

**Proposal for a scientific re-evaluation of
Zirconia Aluminosilicate Refractory Ceramic Fibres and
Aluminosilicate Refractory Ceramic Fibres**

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1 Introduction

Zirconia Aluminosilicate Refractory Ceramic Fibres and Aluminosilicate Refractory Ceramic Fibres also described as aluminosilicate wools (according EN 1094-1: 2008) were recently included in the REACH candidate list with far reaching consequences for the respective industry. Since the classification of the above mentioned fibers as carcinogen category 2 according to directive 67/548/EEC (Category 1B according to the criteria of the CLP regulation, EC (No) 1272/2008) in 1997 a considerable number of new data and reviews has been published. Open questions with regard to the definition of substance identity and with regard to classification, including the evaluation of the newly generated data, prompted the respective industry to search for further steps to be taken for a scientific and regulatory re-evaluation and cooperation with regulators.

The Austrian Association for Building Materials & Ceramic Industries contracted the Umweltbundesamt GmbH for a proposal for a scientific re-evaluation of Zirconia Aluminosilicate Refractory Ceramic Fibres and Aluminosilicate Refractory Ceramic Fibres.

Given the large amount of primary literature and reviews thereof we recommend not to provide another standard scientific review. A multi-stakeholder process is needed to identify the core questions and find agreement on them. At this stage we provide a comprehensive summary of the state of discussion with regard to the animal test data based on systematically identified most recent reviews as well as an analysis of the most recent epidemiological data. Furthermore we recommend a transparent structure which goes beyond standard requirements which should build the basis for an efficient multi-stakeholder discussion.

2 Substance identity (chemical and physical form) – which fibers are in focus and why?

Refractory Ceramic Fibers (RCFs) are a type of man-made vitreous fibers (MMVF) produced from a mixture of alumina, silica and other oxides or kaolin. The raw materials are melted and subsequently made into fibers through a blowing of spinning process that produces fibers with a length weighted mean diameter of 2-3 μ m. The bulk wools and manufactured products can generate and liberate small fibers of varying diameter and length due to breakage of fibers induced by mechanical stress (e.g. during manufacture and handling of bulk wool or products). These fibers can be respirable depending inter alia on their dimensions. Amorphous aluminosilicate RCFs can withstand temperatures up to 1400°C and are primarily used as high temperature insulating material.

The evaluation shall be focused on amorphous aluminosilicate wools (ASW), which are also referred to as amorphous aluminosilicate RCFs and cover amorphous aluminosilicate and zirconia aluminosilicate wools as defined in EN 1094-1: 2008. ASWs belong to the group of high temperature insulation wools (HTIW), which reveal a classification temperature greater than 1000°C (EN 1094-1: 2008). This differentiates technically HTIW from other mineral wools, which are used in different applications. HTIW are man-made mineral wools suitable for use as heat-insulating materials above a temperature of 600°C. Amorphous alkaline earth silicate wools (AES) as well as polycrystalline wools (PCW) also belong to the group of HTIW, but they are not subject of actual considerations.

Chemical composition

Amorphous aluminosilicate wools are subdivided into aluminosilicate wools containing Al₂O₃ and SiO₂ and zirconia aluminosilicate wools containing Al₂O₃, SiO₂ and ZrO₂ as main constituents (see table. 1). Small amounts of other oxides are sometimes incorporated to change the fiber properties or can be present due to “unavoidable” impurities (e.g. potassium-, sodium-, magnesium-, calcium-, titanium-, zirconium-, iron-, chromium oxides) (see tables 1, 2: EN 1094-1: 2008). Both wools are amorphous, fibrous, inorganic materials formed by high temperature fusion of the raw materials into a mass which is fiberised and cooled to a rigid condition without crystallization. A complete chemical identification of both materials is not possible as they are UVCB substances (substances of Unknown or Variable composition, Complex reaction products or Biological materials). According to the REACH guidance for identification and naming of substances these UVCB substances are specified with the IUPAC-name of their constituents.

Table 1 - Typical composition range of Aluminosilicate Wool (EN 1094-1: 2008)

Component	Percentage by mass
Al ₂ O ₃	46 to 56
SiO ₂	44 to 54
Other oxides	< 1

Table 2 - Typical composition range of Zirconia Aluminosilicate Wool (EN 1094-1: 2008)

Component	Percentage by mass
Al ₂ O ₃	< 37
SiO ₂	> 48
ZrO ₂	< 20
Other oxides	< 1

The different concentrations for constituents of fibers as defined in EN 1094-1: 2008 have to be regarded as “typical” values. This means that minor variations are possible, without leading to a different substance identity (~1%). This definition also describes those aluminosilicate wools which are on the market and it shall therefore be the basis of evaluation.

In contrast, the substance identity defined by CAS number 142844-00-6 may be too broad with regard to chemical composition of the fibers. Chemical composition may have an impact on solubility and biopersistence of the fibers, and therefore on their potential carcinogenic effects (see explanation next section). Hence the stricter definitions of EN 1094-1: 2008 may be more appropriate for toxicity evaluation.

Also the definitions characterizing the entry into Annex VI of the CLP-regulation ((EU) No 1272/2008) under index number 650-017-00-8 may be too broad for the evaluation of potential carcinogenic effects of the fibers. It covers fibers with an alkaline oxide and alkali earth oxide (Na₂O+K₂O+CaO+MgO+BaO) content of up to 18%. These oxides may have a strong impact on fiber solubility.

Fiber dimensions

Aluminosilicate Wools¹ are applied as bulk wool, blankets, felts, mats, boards, preformed shapes, papers, etc. As described above, they can release smaller fibers, which fulfill the World Health Organization (WHO) criteria for respirable fibers.

¹ Wool: non-directional agglomeration of fibers with varying diameter and length distributions

The WHO has defined a fiber as having an aspect length to diameter ratio greater than or equal to 3:1 and a length greater or equal to 5 µm (for the purpose of optical counting). Fibers of a diameter less than 3 µm are considered to be respirable for human, whereas those of diameters greater than or equal to 3 µm are regarded as non-respirable (WHO, 1985)². The mean length and diameter of airborne aluminosilicate fibers in occupational samples was 20.6 and 1.05µm, respectively (Mast et al., 2000, Maxim et al., 2000).

The WHO definition only considers fibers equal to or longer than 5µm, a cut off which was introduced in order to facilitate microscopical counting. It was assumed that fibers with a length < 5µm would not induce adverse effects. However, a recent ATSDR expert panel (<http://www.atsdr.cdc.gov/hac/asbestospanel/index.html>) has discussed potential toxicity of fibers < 5µm and proposed further investigation of these small fibers.

Fiber dimensions used in animal studies

According to the standard methods published by JRC 1999³ fibers with the following dimensions shall be tested to evaluate the potential carcinogenic effects.

In rat inhalation studies (for toxicity and biopersistence): A mean aspect ratio of at least 3:1. At least 100 fibers/cm³ longer than 20 µm in length with a geometric mean diameter (GMD) as close as possible to 0.8 µm (for fibers with a density $\rho \sim 2.4$. For fibres with densities different from this the corresponding GMD should be determined).

In rat intraperitoneal injection studies for toxicity and intratracheal injection for biopersistence: A mean aspect ratio of at least 3:1. 95% of the diameters shall be less than 3µm. At least 20% of the WHO (L > 5µm, D < 3µm) fibers in suspension should have a length > 20µm and for this length fraction a GMD as close as possible to 0.8µm (if technically feasible).

This means that in rat studies the focus is on fibers longer than 20 µm with a GMD of 0.8 µm. This is considered to represent the rat respirable fraction of fibers (GMD ~ 0.8 µm) that is most critical in terms of biopersistence (L > 20µm).

For fibers with densities of about 2.7 g/cm³ and an aspect ratio from 3 to 20 a GMD of about 0.8µm corresponds to an aerodynamic diameter between 1 and 2µm. The aerodynamic diameter is considered the best variable for estimating inhalability and respiratory deposition. For all species alveolar peak deposition occurs with particles with an aerodynamic diameter between 1 and 2 µm. Increasing the aspect ratio of the fibre results in a decrease of the corresponding aerodynamic diameter and decreasing alveolar peak deposition. For rats and hamsters, alveolar deposition is essentially zero when the aero-dynamic diameter of the fibers exceeds 3.5µm and the aspect ratio is > 10. In contrast, considerable alveolar deposition occurs in humans breathing at rest even when the aerodynamic diameter of the fibers approaches 5 µm (WHO-IARC 2002, p 342f, citing Dai and Yu 1998 and indicating uncertainties with these theoretical results which are not addressed)

Testing this subfraction of the fiber distribution (GMD ~ 0.8µm, L > 20µm) seems to represent a worst case exposure for the rat, which may be regarded adequate in terms of hazard assessment for potential carcinogenic effects (model uncertainties are summarized in chapter 4, special attention for this consideration needs the fact that alveolar deposition seems the most critical fraction in rats but in humans the tracheobronchiolar deposition might also be a very or even the most critical fraction).

² WHO (World Health Organization). (1985). Reference methods for measuring airborne man-made mineral fibers (MMMMF). Prepared by WHO/EURO Technical Committee for evaluating MMMF, WHO Regional Office, Copenhagen, Denmark.

³ Methods for the Determination of the Hazardous Properties for Human Health of Man Made Mineral Fibres (MMMMF). 1999. Bernstein D.M., Riego Sintes J.M. JRC. EUR 18748 EN

In terms of risk assessment fully valid exposure measurements of the fiber dust concentration and dimension distribution would be necessary in order to estimate the exposure fractions that are inhalable (upper to lower regions) or just respirable (to alveolar region) and fulfill the criteria responsible for carcinogenic effects (see chapter 3). This should be considered when estimating potential human carcinogenic effects on the basis of animal and in vitro data. When monitoring human exposure it would be useful to sample using a size selective sampling technique. The estimation of rat NOAECs and the derivation of safe human exposure levels (DNEL/DMEL) and respective risk assessment needs further modeling with additional respective uncertainties.

However, with regard to classification of amorphous alumino silicate RCFs hazard assessment for potential carcinogenic effects should be the main focus.

3 Starting point for a scientific re-evaluation

3.1 Mechanisms of fiber carcinogenicity

The prevailing paradigm for fiber toxicity is that it is chiefly a function of dose, dimension, and durability (ATSDR, 2004, IARC, 1999, NIOSH, 2006, Warheit et al., 2001, Utell & Maxim, 2010). While fiber dimensions affect deposition and clearance and influence the interaction with target cells, biodurability and biopersistence determine the duration of exposure dose. For a review of underlying molecular and inflammatory mechanisms of fiber induced carcinogenesis see NTP, 2009.

3.2 Animal data

Starting point for the discussion is the IARC 2002 review. Most recent reviews are available from Bernstein 2007, NTP 2009 and Utell and Maxim 2010 that summarize and discuss a large number of primary and secondary literature which shall not be reproduced here. These reviews were identified by a systematic literature search as indicated in chapter 4.3.1 and in addition in Pubmed (Search terms: Refractory ceramic fiber OR Man made vitreous fiber OR MMVF OR "Fiber and inhalation" [title/abstract] AND 2007 to present [publication date]). In addition the Annex XV Dossier for Zirconia Aluminosilicate Refractory Ceramic Fibres and on Aluminosilicate Refractory Ceramic Fibres recently submitted by the German CA (September 2009) provide a comprehensive summary.

In short these latest reviews mirror the current state of discussion: amorphous alumino silicate RCF were classified by IARC 2002 in class 2B (IARC, inadequate epidemiologic evidence and sufficient animal evidence) based on positive long term inhalation studies with rats and positive intraperitoneal studies with rats and hamsters. Also EU classified amorphous alumino silicate RCF, in GHS class 1B, that may be considered analogous to IARC class 2B, depending on the arguments, if there is sufficient confirmation that the mode of action in the animal is relevant for humans. The inhalation studies were criticized for having been carried out under conditions of lung overload, this issue was recognized also within the IARC evaluation. However with the data available so far, it is not possible to

determine the extent to which the experimental outcome was due to the fibers, the particles or to some combination of both. However, the intraperitoneal studies were positive, in terms of carcinogenic potency – in the range with or below some other MMVF – or depending on the evaluation also comparable with crocidolite. Short term inhalation biopersistence studies show a similar picture with the exception that for asbestos comparably much longer weighted half-times for fibers longer than 20 µm are reported.

Some of the –compared to amorphous alumino silicate RCF - more potent or biopersistent MMVF show a higher alkaline oxide and alkali earth oxide content compared to amorphous alumino silicate RCF, however their aluminum oxide content is lower compared to amorphous alumino silicate RCF. Therefore the latter comparative data of MMVFs with regard to i.p. carcinogenic potency and inhalation biopersistence appear to be in disagreement with the European classification scheme: MMVF are classified per default as GHS/CLP class 1B, if the alkaline oxide and alkali earth oxide ($\text{Na}_2\text{O}+\text{K}_2\text{O}+\text{CaO}+\text{MgO}+\text{BaO}$) content is less or equal to 18 % by weight and as GHS/CLP class 2, if above 18%. This classification rule is based on the assumption that a higher alkaline oxide and alkali earth oxide may reduce biodurability and consequently evidence for carcinogenicity.

3.3 Epidemiological data

Amorphous alumina silica RCFs have been in use for more than 50 years (www.dkfg.de). A series of epidemiologic studies was conducted to evaluate the effects from occupational exposure to amorphous alumina silica RCFs. The most relevant investigations are two cohort studies initiated at the University of Cincinnati in the United States and at the Institute of Occupational Medicine (IOM) in Europe, which are still ongoing.

These studies investigated respiratory symptoms (dyspnea, wheezing, asthma, chronic cough, chronic phlegm, pleuritic pain), pulmonary function parameters (forced expiratory volume in one second, FEV_1 and forced vital capacity, FVC), pulmonary changes based on chest X-ray examinations (pleural plaques, pleural changes) and mortality.

LeMasters et al. (2003) describe the US cohort study of the University of Cincinnati which covers current and former male workers between 1952 and 2000 in order to investigate possible excess in mortality. No significant excess mortality related to all deaths, all cancers, malignancies or diseases of the respiratory system, including mesothelioma was observed. However, there was a statistically significant association with cancers of the urinary organs. (These findings might be of interest as an association between exposure to crystalline silica and renal disease has been reported (Calvert et al., 1997, JAMA, 277:1219-1223).)

Considering cumulative exposure did not change the outcome of the evaluation.

The quality of the data for job history, exposure, and smoking history was very high. Limitations of the study are the relative youth of the cohort and its small size. The mortality analysis did have a 95% power to detect a 2-fold increase in all deaths and all cancers and a 40% power to detect 2-fold increase in lung cancer. As noted above this mortality study is still ongoing.

Based on data from the same cohort Walker et al. (2002) found that the mortality data permitted a statistical rejection of the hypothesis that amorphous alumina silica RCF are as potent as either crocidolite or amosite. However, the possibility that RCF are as potent as chrysotile asbestos could not be excluded.

Approximately 10% of 61 subjects having amorphous alumina silica RCF exposure greater than 135 fiber-months/cc had pleural plaques (OR = 6.0) and 8% had interstitial changes of profusion $\geq 1/0$ (OR = 4.7).

These findings of pleural changes are consistent with the findings of the IOM study in Europe. The IOM study found statistically significant increase in pleural changes in amorphous alumina silica RCF workers not occupationally exposed to asbestos with (OR = 2.22) or without (OR = 3.88) adjustment for age (Cowie et al., 2001). The occurrence of pleural plaques raises concern that amorphous alumina silica RCF has the ability to reach the pleural surface and cause localised pleural reactions.

In conclusion these two studies are of relatively high quality but still do not allow a final conclusion on pulmonary effects of amorphous alumina silica RCFs which is mainly caused by too small group numbers and too short latency periods.

For asbestos 25 fiber-years were concluded to double the risk to require lung cancer (BK-Report 1/2007). In the cohorts under investigation exposures did not reach this level. LeMasters et al. (2003) conclude that the preliminary findings warrant the continuation of this mortality study for future analysis.

3.4 *In vitro data*

No evaluation of in vitro toxicity studies was performed at the present stage.

4 Further steps to be considered for a scientific re-evaluation

4.1 Identification of the key questions

As a first step the need to clearly frame the most relevant questions was identified, in order to allow a discussion among all stakeholders involved. From the literature consulted we would expect the following questions to be of core interest for further discussion and agreement:

- What is the evidence for considering MMVFs with alkaline oxide and alkali earth oxide content $\leq 18\%$ or $> 18\%$ as different with regard to their carcinogenic potency? What is the influence of aluminum oxide?
- What is the evidence for the European exclusion criteria for not classifying MMVFs with alkaline oxide and alkali earth oxide content $> 18\%$?
- What is the evidence for a carcinogenic hazard of amorphous aluminosilicate RCF and classification into GHS/CLP category 1B?

4.2 Systematic and transparent literature search

As a second step it is proposed to transparently document a systematic literature search for the specific questions addressed, defining the criteria for searching, selecting and in/excluding references. Primary literature data should be separated from secondary literature and reviews.

The importance of a systematic and transparent literature search and evaluation is demonstrated by Ruden et al. (2001) who summarize the heterogeneity of review results from international and well recognized institutions with regard to the carcinogenicity of trichloroethylene. In summary 29 risk assessments were carried out between 1973 and 1997. The conclusions drawn were broadly distributed over 4 categories from clear negative to clear positive for carcinogenicity. The reference coverage (cited/available) varied from 5 to 81% (average 18%) for all references and from 29 to 100% (average 78%) for the 14 carcinogenicity experiments. There was no carcinogenicity experiment that was cited in all risk assessments. 58% of the total references were cited only in one risk assessment. 27% of the 19 most cited bioassays were interpreted for both as showing positive and as showing negative findings by different risk assessments. 26% of the 19 most cited bioassays were considered both as adequately and as inadequately designed (quality of the study).

Table 4.2. Ruden et al. 2001: The categorization of trichloroethylene risk assessment documents

The Categorization of the TCE Risk Assessment Documents According to the CRAI ^a			
---	+ --	+ - +	+++
<ul style="list-style-type: none"> • NIOSH (1973), agency, U.S.A. • Health and Safety Executive (1982), agency, UK. • Ministerie van Volkshuisvesting, Ruimtelijke Ordening en Milieubeheer (1984), agency, The Netherlands. • ACGIH (1989), mixed group, U.S.A. • ACGIH (1992), mixed group, U.S.A. • ACGIH (1996), mixed group, U.S.A. 	<ul style="list-style-type: none"> • IARC (1976), Int. org. • IARC (1979), Int. org. • National Board of Occupational Safety and Health (1981), agency, Sweden. • WHO (1985), Int. org. • IARC (1987), Int. org.^b • Commission of the European Communities (1990), Int. org., Europe.^c • Nordic Expert Group (1991), occ. agency, Nordic countries • ECETOC (1994), Industry, Europe. • GDCh-Advisory Comm. on Exist. Chem. of Env. Relev. (1994), mixed group, Germany. • HSIA (1996) (online 05-05-99), Industry. 	<ul style="list-style-type: none"> • NIOSH (1978), agency, U.S.A. • Nordic Expert Group (1979), occ. agency, Nordic countries • EPA (1985), agency, U.S.A. • National Inst. of Env. Medicine (1986), academics, Sweden. • EPA (1988), agency, U.S.A. • ATSDR, U.S. Public Health Service and the EPA (1989), agency, U.S.A. • Canadian EPA (1993), agency, Canada. • OECD/European Union (1996), Int. org. (UK).^d • U.S. Public Health Service ATSDR (1997), agency, U.S.A. 	<ul style="list-style-type: none"> • National Inst. of Env. Medicine (1990), academics, Sweden • IARC (1995), Int. org. • Deutsche Forschungsgemeinschaft, DFG (1996), Germany. • Mak (1996), occ. agency, Germany.

^a The CRAI has four different groups: ---, not carcinogenic in animals, negative epidemiology, no/negligible human cancer risk; + --, carcinogenic in animals, negative epidemiology, no/negligible human risk; + - +, carcinogenic in animals, negative epidemiology, a possible, nonnegligible, human cancer risk; +++ , carcinogenic in animals, positive epidemiology, a nonnegligible human cancer risk).

^b The IARC assessed the evidence in animals as "limited" in this risk assessment.

^c Consists of short consensus summaries on carcinogenic effects of several chemical substances.

^d The comprehensive risk assessment document prepared for the OECD and the European Union's existing chemicals programs.

4.3 Uncertainty analysis of testing methods

As a third step the uncertainties of the most important testing methods within the literature retrieved shall be identified and transparently reported such that focused and traceable discussion on the specific aspects of uncertainty is possible.

One possible option would be to apply the recommendations of the REACH guidance document on uncertainty analysis (ECHA guidance on Information Requirements and Chemical Safety Assessment, Chapter R19; http://guidance.echa.europa.eu/docs/guidance_document/information_requirements_en.htm?time=1231750812). The summary table proposed therein may serve for focusing discussion on aspects of disagreement with regard to methods uncertainty and their impact on the overall hazard and risk assessment.

4.3.1 Animal studies

In the following pages methods uncertainties of inhalation and intraperitoneal studies as well as uncertainties of general carcinogenicity methods are summarized using a table adapted from chapter R.19 (ECHA guidance documents). The table may be considered as a living document, it may be adapted throughout the discussion of the sponsor with various stakeholders of the RCF evaluation process. The discussion as well as the data basis for other or changed considerations and conclusions shall remain transparent and traceable. So far only qualitative considerations (+/-) of the uncertainties with regard to biopersistence and carcinogenicity potential are provided. It is recommended that the qualitative analysis shall only be extended to a (semi-)quantitative one for those aspects for which transparent data based evidence is available.

So far the focus of the document was to transparently summarize the scientific information available on distinct aspects of uncertainty. Integration of this scientific information to an overall conclusion on the reliability of the various methods will necessarily contain complex value judgements. Therefore the latter was not considered as a primary aim at this stage of the discussion.

For the compilation of this table the internet-sites from the following international organizations were visited: EU-REACH, OECD, WHO/IPC, IARC, NTP, ECETOC, ECVAM/ESAC and documents were searched for the following terms: fibre, fiber, inhalation, respiratory, RCF, ceramic, uncertain, interspecies, assessment factor. The following documents relevant for this chapter were retrieved:

ECETOC 2003. Derivation of assessment factors for human health risk assessment. ECETOC technical report 86 (includes information on interspecies extrapolation for inhalation)

ECETOC 1996. Toxicology of Man-Made organic Fibres. ECETOC Technical Report No. 69 (includes information on methods discussion)

NTP 2009. Report on carcinogens. Background Document for Glass wool Fibers (includes information on methods discussion)

WHO- IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS 2002. VOLUME 81 MAN-MADE VITREOUS FIBRES (includes information on methods discussion)

WHO- IPCS. Environmental Health Criteria 77: Man-made Mineral Fibers. Geneva:World Health Organization, 1988 (includes information on methods discussion)

In addition the publication from Pott et Roller 1998 was taken into consideration for this summary with regard to biopersistence tests, since from the literature available after a first (not systematic) literature search it discussed these aspects most comprehensively.

This summary should be accomplished by a systematic search for primary literature (eventually using the same search terms) in Toxnet and Pubmed.

SUMMARY OF UNCERTAINTY FROM INHALATION AND INTRATRACHEAL INSTILLATION STUDY RESULTS

	Sources of Uncertainty	Variability or Uncertainty	Influence on estimate for biopersistence in humans ⁴	Influence on estimate for carcinogenicity in humans ⁵	
Hazard assessment	Inhalation Model	<p>Inter-species difference in fundamental anatomy:</p> <p>Rat = nose breathing Δ</p> <p>Human = mouth breathing</p> <p>(ECETOC 1996 citing Warheit 1989: anatomical differences result in enhanced particle penetration to the human lungs)</p>	VAR	-	-
	Instillation model	<p>The intratracheal bolus exposure is kinetically different from an inhalation exposure, since according to NTP 2009 citing Oberdörster 1996 it bypasses the defense mechanisms of extrathoracic region: <i>Pott et Roller 1998: With intratracheal injection longer and thicker (1-3 μm) fibers may enter the respiratory tract. Thicker fibers may show prolonged biopersistence due to cation exchange in periphery and reduced breaking. 1-3 μm fibers may enter human bronchiols and alveoli.</i></p> <p>lacks even distribution of the dose within the lung,</p> <p>usually is carried out in weekly intervals</p> <p><i>Pott et Roller 1998 citing Bernstein 1997 argues that confidence for halftimes limits were smaller with intratracheal tests compared to inhalation tests which may support that uneven distribution and agglomeration does not affect reproducibility of the method.</i></p> <p><i>Furthermore they argue that halftimes for WHO fibers of low biopersistence versus fibers with high biopersistence range from 17 to 104 days with the inhalation test but from 16 to 296 days with the intracheal instillation test. This should support higher sensitivity of the intratracheal instillation test.</i></p>	VAR and UNC	<p>+</p> <p>+/-</p> <p>+/-</p> <p>+</p>	<p>+</p> <p>+/-</p> <p>+/-</p> <p>+</p>

⁴ + ...Aspect of uncertainty is likely to overestimate potential for biopersistence or carcinogenicity; - Aspect of uncertainty is likely to underestimate potential for biopersistence or carcinogenicity; +/- Aspect of uncertainty may over- or underestimate potential for biopersistence or carcinogenicity

Both models	<p>Inter-species difference in airway diameters, branching pattern, symmetry, respiratory rate and air flow leading to species-difference in fibre deposition</p> <p>ECETOC 2003: Alveolar ventilation related to body mass to the power of 0.75 – means higher for laboratory animals compared to humans.</p> <p>WHO-IARC 2002 citing Dai and Yu 1998: Increasing the work load in humans reduces the deposition fraction in the alveolar region because more fibres are deposited in the extra-thoracic and trachea-bronchial regions.</p>	VAR and UNC	+	+
	<p>ECETOC 1996 citing Brody and Roe 1983: in humans compared to rat more bifurcations in bronchial and bronchiolar regions, in addition the bifurcations in humans are symmetric, in rodents asymmetric. As a consequence fibers tend to deposit on alveolar duct bifurcations in rodents whereas in humans they concentrate on the final respiratory duct bifurcation (<i>comment: this would indicate for humans a higher fraction of fibers clearable by mucociliary elevator, reducing biopersistence</i>)</p> <p>NTP 2009 citing Oberdörster 1996: Most of the lung tumors develop in the conducting airways of humans but develop only in the peripheral regions in rats; therefore alveolar respirable fibers appear to be more important in the rat.</p> <p><i>Pott et Roller 1998 mention the considerations of the last two paragraphs and that the biopersistence depends on the specific locus in the respiratory tract: mucos of bronchi, epithelia of bronchi and bronchiols in which fibers may penetrate/adhere, surfactant of alveoli, inside of alveolar macrophages or tissue between alveoli. This indicates in qualitative terms the uncertainty of rat biopersistence tests: With intraperitoneal tests the relevance of its focus (macrophage mediated clearance, clearance via lymph) is uncertain. With inhalation tests the biopersistence results as an integral of late clearance (measurements not immediately after application to exclude fast mucociliary export) from all sites of the respiratory tract, however local deposition sites and local clearance rats differ between human and rat.</i></p>		+	+
	<p>NTP 2009 citing Maxim and McConnell 2001: Modeling studies that normalize for lung weight show that the relative deposition of synthetic vitrous fibres (number of fibers per unit time) in humans is smaller than that for rats.</p> <p>WHO-IPCS 2002, p244 citing Dai and Yu 1998: for a given concentration in air a larger fraction of inhaled long fibres deposited in the <u>alveolar</u> region in humans than in that of</p>		+	+
			+/-	+/-
			-	-

		rats (factor ~10, model uncertainties not described). ECETOC 2003 citing Martonen et al. 1992: <u>Alveolar</u> deposition fractions for most mass median aerodynamic diameters of aerosols (not fibre specific!) lower in rodents than in humans, 10% in rats and mice, 30% in humans.			
		<p>Inter-species difference in clearance</p> <p>WHO-IPCS 2002 and NTP 2009: Biodurability (dissolution, leaching, breaking, splitting intra- and extra-cellular) is expected to be similar in rats and humans, but physiological clearance mechanisms (translocation mediated by macrophages, cilia, penetration to pleura) may be substantially different leading to species specific (total) biopersistence.</p> <p>WHO-IPCS citing Snipes 1989 and Bailey et al 1982: in rats undisturbed overall retention half time of particles or fibers subject to macrophage mediated clearance 60-80 days; for humans the average overall retention half-time in the alveolar region is several 100 days.</p> <p>NTP 2009 citing Maxim and McConnell 2001: Fiber clearance (based on models and data using refractory ceramic fiber) is faster in rats than humans.</p> <p>ECETOC 1996 cites Kreyling 1990: Transport from deeper lung regions towards the tracheobronchial mucociliary escalator is a slow macrophage-mediated process that may be less effective by an order of magnitude in humans and large animal species than in rodents.</p> <p>ECETOC 1996 cites Warheit et al 1994: Interspecies differences in vivo with regard to alveolar macrophage activity and phagocytosis and cellular inflammatory responses.</p> <p>NTP 2009 p174, citing Kane 1996: Although the rat model is the most common, there is some evidence that the hamster might be more appropriate for detecting mesothelioma.</p> <p>WHO-IARC 2002 citing various authors: Diameters of alveolar macrophages differ between rats and humans: rat 10.5-13 µm, humans 14-21 µm, allowing humans to clear longer fibers more easily</p>	VAR and UNC	-	-
				+/-	+/-
				+	+

	<p>Inter-species difference for influence of fibre solubility on biopersistence</p> <p>WHO-IPCS 2002 citing Berry 1999: Because the retention half-time due to mechanical clearance is much longer in humans than in rats, higher fibre solubility reduces persistence more in the human lung than in the rat lung.</p>	VAR and UNC	+/-	+/-
	<p>Inter-species difference in cellular/inflammatory response</p> <p>NTP 2009 citing: Maxim and McConnell 2001 concluded from their data review that human and rodent cells appear to have comparable sensitivity with regard to fibre-induced cytotoxicity, production of inflammatory components (cytokines), transformation and proliferation. Furthermore they conclude</p> <p>Lung fiber burdens associated with fibrosis are similar in rats and humans, although exact comparisons are limited by the paucity of information on the asbestos fibers length, diameter and distribution in the lung.</p>	VAR and UNC	+/- +/-	+/- +/-
	<p>Strain variation</p> <p>ECETOC 1996 cites Gavett et al 1992, Warheit and Hartsky 1994: Strain variation of pulmonary responses have been observed with Sprague-Dawley (CD) and Fischer 344 rats to crystalline silica.</p>	VAR and UNC	+/-	+/-
	<p>Inter-species difference in overall effect</p> <p>NTP 2009 citing Wardenbach et al. 2005: Rats required more than 100 times higher fibre concentration to match the lung cancer risk of asbestos workers and 1000 times higher to match the mesothelioma risk. This supports that the rat inhalation model has a low sensitivity.</p> <p>The earlier Maxim and McConnell 2001 publication is in disagreement with this assumption considering exposure duration differences (and not accepting comparison on basis for lifespan) and other data reported above.</p>	VAR and UNC	- +	- +

<p>Summary: Model uncertainty seems to stem largely from interspecies differences of the deposition patterns and local clearance rates in the respiratory tract.</p> <p>Deposition in the rat may result predominantly in the in the alveolar region and with inhalation experiments also in the nasopharyngeal region whereas in humans the tracheobronchiolar region may be more important.</p> <p>Clearance from especially the alveolar region seems faster in rats compared to humans. However the tracheobronchiolar region may be more important for human tumor development.</p> <p>With the intratracheal instillation test more and thicker fibers may be administered which may increase the sensitivity of the method. However reduced application intervals and uneven fiber distribution in the lung are additional uncertainties to those mentioned for all respiratory tests.</p> <p>In summary no direction and/or quantification of the model uncertainty can be given so far.</p>				
<p>Input Parameters</p>	<p>Was fiber composition generated for the animal test respirable to upper and deeper respiratory compartments of the animal? (representing worst case exposure)</p> <p>NTP 2009 citing Wardenbach et al. 2005: Insulation glass fiber (preparations) were less respirable compared to special purpose glass fibres, only the latter induced tumors. There is uncertainty with regard to respirability.</p> <p>Different fiber preparations may affect test sample composition and effects.</p>	<p>UNC</p>	<p>+/-</p>	<p>+/-</p>
	<p>Was fiber exposure below animal lung overload?</p> <p>WHO-IPCS 2002 citing ILSI 2000 and others: Lung overload would lead to lung burden increasing with experimental time and would lead to adverse effects including carcinogenicity also with chemically inert fibers.</p> <p>Overload occurs when 1-3 mg of particles per gram of rat lung have been deposited (Morrow 1988). Others studies suggest that surface area is a better parameter for estimating lung overload. Uncertainty results from differentiating particle and fibre unspecific overload effects (retarded clearance, alveolar inflammation, fibrosis, lung tumors due to exaggerated lung burden) from particle/fibre specific cytotoxic effects that would also retard clearance, but at lower dose levels compared to non-cytotoxic particles/fibres.</p> <p>(WHO-IPCS 2002 notes that from the Bellmann et al 2001 publication -suggesting overload effect for the RCF1 study- particle/fibre specific cytotoxicity or fibre/particle</p>	<p>UNC</p>	<p>+/-</p>	<p>+/-</p>

		synergistic effects are apparent) NTP 2009 citing: Muhle et al. 1990 introduced the concept of the maximally functionally tolerated dose (MFTD) for particulates. The MFTD was defined as the lung burden associated with a two to four fold decrease in particle clearance. Other indicators that could be useful include: increased lung weight, increased inflammatory parameters, increased target cell proliferation, altered histopathology other than carcinogenicity, impaired lung clearance function, non-linear fibre retention kinetics (Greim 2004, Oberdörster 1996).		+/-	+/-
		Exposure time extrapolation WHO-IPCS 2002: Retention time for a fiber derived from a short-term (e.g. 5 day) inhalation assay may be lower than the value determined from a subchronic or chronic inhalation study. In order to exclude the influence of mucociliary clearance, measurements of retention are generally started some time after exposure. NTP 2009: Wardenbach et al. 2005 supports that consideration of life span is adequate for inter-species sensitivity comparison. In contrast Maxim and McConnell 2001 supports considering exposure duration differences. NTP 2009 citing Maxim and McConnell 2001: Rate of dissolution of fibers is similar in rats and humans, and since humans live longer the rat model might not take into account the effects of clearance.	UNC	- -	- +
	Overall effect on hazard estimate				
Exposure assessment		Does distribution of fiber dimensions and fiber concentrations occurring at work place justify the assumption of human exposure of upper and deeper respiratory compartments, quantitatively relevant for carcinogenic effects?	UNC		
Risk Characterization	Overall effect on risk estimate				

SUMMARY OF UNCERTAINTY FROM INTRAPERITONEAL STUDY RESULTS

	Sources of Uncertainty	Variability or Uncertainty	Influence on estimate for biopersistence in humans ⁵	Influence on estimate for carcinogenicity in humans ⁶
Hazard assessment	<p>Model Different kinetics (compared to respiratory tract exposure)</p> <p>NTP 2009 citing Oberdörster 1996 and Kane 1996:</p> <p>Defense mechanisms are different in lung and peritoneum: <i>Pott et Roller 1998: Also longer and thicker fibers reach tissue critical for tumor development in rats (no deposition in upper/medium respiratory tract). In peritoneum no bronchial-like clearance by cilia.</i></p> <p><i>No qualitative difference of macrophages of peritoneum vs. alveolar region known, but quantitative differences expected. However macrophage clearance might not be the most important for humans considering the tracheobronchial region as dominant site of deposition and tumor development.</i></p> <p>Intracavity injection circumvents the fiber selection process that occurs during translocation of fibers from the alveolar region of the lung to the pleura. <i>Pott et Roller 1998: Clearance from peritoneum for very thin fibers via lymph.</i></p> <p>Kinetics for buildup of body burden is different between i.p. injection (<i>high local doses, fast delivery</i>) and inhalation exposure (<i>more evenly distributed and slower delivery</i>)</p>	VAR and UNC	<p>+</p> <p>+/-</p> <p>+/-</p> <p>+</p>	<p>+</p> <p>+/-</p> <p>+/-</p> <p>+</p>

⁵ + ...Aspect of uncertainty is likely to overestimate potential for biopersistence or carcinogenicity; - Aspect of uncertainty is likely to underestimate potential for biopersistence or carcinogenicity; +/- Aspect of uncertainty may over- or underestimate potential for biopersistence or carcinogenicity

	<p>Different dynamics (compared to respiratory tract exposure)</p> <p><i>Local inflammatory dynamics including self-amplifying mechanisms in lung may be different from those in the peritoneum.</i></p> <p><i>Pott et Roller 1998 citing Muhle et al. 1998 and Collier 1997: Good correlation between halftimes of fibers after intraperitoneal injection and intratracheal instillation support suitability of method.</i></p> <p><i>Pott et Roller 1998 citing Pott 1976: Good correlation between biopersistence and carcinogenicity in intraperitoneal studies reported – supporting suitability of method.</i></p> <p>NTP 2009 citing Ellouk and Jaurand 1994: The relationship of fibre durability to the incidence of peritoneal tumors needs to be addressed.</p> <p>NTP citing Wardenbach et al. 2005: i.p. route shows increased sensitivity. There is no evidence that i.p. injection studies would be biased towards producing false positive results, since no mesotheliomas were induced in rats given high mass of granular silicon carbide dust by i.p. injection.</p>	VAR and UNC		+/-
<p>Summary: Model uncertainty stems from different kinetics and dynamics compared to inhalation exposure. However absence of kinetic confounders in rat studies (deposition pattern, clearance rates) and independence from local inhalatory MFTD (overload effect) is used as an argument for a more reliable comparison of carcinogenic potency of fibers.</p>				
Input Parameters	<p>Fiber composition generated for the animal test should be respirable to deeper respiratory compartments of the animal.</p> <p>Different fiber preparations may affect test sample composition and effects</p>	UNC	+/-	+/-
	<p>Was fiber exposure below local MTD?</p> <p>At very high doses carcinogenicity may be induced by mechanisms not relevant at realistic low dose exposure.</p> <p>NTP citing Oberdörster 1996: Peritoneal and pleural cavities might be overwhelmed by intracavity injections of large doses. Bolus delivery may result in high local doses above MTD</p>	UNC	+/-	+/-
Overall effect on hazard estimate				

Exposure assessment		Does distribution of fiber dimensions and fiber concentrations occurring at work place justify the assumption of human exposure of upper and deeper respiratory compartments, quantitatively relevant for carcinogenic effects?	UNC		
Risk Characterisation	Overall effect on risk estimate				

SUMMARY OF UNCERTAINTY **OTHER THAN RESPIRATORY TRACT SPECIFIC ONES**

	Sources of Uncertainty		Variability or Uncertainty	Influence on estimate for carcinogenicity ⁶
Hazard assessment	Model	Interspecies differences: background tumor incidences damage and repair mechanisms (~ toxico-dynamics) human life time longer compared to test-animal	VAR and UNC	+/- +/- -
		Other model uncertainties: Sensitivity of the model (related i.a. to number of animals/group and background incidence) reproducibility of study results use of young animals from inbred strains for total human population estimate Trends in strain specific background tumor incidences	VAR and UNC	- +/- - +/-
		Ignorance (= unknown differences)	VAR and UNC	+/-
		Input Parameters	High dose to low dose extrapolation	VAR and UNC
		Potential mixture effects (e.g. smoking)	VAR and UNC	-
		Ignorance (= unknown differences)	VAR and UNC	+/-
		Overall effect on hazard estimate		
	Exposure assessment			
Risk Characterization	Overall effect on risk estimate			

⁶ + ...Aspect of uncertainty is likely to overestimate potential for carcinogenicity; - Aspect of uncertainty is likely to underestimate potential for carcinogenicity; +/- Aspect of uncertainty may over- or underestimate potential for carcinogenicity

4.3.2 In vitro studies

For the uncertainty analysis of vitro studies the same approach as shown above for the in vivo studies may be followed. So far a simple text summary is provided below.

In vitro studies can be used to determine fiber solubility. In this context it is important to discriminate the following terms.

Biodurability: According to NTP (2009) biodurability describes the rate of removal of a fiber from the lungs by dissolution or disintegration, the latter due to partial dissolution. It is assumed that biodurability is similar in rats and humans since the ionic milieu in the lung is also relatively similar.

Biopersistence: The ability of a material to persist in the lung in spite of the lung's physiological clearance mechanisms and environmental conditions (definition according to Bernstein and Sintès, 1999). It includes the removal of fibers from the lung by physical clearance of entire fibers, e.g., by ciliary or macrophage-mediated clearance and can be therefore also be described as biodurability plus physiological clearance (Hesterberg and Hart, 2001).

As a screening tool and for mechanistic analysis in vitro tests can provide information about the potential of a fiber to dissolve in environments reflecting different lung environments. Particularly, for fibers with a length > 20 µm, dissolution is an important parameter for clearance as clearance of such fibers by macrophages is reported to be insignificant. The solubility and in vitro dissolution rates are strongly influenced by chemical composition as certain components dissolve more rapidly than others (Eastes et al, 2000a).

Cell free systems use balanced salt solutions and are conducted at a near neutral pH to simulate extracellular fluid or at pH 4.5 to simulate pH of phagolysosomes of macrophages. pH has a major role in these systems (NTB, 2009, IARC, 2002).

For limitations of such tests the IARC Working Group noted that dissolution of solids may be strongly influenced by the presence of surfactant and molecules that selectively bind to surface ions. Particularly, silica and aluminium compounds vary widely with regard to leaching. Moreover, great variability for dissolution rate constants is observed in different studies. The authors assumed that experimental methods or the sensitivity of the analytical method - comparing weights of residual fiber samples to weights of initial fiber samples - may account for these discrepancies (IARC, 2002). On the other hand correlations between in vivo lung clearance and pathogenicity (Hesterberg et al, 1998) as well as in vivo retention time of long fibers are reported (Searl et al. 1999, Maxim et al, 1999a, 1999b) Eastes et al 2000a,b,c used regression techniques and measured in vitro and in vivo data to develop equations for the estimation of the dissolution rate of a wide variety of MMVFs. The overall correlation between dissolution rate constants measured in vivo and in vitro for the same fibers was 0.727. The IARC Working Group noted that the kinetic and thermodynamic approach used by Eastes et al. may not fully apply to the complex multiphase system of a multicomponent fiber in a biological fluid, where leaching and redeposition also occur (IARC, 2002).

Generally standardization needs in this field are recognized. Guldberg et al., 1998, propose to use standardized in vitro methods to avoid in vivo testing in case fibers have chemical composition close to and dissolution rates equivalent to fibers which have already been tested in vivo.

Also several cell culture systems are proposed to determine fiber durability: Nguea et al, 2008, use human monocytic cell line (U-037). While phagocytosis was observed in this study, dissolution was not observed for the fibers used.

Luoto et al, 1994a,b, 1995,1996,1998) studied fibers in a flow-through cell- culture system using macrophages. Long, partly phagocytosed or non-phagocytosed fibers seemed to disappear more rapidly than short phagocytosed fibers. This situation might arise as long fibers brake into short fibers (IARC, 2002).

There are some publications dealing with in vitro protocols evaluating fiber toxicity, however, they were not evaluated for the present report.

4.4 Reliability and Relevance Assessment of individual publications

4.4.1 ToxRtool: in vitro and in vivo studies

As a fourth step the reliability and relevance of primary literature data may be documented using the ToxRtool (Schneider et al. Tox. Lett 189, 138, 2009). This tool allows transparent documentation of aspects of reliability relating to documentation of study conduct/reporting and aspects of relevance relating to the specific question addressed. This may be helpful to identify aspects of disagreement and allow focusing respective discussion between the stakeholders of the MMVF evaluation process.

Examples for the use of ToxRtool are attached with regard to a comparative study of MMVFs on biopersistence and comparative studies on MMVFs on carcinogenicity, all referenced in Brown 2005 (Hesterberg et al. 1998, Miller et al. 1999, Adachi et al. 2001).

For the evaluation of fiber toxicity the ToxRtool needs a specific adaptation with regard to a clear substance definition⁷. These fiber specific identity considerations might need agreement between stakeholders before the tool can be adapted. The most urgent criteria have to be defined: How detailed should the definition of substance identity be? What are appropriate methods to define fiber identity? Should identity be described on a molar basis of the constituents, rather than on weight? As mentioned in chapter 4.1. a multi-stakeholder process may be necessary already for the first step of a re-evaluation – that is “framing the questions”.

⁷ Within the ToxRtool a table with detailed criteria for reliability evaluation is provided. The tool contains comments fields which explain the criteria to be evaluated. These comments fields could easily be amended with specific information on fiber identity criteria.

It has to be highlighted that application of the ToxRtool will not immediately lead to a more harmonized view, but it may help to make different reliability and relevance judgements transparent which may assist for focusing discussion and proceeding more efficiently to a harmonized view. Divergent opinions seem to be a natural phenomenon in science. Figure 1 and 2 show the result of reliability assessments carried out by 12 and 17 experienced, professional assessors for 11 studies.). These results may highlight the need for tools for an efficient dialog.

Fig.1 Results of an inter-rater experiment: total scores (TS) for in vivo studies (each point represents a result obtained by a rater for a specific case study); Schneider et al. (2009).

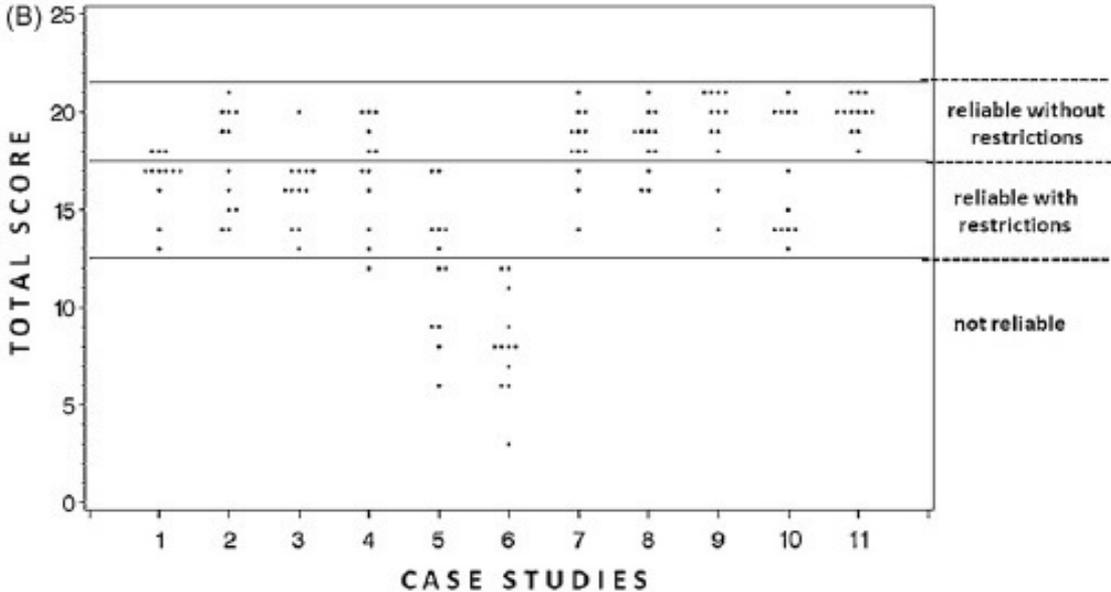
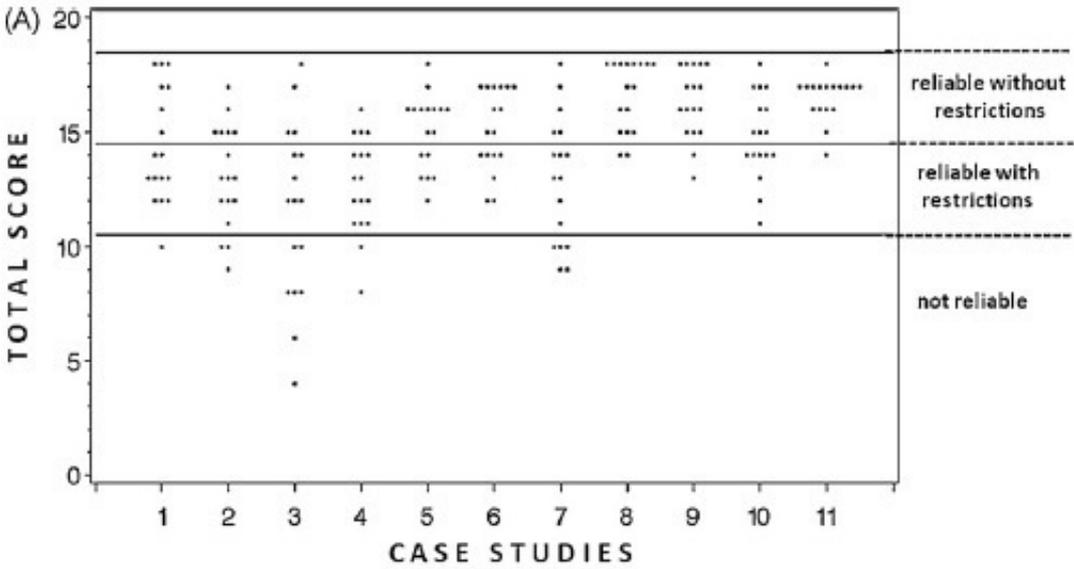


Fig. 2. Results of an inter-rater experiment: total scores (TS) for in vitro studies (each point represents a result obtained by a rater for a specific case study); Schneider et al. (2009).



4.4.2 REACH guidance: human data

With regard to the evaluation of reliability and relevance of human data the European Chemicals Agency (ECHA) has developed three relevant guidance documents for REACH:

i) Chapter 7 &

ii) Chapter 8 from the Guidance on Information Requirements and Chemical Safety assessment Chapters 7 & 8 (http://guidance.echa.europa.eu/docs/guidance_document/information_requirements_en.htm?time=1231750812)

iii) Guidance on the Application of the CLP Criteria (http://guidance.echa.europa.eu/docs/guidance_document/clp_en.htm?time=1276092641). The following is extracted from all three guidance documents.

In general human data are valuable as a source of hazard information because they apply directly to the human species, and the mode of action (MoA) is usually relevant. Furthermore, human data have in most cases been obtained from relevant exposure conditions and are based on an adequate route of exposure. In the case of (aerosol) inhalation data it is especially relevant if human data are available, because the differences of the respiratory tract among different species can have a strong impact on the results of such studies. Differences in clearance and respiratory fraction of the agent (aerosol) reaching the site of action is of high relevance in these studies.

In addition, human data most often come from studies covering a more heterogeneous sample of the population than animal studies carried out on inbred strains. Nevertheless, the quality of the human data needs to be ensured.

Quality considerations in particular differ from those for experimental studies. There are specific uncertainties that deserve attention when using human data. These include the influence of bias, confounding from mixed exposures and other risk factors and accuracy of the exposure information. Given the long latency between exposure to a carcinogen and the onset of clinical disease, robust estimates of carcinogenic potency can be difficult to generate. Recent strategies in improving the methodology of epidemiological assessments include the assessment of biological effects which precede the malignant stages of disease. Such strategies require detailed knowledge on the agent specific underlying MoA.

Since there may be limitations in reliability of human studies (e.g. problems in study design, analysis and reporting as well as limited coverage of the different target organs, too small sample size), they are normally considered together with animal and other data.

The above listed factors can limit the sensitivity of a given study – unequivocal demonstration that a substance is not a human carcinogen is difficult and requires detailed and exact measurements of exposure, appropriate cohort size, adequate intensity and duration of exposure, sufficient follow-up time and sound procedures for detection and diagnosis of cancer of potential concern. A proposal for a reliability assessment of epidemiological data is provided in Annex 3.

4.4.3 Evaluation of reviews:

Reviews comparatively discussing primary literature may be an important source of information but should be evaluated separately and clearly indicated as such, since this may help to increase the transparency of the evaluation. Since the Klimisch Scores are not attributable to reviews a module recommended which might be useful for this task. One example for the use of this module is attached in annex 3 (Wardenbach et al. 2000, referenced in Brown 2003)

4.5 *Synthesis and presentation*

For completeness it shall be mentioned that the last step of the analysis proposed is the synthesis of the results: A transparent well structured scientific text seems appropriate.

5 Conclusion

Given the large amount of primary literature and reviews thereof it is recommended not to provide another standard scientific review. Instead a multi-stakeholder process is needed to identify the core questions and find agreement on them.

Based on the most recent reviews, systematically selected animal studies as well as epidemiological data a comprehensive summary of the state of the discussion is provided. It can be concluded that the results of the ongoing epidemiological studies in worker cohorts in Europe and the United States will contribute important information which shall be considered for future evaluations. With regard to animal and in vitro test data the need for a transparent description of the evidence for the European classification criteria for MMVFs based on alkaline oxide and alkaline earth oxide content was identified. In addition, the evidence for the European exclusion criteria for not classifying MMVFs with alkaline oxide and alkali earth oxide content > 18% as well as the evidence for carcinogenic properties of amorphous alumino silicate RCF and their classification shall be transparently described.

Based on the identified key questions a targeted systematic and transparent literature search documenting the criteria for searching and in/excluding references and clearly separating primary literature data from reviews should be conducted. In a third step a formal analysis of method uncertainty is recommended, a respective working document is provided (chapter 4.3.1). This may also be very helpful before any further testing proposals are discussed. New mechanistic in vitro methods lacking some of the kinetic uncertainties from in vivo studies shall be taken into due consideration. A transparent evaluation of reliability and relevance of the selected publications is recommended.

For this purpose different tools are suggested and exemplarily demonstrated, which may be helpful in this process: Method uncertainty analysis based on chapter R.19 of the REACH guidance documents (chapter 4.3.1.), ToxRtool for reliability and relevance analysis of individual study results (chapter 4.4.1) and two grids for reliability and relevance analysis of reviews and epidemiological data (Annex 2). Valuable methodological input may be gained from the initiative for Evidence Based Toxicology (www.ebtox.org).

A multi-stakeholder process, including representatives from science, policy and industry, is recommended for such a data based, -more than standard- transparent and efficient re-evaluation.

6 Declaration of interest

This project was financed by the Austrian Association for Building Materials & Ceramic Industries The authors confirm that a fully independent scientific assessment and proposal was elaborated.

Annex 1: Criteria for carcinogen classification according to GHS/CLP and IARC

Annex 2: ToxRtool – example evaluations

Annex 3: Proposal for evaluation grids for review and epidemiological studies

Annex 4: Reference list

Annex 5: List of abbreviations

Annex 1

Classification according to the CLP-regulation:

How are carcinogens defined according to CLP?

Annex I, p103: Carcinogen means a substance or a mixture of substances which induce cancer or increase its incidence. Substances which have induced benign and malignant tumours in well performed experimental studies on animals are considered also to be presumed or suspected human carcinogens unless there is strong evidence that the mechanism of tumour formation is not relevant for humans.

For the purpose of classification for carcinogenicity, substances are allocated to one of two categories based on strength of evidence and additional considerations (weight of evidence). In certain instances, route-specific classification may be warranted, if it can be conclusively proved that no other route of exposure exhibits the hazard.

What are the criteria for classifying substances as carcinogenic according to the EU-GHS Regulation?

CATEGORY 1: Known or presumed human carcinogens - 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 (sufficient) **human evidence**, or

Category 1B: presumed to have carcinogenic potential for humans, classification is largely based on (sufficient) **animal evidence**.

In addition, on a case-by-case basis, scientific judgement may warrant a decision of presumed human carcinogenicity derived from studies showing limited evidence ... in humans together with limited evidence ... in experimental animals.

CATEGORY 2: Suspected human carcinogens - 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 ... in human studies or from limited evidence ... in animal studies.

The classification is based on strength of evidence (sufficient/limited) together with additional considerations.

Strength of evidence in humans:

sufficient evidence: ... a positive relationship has been observed between the exposure and cancer in studies in which chance, bias and confounding could be ruled out with reasonable confidence

limited evidence: a positive association has been observed between exposure to the agent and cancer for which a causal interpretation is considered to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence

Strength of evidence in experimental animals:

sufficient evidence : ... an **increased incidence** of malignant neoplasms or of an appropriate combination of benign and malignant **neoplasms** in

- **two or more species** of animals or
- **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**

- a single study in one species and sex 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: because, e.g.

- the evidence of carcinogenicity is restricted to a **single experiment**;
- **unresolved questions** regarding the adequacy of the design, study conduct or interpretation
- Increased incidence only of benign neoplasms or lesions of **uncertain neoplastic potential**
- the evidence of carcinogenicity is restricted to studies that demonstrate **only promoting activity in a narrow range of tissues or organs**

Additional considerations influencing the overall level of concern:

- tumour type and background incidence, multi-site responses, progression of lesions to malignancy; reduced tumour latency; whether responses are in single or both sexes; whether responses are in a single species or several species; structural similarity to a substance(s) for which there is good evidence of carcinogenicity; **routes of exposure; comparison of absorption, distribution, metabolism and excretion between test animals and humans; the possibility of a confounding effect of excessive toxicity at test doses; mode of action and its relevance for humans**, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity.

For details see CLP-regulation, **(EC) No 1272/2008**, p353ff,
http://ec.europa.eu/enterprise/reach/ghs/index_en.htm

Classification according to IARC: Compounds or physical factors assessed by IARC (International Agency for Research on Cancer) are classified in four groups based on the existing scientific evidence for carcinogenicity.

Group 1: "Carcinogenic to humans" There is enough evidence to conclude that it can cause cancer in humans.

Group 2A: "Probably carcinogenic to humans" There is strong evidence that it can cause cancer in humans, but at present it is not conclusive.

Group 2B: "Possibly carcinogenic to humans" There is some evidence that it can cause cancer in humans but at present it is far from conclusive.

Group 3: "Unclassifiable as to carcinogenicity in humans" There is no evidence at present that it causes cancer in humans.

Group 4: "Probably not carcinogenic to humans" There is strong evidence that it does not cause cancer in humans.

Standard IARC classification categorization descriptions

Group 1: "The agent (mixture) is *carcinogenic to humans*. The exposure circumstance entails exposures that are carcinogenic to humans."

"This category is used when there is sufficient evidence of carcinogenicity in humans. Exceptionally, an agent (mixture) may be placed in this category when evidence of carcinogenicity in humans is less than sufficient but there is sufficient evidence of carcinogenicity in experimental animals and strong evidence in exposed humans that the agent (mixture) acts through a relevant mechanism of carcinogenicity."

Examples include asbestos, benzene and ionizing radiation.

Group 2 (A and B): "This category includes agents, mixtures and exposure circumstances for which, at one extreme, the degree of evidence of carcinogenicity in humans is almost sufficient, as well as those for which, at the other extreme, there are no human data but for which there is evidence of carcinogenicity in experimental animals. Agents, mixtures and exposure circumstances are assigned to either group 2A (probably carcinogenic to humans) or group 2B (possibly carcinogenic to humans) on the basis of epidemiological and experimental evidence of carcinogenicity and other relevant data."

Group 2A: "The agent (mixture) is *probably carcinogenic to humans*. The exposure circumstance entails exposures that are probably carcinogenic to humans."

"This category is used when there is limited evidence of carcinogenicity in humans and sufficient evidence of carcinogenicity in experimental animals. In some cases, an agent (mixture) may be classified in this category when there is inadequate evidence of carcinogenicity in humans and sufficient evidence of carcinogenicity in experimental animals and strong evidence that the carcinogenesis is mediated by a mechanism that also operates in humans. Exceptionally, an agent, mixture or exposure circumstance may be classified in this category solely on the basis of limited evidence of carcinogenicity in humans."

Examples include diesel engine exhaust, Formaldehyde and PCBs.

Group 2B: "The agent (mixture) is *possibly carcinogenic to humans*."

"The exposure circumstance entails exposures that are possibly carcinogenic to humans.

This category is used for agents, mixtures and exposure circumstances for which there is limited evidence of carcinogenicity in humans and less than sufficient evidence of carcinogenicity in experimental animals. It may also be used when there is inadequate evidence of carcinogenicity in humans but there is sufficient evidence of carcinogenicity in experimental animals. In some instances, an agent, mixture or exposure circumstance for which there is inadequate evidence of carcinogenicity in humans but limited evidence of

carcinogenicity in experimental animals together with supporting evidence from other relevant data may be placed in this group."

Examples include glass wool, styrene and gasoline exhaust.

Group 3: "The agent (mixture) is ***unclassifiable as to carcinogenicity in humans.*** "

"This category is used most commonly for agents, mixtures and exposure circumstances for which the evidence of carcinogenicity is inadequate in humans and inadequate or limited in experimental animals. Exceptionally, agents (mixtures) for which the evidence of carcinogenicity is inadequate in humans but sufficient in experimental animals may be placed in this category when there is strong evidence that the mechanism of carcinogenicity in experimental animals does not operate in humans.

Agents, mixtures and exposure circumstances that do not fall into any other group are also placed in this category."

Examples include anthracene, caffeine and fluorescent lighting.

Group 4: "The agent (mixture) is ***probably not carcinogenic to humans.***"

"This category is used for agents or mixtures for which there is evidence suggesting lack of carcinogenicity in humans and in experimental animals. In some instances, agents or mixtures for which there is inadequate evidence of carcinogenicity in humans but evidence suggesting lack of carcinogenicity in experimental animals, consistently and strongly supported by a broad range of other relevant data, may be classified in this group."

The only agent in that group is: Caprolactam

Source: <http://www.greenfacts.org/glossary/ghi/iarc-classification.htm>

Annex 2

ToxR-Tool – see next pages



EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection
In Vitro Methods Unit
European Centre for the Validation of Alternative Methods (ECVAM)

ToxRTool - Toxicological data Reliability Assessment Tool

Instructions for use

Structure of the ToxRTool

The ToxRTool has been developed in the Microsoft Excel[®] programme and consists of two different parts, one for *in vivo* and one for *in vitro* data.

The tool comprises a list of 21 criteria for *in vivo* studies and 18 criteria for *in vitro* studies. Each criterion can be assigned either a '1' (one point), i.e. 'criterion met', or a '0' (no point), i.e. 'criterion not met'.

Criteria for evaluating *in vivo* or *in vitro* studies were established congruently to the largest extent possible and are grouped into five groups of criteria in both cases:

- I: Test substance identification
- II: Test system characterisation
- III: Study design description
- IV: Study results documentation
- V: Plausibility of study design and results.

Groups I to IV contain criteria mainly related to documentation, whereas group V goes beyond documentation and asks for an assessment of the internal plausibility of the approach used in the study.

All criteria must be answered (either by 0 or 1) by the user when evaluating a study. Some minimum information requirements are thought to be indispensable for a study to be considered reliable. In the tool, these minimum requirement criteria are highlighted in red. Only if these criteria are met, i.e. rated as '1', the tool will assign a data reliability of Category 1 or 2, irrespective of the total score obtained. Due to their high impact on the final outcome, careful evaluation of the "red" criteria is of crucial importance.

Explanations are available for most of the criteria. These explanations are implemented in Excel as comments to criteria fields. Please read carefully these explanations. This will ensure a common understanding of the criteria.

The total points assigned to a given study lead to a proposal of a reliability category (1 to 3, see result **A** in table below). This result A may be modified to category 3, if A is category 1 or 2, but not all red criteria were met (result **B**). The evaluator is free to deviate from the tool's categorisation (**C**), but is asked to give his or her reasons in such a case (**D**).

A. Numerical result leads to initial Category:	Categories 1, 2 or 3, calculated by tool
B. Checking red scores leads to revised Category:	Categories 1, 2 or 3, calculated by tool
C. Evaluator's proposal of Category:	Categories 1, 2 or 3, by the user
D. Justification in case the category proposed by the evaluator deviates from B:	Explanatory text by the user (in case C deviates from B)

How to use the ToxRTool - step by step

1. Open the Microsoft Excel[®] file.
2. Carefully read the worksheet "Explanations" in the Microsoft Excel[®] file, which contains explanations on the tool (similar to the information given above).
3. There is one worksheet for *in vitro* studies and one for *in vivo* studies. **Please choose the worksheets suitable for the study you plan to evaluate!**
4. After reading the case study, please start by answering the criteria.
5. Each case study worksheet contains all criteria. Address the criteria one by one and rate each criterion: Click on the small green field to the right of the criterion. A drop-down list allows selecting either '0', i.e. criterion not met, or '1', i.e. criterion met, for each criterion. **Please make sure that all criteria are answered!** Explanations for individual criteria show up as a comment for all fields with a red mark in the upper right corner, when you move the mouse into the criteria fields.
6. Points are automatically summed up both for each of the five criteria groups and the overall total (light blue fields). At the bottom of the criteria list (orange fields) the reliability category resulting from the total points is automatically calculated.

7. Under (A) the category is derived from the sum of points (regardless of whether all red criteria have been met), whilst under (B) the category is derived considering the red criteria as essential for the assignment of category 1 and 2.
8. Please fill in the green fields, reading:
 - (C) Evaluator's proposal: Category:
(in case you do not see any specific reason to deviate from the proposed category, just enter category proposed by the tool in (B)
 - (D) Justification in case evaluator deviates under C from B:
(leave empty if you do not deviate)
9. During the course of the quality assessment observations may be made which are important for discussing the relevance of the data for specific purposes. If you would like to document any observation with respect to relevance of the data, please read the text below the list of criteria and use the following green fields.

We are very interested to receive your opinion on the ToxrTool tool. Please use the opportunity to give your observations, opinions, remarks on specific criteria or on general issues by completing the questionnaire available for download from the ECVAM website and send it back to ECVAM (by email to agnieszka.kinsner@jrc.ec.europa.eu). Your comments and suggestions are welcome.

Explanations to ToxRTool

Objective: ToxRTool is designed to assess the inherent quality, also called reliability, of toxicological data as reported in a publication or a test report.

This tool essentially comprises a list of evaluation criteria. Criteria are subdivided in five groups:

I: Test substance identification, II: Test system characterisation, III: Study design description,
IV: Study results documentation, V: Plausibility of study design and data

Per criterion either one ('1') or no ('0') point can be assigned. If a criterion is met, assign '1', if not assign '0'. Please choose from the respective drop-down list.

All criteria must be answered!

In total 21 points for in vivo studies, 18 points for in vitro studies can be assigned. A reliability categorisation based on the total number of points is given below.

Criteria written in red have special importance: points for each of the red criteria are necessary to achieve Reliability category 1 or 2. **Please evaluate with special care!**

Data entry is requested in (and is also restricted to) the fields shaded in green.

Reliability categorisation (definition of categories according to Klimisch et al. 1997)

(Proposed) consequence

	in vivo	in vitro		
1	18-21	15-18	reliable without restrictions	useful, check relevance for intended purpose
2	13-17	11-14	reliable with restrictions	potentially useful, check relevance for intended purpose
3	<13 or not all red criteria met	<11 or not all red criteria met	not reliable	generally not to be used as key study, but depending on the shortcomings of the study it may still be useful in weight-of-evidence approaches or as supportive information
4			not assignable: documentation insufficient (reviews, handbooks, other secondary sources)	generally not to be used as key study, but depending on the shortcomings of the study it may still be useful in weight-of-evidence approaches or as supportive information. (This category is not an outcome of this evaluation tool!)

In addition to the criteria for assessing data reliability, at the bottom of the worksheets there are some questions to be answered optionally ("Optional documentation of observations with importance to relevance"). These questions allow to document observations in a non-formalised way, which may be of importance for the further use of the information for regulatory or other purposes.

Reliability assessment of in vivo toxicity studies

Study under evaluation

Authors:

Adachi S, Kawamura K, Takemoto K

Titel:

A trial on the quantitative risk assessment of man-made mineral fibers by the rat intraperitoneal administration assay using the JFM standard fibrous samples.

Testing facility, year, sponsor, study no. or bibliographic reference:

Ind Health 39 (2001):168-74 (language in Japanese, only abstract available)

Explanations are available for most criteria and show up, when the cursor is moved over the criteria field. Please read carefully!

Red criteria: the maximum score is needed for these criteria to achieve reliability category 1 or 2 (see worksheet Explanations): Please evaluate with special care!

Criteria

Evaluator's explanations, comments on criteria, etc.

No. Criteria Group I: Test substance identification

Score

1 Was the test substance identified?

1

2 Is the purity of the substance given?

0

9 fiber samples, no chemical substance composition explicit (especially important with regard to alkaline oxide and alkali earth oxides), but likely to be retrieved from authors

3 Is information on the source/origin of the substance given?

1

4 Is all information on the nature and/or physico-chemical properties of the test item given, which you deem indispensable for judging the data (see explanation for examples)?

0

no information on fiber dimensions

2

Criteria Group II: Test organism characterisation

5 Is the species given?

1

rats

6 Is the sex of the test organism given?

1

- 7 Is information given on the strain of test animals plus, if considered necessary to judge the study, other specifications (see explanation for examples)?
- 8 Is age or body weight of the test organisms at the start of the study given?
- 9 For repeated dose toxicity studies only (give point for other study types): Is information given on the housing or feeding conditions?

1 F344
1 5 weeks
0

4

Criteria Group III: Study design description

- 10 Is the administration route given?
- 11 Are doses administered or concentrations in application media given?
- 12 Are frequency and duration of exposure as well as time-points of observations explained?
- 13 Were negative (where required) and positive controls (where required) included (give point also, when absent but not required, see explanations for study types and their respective requirements on controls)?
- 14 Is the number of animals (in case of experimental human studies: number of test persons) per group given?
- 15 Are sufficient details of the administration scheme given to judge the study (see explanation for examples)?
- 16 For inhalation studies and repeated dose toxicity studies only (give point for other study types): Were achieved concentrations analytically verified or was stability of the test substance otherwise ensured or made plausible?

1 intraperitoneal injection
0 dose but not application concentrations given
0 single dose - full lifespan observation
1 no indication that negative control included, evaluation relative to chrysotile asbestos
0 330 animals in total, but not explicit no/dose group
0 no information on sample preparation in abstract
0 no information in abstract

2

Criteria Group IV: Study results documentation

- 17 Are the study endpoint(s) and their method(s) of determination clearly described?
- 18 Is the description of the study results for all endpoints investigated transparent and complete?
- 19 Are the statistical methods applied for data analysis given and applied in a transparent manner (give also point, if not necessary/applicable, see explanations)?

0
0
0

0

Criteria Group V: Plausibility of study design and results

- 20 Is the study design chosen appropriate for obtaining the substance-specific data aimed at (see explanations for details)?
- 21 Are the quantitative study results reliable (see explanations for arguments)?

1 probably yes, but not sufficient information to evaluate. For principal methods uncertainties see separate chapter

0

1

WARNING: check for unprocessed criteria! (for each criterium a score has to be selected)

6

9

A Numerical result leads to initial Category:

3

however just abstract considered, with full publication most likely different result

B Checking red scores leads to revised Category:

3

C Evaluator's proposal: Category:

D Justification in case evaluator deviates from B:

Optional documentation of observations with importance to relevance (not part of the reliability assessment)

During the course of the quality assessment observations may be made which are important for discussing the **relevance** of the data for specific purposes. The optional possibility is provided here to document these observations for future use.

What is the purpose of this quality evaluation (data documentation for use under REACH, classification activity under GHS, ECVAM validation activities, other)?

Study conducted according to recent OECD or EU guidelines (or other, e.g. national guidelines)?

If yes, which ones? Study conducted under GLP conditions?

(If not a guideline study): Does a guideline exist for the study endpoint(s) under investigation?

Are you aware of relevant deviations from the guideline(s) in the study evaluated? If yes, which one?

Did you make observations with importance to the regulatory use of the data (example 1: evaluator may hint that a whole body inhalation study was performed with a substance, for which profound percutaneous absorption is expected or known, leading to substantial percutaneous uptake in addition to inhalation uptake; example 2: an Ames reversion assay was performed with strains able to identify frame-shift mutations only or without external metabolic activation; example 3: evaluator is in possession of positive evidence that the results obtained with the in vitro study under evaluation, in conjunction with known toxicokinetic data, are useful to assess the nephrotoxicity of the substance in humans)?

Would you like to make other/general comments on the usability of the data?

Reliability assessment of in vivo toxicity studies

Study under evaluation

Authors:

Hesterberg TW, Chase G, Axten C, Miiller WC, Musselman RP, Kamstrup O, Hadley J, Morscheidt C, Bernstein DM, Thévenaz P.

Titel:

Biopersistence of synthetic vitreous fibers and amosite asbestos in the rat lung following inhalation.

Testing facility, year, sponsor, study no. or bibliographic reference:

Toxicol. Appl. Pharmacol 151 (1998): 262–275

Explanations are available for most criteria and show up, when the cursor is moved over the criteria field. Please read carefully!

Red criteria: the maximum score is needed for these criteria to achieve reliability category 1 or 2 (see worksheet Explanations): Please evaluate with special care!

Criteria

Evaluator's explanations, comments on criteria, etc.

No. Criteria Group I: Test substance identification

Score

1 Was the test substance identified?

1 6 test substances evaluated: chemical composition and fiber dimension

2 Is the purity of the substance given?

1 see above

3 Is information on the source/origin of the substance given?

1

4 Is all information on the nature and/or physico-chemical properties of the test item given, which you deem indispensable for judging the data (see explanation for examples)?

0 no particle count presented

3

Criteria Group II: Test organism characterisation

5 Is the species given?

1

6 Is the sex of the test organism given?

1

- 7 Is information given on the strain of test animals plus, if considered necessary to judge the study, other specifications (see explanation for examples)?
- 8 Is age or body weight of the test organisms at the start of the study given?
- 9 For repeated dose toxicity studies only (give point for other study types): Is information given on the housing or feeding conditions?

1 SPF conditions not explicit - may be retrieved from author
 0
 1
 4

Criteria Group III: Study design description

- 10 Is the administration route given?
- 11 Are doses administered or concentrations in application media given?
- 12 Are frequency and duration of exposure as well as time-points of observations explained?
- 13 Were negative (where required) and positive controls (where required) included (give point also, when absent but not required, see explanations for study types and their respective requirements on controls)?
- 14 Is the number of animals (in case of experimental human studies: number of test persons) per group given?
- 15 Are sufficient details of the administration scheme given to judge the study (see explanation for examples)?
- 16 For inhalation studies and repeated dose toxicity studies only (give point for other study types): Were achieved concentrations analytically verified or was stability of the test substance otherwise ensured or made plausible?

1 inhalation - nose only
 1 < 60 mg/m³
 1 6h/day for 5 days; time points of observation: 1,2,7,14,30,60,90,180,365 days
 1
 1 74 test animals/group; 29 control animals
 1
 1
 7

Criteria Group IV: Study results documentation

- 17 Are the study endpoint(s) and their method(s) of determination clearly described?
- 18 Is the description of the study results for all endpoints investigated transparent and complete?
- 19 Are the statistical methods applied for data analysis given and applied in a transparent manner (give also point, if not necessary/applicable, see explanations)?

1
 1
 1
 3

Criteria Group V: Plausibility of study design and results

- 20 Is the study design chosen appropriate for obtaining the substance-specific data aimed at (see explanations for details)?
- 21 Are the quantitative study results reliable (see explanations for arguments)?

1 for principal methods uncertainties see chapter 2

1

2

WARNING: check for unprocessed criteria! (for each criterium a score has to be selected)

6

19

A Numerical result leads to initial Category:

1

there may be a difference between quality of raw data and published data.

B Checking red scores leads to revised Category:

1

C Evaluator's proposal: Category:

2

D Justification in case evaluator deviates from B:

no particle counts?

Optional documentation of observations with importance to relevance (not part of the reliability assessment)

During the course of the quality assessment observations may be made which are important for discussing the **relevance** of the data for specific purposes. The optional possibility is provided here to document these observations for future use.

What is the purpose of this quality evaluation (data documentation for use under REACH, classification activity under GHS, ECVAM validation activities, other)?

Study conducted according to recent OECD or EU guidelines (or other, e.g. national guidelines)?

If yes, which ones? Study conducted under GLP conditions?

(If not a guideline study): Does a guideline exist for the study endpoint(s) under investigation?

study design largely according to Bernstein und Sintes 1999;

Are you aware of relevant deviations from the guideline(s) in the study evaluated? If yes, which one?

Did you make observations with importance to the regulatory use of the data (example 1: evaluator may hint that a whole body inhalation study was performed with a substance, for which profound percutaneous absorption is expected or known, leading to substantial percutaneous uptake in addition to inhalation uptake; example 2: an Ames reversion assay was performed with strains able to identify frame-shift mutations only or without external metabolic activation; example 3: evaluator is in possession of positive evidence that the results obtained with the in vitro study under evaluation, in conjunction with known toxicokinetic data, are useful to assess the nephrotoxicity of the substance in humans)?

Would you like to make other/general comments on the usability of the data?

deviations: clinical data not given (minor deviation); no recovery control for lung ashing; no particle counts;

Germany disagrees with the suitability of inhalation studies for measuring biopersistence as an indicator for carcinogenicity.

Reliability assessment of in vivo toxicity studies

Study under evaluation

Authors:

Miller BG, Searl A, Davis JMG, Donaldson K, Cullen RT, Bolton RE, Buchanan D,
Sou-tar CA

Titel:

Influence of fiber length, dissolution and biopersistence on the produc-tion of mesothelioma in the rat peritoneal cavity

Testing facility, year, sponsor, study no. or bibliographic reference:

Ann Occup Hyg 43 (1999):155-66

Explanations are available for most criteria and show up, when the cursor is moved over the criteria field. Please read carefully!

Red criteria: the maximum score is needed for these criteria to achieve reliability category 1 or 2 (see worksheet Explanations): Please evaluate with special care!

Criteria

Evaluator's explanations, comments on criteria, etc.

No. Criteria Group I: Test substance identification

Score

1 Was the test substance identified?

1

2 Is the purity of the substance given?

0

no chemical substance composition explicit (especially important with regard to alkaline oxide and alkali earth oxides), but likely to be retrieved from authors

3 Is information on the source/origin of the substance given?

1

4 Is all information on the nature and/or physico-chemical properties of the test item given, which you deem indispensable for judging the data (see explanation for examples)?

1

fiber dimensions are detailed

3

Criteria Group II: Test organism characterisation

- 5 Is the species given?
- 6 Is the sex of the test organism given?
- 7 Is information given on the strain of test animals plus, if considered necessary to judge the study, other specifications (see explanation for examples)?
- 8 Is age or body weight of the test organisms at the start of the study given?
- 9 For repeated dose toxicity studies only (give point for other study types): Is information given on the housing or feeding conditions?

1
1
1 SPF, Wistar - Charles River
1 12 weks
0

4

Criteria Group III: Study design description

- 10 Is the administration route given?
- 11 Are doses administered or concentrations in application media given?
- 12 Are frequency and duration of exposure as well as time-points of observations explained?
- 13 Were negative (where required) and positive controls (where required) included (give point also, when absent but not required, see explanations for study types and their respective requirements on controls)?
- 14 Is the number of animals (in case of experimental human studies: number of test persons) per group given?
- 15 Are sufficient details of the administration scheme given to judge the study (see explanation for examples)?
- 16 For inhalation studies and repeated dose toxicity studies only (give point for other study types): Were achieved concentrations analytically verified or was stability of the test substance otherwise ensured or made plausible?

1 intraperitoneal injection
1
1 single dose - full lifespan observation
1 no negative controls, justification: not necessary due to low spontaneous mesothelioma risk (no reference given)
1 24 animals/group
1
1

7

Criteria Group IV: Study results documentation

- 17 Are the study endpoint(s) and their method(s) of determination clearly described?
- 18 Is the description of the study results for all endpoints investigated transparent and complete?
- 19 Are the statistical methods applied for data analysis given and applied in a transparent manner (give also point, if not necessary/applicable, see explanations)?

1
1
1

3

Criteria Group V: Plausibility of study design and results

- 20 Is the study design chosen appropriate for obtaining the substance-specific data aimed at (see explanations for details)?
- 21 Are the quantitative study results reliable (see explanations for arguments)?

1 for principal methods uncertainties see separate chapter
1
2

WARNING: check for unprocessed criteria! (for each criterium a score has to be selected)

6

19

A Numerical result leads to initial Category:

1 there may be a difference between quality of raw data and published data.

B Checking red scores leads to revised Category:

1

C Evaluator's proposal: Category:

D Justification in case evaluator deviates from B:

Optional documentation of observations with importance to relevance (not part of the reliability assessment)

During the course of the quality assessment observations may be made which are important for discussing the **relevance** of the data for specific purposes. The optional possibility is provided here to document these observations for future use.

What is the purpose of this quality evaluation (data documentation for use under REACH, classification activity under GHS, ECVAM validation activities, other)?

Study conducted according to recent OECD or EU guidelines (or other, e.g. national guidelines)?
If yes, which ones? Study conducted under GLP conditions?

study carried out largely according to Bernstein et Sintes 1999

(If not a guideline study): Does a guideline exist for the study endpoint(s) under investigation?

Are you aware of relevant deviations from the guideline(s) in the study evaluated? If yes, which one?

deviations: 24 instead of 50 rats/group; applied concentrations were >> 5 mg/ml; no detailed description of sample preparation; no particle count; no negative control group; no information on microbiological characterisation; no clinical data/body weight results;

Did you make observations with importance to the regulatory use of the data (example 1: evaluator may hint that a whole body inhalation study was performed with a substance, for which profound percutaneous absorption is expected or known, leading to substantial percutaneous uptake in addition to inhalation uptake; example 2: an Ames reversion assay was performed with strains able to identify frame-shift mutations only or without external metabolic activation; example 3: evaluator is in possession of positive evidence that the results obtained with the in vitro study under evaluation, in conjunction with known toxicokinetic data, are useful to assess the nephrotoxicity of the substance in humans)?

Would you like to make other/general comments on the usability of the data?

Annex 3

Evaluation grid for epidemiological studies:

Exposure:	
Were fibers identified?	
Was duration (years) of exposure reported?	
Were the methods for determining the exposure described?	
Were these methods validated?	
Was a sufficient number of samples taken to appropriately describe exposure?	
Were work tasks sufficiently described in order to allocate individuals to an appropriate exposure group?	
Could exposures to other potential harmful agents be excluded?	
For meta-analyses:	
Were exposure data transformed to allow comparison of different studies?	
Was the method sufficiently described?	
Was the method appropriate?	
Was the potential impact of these transformations assessed and considered for the final conclusion?	
Follow-up:	
Was the length of the follow-up period appropriate?	
Valid method for observing an effect:	
Was the method described?	
Was it an appropriate method to detect the effect?	
Was the same method used for all individuals?	
Bias:	
Selection bias: yes / no?	
Information bias: yes / no?	
Other bias?	
Was bias that could not be avoided identified?	
Was the impact of bias that could not be avoided assessed and considered for the interpretation of the results?	
Confounders	
Were possible confounders described?	
Could confounders be controlled?	
If not, was the impact of confounders considered for the interpretation of the data?	
Statistical reliability:	
Was the sample size large enough to draw a conclusion?	
Was the applied statistical method described?	

Evaluation grid for reviews:

Wardenbach P. Pott F and Wotowitz H-J (2000) Differences between the classification of man-made vitreous fibres (MMVF) according to the European directive and German legislation: analysis of scientific data and implications for worker protection Eur.J.Oncol. 5(suppl.2), 111-118

Reliability

Potential bias

Was the publication peer reviewed?	Y	
Does the publication contain a declaration of interest?	N	
In case of more than one author – do authors stem from multiple stakeholders (Government, Industry, NGO, and University)?	N	BAUA; Justus Liebig University Gießen/Germany – no co-author representing the European approach?
Is the publication less than one year old – if no how many years?	N	From 2000
Are references to primary data traceable?	Y	
Are the criteria for including publications into the review described?	N	

Transparency

Are the critical aspects of the substance identities described?	N	Probably traceable in referenced publications
Are the critical aspects of test systems and study designs described?	N	Probably traceable in referenced publications
Are limitations of the review discussed?	N	Uncertainties of i.p. methods not discussed
Are the reviewed data and the synthesis thereof transparent – if not in which aspect?	Y	
Is the synthesis of the reviewed data plausible– if not in which aspect?	Y	With limitations mentioned above

Relevance

Are the substance identities relevant for the purpose of our review?	Y	With limitations mentioned above
Are the test systems/study designs relevant for the purpose of our review?	Y	With limitations mentioned above

Any other comment		
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Annex 4

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Annex 5

List of abbreviations:

AES	Alkaline Earth Silicate Wools
ATSDR	Agency for Toxic Substances and Disease Registry
CLP	EU regulation on Classification, Labelling and Packaging of substances and mixtures
DMEL	Derived Minimum Effect Level
DNEL	Derived No Effect Level
EBT	Evidence Based Toxicology
ECHA	European Chemicals Agency
FVC	Forced Vital Capacity
FEV ₁	Forced Expiratory Volume in One Second
GHS	Globally Harmonized System of Classification and Labelling of Chemicals
GMD	Geometric Mean Diameter
HTIW	High Temperature Insulation Wool
IARC	International Agency for Research on Cancer
IOM	Institute of Occupational Medicine
IUPAC	International Union of Pure and Applied Chemistry
JRC	Joint Research Center
MMMMF	Man-Made Mineral Fibers
MMVF	Man-Made Vitreous Fibers
NIOSH	The National Institute for Occupational Safety and Health
NOAEC	No Observed Adverse Effect Concentration
NTP	National Toxicology Program
OR	Odds Ratio
PCW	Polycrystalline Wools
RCF/ASW	Refractory Ceramic Fibers / Aluminosilicate Wools
UVCB	Substances of Unknown or Variable composition, Complex reaction products or Biological Materials
WHO	World Health Organisation