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A comparison of the results from intra-pleural and intra-peritoneal studies with those from inhalation and intratracheal tests for the assessment of pulmonary responses to inhalable dusts and fibres.

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Abstract

The aim of this paper is to compare results from inhalation studies with those from intraperitoneal and intrapleural tests, where available, for a number of fibrous and particulate test materials. The objective is to determine how well intraperitoneal/intrapleural studies predict the pathological responses observed in more standard *in viv*o studies of pulmonary toxicity, with a particular focus on carcinogenicity.

Published toxicity data was obtained for a number of materials including asbestos, wollastonite, MMVFs (including glass fibres, stone wools and RCF), silicon carbide whiskers, potassium octatitanate, quartz, kevlar, polypropylene and titanium dioxide.

For some of the fibrous material reviewed, there is conformity between the results of intraperitoneal and inhalation tests such that they are either consistently positive or consistently negative. For the remaining fibrous materials reviewed, intraperitoneal and inhalation tests give different results, with positive results in the intraperitoneal test not being reflected by positive inhalation results.

It is suggested that the intraperitoneal test can be used to exonerate a dust or fibre (because if negative in the intraperitoneal test it is extremely unlikely to be positive in either inhalation or intratracheal tests) but should not be used to positively determine that a dust or fibre is carcinogenic by inhalation. We would argue against the use of intraperitoneal tests for human health risk assessment except perhaps for the purpose of exoneration of a material from classification as a carcinogen.

Key Words: intra-pleural; intra-peritoneal; inhalation; intra-tracheal; dusts; fibres; pathological response; carcinogenic response; pulmonary toxicity; in vivo.

1. Introduction

Hazard characterisation of the toxicity and carcinogenic potential of airborne dusts and fibres is usually carried out using *in vivo* test methods, although *in vitro* approaches are increasingly being utilised to investigate specific relevant toxicological parameters. The *in vivo* models used include inhalation (IH; whole body or nose-only), intratracheal instillation (IT), intraperitoneal injection (IP) and intrapleural injection (IPl). The intratracheal test essentially aims to replicate exposure by inhalation, while intraperitoneal/ intrapleural injection tests investigate the toxicity of fibres to the mesothelium and have also been used to assess carcinogenic potency. As explained more fully below, although expensive to conduct, inhalation toxicity studies are still generally viewed as the 'gold standard' for airborne dusts and fibres (McLellan et al., 1992; Pauluhn & Mohr, 2000).

The toxicity of inhaled fibres is described by the so called '3Ds' paradigm which recognises that the most important parameters are **d**ose, **d**imension (fibre length and diameter, which determine both respirability and pathogenicity in the lung) and **d**urability (or, more properly, biopersistence) (Bernstein et al., 2001a; Bernstein et al., 2001b; Brown & Harrison, 2012; Donaldson et al., 2013). For dusts (rather than fibres) the fundamental concerns are fibrogenicity and cancer due either to inherent pulmonary toxicity or to lung overload effects (Oberdorster, 1995; Pauluhn, 2014; Borm et al., 2015; Morfeld et al., 2015).

The heterogeneity of toxic responses to fibres at different locations in the respiratory tract has been described, for example, by Donaldson et al. (2013) using the well documented example of asbestos. The principal pathologies are as follows:

- Lung parenchyma – interstitial fibrosis with accumulation of fibrous/scar tissue;
- Bronchi/bronchioles – bronchogenic carcinoma (malignant cancer of the cells lining the airways);

- Pleurae (visceral and parietal surfaces) – pleural fibrosis (diffuse accumulation of scar tissue in the pleura) and mesothelioma (malignant tumour arising from the mesothelium lining the pleural space);
- Parietal pleura – pleural plaque (deposits of hyalinised collagen fibres).

As all inhaled fibres and dusts are not equally pathogenic, only some, or possibly none, of these effects will arise following inhalation of any particular material. In this paper the end-point of particular interest is cancer of the lung and/or mesothelium.

This review aims to compare findings from inhalation studies with those using the same fibrous or particulate test materials delivered by the intraperitoneal or intrapleural injection routes. The specific objective is to assess how well intraperitoneal/intrapleural injection studies can predict the pathological responses to airborne dusts and fibres that are observed in inhalation studies of pulmonary toxicity, in particular with respect to carcinogenicity. Where intratracheal studies have also been carried out, these are included for completeness; however, for reasons detailed below (Section 3) for some materials the intratracheal test may not be particularly informative regarding cancer endpoints.

It is emphasised that this review is not intended to be an exhaustive collection of all published studies, but rather a focused comparison of inhalation and IP/IPl test data for a range of dusts and fibres. A good understanding of this relationship is important because of the continued use by some jurisdictions of IP test results for human cancer risk assessment (Harrison et al., 2015).

2. Inhalation methods

The overall aim of an IH study is to administer a well characterised exposure concentration that can be related to inhaled dose and any subsequent response in the animal (Wong, 2007). Inhalation

studies can, however, be subject to variability in several areas including: animals' environment and surroundings; exposure atmosphere; the applied dose; and individual animal biological sensitivity. High degrees of standardisation and control are therefore required to make test results reproducible and comparable, and to fulfil general regulatory requirements (Pauluhn and Mohr, 2000).

Systems have been developed to provide a uniform, controlled environment for inhalation studies for all types of experimental animals in terms of temperature, humidity, air flow, oxygen content, and other major environmental factors (Wong, 2007). Exposure techniques include whole-body chambers, and head and nose-only chambers, which are described briefly below.

Whole-body chambers: In these the animal is immersed in the atmosphere of the chamber. This approach has the advantage of simulating 'natural' workplace or environmental exposures with unrestrained animals. It is the most efficient approach for testing large numbers of animals and/or for long duration studies as the animals can be housed in the chambers. However, this approach uses a large amount of material, and good air mixing in the chamber is essential. In addition, co-exposure through oral and dermal routes cannot be excluded.

Head and nose-only chambers: In these the animal is restrained so that only the head or nose is exposed to the test material. This has the advantage that co-exposure through other routes is unlikely and less material is needed. Additionally, it is easier to contain the test material and allows flexibility in removing animals without the rest of the test group being affected. Disadvantages of this approach include stressing the animals during restraint, and lack of food and water during the exposure period.

3. Pulmonary deposition methods

These alternative methods act as surrogates for inhalation testing and allow instantaneous delivery of a precise dose of test material suspended in a small volume of vehicle to the lungs. As the material is delivered directly to the lower respiratory tract, the potential for deposition in the nasal passsages and/or on fur that can occur in nose-only or whole-body inhalation exposure systems is avoided (Osier & Oberdörster, 1997). Pulmonary deposition methods include the following:

Intratracheal Instillation (IT): This much used technique deposits a precise dose (bolus) of material into the lungs, although the test substance may not be distributed in and cleared from the lungs in the same way as would occur via nose-only inhalation. Intratracheal instillation has been used for repeated dose studies, for example to test carcinogenicity and establish relative potency of fibrous and non-fibrous particulates (Pott, 1993 – cited by Wong, 2007), and to evaluate particulate material that is not readily inhaled by rodents (Driscoll et al., 2000 – cited by Wong, 2007). However, as this technique usually involves a course of injections over a period of several weeks (only) it cannot strictly be considered a 'chronic' exposure as would be achieved in a 24 month IH study; thus for materials with low biopersistence the IT test may not be especially informative regarding the cancer endpoint (Driscoll et al., 2000 – cited by Wong, 2007).

Oropharyngeal Aspiration: This deposits a specific dose of material on the base of the animal's tongue which is then aspirated into the lungs during inhalation. This has the advantage over intratracheal instillation in that material is distributed throughout the lungs, however Foster et al. reported that the clearance of material from the lungs is altered from nose-only inhalation (Foster et al., 2001 – cited in Wong, 2007).

- **Endotracheal Inhalation:** This method utilises a tight-fitting catheter inside the animal's trachea to inflate the lungs, during which the test material is delivered. This has been used to assess ultrafines and non-inhalable aerosols (Oberdorster et al., 1995 – cited in Wong, 2007).
- **Tracheostomy:** A surgical opening in the trachea allows delivery of the test material via a cannula. This method can also be used to obtain fluid or cells from the lungs.

The disadvantages of such techniques is that animals require anaesthesia (and, for some, surgical procedures), making then unsuitable for long-term repeat-dose studies. In addition, normal defensive mechanisms are by-passed.

4. Intracavity injection methods

In addition to the inhalation and pulmonary deposition methods, two other routes of administration have become commonly associated with toxicological assessment of airborne dusts and fibres, namely intrapleural and, especially, intraperitoneal injection.

4.1 Intrapleural injection (IPl)

The most relevant intracavity method for the assessment of pleural toxicity involves delivery of the test material directly into the pleural space (Murphy et al., 2011). This model has been validated for studying pre-mesothelioma processes, for example (Wagner, 1984). Unfortunately it is a technically difficult procedure, as the injection needle has to be positioned exactly within the pleural space, and there are associated ethical issues; these factors limit its use for routine testing purposes.

4.2 Intraperitoneal injection (IP)

Use of the peritoneal cavity as a model for the assessment of fibre pathogenesis has been well documented for many fibre types including asbestos, synthetic vitreous fibres and nanofibres (Donaldson et al., 2013). As the pleural space has, at least until recently, been difficult to utilise directly for toxicity testing due to technical issues associated with effective delivery of test material (see 4.1 above), injection directly into the peritoneal cavity, which is also lined with mesothelium, has proved an accessible and viable alternative. As with the pleural cavity, fibres or particles introduced into the peritoneal cavity which cannot pass through the mesothelial stomata will be retained, potentially leading to a pathogenic response (Donaldson et al., 2010).

Advantages of the intrapleural method include: use of a small amount of test substance, ease of delivering the same dose to all animals, reduced cost, and in some respects a more 'sensitive' test in comparison to inhalation methods (Miller *et al.*, 1999). However, certain important limitations also need to be considered:

- The natural filtering and clearance mechanisms of the lung are by-passed, meaning that material is injected that might never reach the pleura following inhalation exposure;
- High doses are delivered at high rates, contrary to that occurring in the pleura under normal physiologic conditions;
- Impacts on the airways and lung parenchyma are not investigated;
- The impact of fibre biopersistence during transit from the deposition site to the pleura is not taken into account;
- The influence of fibre diameter on pulmonary uptake and deposition is not taken into account;
- The peritoneal mesothelium is assumed to respond in the same way as the pleural mesothelium.

5. Method comparison

In general, inhalation testing is regarded as the most appropriate method for assessing the toxicity of airborne materials as it gives a realistic exposure scenario that can be extrapolated to humans (Pauluhn and Mohr, 2000). This view has been reflected in reviews conducted by various national and international agencies, including ILSI (2005) and the National Research Council (2000). Although inhalation testing can be expensive, time consuming and lack specificity in dose (Bernstein, 2007; Grimm *et al.*, 2002) compared to intratracheal and intrapleural/intraperitoneal studies, there are a number of important problems associated with the alternative methods, as discussed for example by Lippmann et al. (2014). These include: bypassing of the natural defence mechanisms of the lung;

ability to inject/implant large fibres and particles that would not normally be inhaled into the deep lung; very high numbers/concentrations of test material at the injection site that may overwhelm defence mechanisms; targeted tissues (e.g. peritoneal mesothelium) are not the same as for inhalation exposure. Moreover, the coincidental injection of large (non-respirable) irritant fibres into the mesothelium may well confound the test results (Harrison et al, 2015). It is true to say that whilst inhalation studies may lead to exposure of the mesothelium, any toxic effect resulting from this would be expected to be picked up by histopathology. However, intraperitoneal studies cannot be considered to result in exposure of lung tissue.

Some advantages and disadvantages of the various methods discussed above are summarised in Table 1.

Table 1. Some advantages and disadvantages of different exposure methods

Exposure Method	Advantages	Disadvantages
Inhalation	• realistic exposure	• expensive • actual applied dose more difficult to measure
Intratracheal	• lower cost • specified doses • long fibres can be delivered effectively to the lung	• high bolus dose needed • uneven distribution; may block smaller bronchioles • overloading may occur • bypasses the upper respiratory tract • less reflective of chronic exposure for materials with low biopersistence
Intraperitoneal/Intrapleural	• lower cost • specified doses • sensitive • relevant to the determination of possible impacts on the mesothelium	• bypasses the mechanical clearance system • may deposit larger diameter fibres than would normally reach the pleural cavity • not administered to the lung, thus effects on lung tissues

		not investigated and fibres are not subject to natural attenuation within the lung

Adapted from Miller *et al*., 1999; Wong, 2007

With regard to dosimetry, uncertainties in inhalation studies can be reduced by lung burden experiments (using, for example, low temperature ashing and electron microscopy). Although in injection type methods the delivered dose can be measured, there remains uncertainty as to the true dose to the target organ.

6. Comparison of toxicity study findings

In the following sections, published toxicity data for a number of fibres and dusts obtained from inhalation (and, for some materials, intratracheal instillation) studies are compared with toxicity data for the same test materials delivered by the intraperitoneal and/or intrapleural routes. The chemical compositions of the fibres discussed in this report are detailed in Table 2 below (Bellmann et al., 1987; Bernstein, 2007; Grimm et al., 2002; Guldberg et al., 2002; Hesterberg et al., 1998; Kamstrup et al., 2001; Kamstrup et al., 2002; Kamstrup et al., 2004; Lambré et al., 1998; Roller et al., 1996; Searl et al., 1999). While full characterisation of the delivered aerosol – especially fibre/particle size distribution – is extremely important, this information is not consistently available; this is acknowledged as a possible limitation when comparing test results.

183 **Table 2. Chemical composition of fibre types**

Chemical Composition (%)	Crocidolite	Chrysotile	Amosite	MMVF10	MMVF11	MMVF21	MMVF22	MMVF34 / HT	Glass Fibres Type 475	RCF1 RCF4	RCF1a	RCF2
B_2O_3				8.75	4.5				10-12.1			
Na_2O	3.7 – 8.5		0.06	14.95	15.5	2.46-2.7	0.4	0.1-1.9	0.1-14.9	0.54	0.04	<0.3
MgO	2.1 – 3.41	55.2	6.02	4.13	2.8	9.25-9.5	10	9.6-10.7	0.05-0.4	0.08	0.06	0.01
Al_2O_3	0.05 - 0.2		0.22	5.1	3.9	13-13.8	10.6	21.5-23.2	4.5-24	48	45.5	35
SiO_2	49 - 53	41.5	51.01	57.5	63.4	45.9-46.2	38.4	38.85-39.6	33-72.3	47.7	51.25	50
K_2O	0.07 – 0.4		0.14	1.06	1.3	1.25-1.3	0.4	0.8	0.6-3	0.16	0.12	<0.01
CaO	0.3-2.7	0.2	0.28	7.5	7.5	17	37.4	15	1.2-33	0.01	0.08	0.05
TiO_2	0.01			0.01	0.1	0.1-3	0.4	2-2.1	<0.1-3	2.05	1.84	0.04
Fe_2O_3	17-42.5	3	36.95	0.07	0.3	6.2-7	0.3	7.52	<0.1-6.4	0.97	0.89	<0.05
ZnO	-								2.8-4			
BaO	-				<0.1	<0.1	<0.1	0.04	3.6-5			
P_2O_5	-					0.26		0.42				
MnO	0.05-0.12		3.44				2.15	0.3				
SO_3	0.12				0.3	0.15-0.3	1.4	0.05				
F												
ZrO_2				0.03		0.03	0.06	0.06		0.11	0.04	15
Other	FeO 13 -20					0.4		0.9				

6.1 Asbestos (Chrysotile, Crocidolite and Amosite)

Muhle *et al.* (1987) carried out two parallel studies using inhalation and intraperitoneal models to assess the carcinogenic potential of chrysotile (UICC and Calidria) and crocidolite. In the intraperitoneal study, female Wistar rats (aged 5 weeks at the start of the trial) were administered a single intraperitoneal injection of 0.5 mg of crocidolite or chrysotile (Calidria) or chrysotile (UICC) in 1 mL of saline, and observed for a median lifetime of 109 and 116 weeks for crocidolite and chrysotile respectively. In the inhalation study, female Wistar rats (aged 12 weeks at the start of the trial) were exposed via nose-only inhalation to 2.2 mg/m^3 crocidolite and 6.0 mg/m^3 chrysotile (UICC) for 5h, four times per week over a 12 month period (total exposure of 1000 h and cumulative exposure 2200 and 6600 mg/h/m^3 respectively) with a 12 month follow-up exposure-free period. Exposure to crocidolite by intraperitoneal injection induced malignant tumours in 55% of animals compared with 84% in those exposed to chrysotile (UICC), and 0 – 6% in controls. Exposure to Calidria chrysotile was associated with malignant tumours in only 6% of animals, which the authors concluded was due to the lower biopersistence of Calidria chrysotile compared with UICC chrysotile. In contrast, no significant tumour incidence was reported for the inhalation studies, with only 2% (1 animal) exposed to crocidolite developing an adenocarcinoma. Muhle *et al.* (1987) expressed doubts about the inhalation study findings.

As part of a large study of 50 test materials, Pott et al. (1987) assessed the potential carcinogenicity of several types of chrysotile using an intraperitoneal model, and crocidolite using intratracheal and intraperitoneal models. Chrysotile (UICC A) given at doses of 6 and 25 mg to female Wistar rats via intraperitoneal injection, resulted in tumour incidences of 77.1 and 80.6% respectively. UICC B chrysotile administered at doses of 0.05, 0.25 and 1 mg by intraperitoneal injection was associated with a dose dependant increase in tumour incidence rate, with 19.4, 61.8 and 84.4% of rats respectively presenting with tumours. Two other forms of chrysotile, PVNO and calidria, were also tested, administered in a single intraperitoneal injection of 1 and 0.5 mg respectively. A high tumour

incidence rate of 80% was observed with the PVNO form, but a low incidence rate of only 6% was noted for the calidria form.

Pott et al. (1987) also reported findings for crocidolite, tested in female Wistar rats using intratrachael and intraperitoneal models. Following intratracheal instillation of 20 doses of 0.5 mg, or a single intraperitoneal injection of 0.5 mg, similar numbers of tumours were evident, with 42.9% and 56.3% of animals, respectively, presenting with tumours. A higher tumour incidence rate was seen at the higher level of exposure of 2 mg crocidolite (87.5%) using the intraperitoneal model.

Grimm *et al*. (2002) used crocidolite at 2 different doses (0.5mg and 5mg) as a positive control in a study of biosoluble insulation glass wool fibres, injected once into the intraperitoneal cavity of female Wistar rats (strain CrL: WiBR). Pathology was carried out to determine presence of the following: mesothelioma with simultaneous abdominal tumours; other abdominal tumours with serosal spread (but no mesothelioma); and abdominal tumours with neither serosal spread nor mesothelioma. Survival numbers were significantly reduced in the high dose crocidolite group, leading to the validity of use of the high dose being questioned. Importantly, the authors concluded that there may be different aetiologies for the production of mesothelioma by soluble and insoluble fibres following intraperitoneal injection (Grimm *et al*., 2002).

This study was an extension of an earlier investigation reported by Lambré *et al*. (1998) to evaluate the potential carcinogenic hazard of five man-made vitreous fibres, which also used crocidolite as a positive control. Three different doses of crocidolite (0.005, 0.05 and 0.5mg), delivered in a single intraperitoneal dose to female Wistar rats, all produced mesotheliomas in a dose dependent manner (Lambré *et al*., 1998). In comparison to these intraperitoneal studies, Smith et al. (1987) used crocidolite as a positive control in a 2 year inhalation study in rats. A dose of 7 mg was associated

235 with fibrosis in half of the animals, with bronchioloalveolar hyperplasia also evident in a smaller
236 number of animals. One mesothelioma and two bronchoalveolar tumours were reported.
237
238 Hesterberg *et al*. (1995) also conducted long term inhalation studies using crocidolite and chrysotile
239 as positive controls to validate the model used in their study. This used both rats (2 year exposure)
240 and hamsters (18 month exposure), with the rats receiving crocidolite and chrysotile, and hamsters
241 chrysotile, by nose only inhalation at a dose of 10mg m^{-3}. The rats showed signs of pulmonary
242 interstitial fibrosis with both fibre types after 3 months, and a single mesothelioma was present for
243 each of the two asbestos fibres. An increase in other lung tumours was also reported, but the exact
244 tumour types were not detailed. Due to the high mortality rate, the crocidolite exposure was
245 stopped after 10 months. The hamster group exposed to chrysotile also showed the presence of
246 pulmonary fibrosis; however there was no evidence of mesothelioma or other lung tumours
247 (Hesterberg *et al*., 1995).
248
249 Both inhalation and intraperitoneal studies were conducted by Cullen *et al*. (2000a) using amosite
250 asbestos in male Wistar rats (12 weeks of age). Inhalation exposure was carried out in a full body
251 chamber, with exposure being equivalent to 1000 fibres/ml for 7 h per day, 5 days per week for up
252 to 12 months, plus a further 12 month post-exposure recovery period. The intraperitoneal study
253 comprised a single injection of 10^9 fibres in a 2ml suspension. In the inhalation exposure group, 4.8%
254 of animals developed mesothelioma, while in the intraperitoneal group almost all (81%) developed
255 mesothelioma. Carcinomas and adenomas of the lung were also present in the inhalation group,
256 totalling 38.1% of animals.
257
258 Intratracheal instillation of amosite (0.65mg/rat) was undertaken in a study by Padilla-Carlin *et al.*
259 (2011). A single dose was instilled, producing a high degree of inflammation and pulmonary injury
260 with thickening of the interstitial areas. This was only a short-term study so no tumour observations

were made (details therefore not included in Table 3). Comparing the results for intrapleural and inhalation exposure of amosite shows the material to be carcinogenic in both assays, but much more strongly so in the IP test.

Summary of findings for asbestos

- Chrysotile asbestos shows positive results for carcinogenicity in both inhalation (levels between 6 – 10 mg m^{-3}) and intraperitoneal studies (levels between 0.05 – 25 mg), but with a much greater potency in the latter.
- Crocidolite asbestos also shows positive results in both inhalation (levels between 2.2 – 10 mg m^{-3}) and intraperitoneal studies (levels between 0.005 – 5 mg), although there appears less difference in potency between the routes of exposure than with chrysotile asbestos.
- Amosite asbestos shows positive results for carcinogenicity in both inhalation (1000 fibres/ $cm^{-3)}$ and intraperitoneal studies (10^9 fibres), although there is an apparent greater potency through the intraperitoneal route.

6.2 Wollastonite

Wollastonite was included in the large study of 50 test materials described by Pott *et al*. (1987). A total dose of 100mg was given by intraperitoneal injection, in five separate 20mg doses, to 54 rats. No tumours were observed 28 months after these injections, nor were any severe adhesions found. Inhalation and intratracheal studies have also been conducted on wollastonite by Warheit *et al*. (1994) and Tátrai *et al*. (2004) respectively. A short term inhalation study was carried out in male Sprague-Dawley rats, exposed to 115 mg/m^3 (800 fibres/cc) for 5 days to assess biopersistence. Rapid clearance of the wollastonite fibres from the lungs was seen, with a low retention half-time of <1 week. The data indicated that wollastonite fibres have low durability, being composed of calcium silicates, resulting in solubilisation in the lung (Warheit et al., 1994). Tátrai *et al.* (2004) used a single 1 mg intratracheal instillation of wollastonite, with crocidolite (UICC) as a positive control, and

examined the lungs at time intervals of 1, 3 and 6 months post exposure. The authors reported that the wollastonite exposed group showed mild inflammation and fibrosis which remained the same at six months as at one month, while the crocidolite showed increased inflammation at 6 months (Tátrai *et al*., 2004). All three exposure methods demonstrated that wollastonite has low toxicity.

6.3 MMVFs (Man Made Vitreous Fibres)

This section details findings on a variety of common MMVFs, including rock, slag and stone wool. Although strictly speaking RCF and special types of glass fibres are also classed as MMVFs, for clarity these are detailed in individual subsections.

In 1972, intracavity experiments by Pott and Friedrichs (also Stanton and Wrench at the same time) indicated that man-made vitreous fibres could be a potential hazard to human health (Pott and Friedrichs, 1972; Stanton and Wrench, 1972); a large number of studies have subsequently been carried out on a variety of MMVFs using both inhalation and intraperitoneal exposure models.

McConnell *et al.* (1994) conducted a long-term study in Fischer 344/N rats (males, eight weeks of age) exposed by nose-only inhalation for 6 h per day, 5 days per week, for 24 months to 3 concentrations (3, 16, and 30 mg/m^3) each of a rock wool (stone wool), and a slag wool (blast furnace). A dose-related non-specific inflammatory response was seen for both test substances, with rock wool also inducing a minimal local pulmonary fibrosis. Although a number of tumours were present (carcinoma and adenoma), their incidence was not considered to be significantly raised. Bronchoalveolar hyperplasia, on the other hand, was seen at significantly greater incidence in the highest dose animals than in the controls (saline) (McConnell *et al*., 1994). Hesterberg *et al*. (1995) reported the toxicity of different MMVFs (including fibrous glass (MMVF10 and 11), rock (stone) wool (MMVF21) and slag wool (MMVF22)) by inhalation in a series of studies with comparable fibre

numbers (WHO fibres[1]) and dimensions in the delivered aerosols. Groups of rats and hamsters were exposed nose-only to 30mg m^3 doses of each of the test substances for 6 h per day, 5 days per week for either 18 months (hamsters) or 24 months (rats). Exposure to the fibrous glasses and slag wool induced an inflammatory response in rats, but no mesotheliomas or increased lung tumour incidence rates were observed. Exposure to rock wool was associated with minimal lung fibrosis; there were no mesotheliomas and no increase in lung tumour rate.

Miller *et al*. (1999) investigated the carcinogenicity and biopersistence of MMVF 10, 21 and 22 by intraperitoneal administration of 10^9 fibres. MMVF21 showed results consistent with those of Kamstrup *et al.* (2001), with 95% of the test group developing mesothelioma. The MMVF10 and MMVF22 groups showed a lower incidence of mesothelioma (59% and 54% respectively); however, there was no control group included in this study, so it is not known if these results were statistically significant (Miller *et al*., 1999). Comparing the results to those from the inhalation study reported by Hesterberg *et al*. (1995), it can be seen that there is no consistency between the findings, with mesotheliomas being produced by the intraperitoneal method but neither mesotheliomas nor lung tumours being found in the inhalation study.

In a later study, Hesterberg et al. (1998), assessed the potential toxicity of a rapidly dissolving Synthetic Vitreous Fibre (X607) in Fischer rats, exposed by nose-only inhalation to X607 at a concentration of 200 fibres/cc for 6 h per day, 5 days per week for 24 months. RCF1 was included at the same exposure level and duration for comparison purposes (see Section 6.3.2). X607 showed low biopersistence and was not associated with fibrogenic or tumorigenic responses above those seen in the controls.

[1] WHO fibres are defined by the World Health Organization as having a length/diameter ratio ≥3, diameter <3 µm, and length >5 µm

Findings from the study reported by McConnell *et al*. (1994) were compared by Kamstrup *et al*. (2001) using an inhalation study to assess the pathology of a low silica/high aluminium content MMVF (34/HT). Male Fischer rats were exposed via nose-only inhalation to MMVF 34/HT at concentrations of 30 mg/m^3 for 6 h per day, 5 days per week for 104 weeks, with a subsequent non-exposure period lasting until survival of animals in the air control group had dropped to approximately 20%. Pathology results were compared to a previous assessment of stone wool (MMVF21) under the same exposure conditions reported by Hesterberg et al. (1995). The authors reported a marked difference in pulmonary pathogenicity, with MMVF21 but not MMVF34/HT[2] causing pulmonary fibrosis. Although tumours were present for both fibre types, these were comparable to control levels.

No study utilising intraperitoneal/intrapleural models for MMVF34 could be identified for comparison to the data from the inhalation study. However, Kamstrup *et al*. (2002) did conduct an intraperitoneal carcinogenicity study using the high aluminium, low silica HT wool RIF39001 and stone wool (D6) with similar chemical compositions to MMVF34 and MMVF21 respectively. This study administered a single dose of 9 mg of the HT wool, and 36 mg of D6, by intraperitoneal injection into female Wistar rats. The mass dose differed for each fibre type due to differing number and size of the fibre. The HT wool dose (2.1 x 10^9 WHO fibres) was twice that since recommended by the EU guidelines (European Commission 1997). The animals administered the HT wool showed a low incidence (6%) of macroscopic nodules in the peritoneal cavity, while the majority of the group (88%) injected with D6 showed the presence of nodules. Histologically, clumps of fibres were found in the D6 groups either adherent to the surface of the viscera or free within the abdominal cavity, but not in the HT wool group. Formation of granulomas was seen in the D6 group, indicating a cellular response with but not in the HT wool group. Fibrosis was evident in the peritoneal cavity of both D6 (93%) and the HT group (58%), most commonly seen between the liver and diaphragm.

[2] Here and elsewhere, in fibre nomenclature HT denotes 'high temperature'.

Three types of tumours (benign pituitary adenomas, mammary adenocarcinomas and mesothelioma) were observed in the treated animals and in the controls, but mesotheliomas were the most common type of tumour in the D6 group (56%). The authors concluded that the low carcinogenic potential of RIF39001 was due to the high biosolubility of the HT wool (Kamstrup *et al*., 2002).

Inhalation, intratracheal and intraperitoneal studies have been carried out to assess the carcinogenic potential of HT stone wool. A single administration of 1.2 mg of stone wool in 0.2 ml by intratracheal instillation study was conducted by Baier et al. (2000) with sacrifices occurring at different time points up to 90 days post-exposure. Pulmonary granulomas were present at the start of the post instillation period but decreased as the post instillation time increased, with most of the granulomas being resolved and only a very small number of fibres remaining embedded in the surrounding tissue by the end of the study. This study would appear to indicate that the HT fibre is unlikely to be carcinogenic, although this is not possible to determine categorically from a 90-day study (details therefore not included in Table 3). No details on chemical composition of the fibres used were given by the authors, making comparison with other studies difficult. However, it is recognised that, due to differences in the processing of the raw materials and the variations that can occur in the starting material, it is not possible in practice to define a unique chemical composition for stone wools (Guldberg *et al*., 2002).

Sub chronic and chronic inhalation studies have been carried out on high-aluminium, low-silica HT stone wools. A sub-chronic biopersistence study tested 3 different stone wools (RIF41001, 42020-6 and 43006-1) at a single dose of 150 fibres/ml (>20 µm) for a period of 3 months delivered by nose-only exposure. A post exposure period of the same length was included. Only minimal histopathological changes were observed for all three stone wools, and therefore all were assessed as non-fibrogenic (Kamstrup *et al*., 2004). A chronic inhalation study was reported for MMVF34,

delivered to male Fischer 344 rats at 30 mg/m^3 by nose-only inhalation for 6 h/day, 5 days/week for 104 week, with a subsequent non-exposure period lasting until survival in the air control group had dropped to approximately 20%. No pulmonary fibrosis was noted with MMVF34 and the incidence of tumours was comparable with the control group (Kamstrup *et al*., 2001).

Summary of findings for MMVFs

Comparing the results of the inhalation and intraperitoneal tests for MMVF21/D6 and MMVF34 (or similar), the intraperitoneal test showed D6 to be carcinogenic, producing a tumour incidence rate of 56%, while the inhalation tests showed both MMVF21 and MMVF34 to be non-tumorigenic. For HT stone wool, all three exposure models indicated no carcinogenic potential.

Other studies of specific types of man-made vitreous fibres have been conducted and these are discussed separately below.

6.4 Glass Fibres

6.4.1 Glass fibre 104/475

Pott *et al*. (1987) included a number of different types of glass fibres in their large study investigating the carcinogenicity of 50 fibres, dusts and metal compounds. Glass fibre 104/475 was assessed using both intraperitoneal and intratracheal models. In the intraperitoneal study, two separate doses of 104/475 (0.5 mg or 2.0 mg) were injected into the peritoneal cavity. A dose-dependent response for tumour induction was seen with incidences of 16.7 and 25.8% respectively. In a further intraperitoneal study, five 1 mg doses were administered, with a post-exposure period of 28 months. Results of this study showed a high degree of fibrous adhesions and a 66% tumour rate. These two studies did not differentiate between tumour types, but combined incidences of sarcoma, mesothelioma or carcinoma in the abdominal cavity to give an overall tumour rate. In the intratracheal study, a 10 mg dose of 104/475 was instilled in 20 weekly injections of 0.5mg each,

resulting in a 14.7% incidence of lung tumours (types not differentiated). Pott *et al*. reported that this was statistically significant when compared to the unexposed control group of animals in which no tumours were evident (Pott et al., 1987). A further study by Muhle et al. (1987) compared findings from intraperitoneal and inhalation studies using 104/475. Female Wistar rats were administered a single intraperitoneal injection of 0.5mg 104/475, while in the inhalation group female Wistar rats were exposed, nose-only, to 104/475 at a concentration of 3.0 ± 1.8 mg/m^3 for 5 hours per day, 4 days a week for 1 year with a total study duration of 2 years. Following intraperitoneal injection, a total tumour incidence rate of 17% was observed (tumour types not specified). This compared with only one primary lung tumour (squamous cell carcinoma) following inhalation exposure, although there was a high incidence of fibrosis (38%), with bronchioloalveolar hyperplasia (11%) and squamous metaplasia (0.9%) in these animals.

6.4.2 Glass fibre 100/475

Glass fibre 100/475 differs from 104/475 in that it has a smaller diameter. Davis et al. (1995) reported findings of a review comparing models to predict the pathogenicity of fibres, utilising 100/475 as an example of a less durable glass microfibre (details not included in Table 3). Cullen et al. (2000a) have reported findings of both inhalation and intraperitoneal studies using 100/475. Rats were exposed to aerosol concentrations of 1000 fibres/ml for 7 h per day, 5 days per week for 12 months, with an additional post-exposure period of 12 months. After 2 years, no fibrosis was apparent, nor were there any carcinomas or mesotheliomas; however adenomas were detected in 10.5% (4/38) of animals. For the intraperitoneal study, a single administration of 10^9 WHO fibres was used, with mesothelioma being detected in 33% of rats (8/24) (Cullen *et al*., 2000a).

These results indicate that in a similar way to glass fibre 104/475, glass fibre 100/475 shows little or no carcinogenic potency by inhalation but induces a strong (33%) mesothelioma response following intraperitoneal injection.

439

440 **6.4.3 E Glass**

441 In the Cullen *et al.* study carried out to assess the carcinogenic potential of glass fibre 100/475
442 discussed above, 104E glass fibres ("E Glass") were also assessed at an inhalation exposure level of
443 1000 fibres/ml for 7 h/day, 5 days/wk over 12 months. Glass fibre 104E was shown to include a large
444 amount of very fine fibres (<0.1um diameter). At the end of the exposure period, 4 of 47 rats in the
445 exposure group were sacrificed; lung histopathology showed alveolar thickening, macrophage
446 infiltration and pulmonary fibrosis. Following the 12 month recovery period, histopathology showed
447 advanced alveolar fibrosis and bronchoalveolar hyperplasia, with 7 carcinomas, 3 benign adenomas
448 and 2 mesotheliomas in the remaining animals. Cullen *et al.*, (2000a) also assessed 104E glass fibres
449 using an intraperitoneal model, with a single injection of 1000 fibres. In this study, the majority of
450 the group (87.5%; 21/24) developed mesothelioma. Thus both the IP and inhalation methods
451 showed 104E to be carcinogenic.

452

453 **6 .4. Refractory Ceramic Fibres (RCF)[3]**

454 The carcinogenicity of Fiberfrax ceramic wool has been assessed using inhalation, intraperitoneal
455 and intratracheal models. An inhalation study in rats did not result in tumours, but was associated
456 with fibrosis in 22% of animals. In the hamster, a mesothelioma incidence of 1% was recorded with a
457 low incidence of fibrosis (1%) (Smith eta l., 1987). Intratracheal studies showed no, or very small
458 tumour incidence rates numbers, whereas intraperitoneal studies reported high incidences of
459 tumours (68.1 and 83%) in two separate studies (Smith et al., 1987; Pott et al., 1987).

460

461 In a one year inhalation study, Mast et al. (1995a) assessed the toxicity/carcinogenicity of three
462 types of size-selected (length 20 µm and diameter 1 µm) RCF fibres - kaolin-based, high purity, and
463 aluminium zirconia silica - delivered at 30 mg (220 fibres/cm^{-3}). Interstitial and pleural fibrosis was

[3] More correctly called aluminium silicate wools (ASW). Composition of these fibres varies according to the specific ingredients and quantities used in their manufacture.

apparent from 6 and 9 months respectively for all fibres. Pulmonary neoplasms (bronchoalveolar adenomas and carcinomas) were observed in 13, 15.7 and 7.4% of animals exposed to kaolin-based, high purity and aluminium zirconia silica fibres respectively; these were statistically significantly higher than unexposed controls. Pleural mesotheliomas were observed in 1.6% of animals exposed to kaolin-based RCF, in 2.5% exposed to high-purity RCF and 1.7% exposed to aluminium zirconia silica RCF.

A two-year multi-dose inhalation study by the same authors using kaolin-based RCF (as described above) at levels of 3, 9 or 16 mg m^{-3} (36, 91 and 162 fibres/cm^{-3} respectively) reported interstitial fibrosis and focal pleural fibrosis at 12 months in the two highest dose groups. Neoplasms (bronchoalveolar adenomas and carcinomas) were observed in 1.6, 3.9 and 1.6% of animals exposed to kaolin-based RCF at 3, 9 and 16 mg m^{-3} respectively; these incidences were not statistically significantly higher than in the unexposed controls. A single pleural mesothelioma was also observed in one animal (0.8%) exposed to 9 mg/m^3 of kaolin-based RCF (Mast et al., 1995b).

In a subsequent review of their single-dose RCF study (Mast et al., 1995a) using mathematical modelling to assess deposition, clearance and retention of RCF fibres and taking into account the concept of 'overload', Mast et al. (2000) suggested that a level of 30 mg m^{-3} may have exceeded the maximum tolerated dose, which would have overloaded the lung and had a major impact on the observed chronic adverse effects (Mast et al., 2000).

The RCF sample used in the Mast et al. studies is believed to contain more non-fibrous materials than MMVFs, which could have had a serious effect on the results obtained, potentially leading to a false positive result. Therefore, in a subsequent study the RCF1 (kaolin-based) sample administered was processed in the same way as MMVF samples, as reported by Hesterberg *et al*. (1995), giving RCF1a.

The size-selected RCF1a sample was used by Bellmann *et al.* (2001) in a short term nose-only inhalation study and compared to the results for the original RCF1. Female Wistar rats were exposed for 6 h/day, 5 days/wk for 3 wk to either RCF1a or RCF1 fibre aerosol at a concentration of about 125 fibres (>20µm long)/ml; due to differences in the nonfibrous particle content, the average gravimetric aerosol concentration differed between the two samples (RCF1, 51.2 mg/m^3; RCF1a, 25.8 mg/m^3). The post-treatment observation period was 12 months. The clearance function of alveolar macrophages was seen to be severely retarded following exposure to RCF1 but not RCF1a. In both groups, a significant increase in polymorphonuclear leukocyte and lymphocyte counts was shown 3 days following the end of exposure, which persisted longer (remaining high at 3 months post exposure) in the RCF1 group than in the RCF1a, indicating persistent inflammation. Histopathology showed the presence of inflammatory changes, with similar fibrotic and hyperplastic changes in both RCF1a and RCF1; however, at the end of the 12 month post exposure period the fibrotic changes were only present in the RCF1 group. The authors suggested that the difference in the results between RCF1 and RCF1a could be explained by an increased number of shorter fibres (falling outside the WHO definition) in RCF1a compared to RCF1, and also noted that the numerous lesions seen in the RCF1 exposure group resembled those seen in a lung overload study. This study casts thus further doubts on the RCC studies on RCF, and also raises the issue that non-fibrous components found in the administered aerosol could lead to inflammation and in turn tumour production (Brown et al, 2000; Bellmann *et al*., 2001).

An intraperitoneal study was conducted as part of the Colt Fibre Research Programme (CFRP) using RCF1, at a target dose of 10^9 WHO fibres (Miller et al., 1999) This study not only looked at mesothelioma production, but also the importance of fibre length, biopersistence and dissolution rate in relation to tumour production. Administration of RCF 1 was associated with an 88% incidence of mesotheliomas. The intraperitoneal exposure method using RCF1 thus produced a high

516 incidence of tumours, differing significantly from the inhalation method. Miller et al. (1999) also
517 reported mesothelioma in rats administered zirconia aluminosilicate RCF2 (188.8 mg) by
518 intraperitoneal injection, with an incidence of 72%.
519

520 **6.5 Titanium Dioxide**

521 Titanium dioxide (anatase) was used as a non-carcinogenic control dust in the large carcinogenicity
522 study of around 50 dusts conducted by Pott *et al*. (1987). Doses ranging from 10 to 100 mg were
523 administered by the intraperitoneal route, resulting in tumour rates of between 0% and 9.4% (see
524 Table 3). Muhle *et al*. (1987) similarly reported an absence of tumours following administration of a
525 single intraperitoneal dose of 10 mg of the anatase form of titanium dioxide. Pott and Roller
526 included the anatase form of titanium dioxide (ultra-fine and fine) in their large study of 19 dusts.
527 Intratracheal instillation of ten 6 mg doses resulted in 69.6% and 29.5% incidence of tumours for the
528 ultra-fine and fine forms respectively (Pott and Roller, 2005). It is likely that these high tumour yields
529 were due to an overload effect consequent to the very high doses delivered (total 60mg).
530

531 The rutile form of titanium dioxide was used by Cullen *et al*. (2000b) in an inhalation study at two
532 doses (25mg/m^3 for 209 days and 50 mg/m^3 for 118 days) designed to produce overload effects. A
533 whole body exposure chamber was used, with animals being exposed for 7 hours per day for 5 days
534 a week and sacrificed at 6 different time points. Histopathological examination showed Type II
535 hyperplasia with thickening of the alveolar walls, and the presence of macrophages containing dust
536 particles, but no significant fibrogenic activity (Cullen *et al*., 2000b). These findings are in agreement
537 with those reported by Donaldson *et al.* (1988) from an inhalation study on titanium dioxide
538 delivered to rats at 10mg/m^3 over periods of 32 and 75 days (Donaldson *et al*., 1988). However, Lee
539 et al. (1986) reported contrasting findings from a 2 year inhalation study involving exposure to 10, 50
540 and 250 mg/m^3 rutile titanium dioxide. At the lowest dose, dust laden macrophages were noted; at

the medium dose, thickening of the alveolar walls in addition to macrophage infiltration and one bronchoalveolar adenoma was found. The highest dose animals showed similar responses to those of the mid dose group in the first year, but went on to develop bronchoalveolar adenomas and 14 cystic keratinizing squamous carcinomas in 25 (from a total of 151) animals, although these were difficult to differentiate from squamous metaplasia. While tumours were seen to develop in this study, it was determined that this was due to the excessive dose used (leading to overload conditions) in the study which is unlikely to occur or be relevant to human exposure (Lee et al., 1986).

For titanium dioxide, the inhalation, intratracheal and intraperitoneal models all generally indicate no or low carcinogenic potential except when exceptionally high doses are administered, leading to overload effects.

6.6 Silicon Carbide

6.6.1 Silicon carbide whiskers

Intraperitoneal, inhalation and intrapleural studies have been conducted on silicon carbide whiskers. In a dose-range finding study, Adachi and colleagues administered a single dose of 10 mg/rat of nine different fibre types including silicon carbide whiskers by the intraperitoneal route to female F344 rats. This resulted in development of peritoneal mesothelioma in all test animals within a year, leading the authors to conclude that a reduced dose of 5 mg/rat of silicon carbide whiskers was appropriate for the main study. A year after administration of 5mg/rat, 70% of the animals had developed mesothelioma (Adachi *et al*., 2001). UICC chrysotile B was used as a positive control, resulting in a 70% incidence of mesothelioma, one year after administration.

In a long-term study, rats were exposed by whole-body inhalation to silicon carbide whiskers for 7 hours/day, 5 days/week, for 41 weeks; amosite asbestos was used as a positive control (Davis et al.,

1996). Following the exposure period some rats (n=42) were assessed for life and in these animals 20 tumours of the lung and pleura were recorded (5 carcinomas, 5 adenomas and 10 malignant mesotheliomas). A few animals had more than one type of tumour so that the number of tumour-bearing animals was reported to be 16. In comparison to amosite, silicon carbide produced fewer tumours in the lung parenchyma, but produced a total of 10 mesotheliomas compared with 2 related to amosite exposure.

In a biopersistence inhalation study, male Wistar rats were exposed to silicon carbide whiskers at a concentration of 2.6 ± 0.4 mg/m^3 (98 ± 19 fibres/ml) for 6 hours a day, 5 days a week for up to 1 year (Akiyama *et al.* 2007). This dose was chosen as it was close to the occupational exposure limit for silicon carbide whiskers at that time. Histopathological examination showed fibrotic changes in the lung including thickening of the alveolar walls, macrophage infiltration, aggregation of fibres, and bronchoalveolar hyperplasia in two animals.

Johnson and Hahn (1996) reported findings of a carcinogenicity study using a single intrapleural administration of 20 mg silicon carbide whiskers of different lengths, containing either 5.6×10^8 fibres/kg bw, 1.2×10^{7}fibres/kg bw or 8×10^8 fibres/kg bw (named SiCW 1, SiCW 2 and SiCW 3 respectively) to female rats. Animals were assessed over their lifetime and those treated with SiCW 1 and SiCW 2 developed pleural mesotheliomas at a rate of 90% and 87% respectively. In comparison, 23% of those treated with SiCW3 and 57% of the positive controls treated with crocidolite developed pleural mesotheliomas.

6.6.2 Granular silicon carbide

The granular, non-fibrous, form of silicon carbide has been assessed through intraperitoneal studies. Roller et al. (1996) examined groups of male or female rats for up to 30 months for tumours in the abdominal cavity after repeated (5 or 20) intraperitoneal injections of 50 mg of granular silicon

carbide (equivalent to approximately 667 mg/kg bw and 2,666 mg/kg bw). From a total of 395 rats, only two mesotheliomas were found. Pott et al. (1994) also reported no increase in tumours in a carcinogenicity study with non-fibrous silicon carbide administered to rats by repeated (5 or 20) intraperitoneal injections of 50mg (equivalent to approximately 667 mg/kg bw and 2,666 mg/kg bw).

6.7 Potassium Octatitanate

The intraperitoneal study carried out by Adachi *et al*. (2001) described above, also investigated potassium octatitanate under the same conditions of exposure. In the dose-range finding study, a 77% incidence of mesothelioma was present, indicating that a reduced dose of 5mg/m^3 was appropriate for the follow-on study. In the second study, exposure to potassium octatitanate resulted in an incidence of 20% mesotheliomas (Adachi *et al*., 2001).

Yamato *et al*. (2003) conducted a low exposure (2.2 ± 0.7 mg/m^3 or 111 ± 34 fibres/ml) long term inhalation study, exposing male Wistar rats to potassium octatitanate for one year for 6 h per day, 5 days per week. Histopathology showed the presence of mild fibrotic changes around macrophages that had engulfed the fibres at 3 days, 6 and 12 months. No malignant pulmonary tumours were observed, although adenomas were found in 2 rats (3.4%; 2/59) at 6 months post exposure and in 1 rat (1.7%; 1/59) at 12 months. Squamous metaplasia was also found in 1 rat (1.7%; 1/59) at the 12 month period. In a chronic inhalation study, Ikegami *et al*., (2004) reported toxicological findings following exposure of male Fischer 344 rats via whole-body inhalation to 0, 20, 60, or 200 WHO fibres/cc of potassium octatitanate for 6 h/day, 5 days/w for 24 months. At the mid dose, alveolar wall thickening and minimal alveolar fibrosis were noted following 18 and 24 months of exposure. At 200 fibres/cc exposure, slight alveolar wall thickening was apparent after 12 months of exposure and slight alveolar fibrosis after 18 and 24 months of exposure. No exposure-related pulmonary neoplasms or mesotheliomas were observed.

An intratracheal instillation study investigating lung burden and biopersistence was carried out by Oyabu et al. (2006) utilising data from the inhalation study reported by Yamato et al. (2003). The authors interpreted that the data showed a threshold and that the dose would lie between 1.5 and 2.4 mg; one of four doses (0.5, 1, 2 and 5mg) were therefore instilled into male Kud:Wistar rats, which were sacrificed at different time points for up to one year. Dose-related fibrotic changes and thickening of the alveolar wall were observed (not included in Table 3 as no quantitative data). Thus inhalation and intratracheal exposure to potassium octatitanate gave rise to no malignant tumours, whilst the intraperitoneal study produced a high tumour yield.

6.8 Quartz

Quartz (a known macrophage toxin) was one of the dusts included in two of the intraperitoneal studies carried out in the large study by Pott *et al*. (1987). The two doses used (10 mg and 40 mg) induced tumours (sarcoma, mesothelioma or carcinoma in the abdominal cavity) at rates of 5.9% and 22% respectively. These results can be compared to those from intratracheal instillation experiments carried out using quartz. In a short-term (1 month) study, Luchtel *et al*. (1989) used quartz as a positive control at a single dose of 5mg, which was associated with fibrotic lesions and increased numbers of macrophages in the alveoli (study not included in Table 3 due to short duration). In 2005, Pott and Roller conducted the "19 dust study" to test the carcinogenicity of a number of dusts using intratracheal instillation. Quartz was used as a positive control due to its known toxicity. Exposure to single instillation doses of 5 and 10 mg resulted in total tumour (adenoma, adenocarcinoma or squamous mixed cell carcinoma in the lung) incidences of 65.7% and 71.4% respectively. Instillation of a higher dose of 20 mg, delivered in two doses of 10 mg each, resulted in a 77.8% incidence of total lung tumours (Pott and Roller, 2005).

Although intraperitoneal and intratracheal studies confirm a tumorigenic response in the lungs following exposure to quartz, a difference in the degree of tumour development is evident. At the

10 mg dose level, intraperitoneal injection resulted in an incidence of 5.9% tumours (Pott *et al*., 1987) while the intratracheal study gave rise to 57.1% tumours (Pott and Roller, 2005).

6.9 Kevlar

The aramid fibre 'Kevlar' was assessed for carcinogenic potential by Pott et al. (1987) using an intraperitoneal model, and by Warheit et al. (1994) in a 3 month inhalation study. In the intraperitoneal study, 5.8% of rats administered 20 mg of Kevlar fibres (5 x 4mg) showed tumour development. In the inhalation study, Crl:CD BR rats were exposed for 5 days to aerosols of Kevlar fibrils (900-1344 f/cc; 9-11 mg/m^3). No pulmonary lesions were observed, which was considered to be due to the rapid clearance of the Kevlar fibres. One chronic inhalation study on Kevlar has been reported by Lee et al. (1988). Rats (male and female) were exposed to Kevlar fibrils at concentrations of 0, 2.5, 25, and 100 fibrils/cc for 6 h per day, 5 days per week for 2 years. One group was also exposed to 400 fibrils/cc for 1 year and allowed to recover for 1 year. Lung tumours were observed in treated animals, however, the authors considered these to be a unique type of experimentally induced tumour (cystic keratinizing squamous cell carcinoma) and not of relevance to the human situation.

Thus both intraperitoneal and inhalation experiments appear to indicate low carcinogenic potential for Kevlar.

6.10 Polypropylene

Polypropylene fibres have been assessed for carcinogenic potential using inhalation and intraperitoneal exposure models. Hesterberg et al. (1992) administered polypropylene fibres at 15, 30, or 60mg/m^3 (actual doses achieved were 13.03, 28.07 and 59.61 mg/m^3) by nose-only inhalation to male Fischer rats for 6 h per day, 5 days per week for 90 days. A dose-dependent increase in pulmonary macrophages and reversible increase in mild cellularity were noted. In an intraperitoneal study carried out by Pott et al. (1987) as part of a large carcinogenic study of around 50 dusts,

672 female Wistar rats were administered 50mg of polypropylene fibres (5 doses of 10 mg), resulting in
673 only a 2% tumour incidence.
674
675 Thus both exposure methods provide evidence that polypropylene fibres are non-carcinogenic (Pott
676 *et al*., 1987; Hesterberg *et al*., 1992).
677
678 Table 3 shows a comparison of all available results for each of the fibre types used in the inhalation,
679 intratracheal and intraperitoneal studies described above. The data have been collated to include
680 the dose and size (distribution given where available in original study) of each fibre type, along with
681 (where available) the types of tumour produced, whether fibrosis was present, and the total
682 percentage of tumours produced. It should be noted that for accuracy, exposure concentration is
683 given as cited in the original study (i.e. fibre number is given only when originally cited), however,
684 should the reader wish to do so, calculations are available to convert gravimetric concentration to
685 fibre number/cm^3. In addition, cumulative exposure is cited if given in the original study, if not cited,
686 exposure durations are detailed should the reader wish to calculate the cumulative dose.
687

688 **Table 3 Summary of toxicity study findings utilising inhalation, intratrachael and intraperitoneal models of exposure**

Reference	Material Exposure Method IH/IT/IP	Exposure Duration (hrs/day, days/wk, total months, INH) or months (IP)[1]	Length (µm)	Diameter (µm)	Mass / Fibre concentration[2,3]	Percentage of animals with tumours / histopathological lesions[4]						
						Mesothelium/abdominal cavity		Lung				
						Mesothelioma	Total abdominal tumours	Carcinoma	Adenoma	Bronchiolo-alveolar hyperplasia	Fibrosis	Total pulmonary tumours
Asbestos												
Hesterberg *et al.*. 1995	Chrysotile IH (rat)	6.5.24	>5	<3	10mg m^{-3} (1.1 ± 1.1 x10^4 WHO fibres/cm^{-3})	1.4	NS	NS	NS	NS	Yes	18.9
	Chrysotile IH (hamster)	6.5.18			10mg m^{-3} (3000 ± 1400 WHO fibres/cm^{-3})	0	0	0	0	0	No	0
Muhle *et al.,* 1987	Chrysotile (UICC) IH	5.4.12 (12 month follow-up)	2.0 - 14	0.28 - 1.6	6.0 mg m^{-3} (131 + 72 fibres 1 > 5 µm) ^{SD} Cumulative exposure of 6000 mg h m^{-3}	-	-	0	0	12	Yes (42)	12
	Chrysotile (UICC) IP	24	0.3 - 3.6	0.08 - 0.18	1.0 mg ^{SD} (single dose)	NS	84	-	-	-	-	-
	Chrysotile (Calidria)	24	0.4 - 5.9	0.02 - 0.10	0.5mg ^{SD} (single dose)	NS	6	-	-	-	-	-

	IP											
Pott *et al.,* 1987	Chrysotile (UICC A) IP	Up to 30	9	0.15	6 mg (single dose)	NS	77.1	-	-	-	-	-
		Up to 30			25 mg (single dose)	NS	80.6	-	-	-	-	-
	Chrysotile (UICC B) IP	Up to 30	0.9	0.11	0.05 mg (single dose)	NS	19.4	-	-	-	-	-
		Up to 30			0.25 mg (single dose)	NS	61.8	-	-	-	-	-
		Up to 30			1 mg (single dose)	NS	84.4	-	-	-	-	-
	Chrysotile (PVNO) IP	Up to 30	0.9	0.11	1 mg (single dose)	NS	80.0	-	-	-	-	-
	Chrysotile (Calidria) IP	Up to 30	1.2	0.03	0.5 mg (single dose)	NS	6.3	-	-	-	-	-
Adachi *et al.,* 2001	Chrysotile (UICC B) IP	24	>5	<3	10 mg (10 x 1 mg)	85	85	-	-	-	-	-
Smith *et al.,* 1987	Crocidolite (UICC) IH	6.5.24	≤5 (95%)	2.5 ± 0.2 µm-	7 mg 3000 fibres/cm^{-3} SD	1.8	NS	NS	NS	8	Yes (53)	3.5
Muhle *et al.,* 1987	Crocidolite (South Africa) IH	5.4.12 (12 month follow-up)	0.72 – 4.5	0.17 – 0.46	2.2 (± 1.3) mg m^{-3} SD Cumulative exposure of 2200 mg h m^{-3}	-	-	1	0	74	Yes (36)	76
	Crocidolite (South Africa) IP	24			0.5 mg SD (single dose)	NS	55	-	-	-	-	-

Hesterberg *et al.,* 1995	Crocidolite IH	6.5.24	>5	<3	10 mg m^{-3} (0.16 ± 0.1 $x10^4$ WHO fibres/cm^{-3})	0.9	0.9	NS	NS	NS	Yes	14.2
Pott *et al.,* 1987	Crocidolite IT	Up to 30	2.1	0.2	10 mg (20 x 0.5)	-	-	31.4	0	-	-	42.9
	Crocidolite IP	Up to 30			0.5 mg	NS	56.3	-	-	-	-	-
					2 mg	-		-	-	-	-	-
Lambre *et al.,* 1998	Crocidolite IP	32.5	>5	<2	0.005 mg (1.9×10^6 fibres) ^{SD} (single dose)	7.8	7.8	-	-	-	-	-
		32.5			0.05 mg (18.9×10^6 fibres) ^{SD} (single dose)	15.7	19.6	-	-	-	-	-
		32.5			0.5 mg (188.6×10^6 fibres) ^{SD} (single dose)	39.2	49.0	-	-	-	-	-
Grimm *et al.,* 2002	Crocidolite IP	31	>5, <15	<3	27 mg (0.5×10^6 WHO fibres) ^{SD} (single dose)	52.9	NS	-	-	-	-	-
		31			45 mg (5.0×10^6 WHO fibres) ^{SD} (single dose)	88.2	NS	-	-	-	-	-
Cullen *et al.,* 2000a	Amosite IH	7.5.12 (12 month follow-up)	>0.4, <20	>0.1, <0.9	1000 fibres/ cm^{-3}	4.8	NS	16.7	21.4	-	Yes	38.1
	Amosite	24			10^9 fibres (single dose)	81	NS	-	-	-	Yes	NS

	IP											
Wollastonite												
Warheit *et al.* 1994	Wollastonite IH	6.5.0 (6 month follow-up)	-	Aero-diam 2.6 (± 2.0) – 4.3 (± 2.2) µm -	59 - 114 mg m^{-3} (123 - 835 fibres/ cm^{-3})	-	-	-	-	-	Yes (mild)	-
Tátrai *et al.* 2004	Wollastonite IT	6	10 – 20 (median)	≤ 1 (median)	1 mg (single dose) ^{SD}	-	-	-	-	-	Yes (mild)	-
Pott *et al.* 1987	Wollastonite IP	Up to 30	5.2	1.1	100 (5x20mg)	NS	0	-	-	-	-	-
Man-made vitreous fibres												
McConnell *et al.* 1994	MMVF rock wool IH	6.5.24	>5	<3	3, 16, 30 mg m^{-3}	No	-	-	-	-	Yes (v mild for all doses)	NS
	MMVF slag wool IH	6.5.24	>5	<3	3, 16, 30 mg m^{-3}	No	-	-	-	-	No (for all doses)	NS
Hesterberg *et al.* 1995	MMVF 10 (fibreglass) IH	6.5.24	0 - > 100	0 - > 3	30 mg $m^{-3\ SD}$	0	0	NS	NS	NS	No	5.9
	MMVF 11 (fibreglass) IH	6.5.24				0	0	NS	NS	NS	No	2.7
	MMVF 21 (rock wool)	6.5.24				0	0	NS	NS	NS	Yes	4.4

	IH											
	MMVF 22 (slag wool) IH	6.5.24				0	0	NS	NS	NS	No	2.6
Hesterberg *et al.* 1998b	Synthetic vitreous fibre X607 IH	6.5.24	11 ± 4	0.9 ± 0.3	30 (± 6) mg m^{-3} (174 ± 72 WHO fibers/cm^{-3}) SD	0	-	0.8	0.8	4.9	-	1.6
Kamstrup *et al.* 2001	Stone wool – HT (MMVF34) IH	6.5.24	11.1	0.98	30 mg m^{-3}	-	7	4.7	5.6	-	No	NS
Kamstrup *et al.* 2004	Stone wool – HT RIF41001 IH	6.5.3 (3 month follow-up)	44.2 ± 1.7	0.75 ± 1.9	15, 50, 150 fibres/ cm^{-3} (>20 µm long)	-	-	-	-	-	No for all doses (after 3 months)	NS
	RIF42020-6 IH	6.5.3 (3 month follow-up)	36.5 ± 1.5	0.72 ± 1.9	15, 50, 150 fibres/ cm^{-3} (>20 µm long)	-	-	-	-	-	No for all doses (after 3 months)	NS
	RIF43006-1 IH	6.5.3 (3 month follow-up)	38.1 ± 1.6	0.63 ± 1.2	15, 50, 150 fibres/ cm^{-3} (>20 µm long)	-	-	-	-	-	No for all doses (after 3 months)	NS
Miller *et al.* 1999	MMVF10 (glass wool) IP	Assessed for life	>0.4 - > 20	< 0.95 > 0.95	144.4 mg (single dose) SD	59	-	-	-	-	-	-
	MMVF21 (stone wool) IP	Assessed for life			183.1 mg (as 2 doses) SD	95	-	-	-	-	-	-

	MMVF 22 (slag wool) IP	Assessed for life			129.6 mg (single dose) SD	54	-	-	-	-	-	-
Glass Fibres 104/475												
Muhle *et al.* 1987	Glass fibre 104/475 IH	5.4.12 (12 month follow-up)	2.0 – 12.4	0.23 – 0.80	3.0 mg m^{-3} SD Cumulative exposure of 3000 mg h m^{-3}	-	-	0.9	0	11	Yes (38)	NS
	Glass fibre 104/475 IP	24	1.4 – 8.4	0.09 – 0.40	0.5 mg SD (single dose)	NS	17	-	-	-	-	-
Pott *et al.* 1987	Glass fibre 104/475 IT	Up to 30	3.2	0.18	10 mg (20 x 0.5 mg)	-	-	11.8	2.9	-	-	14.7
	Glass fibre 104/475 IP	Up to 30			0.5 mg (single dose)	NS	16.7	-	-	-	-	-
		Up to 30			2 mg (single dose)	NS	25.8	-	-	-	-	-
		Up to 30			5 mg (5 x 1 mg)	NS	66.0	-	-	-	-	-
Glass Fibre 100/475												
Cullen *et al.* 2000a	Glass fibre 100/475 IH	7.5.12 (12 month follow-up)	>0.4, < 20	<0.1, <0.9	1000 fibres/ cm^{-3} (single dose)	0	NS	-	10.5	-	No	NS
	Glass fibre 100/475 IP	24			10^9 WHO fibres (single dose)	33	NS	-	-	-	-	NS
E Glass												
Cullen *et al.* 2000a	E Glass microfiber	7.5.12 (12 month	>0.4, <20	<0.1, <0.9	1000 fibres/ cm^{-3}	4.7	-	16.2	6.9	-	Yes	23.2

	104E IH	follow-up)										
	E Glass microfiber 104E IP	24			10^9 WHO fibres (single dose)	87.5	NS	-	-	-	-	-
Refractory Ceramic Fibres												
Smith *et al.* 1987	Ceramic wool Fiberfrax IH (rat)	6.5.24 (follow-up for life)	25	1.8	200 fibres/cm^{-3} 12 mg $m^{-3\ SD}$	0	-	-	-	2	Yes (22)	0
	Ceramic wool Fiberfrax IH (Hamster)	6.5.24 (follow-up for life)	25	1.8	200 fibres/cm^{-3} 12 mg $m^{-3\ SD}$	1	-	-	-	3	Yes (1)	0
	Ceramic wool Fiberfrax IT (rat)	assessed for life	25	1.8	10 mg ^{SD} (2 x 5 mg)	-	-	-	-	27	Yes (9)	0
	Ceramic wool Fiberfrax IP (rat)	assessed for life	25	1.8	25 mg ^{SD} (single dose)	83	NS	-	-	-	Yes (100)	NS
Pott *et al.* 1987	Ceramic wool, Fiberfrax IP	Up to 30	8.3	0.91	45 mg (5 x 9 mg)	NS	68.1	-	-	-	-	-
Hesterberg *et al.* 1995	RCF1 IH (rat)	6.5.24	0 - > 100	0 - >3	30 mg $m^{-3\ SD}$	1.6	1.6	NS	NS	NS	Yes	13
	RCF1 IH (Hamster)	6.5.18	0 - > 100	0 - >3	30 mg $m^{-3\ SD}$	41	41	NS	NS	NS	Yes	0
Mast et al., 1995a	RCF1 (Kaolin based)	6.5.24	12.8 – 17.4	0.8	30 mg m^{-3} (187 WHO	1.6	1.6	Yes	Yes	NS	Yes	13 (bronchoal

	IH	(Up to 6 months follow-up)			fibres/cm^{-3})$_{SD}$							veolar adenoma and carcinoma combined)
	RCF2 (alumina zirconia silica) IH	6.5.24 (Up to 6 months follow-up)			30 mg m^{-3} (220 WHO fibres/cm^{-3})$_{SD}$	2.5	2.5	Yes	Yes	NS	Yes	7.4 (bronchoal veolar adenoma and carcinoma combined)
	RCF3 (high purity) IH	6.5.24 (Up to 6 months follow-up)			30 mg m^{-3} (182 WHO fibres/cm^{-3})$_{SD}$	1.7	1.7	Yes	Yes	NS	Yes	10.7 (bronchoal veolar adenoma and carcinoma combined)
Mast et al., 1995b	RCF1 (Kaolin based) IH	6.5.24 (Up to 6 months follow-up)	20	1	3 mg m^{-3} (36 WHO fibres/cm^{-3})$_{SD}$	0	0	Yes	Yes	NS	No	1.6 (bronchoal veolar adenoma and carcinoma combined)
		6.5.24 (Up to 6 months follow-up)			9 mg m^{-3} (91 WHO fibres/cm^{-3})$_{SD}$	0.8	0.8	Yes	Yes	NS	Yes	3.9 (bronchoal veolar adenoma and carcinoma combined)
		6.5.24 (Up to 6 months follow-up)			16 mg m^{-3} (162 WHO fibres/cm^{-3})$_{SD}$	0	0	Yes	Yes	NS	Yes	1.6
Bellmann *et al.* 2001	RCF1 IH	6.5.3wk (12 month	10.5	0.94	51.2 mg m^{-3}$_{SD}$	-	-	-	-	-	Yes (5 within	NS

		follow-up)									12 months)	
	RCF1a IH	6.5.3wk (12 month follow-up)	13.3	0.86	25.8 mg m^{-3} $_{SD}$	-	-	-	-	-	Yes (8 within 12 months)	NS
Titanium Dioxide												
Lee *et al.* 1986	Titanium dioxide (rutile) IH	6.5.24	-	Aero-diam 1.5 – 1.7	10 mg	-	-	0.7	0.7	95.2	Yes (7.5)	NS
		6.5.24			50 mg	-	-	0	0.7	100	Yes (60.4)	NS
		6.5.24			250 mg	-	-	9.3	16.6	100	Yes (98.7)	NS
Donaldson *et al.* 1988	Titanium dioxide (rutile) IH	7.5.15(wk) (up to 2 months follow-up)	-	-	10 mg	-	-	-	-	-	No (after 3 months)	-
Cullen *et al.* 2000b	Titanium dioxide (rutile) IH	7.5.8	-	Aero-diam 2.1 (±2.2)	25 mg	-	-	-	-	-	No (after 7 months)	-
		7.5.8			50 mg	-	-	-	-	-	No (after 7 months)	-
Pott and Roller 2005	Titanium dioxide P25 hydrophilic (anatase) IT	28	-	0.025	5 x 3 mg	-	-	NS	NS	NS	NS	52.4
		28			5 x 6 mg	-	-	NS	NS	NS	NS	67.4
		28			10 x 6 mg	-	-	NS	NS	NS	NS	69.6
Pott and Roller 2005	Titanium dioxide P805, AL 90, hydrophobic IT	28	-	0.021	10 x 6 mg	-	-	NS	NS	NS	NS	0
		28			20 x 6 mg	-	-	NS	NS	NS	NS	6.7
Pott and	Titanium	28	-	0.2	10 x 6 mg	-	-	NS	NS	NS	NS	29.5

Roller 2005	dioxide AL 23 203-3 hydrophilic (anatase) IT	28			20 x 6 mg	-	-	NS	NS	NS	NS	63.6
Pott *et al.* 1987	Titanium dioxide (anatase) IP	Up to 30	granular		10 mg (over 3 inj.)	NS	0	-	-	-	-	-
		Up to 30			90 mg (over 5 inj.)	NS	5.3	-	-	-	-	-
		Up to 30			100 mg (5x20 mg)	NS	9.4	-	-	-	-	-
Muhle *et al.* 1987	Titanium dioxide (anatase) IP	24	granular		10 mg (single dose)	NS	9.4	-	-	-	-	-
Silicon Carbide												
Adachi *et al.* 2001	Silicon carbide whiskers IP	24	6.4 ± 2.45	0.3 ± 1.58	5mg (5 x 1 mg) 414 x 10^3 fibres/µg	70	70	-	-	-	-	-
		24			10mg (10 x 1 mg)	100 (within 12 months)	100	-	-	-	-	-
Johnson and Hahn, 1996	Silicon carbide – granular IP	Assessed for life	4.5 (± 0.23)	<1	5.6×108 fibres/kg bw SD	90	-	-	-	-	-	-
		Assessed for life	20.1 (± 1.01)	<1	1.2×107 fibres/kg bw SD	87	-	-	-	-	-	-
		Assessed for life	6.6 (± 0.40)	<1	8×108 fibres/kg bw SD	23	-	-	-	-	-	-
Davis et al., 1996	Silicon carbide whiskers	Assessed for life	5 - 20	0.45	1000 fibres/ cm^{-3}	10	20	-	-	-	Yes	NS

	IH											
Akiyama *et al.,*2007	Silicon carbide whiskers IH	6.5.12 (12 months foloow-up)	2.8 ± 2.3	0.5 ± 1.5 (Aero-dynamic diameter 2.4 ± 2.4)	2.6 (± 0.4) mg m^{-3} (98 ± 19 fibres/cm^{-3})	-	-	-	-	4.7	Yes (severe)	NS
Potassium Octatitanate												
Yamato *et al.* 2003	PT1 potassium octatitanate whiskers IH	6.5.12 (12 months follow-up)	3.4 ± 2.7	0.44 ± 1.4	2.2 ± 0.7 mg m^{-3} (111 ± 34 fibre/cm-^{3})	-	-	-	10	-	Yes (mild)	NS
Ikegami et al. 2004	potassium octatitanate fibres IH	6.5.24	>5	<3	200 WHO fibers/ cm^{-3}	-	-	-	-	-	Yes (mild)	NS
Adachi *et al.* 2001	Potassium Octatitanate (whiskers) IP	24	6 ± 2.04	0.35 ± 1.51	5 mg (5 x 1 mg) 594 x 10^{3} fibres/µg	20	NS	-	-	-	-	-
		24			10 mg (10x 1 mg)	77	NS	-	-	-	-	-
Quartz												
Pott and Roller 2005	Quartz IT	28	granular		5 mg	-	-	NS	NS	NS	NS	65.7
					10 mg	-	-	NS	NS	NS	NS	71.4
		28			10 x 2mg	-	-	NS	NS	NS	NS	77.8

Pott *et al.* 1987	Quartz (DQ12) IP	Up to 30	granular	10 mg (single dose)	NS	5.9	-	-	-	-	-
		Up to 30		40 mg (2x20 mg)	NS	22.0	-	-	-	-	-
Kevlar											
Lee *et al.* 1988	Kevlar IH	6.5.24	< 100	< 3	0.08 (± 0.04) mg 2.4 (±0.8) fibrils/cm^{-3} SD	-	-	0	0.7	0.7	No	NS
		6.5.24			0.32 (± 0.08) 25.5 (± 9.9) fibrils/cm^{-3} SD	-	-	0	0.7	96.9	Yes (93.9)	NS
		6.5.24			0.63 (± 0.14) 100 (± 37) fibrils/cm^{-3} SD	-	-	2.9	2.9	98.5	Yes (96.3)	NS
		6.5.24			- (± 0.46) 411 (± 109) fibrils/cm^{-3} SD	-	-	7.6	7.6	93.5	Yes (96.7)	NS
Warheit et al. 1994	Kevlar Fibrils IH	6.5.0 (6 month follow-up)	-	Aero-dynamic diameter 3.2 (±2.7) – 4.7 (±3.2)	613 – 1344 f/cm^{-3} (2.9– 11.1 mg m^{-3})	-	-	-	-	-	No	-
Pott *et al.* 1987	Kevlar IP	Up to 30	3.9	0.47	20 mg[a] (5x4mg)	NS	5.8	-	-	-	-	-
Polypropylene											
Hesterberg *et al.* 1992	Polypropyl-ene fibers IH	6.5.3 (up to 1 month follow-up)	11.6 – 14.7	1.2	13.03 (± 2.21) mg m^{-3} (12.1 (± 3.5) fibers/ cm-^{3})	-	-	-	-	-	No	-

		6.5.3 (up to 1 month follow-up)			28.07 (± 5.91) mg m^{-3} (20.1 (± 7.1) fibres/ cm^{-3})	-	-	-	-	-	No	-
		6.5.3 (up to 1 month follow-up)			59.61 ± 6.46 mg m^{-3} (48.1 (± 17.2) fibres/ cm^{-3})	-	-	-	-	-	No	-
Pott *et al.* 1987	Polypropyl-ene fibers IP	Up to 30	7.4	1.1	50 mg (5 x 10 mg)	NS	2.0	-	-	-	-	-

[1] Exposure durations are detailed to allow calculation of cumulative dose, if required.
[2] For accuracy, exposure concentration is given as cited in original study. Should the reader wish to do so, calculations are available to convert gravimetric concentration to fibre number/cm^3.
[3] SD indicates that fibre size distribution data is included in the original citation
4 percentage of rats examined with sarcoma, mesothelioma or carcinoma in the abdominal cavity (excluding tumours of the uterus)
' – ' not applicable to study / not identified; NS – identified but numbers not specified; IH – inhalation; IP – intraperitoneal; IT – intratrachael; SD – standard deviation; Aero-diam – aerodynamic diameter ; HT – high aluminium/low silica type wool; WHO fibres are defined by the World Health Organization as having a length/diameter ratio ≥3, diameter <3 µm, and length >5 µm; **a** – non-homogeneous suspension.

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7. Discussion and Conclusions

Following review of all identified data, it is evident that, for a number of the fibres tested, the same or similar carcinogenic potential is exhibited whether inhalation and/or intratracheal, or intraperitoneal exposure models are used. The following fibres demonstrated consistently negative (or equivocally negative) results:

- Wollastonite
- 104/475 glass fibres
- HT stone wool
- Titanium dioxide, except at extremely high doses
- Kevlar, notwithstanding a query about the relevance of certain tumours observed in an inhalation study
- Polypropylene

Only for amosite, silicon carbide whiskers, E-Glass, and possibly crocidolite and quartz, were results consistently positive for both inhalation and intraperitoneal or intrapleural study methods. Crocidolite and quartz were also consistently positive in intratracheal studies.

For other fibre types, markedly different results for carcinogenic potential were obtained with different exposure models. These included:

- Chrysotile
- MMVFs (various types) including specifically:
    - 100/475 glass fibres
    - 104E glass fibres
    - RCF[4]

[4] RCF has tested positive in inhalation and intraperitoneal tests, however there is uncertainty about the positive IH results because of concerns about overload resulting from the high doses used and high particulate to fibre ratio.

- Potassium octatitanate

For silicon carbide, marked differences were noted in the results for whiskers and the granular form in the intraperitoneal studies. Whilst silicon carbide whiskers showed positive findings, the non-fibrous granular form was negative.

A number of important caveats apply in considering the results presented here. The majority of experiments reviewed here were on fibres rather than dusts, and tumour type/location is often different for dusts and fibres. Where the same fibre type has been used in more than one study, there is not always consistency of manufacturer/producer. This could mean that fibres with slightly different chemical compositions are being compared. Even with the same manufacturer, differences may still occur due to inter-batch variations. This issue was highlighted by Guldberg *et al*. (2002) who suggested that stone wool fibres cannot have a defined chemical composition, as variations will necessarily occur during the processing of raw materials. This reasoning would apply to the majority of fibres that are produced from natural raw materials of variable composition. Due to the lack of definitive chemical compositions, it is difficult to confidently compare the study results on the same fibre types, which in turn makes it problematic to definitively compare the results from studies using different exposure methods of the same fibres. Also, not all of the papers reviewed here included sufficient information on the chemical composition of the fibre test materials to allow a truly robust comparison of results. The same is true of fibre/particle size distribution data. There is also a problem with fibre nomenclature. For example, MMVF10 and 11 are glass fibres, but some studies refer to glass wool or different types of glass fibre, which may or may not be the same; it is difficult to confidently compare these studies without the specific chemical composition data to determine if they are indeed the same fibre type.

Finally, the issue of dose is problematic in an exercise such as this. The majority of intraperitoneal studies and some intratracheal studies use one single large dose (or a limited series of smaller doses), while in the inhalation studies exposure is to a low concentration extended over a longer period of time. As a result, doses are difficult to directly compare. In addition, lung overload can occur and can lead to false positives, as shown for example in the study reported by Lee et al. (1986) and implied for the RCF experiment by Mast (1995a). This underlines the importance of determining a relevant and appropriate dose when designing studies, in order to be confident in the validity of the findings. The same argument no doubt applies to intrapleural/intraperitoneal testing where the basis and validity of the amount of material injected is subject to even greater uncertainty. In all examples, the question of relevance to human exposures remains a source of uncertainty.

To summarise, for some of the dusts and fibres reviewed, there is conformity between the results of intraperitoneal and inhalation such that they are either consistently positive (a few only) or consistently negative. For the remaining dusts and fibres reviewed, intraperitoneal and inhalation tests give different results, with positive results in the intraperitoneal test not being reflected by positive inhalation test results. In no circumstances was a positive inhalation study reflected by a negative intraperitoneal study.

Intraperitoneal studies appear to be more 'sensitive' to the carcinogenic potential of injected materials, but as noted earlier this is a highly non-physiological route of exposure and false positive results cannot be discounted. As shown in this paper, positive IP/IPl study results for carcinogenicity are not consistently reflected by positive results in inhalation studies.

763 The German Committee on Hazardous Substances (AGS) document 'ERR (exposure-risk relationship)
764 for aluminium silicate fibres[5]' makes the assumption that IP tests are able to accurately and in a
765 quantitative fashion discriminate fibres in relation to their carcinogenic potency in the lung. As a
766 consequence the AGS reaches the conclusion that certain types of MMMF pose a carcinogenic risk
767 the same order of magnitude as crocidolite asbestos (Harrison et al., 2015). For the application of
768 animal test results to human cancer risk assessment, it is very important to understand the strengths
769 and weaknesses of the different methods used and the consistency or otherwise of the results
770 obtained. Pott (1991) argued that while the inhalation method was the best to use for
771 carcinogenicity testing of airborne *particles*, this is not the case in relation to respirable *fibres* and
772 that false negatives should be expected. In line with this argument, Wardenbach et al. (2000)
773 expressed reservations about the results obtained with asbestos in rodent inhalation studies
774 compared to the human experience. Pott recommended that intratracheal, intrapleural and
775 intraperitoneal instillation rather than inhalation should be used to determine the carcinogenicity of
776 respirable fibres (Pott, 1991; Pott *et al.*, 1992). However, the opposite view has been expressed by
777 many researchers. For example, Lippmann (2014), noting the findings of a review by Hesterberg and
778 Hart (2001) which showed that positive results for carcinogenicity with a number of MMVF in
779 injection/instillation studies were not replicated in well-conducted inhalation tests, concluded that
780 "implantation studies are not appropriate for assessing the potential hazard of SVFs in humans
781 exposed by inhalation". This is in line with the conclusions by McClellan et al. (1992) regarding the
782 superiority of inhalation testing.
783
784 From the results of this survey it may be concluded that the intraperitoneal test can be used to
785 exonerate a dust or fibre (because if negative in the intraperitoneal test it is extremely unlikely to be

[5] Exposure-risk relationship for aluminium fibres. Committee on Hazardous Substances (AGS) - AGS Management - BAuA - www.baua.de. May 2010.

positive in either inhalation or intratracheal tests)[6] but it should not be used to determine that a dust or fibre would be carcinogenic by inhalation (Bernstein et al., 2001b). We would argue against the use of intraperitoneal tests for human health risk assessment except perhaps for the purpose of exoneration of a material from classification as a carcinogen.

Conflict of Interest Statement

The named authors all contributed to this paper. Gail Drummond is a PhD student at the University of Hertfordshire with research interests in this area. Paul Harrison and Ruth Bevan are independent toxicology/risk assessment consultants; they are not involved in legal testimony related to the materials and products discussed and do not have any form of commercial interest in them. Paul Harrison acts as an advisor to ECFIA (an association representing the high temperature insulation wool industry) in matters relating to health and safety.

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[6] This is in line with an earlier statement by Pott et al. (1987) that *"…if a high dose [of a dust] does not induce tumours in [the intraperitoneal] test, no suspicion of carcinogenic potency can be substantiated".*

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A comparison of the results from intra-pleural and intra-peritoneal studies with those from inhalation and intratracheal tests for the assessment of pulmonary responses to inhalable dusts and fibres.

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Highlights

- Comparison of findings from inhalation, intraperitoneal and intrapleural assays.
- Focus on fibrous and particulate materials.
- Assessment of the prediction of carcinogenicity using IT/IP studies.
- It is suggested that IP studies can only be used to exonerate a dust or fibre.
- Carcinogenicity of these should not be positively identified using IP studies.