had died of stomach cancer compared with 0.44 expected. - The study is too small to obtain meaningful results (seven cases).	Järvholm B. *et al.,* 1982

	and chalk).					
Cohort	Dust levels of aluminium oxide, SiC, and formaldehyde.	mortality and cancer morbidity	911 individuals (521 were blue collar workers) with at least five years employment sometime between 1955 and 1983	Workers could be divided into heavy (> 5mg/m^3) or low exposure (< 5mg/m^3) to abrasives.	- Among the blue collar workers were four cases of mortality due to non-malignant respiratory diseases (pneumonia (1), chronic bronchitis (2), and asthma (1)), whereas 3.2 cases of respiratory diseases would have been expected for the general population. This is no significant increase. - No case of silicosis. - Two of the four cases of respiratory disease had occurred in men with heavy exposure. - The study did not have the power to exclude a moderately increased incidence of cancer of certain sites or of mortality from certain causes.	Edling C. *et al.* (1987)
Case study	Metal dusts	Pneumoconiosis	An active male worker who worked 42 years in SiC plant and had a carborundum pneumoconiosis.	Exposed to carborundum dust. Particle retention was examined from the lung parenchyma of the lobectomy.	- SiC fibres (longer than 5 microns) was found with a concentration of 39,300 fibres/mg dry lung	Dufresne A. *et al.*, 1993
Case study	SiC dust	Pneumoconiosis	Three patients with history of working at a SiC plant.	Long-term exposure to SiC dust.	- Pneumoconiosis was induced by prolonged exposure to SiC	Massé S. *et al.*, 1988

The most recent study of Bugge *et al.* (2012) examined the relative importance of the exposures including quartz, cristobalite, SiC particles and SiC fibres, with respect to lung cancer risk, by using a comprehensive historic job exposure matrix based on about 8000 measurements (Føreland S. *et al.,* 2012). Cumulative exposure to total and respirable dust, respirable quartz, cristobalite, and SiC particles and SiC fibres was assessed for 1687 long-term workers employed during 1913 – 2003 in the Norwegian SiC industry. The study cohort was based on a previously established cohort in the Norwegian SiC industry (Bugge M.D. *et al.,* 2010; Romundstad P. *et al.,* 2001). SIR for lung cancer, with follow-up during 1953 – 2008, were calculated stratified by cumulative exposure categories. Poisson regression analyses were performed using both categories and log-transformed cumulative exposure variables. The lung cancer incidence was increased at the highest level of exposure to SiC particles and SiC fibres (Table 27).

Table 27 Observed number of cases (Obs) and standardized incidence ratio (SIR), with 95% 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

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

Internal analyses showed associations between exposure level and lung cancer incidence for SiC particles and SiC fibres. In multivariate analyses, fibres adjusted for SiC showed the second most consistent associations (following cristobalite) (Table 28). The results indicated 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).

Table 28 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

	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.
†$\mathbf{r_{Pearson}}$: Pearson's correlation coefficient.
PYR, person - years; AIC, Akaike's Information Criterion; SiC, silicon carbide.

A study among workers in the Norwegian SiC industry, followed until 2005, revealed an excess incidence of lung and total cancer (Table 29) (Bugge M.D. *et al.,* 2010). The total cohort for this study was based on the cohort population of Romundstad P. *et al.* (2010) which comprised 2612 men employed for >6 months between 1913–2003. The follow-up period for cancer was 1953–2005. Short-term workers were defined as having <3 years of total employment in the industry. Among the short-term workers, an overall excess incidence of cancer (SIR 1.4, 95% CI 1.2–1.6), with an excess of lung cancer (SIR 2.6, 95% CI 1.9–3.5) as the most important contributing factor, was observed. The long-term workers also had an excess incidence of total cancer (SIR 1.2, 95% CI 1.1–1.3) and lung cancer (SIR 1.7, 95% CI 1.3–2.2). An increased risk of cancers at other sites was also observed, specifically among short-term workers.

The highest SIR for lung cancer were seen among the short-term workers, and among the long-term workers the SIR were fairly stable with increasing employment duration (Bugge M.D. *et al.*, 2010). Ideally, for a causal association, one would have expected an increasing trend in risk with increasing duration of employment, but as employment duration is an imperfect exposure indicator, results should be interpreted with caution. The results indicate that differential selection bias and confounding between short- and long-term workers may distort the assessment of exposure–response relationships in cohorts of occupationally exposed workers. The causative agents in dust for increased risk of cancer could not be identified in this study

Table 29 Observed (Obs) number of cases and standardized incidence ratio (SIR) of cancer, all sites, with 95% confidence intervals (95% CI), 1953-2005, among 2612 male Norwegian SiC short- and long-term workers employed > 6 months 1913-2003. [ICD-7=International Classification of Diseases, 7th revision].

		Short-term workers (N=925)			Long-term workers (N=1687)		
Site	**ICD-7 code**	**Obs**	**SIR**	**95%CI**	**Obs**	**SIR**	**95%CI**
Lip	140	3	2.1	0.7-6.7	7	2.4	1.2-5.1
Oral cavity, pharynx	141, 143 – 148	6	2.5	1.1-5.6	10	2.1	1.1-3.9
Digestive organs	150-159	37	1.0	0.8-1.4	82	1.1	0.9-1.3
Esophagus	150	3	1.9	0.6-5.8	3	0.9	0.3-2.7
Stomach	151	13	1.4	0.8-2.4	25	1.3	0.9-1.9
Small intestine	152	0	0.0	0.0-8.0	2	2.1	0.5-8.3
Colon	153	11	1.0	0.5-1.8	26	1.0	0.7-1.5
Rectum	154	3	0.4	0.1-1.3	15	1.0	0.6-1.7
Liver	155	2	2.3	0.6-9.1	2	1.1	0.3-4.2
Pancreas	157	5	1.2	0.5-2.8	9	1.0	0.5-1.9
Nose, sinuses, etc	160	0	0.0	0.0-9.7	2	2.6	0.6-10.4
Larynx	161	2	1.3	0.3-5.1	3	0.9	0.3-2.8
Trachea, bronchus, and lung	162	43	2.6	1.9-3.5	60	1.7	1.3-2.2
Pleura	163	2	3.7	0.9-14.7	1	0.8	0.1-6.0
Prostate	177	26	0.9	0.6-1.3	77	1.2	1.0-1.5
Testis	178	1	0.5	0.1-3.9	2	0.6	0.2-2.4
Kidney, ureter	180	4	0.8	0.3-2.2	10	1.0	0.5-1.9
Bladder and other urinary organs	181	13	1.4	0.8-2.4	19	0.9	0.6-1.5
Melanoma of skin	190	6	1.2	0.5-2.7	15	1.5	0.9-2.5
Other skin (non-melanoma)*	191	11	2.1	1.1-3.7	18	1.5	0.9-2.3
Brain, nervous system	193	3	0.8	0.3-2.5	5	0.7	0.3-1.7
Thyroid gland	194	4	5.8	2.2-15.4	1	0.7	0.1-5.2
Hodgkin lymphoma	201	4	5.2	2.0-13.9	1	0.7	0.1-5.1
Non-hodgkin lymphoma	200 + 202	1	0.3	0.0-2.3	8	1.2	0.6-2.4
Multiple myeloma	203	4	1.8	0.7-4.7	3	0.6	0.2-1.9
Leukemia	204	2	1.8	0.5-7.4	6	2.8	1.2-6.1
Unspecified sites	199	10	2.1	1.2-4.0	11	1.1	0.6-2.0
Other specified sites		2	0.8	0.2-3.4	6	1.3	0.6-2.8
All sites	140 - 204	184	1.4	1.2-1.6	347	1.2	1.1-1.3

*Except basal cell carcinoma.

Romundstad and co-workers (Romundstad P. et al, 2001) studied cancer incidence among 2,620 men (26% never smokers, 63% current smokers and 11% former smokers) employed for more than 6 months in three Norwegian SiC smelters. The cohort's incidence of lung cancer was observed to increase (74 observed versus 39.9 expected; SIR= 1.9; 95% CI 1.5-2.3). In addition, the study found an increased risk of cancer of the stomach (39 observed versus 26.5 expected; SIR= 1.5; 95% CI 1.1-2.0) and the upper respiratory tract (16 observed cases versus 9.6 expected; 95% CI 1.0-2.7), together with a borderline increased risk of lip cancer (SIR 2.0, 95% CI 0.9-3.9) and non-melanoma skin cancer (SIR 1.5, 95% CI 0.9-2.5) (Table 30). For lag times of 20 years or more the association diminished gradually. The incidence of stomach cancer was highest (SIR 2.6 95% CI 1.5-4.1) among workers employed in a refinery department, where the SiC products were crushed, cleaned, and packed. However, no further increment in risk was observed with increasing duration of employment in these departments. In addition, no association was observed between exposure to various particulates and the incidence of upper respiratory tract cancer. The authors suggested that the approximate nature of the exposure estimates and chance may have led to errors that easily could have biased the shape of the exposure-response relation.

Table 30 Observed and expected numbers of lung cancers and SIR, by cumulative exposure to SiC fibres in the period of 1953-1996, and in different calendar period of first employment (before 1960; in 1960 or later)

	Cumulative SiC fibre exposure (fibres/ml·y)	1953-1996				First employment before 1960				First employment in 1960 or later			
		Observed no.	Expected no.	SIR	95% CI	Observed no.	Expected no.	SIR	95% CI	Observed no.	Expected no.	SIR	95% CI
No lag	**0**	5	9.4	0.6	0.2-1.5	2	4.9	0.4	0.0-3.4	3	5.0	0.6	0.1-1.8
	0.1-0.9	25	11.7	2.0	1.3-3.0	16	6.3	2.5	1.2-3.0	9	5.4	1.7	0.8-3.2
	1-4.9	19	10.1	1.8	1.1-2.8	15	6.1	2.5	1.5-4.1	4	4.0	1.0	0.3-3.2
	≥5	25	8.8	2.9	1.9-4.2	18	6.0	3.0	2.2-6.0	7	2.8	2.5	1.0-5.2
20-y lag	**0**	15	17.1	0.9	0.5-1.5	7	5.4	1.3	0.5-2.7	8	11.7	0.7	0.3-1.4
	0.1-0.9	23	9.9	2.3	1.5-3.5	15	6.9	2.2	1.2-3.6	8	3.0	2.6	1.0-5.2
	1-4.9	18	7.8	2.3	1.4-3.6	13	5.9	2.2	1.2-3.8	5	1.9	2.7	0.9-6.2
	≥5	18	5.1	3.5	2.1-5.6	16	4.6	3.5	2.0-5.6	2	0.5	4.0	0.5-14.5

A paper of Bugge *et al.* in 2011 (Bugge MD *et al.,* 2011) presented an update of the previous Norwegain mortality study (Romundstad P. *et al.*, 2002), with an additional 11 years of follow-up and improved exposure estimates from the revised job-exposure matrix (JEM). In this study, Bugge and co-workers found that exposure to SiC and crystalline silica may contribute to obstructive lung diseases (OLD) development among SiC industry workers. In this study, 1687 long-term workers employed in 1913-2003 in the Norwegian SiC industry were characterized with respect to cumulative exposure to quartz, cristobalite, SiC particles and SiC fibres. SMRs for underlying causes of death, 1951-2007, were calculated stratified by category of cumulative exposure, and Poisson regression analyses of were performed using cumulative exposure variables. An increased total mortality (SMR 1.1, 95% CI 1.0 to 1.2) and increased mortality from cancer (SMR 1.2, 95% CI 1.0 to1.4), non-malignant respiratory diseases (SMR1.6, 95% CI 1.3 to 2.0) and external factors (SMR1.5, 95% CI 1.1 to 2.0), were observed (Table 31). The SMR of OLD was increased at the highest level of cumulative exposure to all investigated exposure factors. In the internal analyses, an increased risk of OLD (SMR 2.0, 95%CI 1.5 to 2.7), pneumonia (SMR 1.4, 95% CI 1.0 to 1.9; 38 cases) and pneumoconiosis (SMR15, 95% CI 7.0 to 31; 7cases) was observed with increasing levels of cumulative exposure to SiC particles. For circulatory diseases the SMR was 1.0 (95% CI 0.9 to 1.2; 347 cases), for digestive diseases 1.1 (95% CI 0.7 to 1.7; 17cases) and for other diagnoses 0.9 (95% CI 0.7 to 1.1; 88cases).

Table 31 Standardised mortality ratios (SMR) with 95% CI of cause of death 1951-2007, by cumulative exposure groups, among Norwegian SiC industry workers employed 1913-920

	Number of cases	**SMR**	**95% CI**
Total mortality	788	1.1	1.0 to 1.2
Cancer	201	1.2	1.0 to 1.4
Respiratory diseases	91	1.6	1.3 to 2.0
OLD	45	2.0	1.5 to 2.7
pneumonia	38	1.4	1.0 to 1.9
pneumoconiosis	7	15	7.0 to 31
External causes	44	1.5	1.1 to 2.0
Circulatory diseases	347	1.0	0.9 to 1.2
Digestive diseases	17	1.1	0.7 to 1.7
Other diagnoses	88	0.9	0.7 to 1.1

In 2002, the associations between exposures in the SiC industry and mortality from non-malignant diseases were further studied by Romundstad and co-workers (Romundstad P *et al.,* 2002). Mortality among 2562 men, working in one of three SiC melters was investigated, giving 52618 person-years of follow up from 1962 to 1996. Dose-response relations were investigated by internal comparisons using Poisson regression and by stratified standardized mortality ratio (SMR) analyses. Mortality from all causes was significantly raised compared with the Norwegian mortalities among men SMR=1.12, (95% CI 1.05 to 1.20) (Table 32). An excess mortality from cancer, SMR 1.18 (95% CI, 1.03 to 1.35) was found. An excess mortality from asthma, emphysema, and chronic bronchitis combined was also found, SMR=2.21(95% CI 1.6 to 2.95), increasing from 1.05 in the unexposed category to 2.64 (95% CI 1.44 to 4.43) in the upper category of exposure to total dust (Table 33). Smoking was found not to act as a confounder. No association was found for circulatory mortality. There was increased mortality from asthma, emphysema, and chronic bronchitis combined among SiC workers exposed to dust.

Table 32 Observed (Obs) and expected (Exp) number of cause specific deaths and SMR among 2562 mal Norwegian SiC smelter workers in the follow up period 1962-96

Cause of death	Obs	Exp	SMR	95% CI
All causes	847	753.6	1.1	1.1 to 1.2
Cancer	204	173.0	1.2	1.0 to 1.4
Circulatory diseases	376	371.7	1.0	0.9 to 1.1
Ischaemic heart disease	208	234.9	0.9	0.8 to 1.0
Cerebrovascular disease	91	71.6	1.3	1.0 to 1.6
Sudden death	37	24.3	1.5	1.1 to 2.1
Respiratory diseases	77	56.6	1.4	1.1 to 1.7
Asthma, chronic bronchitis, emphysema	45	20.4	2.2	1.6 to 3.0
Pneumoconiosis	6	0.8	7.9	2.9 to 17.1
Pneumonia	24	30.7	0.8	0.5 to 1.2
Digestive diseases	17	18.0	0.9	0.5 to 1.4
External causes	58	49.1	1.2	0.9 to 1.5

Table 33 Observed (Obs) and expected (Exp) number of deaths from chronic obstructive lung diseases (asthma, chronic bronchitis, and emphysema) and SMR by cumulative exposure to total dust (mg/m^3·y) and duration of employment

Cumulative exposure to	All employees				Employment > 3 y			
total dust (mg/m3·y)	Obs	Exp	SMR	95% CI	Obs	Exp	SMR	95% CI
0	3	2.9	1.1	0.2 to 3.1	1	2.2	0.5	0.0 to 2.5
0 – 14.9	13	5.0	2.6	1.4 to 4.4	2	1.1	1.8	0.2 to 6.6
15 – 69.9	15	7.2	2.1	1.2 to 3.4	12	5.6	2.1	1.1 to 3.7
>70	14	5.3	2.6	1.4 to 4.4	14	5.3	2.6	1.4 to 4.4

Infante-Rivard *et al.* (1994) published a retrospective cohort study among 585 Québec SiC production workers who had worked at any time between 1950 and 1980 at the three Québec silicon production plants. Infante-Rivard *et al.* (1994) showed an increased mortality from lung cancer (24 observed versus 14.14 expected; standardized mortality ratio (SMR) 1.69; 95% CI 1.09-2.52; significant $p < 0.05$) and stomach cancer (7 observed versus 3.19 expected; (SMR) 2.18; 95% CI 0.88-4.51; not significant). However the power of the study was low, because of small sample size and use of cumulative total dust exposure variable, which may be poor indicator of lung irritants and other potential carcinogens in this industry. Firm conclusions on an increased risk of non-malignant respiratory diseases and lung cancer among production workers in the silicon carbide industry could not be reached.

Jakobsson K. and co-workers have studied the cause specific mortality and cancer morbidity in workers exposed to the dust of grinding materials, grinding agents, and stainless steel, especially with regard to a possibly increased risk of respiratory, stomach, and colorectal cancer (Jakobsson K *et al.,* 1997). The exposed cohort comprises workers with at least 12 months employment time at two plants, producing stainless steel sinks and saucepans (n=727). Reference cohorts of other

industrial workers (n=3965) and fishermen (n=727) were also analysed. Standardised Incidence Ratios (SIRs) were calculated for cancer morbidity between 1958 and 1992. In the exposed cohort, there was an increase of morbidity from colon cancer ((Table 34), which was explained by an excess of tumours in the sigmoid part only (Table 35). A slight nominal excess of rectal cancers (nice cases, SIR 1.4 95% CI 0.6-2.6) and a significant excess of prostate cancer morbidity (thirty-six cases, SIR 1.7 95% CI 1.2-2.4) were found. Increased risk was especially pronounced among those who were employed longer (1-14 years: four cases, SIR 1.7 95% CI 0.4-4.5; ≥15 years: three observed, SIR 4.3 95% CI 0.9-13).

Table 34 Tumour morbidity 1958-1992 in a cohort of workers grinding stainless steel and in control workers

					Control cohorts							
	Exposed cohort (n=719)				Industrial workers (n=3965)				Fishermen (n-8078)			
Tumour	**O**	**E**	**SIR**	**95% CI**	**O**	**E**	**SIR**	**95% CI**	**O**	**E**	**SIR**	**95% CI**
All malignant tumours	112	119	0.9	0.7-1.2	478	435	1.1	1.0-1.2	832	856	1.0	0.9-1.1
Oropharyngeal	3	1.8	1.6	0.3-4.8	13	8.2	1.6	0.8-2.7	16	14.8	1.1	0.6-1.8
Stomach	8	9.6	0.8	0.3-1.7	33	30.4	1.1	0.7-1.6	50	57	0.9	0.6-1.2
Colon	12	8.3	1.4	0.7-2.6	29	32.8	0.9	0.6-1.3	68	70.7	1.0	0.7-1.3
Rectum	9	6.7	1.4	0.6-2.6	32	25.0	1.3	0.8-1.9	44	43.6	1.0	0.7-1.4
Pancreas	3	3.7	0.8	0.1-2.4	15	14.5	1.0	0.5-1.8	34	29	1.2	0.8-1.7
Sinonasal	0	0.5	0.0	0.0-8.0	3	1.1	2.7	0.5-7.8	4	2.2	1.8	0.4-4.6
Larynx	1	1.4	0.7	0.0-3.9	4	5.6	0.7	0.1-1.9	6	8.6	0.7	0.2-1.6
Primary lung	7	12.4	0.6	0.2-1.2	48	45.0	1.1	0.7-1.5	72	69.7	1.0	0.8-1.4
Prostate	36	21.2	1.7	1.2-2.4	105	87.0	1.2	0.9-1.5	200	197	1.0	0.8-1.2
Renal	5	3.8	1.3	0.4-3.2	15	13.1	1.1	0.6-1.9	22	25.2	0.9	0.5-1.4
Uroepitheal	5	10.0	0.5	0.1-1.2	42	36.3	1.2	0.8-1.6	45	59.7	0.7	0.5-1.1
Lymphoma, myeloma	3	5.7	0.5	0.1-1.6	22	20.8	1.1	0.6-1.7	41	41.6	1.0	0.7-1.4

O = observed; E = expected; SIR = Standardised Incidence Ratio

Table 35 Site specific colorectal cancer morbidity 1958-1992 in a cohort of workers grinding stainless steel and in control workers (a minimum employment time of one year was required; the observation period began 15 years after the start of employment)

	Exposed cohort								Control cohorts							
	Employed 1-14 y (n=537)				Employed ≥ 15 y (n=182)				Industrial workers (n=3965)				Fishermen (n=8078)			
Tumour	**O**	**E**	**SIR**	**95% CI**	**O**	**E**	**SIR**	**95% CI**	**O**	**E**	**SIR**	**95% CI**	**O**	**E**	**SIR**	**95% CI**
Caecum and colon ascendens	1	2.3	0.4	0.0-2.5	0	0.6	0.0	0.0-6.0	3	9.1	0.3	0.0-1.0	23	22.2	1.0	0.6-1.6
Colon transversum including flexure	1	0.6	1.8	0.0-10	0	0.2	0.0	0.0-24	3	4.2	0.7	0.1-2.1	9	8.9	1.0	0.4-2.0
Colon descandens	0	0.3	0.0	0.0-11	0	0.1	0.0	0.0-38	1	1.8	0.5	0.0-3.1	2	3.2	0.6	0.0-2.3
Colone sigmoideum	4	2.3	1.7	0.4-4.5	3	0.7	4.3*	0.9-13	13	12.3	1.0	0.5-1.8	19	25.8	0.7	0.4-1.2
Colon, multiple or not specified	2	0.9	2.3	0.2-8.2	1	0.2	4.2	0.1-24	9	4.5	2.0	0.9-3.3	13	10.2	1.3	0.6-2.2
Rectum (anus included)	7	5.2	1.3	0.5-2.8	2	1.4	1.4	0.1-5.0	32	25.0	1.3	0.8-1.9	44	43.6	1.0	0.7-1.4

The mortality pattern among 86 men was determined in a Swedish study (Järvholm B. *et al.,* 1982) to investigate the possible hazards of polishing steel with polishing pastes (containing tallow, beeswax, carnauba wax, alundum, SiC, ferric oxide and chalk). A total of 18 men had died compared with 13.3 expected death rates of Swedish male population. 4 had died of stomach cancer compared with 0.44 expected ($p < 0.005$). The mortality for other causes of death was found not increased. The results of this study do not permit any definite conclusions, but nevertheless indicate a possible cancer hazard among polishers who are exposed to polishing pastes containing SiC.

Dufresne and co-workers (Dufresne A. *et al.*, 1993) evaluated on pulmonary dust retention in a man who worked 42 years in the vicinity of an Acheson furnace of a SiC plant and had a carborundum pneumoconiosis. In this study, special attention was given the retained SiC fibres in the lung parenchyma. The concentration of SiC fibres longer than 5 microns is 39,300 fibres/mg dry lung. These fibres have been found to have a similar morphology to fibres observed in the working environment.

The case studies of Massé S and co-workers (Massé S. *et al.,* 1988) suggest that the exposure to SiC dust may cause a distinctive pneumoconiosis. When the tissues from three workers with the history of long-term exposure to SiC dust were examined for light microscopy after they had been admitted to a hospital, a mixed pneumoconiosis was found. The lesions can be summarized as follows: (a) abundance of intra-alveolar macrophages associated with a mixture of inhaled particles including carbon, silicon, pleomorphic crystals, SiC, and ferruginous bodies showing a thin black central core; (b) nodular fibrosis, generally profuse, containing silica and ferruginous bodies and associated with large amount of carbon pigment; (c) interstitial fibrosis, less prominent than the nodular form; (d) carcinoma in two cases. These case studies suggest that he Stanton hypothesis on fibre properties and carcinogenesis (Stanton M.F. *et al.*, 1981) could be applied to SiC dust.

In a cohort study (Edling C. *et al,* 1987) more than 500 individuals exposed to SiC dusts working in the manufacture of abrasives were followed up from 1958 until 1983. The study revealed no significant increase in total mortality, cancer mortality, or incidence of non-malignant respiratory diseases ascribable to SiC dusts. The study did not, however, have the power to exclude a moderately increased incidence of cancer of certain sites or of mortality from certain causes.

4.10.3 Other relevant information

ACGIH has defined fibrous forms of SiC (including whiskers) as A2; Suspected human carcinogen (ACGIH TLVs and BEIs, 2008).

The carcinogenicity of silicon carbide fibres and whiskers was recently assessed by IARC (Grosse et al, 2014). IARC concluded that occupational exposure associated with the Acheson process were classified as carcinogenic to humans (Group 1) on the basis of sufficient evidence in humans that they cause lung cancer. Since the correlation between exposures to SiC fibres and cristobalite made it difficult to disentangle their independent effects, the Working Group concluded that fibrous SiC is possibly carcinogenic to humans (Group 2B) based on limited evidence in humans that it causes lung cancer. Although not unanimous, the Working Group classified SiC whiskers as probably carcinogenic to humans (Group 2A) rather than possibly carcinogenic to humans (Group 2B), on the basis that the physical properties of the whiskers resemble those of asbestos and erionite fibres, which are known carcinogens. In addition, the results of available mechanistic studies were consistent with proposed mechanisms of fibre carcinogenicity. The majority of the Working Group considered that differences in the nature of SiC fibres and SiC whiskers warranted separate evaluations.

In 2012, the Health Council of the Netherlands has derived classification as carcinogenic for SiC. Based on the available information, the Committee concluded that fibrous SiC (fibres, whiskers) may cause cancer according to a non-stochastic mechanism and should be classified as carcinogenic to humans (in category 1A) (Evaluation of the carcinogenicity and genotoxicity of SiC, 2012). The limited data on the non-fibrous form of SiC are considered insufficient to classify the carcinogenic properties of this substance. This classification was taken over in the Dutch national list of CMR substances relevant for worker legislation.

The carcinogenicity of certain fibres is known for a long period and started with the observation of an increase in mesotheliomas after exposure to asbestos fibres. In respiratory toxicology, it is generally accepted that high aspect ratio particles (fibers) pose an additional hazard beyond that produced by conventional compact particles. A high aspect ratio is defined by the WHO as a ratio of fiber length to diameter ≥3 (WHO 1988). The toxic potential of fibers is often described with the 3Ds of particle toxicology: Dose, Dimension, and Durability (Bernstein DM 2007). The mechanism of these factors and their contribution to the toxicity of fibers after implantation will be shortly discussed.

- The dose usually refers to the number of long fibers that reach the lung parenchyma and cannot be removed by macrophages or by other clearance mechanisms of the lungs.

- The dimension refers to the length and diameter of the fibers. The dimension influences both the uptake of the fibers through inhalation and the durability in the body. The influence of the diameter on the uptake is specifically relevant to inhalation, as fibers with a diameter over 3 µm cannot be inhaled into the deep lung. The length determines whether the fiber can be engulfed and removed by the macrophage.

- The durability determines how fast the fiber can dissolve and/or break down once deposited in the lung. The durability depends on the dimension and composition of the fibers and the characteristics of the local environment.

When long, thin, biopersistent fibres enter the body they cannot be cleared by macrophages or by other clearance mechanisms. Hence they accumulate and cause chronic inflammation reactions, which lead in time to the formation of fibrosis and granuloma. Although this mechanism was first described for asbestos, it occurs irrespective of the chemical composition of the fibres, as long as they are biopersistent and of the right shape.

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 engolved by microphages and behave comparable to non-fibrous particles. Except for overload conditions, the involvement of these short fibres in carcinogenesis is considered low (Bernstein, 2007). A plausible hypothesis for the induction of mesothelioma was proposed by Donaldson (2010). 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 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 for stoma retention and inflammation (Lippmann, 2014).

A third mechanism for fibre carcinogenicity is mesothelial piercing of the pleura. The available in vitro data show that the diameter, with smaller diameters (50 nm) being more toxic than wider diameter (150 nm), is more important than length. 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 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. More in general, fibres with a diameter above 3 µm are not considered respirable (Harrison, 2015).

4.10.4 Summary and discussion of carcinogenicity

From the available animal data it can be concluded that the tested fibre like forms of SiC is able to induce tumours upon inhalation, as well as upon intrapleural and intraperitoneal administration.

Upon inhalation of SiCW (single crystal, mean diameter of 0.45 µm and > 5 µm in length) Davis *et al.* (1996) reported the clear increase of carcinomas, adenomas and mesotheliomas in lungs of rats exposed to SiC whiskers. This study is considered to be the key study as this is the only study with a route of exposure normally relevant to humans. In addition, no tumor induction was found in the inhalation study of Akiyama I. *et al.* (2007) when rats were exposed to SiCW (mean diameter of 0.5 µm and length of 2.8 µm) although broncho-alveolar hyperplasia and advanced fibrosis of the lung parenchyma were found. This result supported the results in previous studies that the carcinogenicity is a function of the fibre length. However, due to the low number of animals observed until 2 years of age, the relatively short observation period and the low exposure levels, no final conclusion can be drawn. Dose-response studies were unfortunately not available.

Stanton *et al.* (1981) reported the increased incidence of pleural carcinomas, resembling mesenchymal mesotheliomas in man, 1 year after intrapleural administration of SiCW (metallic crystalline, strongly variable in diameters and length). The probability of pleural sarcoma correlates best with general fibres in general that measure ≤ 0.25 µm x > 8 µm. The overall frequency of mesotheliomas in rats injected with SiCW was found to be comparable to that of rats injected with asbestos, used as a positive control in some studies (Vasil'eva L.A. *et al.*, 1989; Adachi S. *et al.*, 2001). The development of adenocarcinomas in combination with mesotheliomas, and development of peritoneal mesotheliomas upon intrapleural administration of SiCW (SiCW 1: diameter of 0.42 and length of 4.5 µm; SiCW 2: diameter of 0.75 and length of 20.1 µm; SiCW 3: diameter of 0.32 and length of 6.6 µm) to rats were also reported (Johnson N.F. and Hahn F.F., 1996). This study also showed that other aspects of a fibre must also be important although fibre dimensions are a critical factor for carcinogenesis. In the case of SiCW, surface chemistry may have a limited influence on their carcinogenic potency. No animal data on intrapleural administration of non-fibrous SiC were retrieved.

Intraperitoneal administration of SiCW (mean diameter of < 0.95 µm and length of > 0.4 µm) and unspecified SiCW to rats could lead to early development of peritoneal mesotheliomas (Miller B.G. *et al.*, 1999b, Adachi S. *et al.*, 2001). A dose response relation for tumour incidence was observed by Pott (1991) for unspecified SiC with dimensions of 3.1 * 0.31 µm. No increased tumour incidence was found in rats which had received an injection of non-fibrous SiC (Pott F. *et al.*, 1994) or granular SiC (Roller M. *et al.,* 1996). The study by Rodelsperger and Brückel (2006) indicates that the potency for carcinogenicity of cleavage products fulfilling the WHO fibre definition is lower than the potency of whiskers. This can be expected based on the different dimensions of these

fibres. This does not show that such fibres have no carcinogenic potential as this requires a study using only SiC fibres.

Table 36 Summary of dependency of tumor formation in rats via different routes on SiC characteristics

Administration route	SiC characteristics	Tumor formation	Reference
Inhalation	Fibrous single crystal mean diameter of 0.45 µm and > 5 µm in length	Yes	Davis J.M.G. et al., 1996
Inhalation	Fibrous mean diameter of 0.5 µm and 2.8 µm in length	No	Akiyama I. et al., 2007
intrapleural administration	Fibrous metallic crystalline mean diameter of 0.05 to > 1.5 µm and >1.5-2.5 to > 8 µm in length	Yes Best correlation with general fibres that measure with diameter ≤ 0.25 µm and length > 8 µm. Relatively high correlations with fibres with a diameter up to 1.5 µm and a length greater than 4 µm.	Stanton M.F. et al., 1981
intrapleural administration	Fibrous mean diameter of 0.42 and 4.5 µm in length	Yes significant	Johnson N. F. and Hahn F.F., 1996
	Fibrous mean diameter of 0.75 and 20.1 µm in length	Yes significant	
	Fibrous mean diameter of 0.32 and 6.6 µm in length	Yes Not significant	
Intraperitoneal administration	Non-fibrous SiC	No	Pott F. et al., 1994
Intraperitoneal administration	Granular SiC	No	Roller M. et al., 1996
Intraperitoneal administration	SiC 3.1 * 0.31 µm	Yes	Pott, F., 1991
Intraperitoneal administration	Fibrous single crystal mean diameter of 0.45 µm and > 5 µm in length	Yes	Davis J.M.G. et al., 1996
Intraperitoneal administration	Fibrous mean diameter of < 0.95 and > 0.4 µm in length	Yes	Miller B.G. et al., 1999b
Intraperitoneal administration	Fibrous Characteristics not specified	Yes	Adachi S. et al., 2001

The epidemiological studies found exposure-response associations between increased risk of cancer and exposure to total dust in SiC industry (Bugge M.D. *et al.*, 2010; Romundstad P. *et al.*, 2001, Bugge M.D. *et al.,* 2011; Romundstad P. *et al.,* 2002; Infante-Rivard C. *et al.,* 1994). However, limited information is available about exposure-response associations between specific dust constituents and increased risk of cancer. The most recent study of Bugge *et al*. (2012) however, examined the relative importance of the exposures including quartz, cristobalite, SiC particles and SiC fibres, with respect to lung cancer risk. The results indicated 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. Exposure to quartz and SiC particles did not seem to influence the lung cancer incidence.

Most epidemiological studies in the SiC industry come from the same source population (Norwegian studies) (Bugge M.D. *et al*., 2012; Bugge M.D. *et al.*, 2010; Romundstad P. *et al.*, 2001, Bugge m.D. *et al.*, 2011; Romundstad P. *et al.*, 2002). This implies that there is no or only limited replication of the results in other populations in the world. The other cohort studies conducted in Canada and Sweden have low power, because of small sample size (Infante-Rivard C. *et al.*, 1994; Jakobsson K. *et al*., 1997; Järvholm B. *et al*., 1982).

Case-control studies did show an association between pneumoconiosis and exposure to SiC dust (Dufresne A. *et al.,* 1993; Massé S. *et al.*, 1988) but the studies included a very small number (4) cases in total.

Reviews of available information on the carcinogenicity of fibres show that this is related to insoluble, persistent fibres with a certain diameter and length. The suggested maximal diameter and length for mesotheliomas is < 0.1µm and > 5 µm and for lung carcinoma 0.1 – 3 µm and > 15 µm (Lippmann, 2014, Harrison, 2015).

4.10.5 Comparison with criteria

The dossier submitter proposes classification as Carc. 1B for SiC. The rationale is as follows:

The CLP criteria for classification in Carc. 1 are as follows:

“Known or presumed human carcinogens

A substance is classified in Category 1 for carcinogenicity on the basis of epidemiological and/or animal data. A substance may be further distinguished as:

Category 1A, known to have carcinogenic potential for humans, classification is largely based on human evidence, or

Category 1B: Category 1B, presumed to have carcinogenic potential for humans, classification is largely based on animal evidence. The classification in Category 1A and 1B is based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be

derived from:

- *human studies that establish a causal relationship between human exposure to a substance and the development of cancer (known human carcinogen); or*
- *animal experiments for which there is sufficient (1) evidence to demonstrate animal carcinogenicity (presumed human carcinogen). In addition, on a case-by-case basis, scientific*

judgement may warrant a decision of presumed human carcinogenicity derived from studies showing limited evidence of carcinogenicity in humans together with limited evidence of carcinogenicity in experimental animals."

In the CLP, sufficient evidence of carcinogenicity is defined as 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 in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide sufficient evidence. A single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites;"*

Limited evidence of carcinogenicity is defined as when *"the data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs."*

According to these criteria, a classification in category 1A is not warranted as the available epidemiologic data only show a limited evidence of carcinogenicity as there is a positive association between exposure to SiC fibres and lung cancer. However, confounding cannot be ruled out as there was also exposure to other carcinogens and this exposure also showed a positive association with lung cancer (Massé S. *et al*., 1988; Romundstad P *et al*., 2001, 2002; Bugge MD *et al*., 2010, 2011, 2012;). Non-fibrous SiC did not seem to contribute to the cancer risk (Bugge M.D. *et al*., 2012).

However, according to these criteria a classification in Carc. Cat. 1B is warranted to SiC fibres with certain diameter and length as experimental animal data has shown that these SiC fibres caused increased incidence of various tumours, including mesotheliomas, after inhalation (Davis J.M.G. et al., 1996), after intrapleural administration (Stanton M.F. *et al*, 1981; Vasil'eva L. A. *et al*., 1989; Johnson N.F. and Hahn F.F., 1996), and after intraperitoneal administration (Miller B.G. et al., 1999b; Adachi S. et al., 2001, Pott, F., 1991). This is further supported by the low dissolution rate (Davis, 1996) of less than 0.2% at pH 7.0, 4.6 and 0.6 in a period of up to 56 days and high in vivo tissue retention (12 month clearance size depend -8 to 45% for fibres above 1 μm). The results suggest that SiC is carcinogenic when present in the fibrous form. In general fibre carcinogenicity is a function of the fibre length, with fibres longer than 20 μm having the greatest effect on carcinogenicity. In addition, SiC with average diameter of 0.5 μm and length of 2.8 μm did not cause tumor induction after one year inhalation study also suggests that fibre characteristics is important for carcinogenesis of SiC fibres. However, this study has some limitations in number of animals, observation period and exposure level. Also, tumour formation was observed with fibres with average dimensions of 3.1 * 0.31 μm (Pott, 1991). Non-fibrous forms of SiC did not induce any adverse effects in a number of short term animal studies or carcinogenicity in long term animal studies after intraperitoneal administration (Pott F. *et al.*, 1994; Roller M. *et al.*, 1996).

Classification with Carc. 1B –H350i for SiC whiskers and some type of fibres is therefore warranted. However, this requires a definition of the fibres. Although most studies seem to be performed with whiskers (monocrystalline), it is considered reasonable to extrapolate this to fibres in general because the dimensions of whiskers and fibres can be comparable and the dissolution and

surface active properties will not be much different because both materials have the same chemical composition. Whiskers and some fibre types are mostly single beta crystals whereas particulates are mostly alpha polycrystalline. No difference in dissolution is expected between mono and poly crystals of the same crystal form (alpha or beta). However, poly crystals may split easier into smaller mono crystals. Both the alpha and the beta form are insoluble in water (Table 9) and at low pH seen its chemical resistance to acids (Davis, 1996) (SILICONCARBIDE (α-SIC) DATASHEET http://www.esd-sic.nl/esd_nl/201_slijpmiddelen/esd_sic-datasheet_en.pdf).

The available data on SiC fibres and more general on durable fibres show that the carcinogenicity increases with increasing length and decreases with increasing diameter. The same results are also shown by the in vivo repeated dose studies and the in vitro studies. However, the available data does not allow making a precise definition of carcinogenic and non-carcinogenic SiC fibre sizes especially because all tests were performed with fibres with a large variability. Recent reviews suggested maximal diameter and length for mesotheliomas is < 0.1 µm and > 5 µm and for lung carcinoma 0.1 – 3 µm and > 15 µm (Lippmann, 2014, Harrison, 2015). Overall this could be translated into a maximal diameter of 3 µm and a minimal length of 5 µm. Seen the resemblance of the effects of SiC fibres with other fibres, the use of the same fibre definition as for other fibres is justified:

- The only fibres with a harmonised classification for carcinogenicity are the mineral wools and the refractory ceramic fibres. For these substances the fibres are defined with Note R which reads: "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." This definition only takes into account the fibre diameter but not the fibre length which was also considered important. In addition, RAC recently advised on the classification for carcinogenicity of E-glass microfibres of representative composition (RAC, 2014a) and Glass microfibres of representative composition (RAC, 2014b). RAC advised note A but not note R and Q.

- Another option would be to use the WHO fibre definition (diameter < 3 µm, length ≥ 5 µm and aspect ratio ≥ 3:1). This option takes into account both diameter and length.

The use of Note R for SiC fibres is considered incorrect as this note was developed for man-made mineral fibres which by definition have a high aspect ratio whereas SiC fibres differ in aspect ratio between intentionally produced whiskers with a high aspect ratio and non-intentionally produced SiC fibres with varying aspect ratio. Also the data show that the carcinogenicity depends on the fibre length. Therefore, it is considered relevant to include the length and the aspect ratio into the definition of the fibre. As there is already an accepted fibre definition, the WHO definition which is further supported by the outcome of recent reviews on the dependence of carcinogenicity on fibre diameter and length, it is proposed to apply this to the classification of SiC fibres. This definition is also in line with the available data showing that fibres with a length below 2.8 µm showing no carcinogenic potential (Akiyama et al, 2007) and fibres above 5 µm do (Davis et al, 1996). However, as these fibres contained also fibres which we much larger than 5 µm this does not exclude the possibility that the larger fibre contribute mainly to the carcinogenicity.

The use of note Q is not proposed as the available data shows high biopersistence of SiC fibres and excessive carcinogenicity in line with the RAC advice on E-glass microfibers and glass microfibers.

The use of note A is not proposed as the proposed international chemical identifier is not for a group entry but for a specific substance with defined physical properties.

Only local tumours after inhalation, i.p. and intrapleural installation were observed indicating that the relevant route for carcinogenicity in the hazard statement could be limited to the inhalation

route. There are no studies available by dermal and oral route. However, seen the proposed mechanism of SiC fibres and fibres in general for carcinogenicity after inhalation, no carcinogenicity via other relevant routes is expected. Therefore, classification with the hazard statement H350i is proposed. This is also in line with the RAC advice on E-glass and glass microfibers (RAC, 2014a and RAC, 2014b).

The CLP criteria for classification in Carc. 2 are as follows:

"Suspected human carcinogens

The placing of a substance in Category 2 is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations. Such evidence may be derived either from limited evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies."

Classification as Carc. 2 is not appropriate as the available epidemiological data and animal studies showed sufficient evidence that exposure to SiC fibres can increase the incidence of tumours in animals.

4.10.6 Conclusions on classification and labelling

Classification as Carc. 1B – H350i: May cause cancer via inhalation is proposed for SiC fibre fulfilling the WHO definition.

4.11 Toxicity for reproduction

Not evaluated in this report

4.12 Other effects

Not evaluated in this report

5 ENVIRONMENTAL HAZARD ASSESSMENT

Not evaluated in this report

6 OTHER INFORMATION

Not evaluated in this report

7 REFERENCES

Adachi S., Kawamura K., Takemoto K. (2001) A trial on the quantitative risk assessment of man-made mineral fibres by the rat intraperitoneal administration assay using the JFM standard fibrous samples. Ind Health; 39(2): 168-174

Akiyama I., Ogami A., Oyabu T., Yamato H., Morimoto Y., Tanaka I. (2003) Clearance of deposited silicon carbide whisker from rat lungs inhaled during a 4-week exposure. J Occup Health. Jan;45(1):31-5

Akiyama I.; Ogami A.; Oyabu T.; Yamato H.; Morimoto Y.; Tanaka I. (2007) Pulmonary effects and biopersistence of deposited silicon carbide whisker after 1-year inhalation in rats. Inhal Toxicol; 19(2):141-7

Bégin R., Dufresne A., Cantin A., Massé S., Sébastien P., Perrault G. (1989) Carborundum Pneumoconiosis - Fibres in the Mineral Activate Macrophages to Produce Fibroblast Growth Factors and Sustain the Chronic Inflammatory Disease. Chest; 95: 842-49

Bernstein, M.D. (2007) Synthetic Vitreous Fibers: A Review Toxicology, Epidemiology and Regulations. Critical Reviews in Toxicology, 37:839–886.

Brown D.M., Fisher C., Donaldson K. (1998) Free radical activity of synthetic vitreous fibres: iron chelation inhibits hydroxyl radical generation by refractory ceramic fibre. J Toxicol Environ Health A; 53(7): 545-561

Bruch J., Rehn B. (1996) Relevant differences in pathogenicity of nuisance dusts; model investigations on samples of silicon carbide dusts. Exp Toxic Pathol; 48: 477-480; Gustav Fischer Verlag Jena

Bruch J., Rehn B., Song H., Gono E., Malkusch W. (1993a). Toxicological investigations on silicon carbide - 1. Inhalation studies. British Journal of Industrial Medicine; 50: 797-806

Bruch J., Rehn B., Song W., Gono E., Malkusch W. (1993b). Toxicological investigations on silicon carbide. 2. In vitro cell tests and long term injection tests. British Journal of Industrial Medicine; 50: 807-813

Bruch J, Rehn B, Duval-Arnould G, Efskind J, Röderer G, Sébastian P. (2014) Toxicological investigations on the respirable fraction of silicon carbide grain products by the in vitro vector model. Inhal Toxicol. 2014 Apr; 26(5): 278-88.

Bugge M.D., Kjuus H., Martinsen J.I., Kjaerheim K. (2010) Cancer incidence among short- and long-term workers in the Norwegian silicon carbide industry. Scand J Work Environ Health; 36(1): 71-79

Bugge M.D., Føreland S., Kjaerheim K., Eduard W., Martinsen J.I., Kjuus H. (2011) Mortality from non-malignant respiratory diseases among workers in the Norwegian silicon carbide industry: associations with dust exposure. Occup Environ Med; 68(12): 863-869

Bugge M.D., Kjaerheim K., Føreland S., Eduard W., Kjuus H. (2012) Lung cancer incidence among Norwegian silicon carbide industry workers: associations with particulate exposure factors. Occup Environ Med; 69(8): 527-533

Davis J.M.G., Brown D.M., Cullen R.T., Donaldson K., Jones A.D., Miller B.G., Mclntosh C., Searl A. (1996) A comparison of methods of determining and predicting the pathogenicity of mineral fibres. Inhalation Toxicol; 8(8): 747-770

Donaldson, K., Murphy, FA, Duffin, R, Poland, CA. (2010) Asbestos, carbon nanotubes and the pleural mesothelium: a review of the hypothesis regarding the role of long fibre retention in the parietal pleura, inflammation and mesothelioma. Particle and Fibre Toxicology 7:5.

Dufresne A., Sébastien P., Perrault G., Massé S., Bégin R. (1992) Pulmonary clearance of fibrous and angular SiC particulates in the sheep model of pneumoconiosis. Ann Occup Hyg; 36(5):519-30

Dufresne A., Loosereewanich P., Harrigan M., Sebastien P., Perrault G., Begin R. (1993) Pulmonary dust retention in a silicon carbide worker. Am Ind Hyg Assoc J; 54(6): 327-330

Edling C., Järvholm B., Andersson L., Axelson O. (1987) Mortality and cancer incidence among workers in an abrasive manufacturing industry. British Journal of Industrial Medicine; 44: 57-59

Føreland S., Bugge M.D., Bakke B., Bye E., Eduard W. (2012) A novel strategy for retrospective exposure assessment in the Norwegian silicon carbide industry. J Occup Environ Hyg; 9(4): 230-241

Yann Grosse, Dana Loomis, Kathryn Z Guyton, Béatrice Lauby-Secretan, Fatiha El Ghissassi, Véronique Bouvard, Lamia Benbrahim-Tallaa, Neela Guha, Chiara Scoccianti, Heidi Mattock, Kurt Straif (2014) Carcinogenicity of fluoro-edenite, silicon carbide fibres and whiskers, and carbon nanotubes. The Lancet oncology Vol. 15: 1427-1428.

Paul Harrison, Philip Holmes, Ruth Bevan, Klaus Kamps, Leonard Levy, Helmut Greim (2015) Regulatory risk assessment approaches for synthetic mineral fibres. Regulatory Toxicology and Pharmacology 73 425-441.

Health Council of the Netherlands (2012) Evaluation of the carcinogenicity and genotoxicity of silicon carbide.

IARC Scientific Publications No. 140 (1999) Mechanisms of fibre Carcinogenesis.

IARC – International Agency for Research on Cancer, 'Man-made Vitreous Fibres' IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 81,2002, pp. 1-418.

Infante-Rivard C., Dufresne A., Armstrong B., Bouchard P., Theriault G. (1994) Cohort study of silicon carbide production workers. Am J Epidemiol; 140(11): 1009-1015

Jakobsson K., Mikoczy Z., Skerfving S. (1997) Deaths and tumours among workers grinding stainless steel: a follow up. Occup Environ Med; 54(11): 825-829

Järvholm B., Thiringer G., Axelson O. (1982) Cancer morbidity among polishers. Br J Ind Med; 39(2): 196-197

Johnson N.F., Hoover M.D., Thomassen D.G. et al. (1992) In vitro activity of silicon carbide whiskers in comparison to other industrial fibres using four cell culture systems. Am J Ind Med; 21: 807-23

Johnson N.F., Hahn F.F. (1996) Induction of mesothelioma after intrapleural inoculation of F344 rats with silicon carbide whiskers or continuous ceramic filaments. Occup Environ Med; 53(12): 813-816

Lapin C.A., Craig D.K., Valerio M.G., McCandless J.B., Bogoroch R. (1991) A subchronic inhalation toxicity study in rats exposed to silicon carbide whiskers. Fundam Appl Toxicol; 16(1): 128-146.

Lippmann M. (2014) Toxicological and epidemiological studies on effects of airborne fibers: Coherence and public health implications. Crit Rev Toxicol, 44(8): 643–695.

Krewski D.; Yokel R.A., Nieboer E., Borchelt D., Cohen J., Harry J., Kacew S., Lindsay J., Mahfouz A.M., Rondeau V. (2007) Human health risk assessment for aluminium, aluminium oxide, and aluminium hydroxide. Journal of Toxicology and Environmental Health. Part B, 10:1-269

Massé S., Bégin R., Cantin A. (1988) Pathology of silicon carbide pneumoconiosis. Mod Pathol. Mar;1(2):104-8

Miller B.G., Jones A.D., Searl A., Buchanan D., Cullen R.T., Soutar C.A., Davis J.M.G., Donaldson K. (1999a) Influence of characteristics of inhaled fibres on development of tumours in the rat lung. Ann Occup Hyg; 43(3): 167-179

Miller B.G., Searl A., Davis J.M., Donaldson K., Cullen R.T., Bolton R.E. et al. (1999b) Influence of fibre length, dissolution and biopersistence on the production of mesothelioma in the rat peritoneal cavity. Ann Occup Hyg;43(3): 155-166.

Nagaia, H., Okazaki, Y., Chew, S.H., Misawa, N., Yamashita, Y., Akatsuka, S., Ishihara, T., Yamashita, Yoshikawa, K., Yasui, H., Jiang, L., Ohara, H., Takahashi, T., Ichihara, G., Kostarelos, K., Miyata, Y, Shinohara, H., and Toyokuni, S. (2011) Diameter and rigidity of multiwalled carbon nanotubes are critical factors in mesothelial injury and carcinogenesis. PNAS 108: 49: E1330-E1338.

Pott F., Roller M., Rippe R.M., Germann P-G, Bellmann B. (1991) Tumours by the intraperitoneal and intrapleural routes and their significance for the classification of mineral fibres. In: Brown RC, Hoskins JA, Johnson NF, editors. Mechanisms in fibre carcinogenesis. NATO ASI Series (Series A Life Sciences Vol 223): New York: Plenum Press; 547-565

Pott F., Roller M., Kamino K., Bellmann B. (1994) Significance of durability of mineral fibres for their toxicity and carcinogenic potency in the abdominal cavity of rats in comparison with the low sensitivity of inhalation studies. Environ Health Perspect; 102 (5): 145-150

RAC (2014a) Opinion proposing harmonised classification and labelling at EU level of E-glass microfibres of representative composition. CLH-O-0000001412-86-34/F. ECHA, Helsinki (http://echa.europa.eu/documents/10162/22633645-4e3f-4cfe-92ce-cc2fa9bf141d)

RAC (2014b) Opinion proposing harmonised classification and labelling at EU level of glass microfibres of representative composition. CLH-O-0000001412-86-35/F. ECHA, Helsinki (http://echa.europa.eu/documents/10162/9e2e8779-4f7e-44d4-90af-11a6b072685f)

Rehn B., Seiler F., Rehn S., Bruch J., Maier M. (2003) Investigations on the inflammatory and genotoxic lung effects of two types of titanium dioxide: untreated and surface treated. Toxicology and Applied Pharmacology; 189: 84-95

Rödelsperger K, Brückel B. (2006) The carcinogenicity of WHO fibres of silicon carbide: SiC whiskers compared to cleavage fragments of granular SiC. Inhal Toxicol; 18(9): 623-631

Roller M., Pott F., Kamino K., Althoff G.H., Bellmann B. (1996). Results of current intraperitoneal carcinogenicity studies with mineral and vitreous fibres. Exp Toxic Pathol; 48: 3-12

Romundstad P., Andersen A., Haldorsen T. (2001) Cancer incidence among workers in the Norwegian silicon carbide industry. Am J Epidemiol; 153(10): 978-986

Romundstad P., Andersen A., Haldorsen T. (2002) Non-malignant mortality among Norwegian silicon carbide smelter workers. Occup Environ Med; 59(5): 345-347

Skogstad A., Føreland S., Bye E. and Eduard W. (2006) Airborne fibres in the Norwegian silicon carbide industry. Ann. Occup. Hyg. 50; 3: 231 – 240

Stanton M.F., Layard M. (1978) The carcinogenicity of fibrous material. In proceedings of the workshop on asbestos: Definitions and measurement methods; held at the National Bureau of Standards, Gaithersburg, MD, 1977. Washington, DC: National Bureau of Standards, pp. 143-51 (NBS special publication no. 506)

Stanton M.F., Layard M., Tegeris A., Miller E., May M., Morgan E. et al. (1981) Relation of particle dimension to carcinogenicity in amphibole asbestoses and other fibrous minerals. J Natl Cancer Inst; 67(5): 965-975

Svensson I., Artursson E., Leanderson P., Berglind R., Lindgren F. (1997) Toxicity in vitro of some silicon carbides and silicon nitrides: whiskers and powders. Am J Ind Med; 31(3): 335-343

Vanderlaan M., Steele V., Nettesheim P. (1983) Increased DNA content as an early marker of transformation in carcinogen-exposed rat tracheal cell cultures. Carcinogenesis; 4(6): 721 -727

Vasil'eva L.A., Pylev L.N., Kiianenko V.V., Nikolaĭshvili A.A., (1989) Carcinogenic properties of silicon carbide whiskers. Eksp Onkol; 11(2):13-15

Vaughan G.L., Jordan J., Karr S.. (1991) The toxicity, in vitro, of silicon carbide whiskers. Environ Res; 56(1): 57-67

Vaughan G.L., Trently S.A., Wilson R.B. (1993) Pulmonary response, in vivo, to silicon carbide whiskers. Environ Res; 63(2): 191-201

WHO (1985) Reference methods for measuring airborne man-made mineral Fiber (MMMF) WHO/EURO MMMF Reference Scheme. Prepared by the WHO/EURO Technical Committee for Monitoring and Evaluating Airborne MMMF, World Health Organisation, Copenhagen

8 ANNEXES