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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 vivo* 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.

2

1   **1. Introduction**

2   Hazard characterisation of the toxicity and carcinogenic potential of airborne dusts and fibres is

3   usually carried out using *in vivo* test methods, although *in vitro* approaches are increasingly being

4   utilised to investigate specific relevant toxicological parameters. The *in vivo* models used include

5   inhalation (IH; whole body or nose-only), intratracheal instillation (IT), intraperitoneal injection (IP)

6   and intrapleural injection (IPl). The intratracheal test essentially aims to replicate exposure by

7   inhalation, while intraperitoneal/ intrapleural injection tests investigate the toxicity of fibres to the

8   mesothelium and have also been used to assess carcinogenic potency. As explained more fully

9   below, although expensive to conduct, inhalation toxicity studies are still generally viewed as the

10 'gold standard' for airborne dusts and fibres (McLellan et al., 1992; Pauluhn & Mohr, 2000).

11

12 The toxicity of inhaled fibres is described by the so called '3Ds' paradigm which recognises that the

13 most important parameters are **d**ose, **d**imension (fibre length and diameter, which determine both

14 respirability and pathogenicity in the lung) and **d**urability (or, more properly, biopersistence)

15 (Bernstein et al., 2001a; Bernstein et al., 2001b; Brown & Harrison, 2012; Donaldson et al., 2013).

16 For dusts (rather than fibres) the fundamental concerns are fibrogenicity and cancer due either to

17 inherent pulmonary toxicity or to lung overload effects (Oberdorster, 1995; Pauluhn, 2014; Borm et

18 al., 2015; Morfeld et al., 2015).

19

20 The heterogeneity of toxic responses to fibres at different locations in the respiratory tract has been

21 described, for example, by Donaldson et al. (2013) using the well documented example of asbestos.

22 The principal pathologies are as follows:

23

24   • Lung parenchyma – interstitial fibrosis with accumulation of fibrous/scar tissue;

25   • Bronchi/bronchioles – bronchogenic carcinoma (malignant cancer of the cells lining the

26   airways);

27   •   Pleurae (visceral and parietal surfaces) – pleural fibrosis (diffuse accumulation of scar tissue

28   in the pleura) and mesothelioma (malignant tumour arising from the mesothelium lining the

29   pleural space);

30   •   Parietal pleura – pleural plaque (deposits of hyalinised collagen fibres).

31

32 As all inhaled fibres and dusts are not equally pathogenic, only some, or possibly none, of these

33 effects will arise following inhalation of any particular material. In this paper the end-point of

34 particular interest is cancer of the lung and/or mesothelium.

35

36 This review aims to compare findings from inhalation studies with those using the same fibrous or

37 particulate test materials delivered by the intraperitoneal or intrapleural injection routes. The

38 specific objective is to assess how well intraperitoneal/intrapleural injection studies can predict the

39 pathological responses to airborne dusts and fibres that are observed in inhalation studies of

40 pulmonary toxicity, in particular with respect to carcinogenicity. Where intratracheal studies have

41 also been carried out, these are included for completeness; however, for reasons detailed below

42 (Section 3) for some materials the intratracheal test may not be particularly informative regarding

43 cancer endpoints.

44

45 It is emphasised that this review is not intended to be an exhaustive collection of all published

46 studies, but rather a focused comparison of inhalation and IP/IPl test data for a range of dusts and

47 fibres. A good understanding of this relationship is important because of the continued use by some

48 jurisdictions of IP test results for human cancer risk assessment (Harrison et al., 2015).

49

50 **2. Inhalation methods**

51
52 The overall aim of an IH study is to administer a well characterised exposure concentration that can

53 be related to inhaled dose and any subsequent response in the animal (Wong, 2007). Inhalation

54 studies can, however, be subject to variability in several areas including: animals' environment and

55 surroundings; exposure atmosphere; the applied dose; and individual animal biological sensitivity.

56 High degrees of standardisation and control are therefore required to make test results reproducible

57 and comparable, and to fulfil general regulatory requirements (Pauluhn and Mohr, 2000).

58

59 Systems have been developed to provide a uniform, controlled environment for inhalation studies

60 for all types of experimental animals in terms of temperature, humidity, air flow, oxygen content,

61 and other major environmental factors (Wong, 2007). Exposure techniques include whole-body

62 chambers, and head and nose-only chambers, which are described briefly below.

63

64 **Whole-body chambers:** In these the animal is immersed in the atmosphere of the chamber. This

65 approach has the advantage of simulating 'natural' workplace or environmental exposures with

66 unrestrained animals. It is the most efficient approach for testing large numbers of animals and/or

67 for long duration studies as the animals can be housed in the chambers. However, this approach

68 uses a large amount of material, and good air mixing in the chamber is essential. In addition, co-

69 exposure through oral and dermal routes cannot be excluded.

70

71 **Head and nose-only chambers:** In these the animal is restrained so that only the head or nose is

72 exposed to the test material. This has the advantage that co-exposure through other routes is

73 unlikely and less material is needed. Additionally, it is easier to contain the test material and allows

74 flexibility in removing animals without the rest of the test group being affected. Disadvantages of

75 this approach include stressing the animals during restraint, and lack of food and water during the

76 exposure period.

77

78 **3. Pulmonary deposition methods**

79 These alternative methods act as surrogates for inhalation testing and allow instantaneous delivery

80 of a precise dose of test material suspended in a small volume of vehicle to the lungs. As the

81 material is delivered directly to the lower respiratory tract, the potential for deposition in the nasal

82 passsages and/or on fur that can occur in nose-only or whole-body inhalation exposure systems is

83 avoided (Osier & Oberdörster, 1997). Pulmonary deposition methods include the following:

84 **Intratracheal Instillation (IT):** This much used technique deposits a precise dose (bolus) of material

85 into the lungs, although the test substance may not be distributed in and cleared from the lungs in

86 the same way as would occur via nose-only inhalation. Intratracheal instillation has been used for

87 repeated dose studies, for example to test carcinogenicity and establish relative potency of fibrous

88 and non-fibrous particulates (Pott, 1993 – cited by Wong, 2007), and to evaluate particulate material

89 that is not readily inhaled by rodents (Driscoll et al., 2000 – cited by Wong, 2007). However, as this

90 technique usually involves a course of injections over a period of several weeks (only) it cannot

91 strictly be considered a 'chronic' exposure as would be achieved in a 24 month IH study; thus for

92 materials with low biopersistence the IT test may not be especially informative regarding the cancer

93 endpoint (Driscoll et al., 2000 – cited by Wong, 2007).

94 **Oropharyngeal Aspiration:** This deposits a specific dose of material on the base of the animal's

95 tongue which is then aspirated into the lungs during inhalation. This has the advantage over

96 intratracheal instillation in that material is distributed throughout the lungs, however Foster et al.

97 reported that the clearance of material from the lungs is altered from nose-only inhalation (Foster et

98 al., 2001 – cited in Wong, 2007).

99 • **Endotracheal Inhalation:** This method utilises a tight-fitting catheter inside the animal's

100 trachea to inflate the lungs, during which the test material is delivered. This has been used to

101 assess ultrafines and non-inhalable aerosols (Oberdorster et al., 1995 – cited in Wong, 2007).

102 • **Tracheostomy:** A surgical opening in the trachea allows delivery of the test material via a

103 cannula. This method can also be used to obtain fluid or cells from the lungs.

104

105 The disadvantages of such techniques is that animals require anaesthesia (and, for some, surgical

106 procedures), making then unsuitable for long-term repeat-dose studies. In addition, normal

107 defensive mechanisms are by-passed.

108

109 **4. Intracavity injection methods**

110 In addition to the inhalation and pulmonary deposition methods, two other routes of administration

111 have become commonly associated with toxicological assessment of airborne dusts and fibres,

112 namely intrapleural and, especially, intraperitoneal injection.

113 **4.1 Intrapleural injection (IPl)**

114 The most relevant intracavity method for the assessment of pleural toxicity involves delivery of the

115 test material directly into the pleural space (Murphy et al., 2011). This model has been validated for

116 studying pre-mesothelioma processes, for example (Wagner, 1984). Unfortunately it is a technically

117 difficult procedure, as the injection needle has to be positioned exactly within the pleural space, and

118 there are associated ethical issues; these factors limit its use for routine testing purposes.

119 **4.2 Intraperitoneal injection (IP)**

120 Use of the peritoneal cavity as a model for the assessment of fibre pathogenesis has been well

121 documented for many fibre types including asbestos, synthetic vitreous fibres and nanofibres

122 (Donaldson et al., 2013). As the pleural space has, at least until recently, been difficult to utilise

123 directly for toxicity testing due to technical issues associated with effective delivery of test material

124 (see 4.1 above), injection directly into the peritoneal cavity, which is also lined with mesothelium,

125 has proved an accessible and viable alternative. As with the pleural cavity, fibres or particles

126 introduced into the peritoneal cavity which cannot pass through the mesothelial stomata will be

127 retained, potentially leading to a pathogenic response (Donaldson et al., 2010).

128

129 Advantages of the intrapleural method include: use of a small amount of test substance, ease of

130 delivering the same dose to all animals, reduced cost, and in some respects a more 'sensitive' test in

131 comparison to inhalation methods (Miller *et al.*, 1999). However, certain important limitations also

132 need to be considered:

133 • The natural filtering and clearance mechanisms of the lung are by-passed, meaning

134 that material is injected that might never reach the pleura following inhalation

135 exposure;

136 • High doses are delivered at high rates, contrary to that occurring in the pleura under

137 normal physiologic conditions;

138 • Impacts on the airways and lung parenchyma are not investigated;

139 • The impact of fibre biopersistence during transit from the deposition site to the

140 pleura is not taken into account;

141 • The influence of fibre diameter on pulmonary uptake and deposition is not taken

142 into account;

143 • The peritoneal mesothelium is assumed to respond in the same way as the pleural

144 mesothelium.

145

146 **5. Method comparison**

147 In general, inhalation testing is regarded as the most appropriate method for assessing the toxicity

148 of airborne materials as it gives a realistic exposure scenario that can be extrapolated to humans

149 (Pauluhn and Mohr, 2000). This view has been reflected in reviews conducted by various national

150 and international agencies, including ILSI (2005) and the National Research Council (2000). Although

151 inhalation testing can be expensive, time consuming and lack specificity in dose (Bernstein, 2007;

152 Grimm *et al.*, 2002) compared to intratracheal and intrapleural/intraperitoneal studies, there are a

153 number of important problems associated with the alternative methods, as discussed for example

154 by Lippmann et al. (2014). These include: bypassing of the natural defence mechanisms of the lung;

155 ability to inject/implant large fibres and particles that would not normally be inhaled into the deep

156 lung; very high numbers/concentrations of test material at the injection site that may overwhelm

157 defence mechanisms; targeted tissues (e.g. peritoneal mesothelium) are not the same as for

158 inhalation exposure. Moreover, the coincidental injection of large (non-respirable) irritant fibres into

159 the mesothelium may well confound the test results (Harrison et al, 2015). It is true to say that

160 whilst inhalation studies may lead to exposure of the mesothelium, any toxic effect resulting from

161 this would be expected to be picked up by histopathology. However, intraperitoneal studies cannot

162 be considered to result in exposure of lung tissue.

163

164 Some advantages and disadvantages of the various methods discussed above are summarised in

165 Table 1.

166

167 **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

9

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

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

169   With regard to dosimetry, uncertainties in inhalation studies can be reduced by lung burden

170   experiments (using, for example, low temperature ashing and electron microscopy). Although in

171   injection type methods the delivered dose can be measured, there remains uncertainty as to the

172   true dose to the target organ.

173   **6. Comparison of toxicity study findings**

174   In the following sections, published toxicity data for a number of fibres and dusts obtained from

175   inhalation (and, for some materials, intratracheal instillation) studies are compared with toxicity

176   data for the same test materials delivered by the intraperitoneal and/or intrapleural routes. The

177   chemical compositions of the fibres discussed in this report are detailed in Table 2 below (Bellmann

178   et al., 1987; Bernstein, 2007; Grimm et al., 2002; Guldberg et al., 2002; Hesterberg et al., 1998;

179   Kamstrup et al., 2001; Kamstrup et al., 2002; Kamstrup et al., 2004; Lambré et al., 1998; Roller et al.,

180   1996; Searl et al., 1999). While full characterisation of the delivered aerosol – especially

181   fibre/particle size distribution – is extremely important, this information is not consistently available;

182   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				

11

184 **6.1 Asbestos (Chrysotile, Crocidolite and Amosite)**

185 Muhle *et al.* (1987) carried out two parallel studies using inhalation and intraperitoneal models to

186 assess the carcinogenic potential of chrysotile (UICC and Calidria) and crocidolite. In the

187 intraperitoneal study, female Wistar rats (aged 5 weeks at the start of the trial) were administered a

188 single intraperitoneal injection of 0.5 mg of crocidolite or chrysotile (Calidria) or chrysotile (UICC) in

189 1 mL of saline, and observed for a median lifetime of 109 and 116 weeks for crocidolite and

190 chrysotile respectively. In the inhalation study, female Wistar rats (aged 12 weeks at the start of the

191 trial) were exposed via nose-only inhalation to 2.2 mg/m^3 crocidolite and 6.0 mg/m^3 chrysotile

192 (UICC) for 5h, four times per week over a 12 month period (total exposure of 1000 h and cumulative

193 exposure 2200 and 6600 mg/h/m^3 respectively) with a 12 month follow-up exposure-free period.

194 Exposure to crocidolite by intraperitoneal injection induced malignant tumours in 55% of animals

195 compared with 84% in those exposed to chrysotile (UICC), and 0 – 6% in controls. Exposure to

196 Calidria chrysotile was associated with malignant tumours in only 6% of animals, which the authors

197 concluded was due to the lower biopersistence of Calidria chrysotile compared with UICC chrysotile.

198 In contrast, no significant tumour incidence was reported for the inhalation studies, with only 2% (1

199 animal) exposed to crocidolite developing an adenocarcinoma. Muhle *et al.* (1987) expressed doubts

200 about the inhalation study findings.

201

202 As part of a large study of 50 test materials, Pott et al. (1987) assessed the potential carcinogenicity

203 of several types of chrysotile using an intraperitoneal model, and crocidolite using intratracheal and

204 intraperitoneal models. Chrysotile (UICC A) given at doses of 6 and 25 mg to female Wistar rats via

205 intraperitoneal injection, resulted in tumour incidences of 77.1 and 80.6% respectively. UICC B

206 chrysotile administered at doses of 0.05, 0.25 and 1 mg by intraperitoneal injection was associated

207 with a dose dependant increase in tumour incidence rate, with 19.4, 61.8 and 84.4% of rats

208 respectively presenting with tumours. Two other forms of chrysotile, PVNO and calidria, were also

209 tested, administered in a single intraperitoneal injection of 1 and 0.5 mg respectively. A high tumour

210   incidence rate of 80% was observed with the PVNO form, but a low incidence rate of only 6% was

211   noted for the calidria form.

212

213   Pott et al. (1987) also reported findings for crocidolite, tested in female Wistar rats using

214   intratrachael and intraperitoneal models. Following intratracheal instillation of 20 doses of 0.5 mg,

215   or a single intraperitoneal injection of 0.5 mg, similar numbers of tumours were evident, with 42.9%

216   and 56.3% of animals, respectively, presenting with tumours. A higher tumour incidence rate was

217   seen at the higher level of exposure of 2 mg crocidolite (87.5%) using the intraperitoneal model.

218

219   Grimm *et al*. (2002) used crocidolite at 2 different doses (0.5mg and 5mg) as a positive control in a

220   study of biosoluble insulation glass wool fibres, injected once into the intraperitoneal cavity of

221   female Wistar rats (strain CrL: WiBR). Pathology was carried out to determine presence of the

222   following: mesothelioma with simultaneous abdominal tumours; other abdominal tumours with

223   serosal spread (but no mesothelioma); and abdominal tumours with neither serosal spread nor

224   mesothelioma. Survival numbers were significantly reduced in the high dose crocidolite group,

225   leading to the validity of use of the high dose being questioned. Importantly, the authors concluded

226   that there may be different aetiologies for the production of mesothelioma by soluble and insoluble

227   fibres following intraperitoneal injection (Grimm *et al*., 2002).

228

229   This study was an extension of an earlier investigation reported by Lambré *et al*. (1998) to evaluate

230   the potential carcinogenic hazard of five man-made vitreous fibres, which also used crocidolite as a

231   positive control. Three different doses of crocidolite (0.005, 0.05 and 0.5mg), delivered in a single

232   intraperitoneal dose to female Wistar rats, all produced mesotheliomas in a dose dependent manner

233   (Lambré *et al*., 1998). In comparison to these intraperitoneal studies, Smith et al. (1987) used

234   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

14

261   were made (details therefore not included in Table 3). Comparing the results for intrapleural and

262   inhalation exposure of amosite shows the material to be carcinogenic in both assays, but much more

263   strongly so in the IP test.

264

265   **Summary of findings for asbestos**

266   •   Chrysotile asbestos shows positive results for carcinogenicity in both inhalation (levels

267   between 6 – 10 mg m^{-3}) and intraperitoneal studies (levels between 0.05 – 25 mg), but with

268   a much greater potency in the latter.

269   •   Crocidolite asbestos also shows positive results in both inhalation (levels between 2.2 – 10

270   mg m^{-3}) and intraperitoneal studies (levels between 0.005 – 5 mg), although there appears

271   less difference in potency between the routes of exposure than with chrysotile asbestos.

272   •   Amosite asbestos shows positive results for carcinogenicity in both inhalation (1000 fibres/

273   cm$^{-3)}$ and intraperitoneal studies (10^9 fibres), although there is an apparent greater potency

274   through the intraperitoneal route.

275

276   **6.2 Wollastonite**

277   Wollastonite was included in the large study of 50 test materials described by Pott *et al*. (1987). A

278   total dose of 100mg was given by intraperitoneal injection, in five separate 20mg doses, to 54 rats.

279   No tumours were observed 28 months after these injections, nor were any severe adhesions found.

280   Inhalation and intratracheal studies have also been conducted on wollastonite by Warheit *et al*.

281   (1994) and Tátrai *et al*. (2004) respectively. A short term inhalation study was carried out in male

282   Sprague-Dawley rats, exposed to 115 mg/m^3 (800 fibres/cc) for 5 days to assess biopersistence.

283   Rapid clearance of the wollastonite fibres from the lungs was seen, with a low retention half-time of

284   <1 week. The data indicated that wollastonite fibres have low durability, being composed of calcium

285   silicates, resulting in solubilisation in the lung (Warheit et al., 1994). Tátrai *et al.* (2004) used a single

286   1 mg intratracheal instillation of wollastonite, with crocidolite (UICC) as a positive control, and

287   examined the lungs at time intervals of 1, 3 and 6 months post exposure. The authors reported that

288   the wollastonite exposed group showed mild inflammation and fibrosis which remained the same at

289   six months as at one month, while the crocidolite showed increased inflammation at 6 months

290   (Tátrai *et al.*, 2004). All three exposure methods demonstrated that wollastonite has low toxicity.

291

292   **6.3 MMVFs (Man Made Vitreous Fibres)**

293   This section details findings on a variety of common MMVFs, including rock, slag and stone wool.

294   Although strictly speaking RCF and special types of glass fibres are also classed as MMVFs, for clarity

295   these are detailed in individual subsections.

296

297   In 1972, intracavity experiments by Pott and Friedrichs (also Stanton and Wrench at the same time)

298   indicated that man-made vitreous fibres could be a potential hazard to human health (Pott and

299   Friedrichs, 1972; Stanton and Wrench, 1972); a large number of studies have subsequently been

300   carried out on a variety of MMVFs using both inhalation and intraperitoneal exposure models.

301

302   McConnell *et al.* (1994) conducted a long-term study in Fischer 344/N rats (males, eight weeks of

303   age) exposed by nose-only inhalation for 6 h per day, 5 days per week, for 24 months to 3

304   concentrations (3, 16, and 30 mg/m^3) each of a rock wool (stone wool), and a slag wool (blast

305   furnace). A dose-related non-specific inflammatory response was seen for both test substances, with

306   rock wool also inducing a minimal local pulmonary fibrosis. Although a number of tumours were

307   present (carcinoma and adenoma), their incidence was not considered to be significantly raised.

308   Bronchoalveolar hyperplasia, on the other hand, was seen at significantly greater incidence in the

309   highest dose animals than in the controls (saline) (McConnell *et al.*, 1994). Hesterberg *et al*. (1995)

310   reported the toxicity of different MMVFs (including fibrous glass (MMVF10 and 11), rock (stone)

311   wool (MMVF21) and slag wool (MMVF22)) by inhalation in a series of studies with comparable fibre

16

312   numbers (WHO fibres[1]) and dimensions in the delivered aerosols. Groups of rats and hamsters were

313   exposed nose-only to 30mg m^3 doses of each of the test substances for 6 h per day, 5 days per week

314   for either 18 months (hamsters) or 24 months (rats). Exposure to the fibrous glasses and slag wool

315   induced an inflammatory response in rats, but no mesotheliomas or increased lung tumour

316   incidence rates were observed. Exposure to rock wool was associated with minimal lung fibrosis;

317   there were no mesotheliomas and no increase in lung tumour rate.

318

319   Miller *et al.* (1999) investigated the carcinogenicity and biopersistence of MMVF 10, 21 and 22 by

320   intraperitoneal administration of 10^9 fibres. MMVF21 showed results consistent with those of

321   Kamstrup *et al.* (2001), with 95% of the test group developing mesothelioma. The MMVF10 and

322   MMVF22 groups showed a lower incidence of mesothelioma (59% and 54% respectively); however,

323   there was no control group included in this study, so it is not known if these results were statistically

324   significant (Miller *et al.*, 1999). Comparing the results to those from the inhalation study reported by

325   Hesterberg *et al.* (1995), it can be seen that there is no consistency between the findings, with

326   mesotheliomas being produced by the intraperitoneal method but neither mesotheliomas nor lung

327   tumours being found in the inhalation study.

328

329   In a later study, Hesterberg et al. (1998), assessed the potential toxicity of a rapidly dissolving

330   Synthetic Vitreous Fibre (X607) in Fischer rats, exposed by nose-only inhalation to X607 at a

331   concentration of 200 fibres/cc for 6 h per day, 5 days per week for 24 months. RCF1 was included at

332   the same exposure level and duration for comparison purposes (see Section 6.3.2). X607 showed low

333   biopersistence and was not associated with fibrogenic or tumorigenic responses above those seen in

334   the controls.

335

---

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

336 Findings from the study reported by McConnell *et al*. (1994) were compared by Kamstrup *et al*.

337 (2001) using an inhalation study to assess the pathology of a low silica/high aluminium content

338 MMVF (34/HT). Male Fischer rats were exposed via nose-only inhalation to MMVF 34/HT at

339 concentrations of 30 mg/m^3 for 6 h per day, 5 days per week for 104 weeks, with a subsequent non-

340 exposure period lasting until survival of animals in the air control group had dropped to

341 approximately 20%. Pathology results were compared to a previous assessment of stone wool

342 (MMVF21) under the same exposure conditions reported by Hesterberg et al. (1995). The authors

343 reported a marked difference in pulmonary pathogenicity, with MMVF21 but not MMVF34/HT[2]

344 causing pulmonary fibrosis. Although tumours were present for both fibre types, these were

345 comparable to control levels.

346

347 No study utilising intraperitoneal/intrapleural models for MMVF34 could be identified for

348 comparison to the data from the inhalation study. However, Kamstrup *et al*. (2002) did conduct an

349 intraperitoneal carcinogenicity study using the high aluminium, low silica HT wool RIF39001 and

350 stone wool (D6) with similar chemical compositions to MMVF34 and MMVF21 respectively. This

351 study administered a single dose of 9 mg of the HT wool, and 36 mg of D6, by intraperitoneal

352 injection into female Wistar rats. The mass dose differed for each fibre type due to differing number

353 and size of the fibre. The HT wool dose (2.1 x 10^9 WHO fibres) was twice that since recommended by

354 the EU guidelines (European Commission 1997). The animals administered the HT wool showed a

355 low incidence (6%) of macroscopic nodules in the peritoneal cavity, while the majority of the group

356 (88%) injected with D6 showed the presence of nodules. Histologically, clumps of fibres were found

357 in the D6 groups either adherent to the surface of the viscera or free within the abdominal cavity,

358 but not in the HT wool group. Formation of granulomas was seen in the D6 group, indicating a

359 cellular response with but not in the HT wool group. Fibrosis was evident in the peritoneal cavity of

360 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'.

361 Three types of tumours (benign pituitary adenomas, mammary adenocarcinomas and

362 mesothelioma) were observed in the treated animals and in the controls, but mesotheliomas were

363 the most common type of tumour in the D6 group (56%). The authors concluded that the low

364 carcinogenic potential of RIF39001 was due to the high biosolubility of the HT wool (Kamstrup *et al.*,

365 2002).

366

367 Inhalation, intratracheal and intraperitoneal studies have been carried out to assess the carcinogenic

368 potential of HT stone wool. A single administration of 1.2 mg of stone wool in 0.2 ml by

369 intratracheal instillation study was conducted by Baier et al. (2000) with sacrifices occurring at

370 different time points up to 90 days post-exposure. Pulmonary granulomas were present at the start

371 of the post instillation period but decreased as the post instillation time increased, with most of the

372 granulomas being resolved and only a very small number of fibres remaining embedded in the

373 surrounding tissue by the end of the study. This study would appear to indicate that the HT fibre is

374 unlikely to be carcinogenic, although this is not possible to determine categorically from a 90-day

375 study (details therefore not included in Table 3). No details on chemical composition of the fibres

376 used were given by the authors, making comparison with other studies difficult. However, it is

377 recognised that, due to differences in the processing of the raw materials and the variations that can

378 occur in the starting material, it is not possible in practice to define a unique chemical composition

379 for stone wools (Guldberg *et al.*, 2002).

380

381 Sub chronic and chronic inhalation studies have been carried out on high-aluminium, low-silica HT

382 stone wools. A sub-chronic biopersistence study tested 3 different stone wools (RIF41001, 42020-6

383 and 43006-1) at a single dose of 150 fibres/ml (>20 μm) for a period of 3 months delivered by nose-

384 only exposure. A post exposure period of the same length was included. Only minimal

385 histopathological changes were observed for all three stone wools, and therefore all were assessed

386 as non-fibrogenic (Kamstrup *et al.*, 2004). A chronic inhalation study was reported for MMVF34,

387   delivered to male Fischer 344 rats at 30 mg/m^3 by nose-only inhalation for 6 h/day, 5 days/week for

388   104 week, with a subsequent non-exposure period lasting until survival in the air control group had

389   dropped to approximately 20%. No pulmonary fibrosis was noted with MMVF34 and the incidence of

390   tumours was comparable with the control group (Kamstrup *et al.*, 2001).

391

392   **Summary of findings for MMVFs**

393   Comparing the results of the inhalation and intraperitoneal tests for MMVF21/D6 and MMVF34 (or

394   similar), the intraperitoneal test showed D6 to be carcinogenic, producing a tumour incidence rate of

395   56%, while the inhalation tests showed both MMVF21 and MMVF34 to be non-tumorigenic. For HT

396   stone wool, all three exposure models indicated no carcinogenic potential.

397

398   Other studies of specific types of man-made vitreous fibres have been conducted and these are

399   discussed separately below.

400

401   **6.4 Glass Fibres**

402   **6.4.1 Glass fibre 104/475**

403   Pott *et al*. (1987) included a number of different types of glass fibres in their large study investigating

404   the carcinogenicity of 50 fibres, dusts and metal compounds. Glass fibre 104/475 was assessed

405   using both intraperitoneal and intratracheal models. In the intraperitoneal study, two separate

406   doses of 104/475 (0.5 mg or 2.0 mg) were injected into the peritoneal cavity. A dose-dependent

407   response for tumour induction was seen with incidences of 16.7 and 25.8% respectively. In a further

408   intraperitoneal study, five 1 mg doses were administered, with a post-exposure period of 28 months.

409   Results of this study showed a high degree of fibrous adhesions and a 66% tumour rate. These two

410   studies did not differentiate between tumour types, but combined incidences of sarcoma,

411   mesothelioma or carcinoma in the abdominal cavity to give an overall tumour rate. In the

412   intratracheal study, a 10 mg dose of 104/475 was instilled in 20 weekly injections of 0.5mg each,

413 resulting in a 14.7% incidence of lung tumours (types not differentiated). Pott *et al*. reported that

414 this was statistically significant when compared to the unexposed control group of animals in which

415 no tumours were evident (Pott et al., 1987). A further study by Muhle et al. (1987) compared

416 findings from intraperitoneal and inhalation studies using 104/475. Female Wistar rats were

417 administered a single intraperitoneal injection of 0.5mg 104/475, while in the inhalation group

418 female Wistar rats were exposed, nose-only, to 104/475 at a concentration of 3.0 ± 1.8 mg/m^3 for 5

419 hours per day, 4 days a week for 1 year with a total study duration of 2 years. Following

420 intraperitoneal injection, a total tumour incidence rate of 17% was observed (tumour types not

421 specified). This compared with only one primary lung tumour (squamous cell carcinoma) following

422 inhalation exposure, although there was a high incidence of fibrosis (38%), with bronchioloalveolar

423 hyperplasia (11%) and squamous metaplasia (0.9%) in these animals.

424

6.4.2 Glass fibre 100/475

426 Glass fibre 100/475 differs from 104/475 in that it has a smaller diameter. Davis et al. (1995)

427 reported findings of a review comparing models to predict the pathogenicity of fibres, utilising

428 100/475 as an example of a less durable glass microfibre (details not included in Table 3). Cullen et

429 al. (2000a) have reported findings of both inhalation and intraperitoneal studies using 100/475. Rats

430 were exposed to aerosol concentrations of 1000 fibres/ml for 7 h per day, 5 days per week for 12

431 months, with an additional post-exposure period of 12 months. After 2 years, no fibrosis was

432 apparent, nor were there any carcinomas or mesotheliomas; however adenomas were detected in

433 10.5% (4/38) of animals. For the intraperitoneal study, a single administration of 10^9 WHO fibres

434 was used, with mesothelioma being detected in 33% of rats (8/24) (Cullen *et al*., 2000a).

435

436 These results indicate that in a similar way to glass fibre 104/475, glass fibre 100/475 shows little or

437 no carcinogenic potency by inhalation but induces a strong (33%) mesothelioma response following

438 intraperitoneal injection.

439

6.4.3 E Glass

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

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

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

In a one year inhalation study, Mast et al. (1995a) assessed the toxicity/carcinogenicity of three types of size-selected (length 20 μm and diameter 1 μm) RCF fibres - kaolin-based, high purity, and 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.

464   apparent from 6 and 9 months respectively for all fibres. Pulmonary neoplasms (bronchoalveolar

465   adenomas and carcinomas) were observed in 13, 15.7 and 7.4% of animals exposed to kaolin-based,

466   high purity and aluminium zirconia silica fibres respectively; these were statistically significantly

467   higher than unexposed controls. Pleural mesotheliomas were observed in 1.6% of animals exposed

468   to kaolin-based RCF, in 2.5% exposed to high-purity RCF and 1.7% exposed to aluminium zirconia

469   silica RCF.

470

471   A two-year multi-dose inhalation study by the same authors using kaolin-based RCF (as described

472   above) at levels of 3, 9 or 16 mg m^{-3} (36, 91 and 162 fibres/cm^{-3} respectively) reported interstitial

473   fibrosis and focal pleural fibrosis at 12 months in the two highest dose groups. Neoplasms

474   (bronchoalveolar adenomas and carcinomas) were observed in 1.6, 3.9 and 1.6% of animals exposed

475   to kaolin-based RCF at 3, 9 and 16 mg m^{-3} respectively; these incidences were not statistically

476   significantly higher than in the unexposed controls. A single pleural mesothelioma was also observed

477   in one animal (0.8%) exposed to 9 mg/m^{3} of kaolin-based RCF (Mast et al., 1995b).

478

479   In a subsequent review of their single-dose RCF study (Mast et al., 1995a) using mathematical

480   modelling to assess deposition, clearance and retention of RCF fibres and taking into account the

481   concept of 'overload', Mast et al. (2000) suggested that a level of 30 mg m^{-3} may have exceeded the

482   maximum tolerated dose, which would have overloaded the lung and had a major impact on the

483   observed chronic adverse effects (Mast et al., 2000).

484

485   The RCF sample used in the Mast et al. studies is believed to contain more non-fibrous materials

486   than MMVFs, which could have had a serious effect on the results obtained, potentially leading to a

487   false positive result. Therefore, in a subsequent study the RCF1 (kaolin-based) sample administered

488   was processed in the same way as MMVF samples, as reported by Hesterberg *et al.* (1995), giving

489   RCF1a.

490

491 The size-selected RCF1a sample was used by Bellmann *et al.* (2001) in a short term nose-only

492 inhalation study and compared to the results for the original RCF1. Female Wistar rats were

493 exposed for 6 h/day, 5 days/wk for 3 wk to either RCF1a or RCF1 fibre aerosol at a concentration of

494 about 125 fibres (>20μm long)/ml; due to differences in the nonfibrous particle content, the average

495 gravimetric aerosol concentration differed between the two samples (RCF1, 51.2 mg/m^3; RCF1a,

496 25.8 mg/m^3). The post-treatment observation period was 12 months. The clearance function of

497 alveolar macrophages was seen to be severely retarded following exposure to RCF1 but not RCF1a.

498 In both groups, a significant increase in polymorphonuclear leukocyte and lymphocyte counts was

499 shown 3 days following the end of exposure, which persisted longer (remaining high at 3 months

500 post exposure) in the RCF1 group than in the RCF1a, indicating persistent inflammation.

501 Histopathology showed the presence of inflammatory changes, with similar fibrotic and hyperplastic

502 changes in both RCF1a and RCF1; however, at the end of the 12 month post exposure period the

503 fibrotic changes were only present in the RCF1 group. The authors suggested that the difference in

504 the results between RCF1 and RCF1a could be explained by an increased number of shorter fibres

505 (falling outside the WHO definition) in RCF1a compared to RCF1, and also noted that the numerous

506 lesions seen in the RCF1 exposure group resembled those seen in a lung overload study. This study

507 casts thus further doubts on the RCC studies on RCF, and also raises the issue that non-fibrous

508 components found in the administered aerosol could lead to inflammation and in turn tumour

509 production (Brown et al, 2000; Bellmann *et al.*, 2001).

510

511 An intraperitoneal study was conducted as part of the Colt Fibre Research Programme (CFRP) using

512 RCF1, at a target dose of 10^9 WHO fibres (Miller et al., 1999) This study not only looked at

513 mesothelioma production, but also the importance of fibre length, biopersistence and dissolution

514 rate in relation to tumour production. Administration of RCF 1 was associated with an 88%

515 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

541   the medium dose, thickening of the alveolar walls in addition to macrophage infiltration and one

542   bronchoalveolar adenoma was found. The highest dose animals showed similar responses to those

543   of the mid dose group in the first year, but went on to develop bronchoalveolar adenomas and 14

544   cystic keratinizing squamous carcinomas in 25 (from a total of 151) animals, although these were

545   difficult to differentiate from squamous metaplasia. While tumours were seen to develop in this

546   study, it was determined that this was due to the excessive dose used (leading to overload

547   conditions) in the study which is unlikely to occur or be relevant to human exposure (Lee et al.,

548   1986).

549

550   For titanium dioxide, the inhalation, intratracheal and intraperitoneal models all generally indicate

551   no or low carcinogenic potential except when exceptionally high doses are administered, leading to

552   overload effects.

553

554   **6.6 Silicon Carbide**

555   **6.6.1 Silicon carbide whiskers**

556   Intraperitoneal, inhalation and intrapleural studies have been conducted on silicon carbide whiskers.

557   In a dose-range finding study, Adachi and colleagues administered a single dose of 10 mg/rat of nine

558   different fibre types including silicon carbide whiskers by the intraperitoneal route to female F344

559   rats. This resulted in development of peritoneal mesothelioma in all test animals within a year,

560   leading the authors to conclude that a reduced dose of 5 mg/rat of silicon carbide whiskers was

561   appropriate for the main study. A year after administration of 5mg/rat, 70% of the animals had

562   developed mesothelioma (Adachi *et al.*, 2001). UICC chrysotile B was used as a positive control,

563   resulting in a 70% incidence of mesothelioma, one year after administration.

564

565   In a long-term study, rats were exposed by whole-body inhalation to silicon carbide whiskers for 7

566   hours/day, 5 days/week, for 41 weeks; amosite asbestos was used as a positive control (Davis et al.,

567   1996). Following the exposure period some rats (n=42) were assessed for life and in these animals 20

568   tumours of the lung and pleura were recorded (5 carcinomas, 5 adenomas and 10 malignant

569   mesotheliomas). A few animals had more than one type of tumour so that the number of tumour-

570   bearing animals was reported to be 16. In comparison to amosite, silicon carbide produced fewer

571   tumours in the lung parenchyma, but produced a total of 10 mesotheliomas compared with 2

572   related to amosite exposure.

573

574   In a biopersistence inhalation study, male Wistar rats were exposed to silicon carbide whiskers at a

575   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

576   (Akiyama *et al.* 2007). This dose was chosen as it was close to the occupational exposure limit for

577   silicon carbide whiskers at that time. Histopathological examination showed fibrotic changes in the

578   lung including thickening of the alveolar walls, macrophage infiltration, aggregation of fibres, and

579   bronchoalveolar hyperplasia in two animals.

580

581   Johnson and Hahn (1996) reported findings of a carcinogenicity study using a single intrapleural

582   administration of 20 mg silicon carbide whiskers of different lengths, containing either 5.6×10^8

583   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

584   respectively) to female rats. Animals were assessed over their lifetime and those treated with SiCW

585   1 and SiCW 2 developed pleural mesotheliomas at a rate of 90% and 87% respectively. In

586   comparison, 23% of those treated with SiCW3 and 57% of the positive controls treated with

587   crocidolite developed pleural mesotheliomas.

588

589   **6.6.2 Granular silicon carbide**

590   The granular, non-fibrous, form of silicon carbide has been assessed through intraperitoneal studies.

591   Roller et al. (1996) examined groups of male or female rats for up to 30 months for tumours in the

592   abdominal cavity after repeated (5 or 20) intraperitoneal injections of 50 mg of granular silicon

593 carbide (equivalent to approximately 667 mg/kg bw and 2,666 mg/kg bw). From a total of 395 rats,

594 only two mesotheliomas were found. Pott et al. (1994) also reported no increase in tumours in a

595 carcinogenicity study with non-fibrous silicon carbide administered to rats by repeated (5 or 20)

596 intraperitoneal injections of 50mg (equivalent to approximately 667 mg/kg bw and 2,666 mg/kg bw).

597

598 **6.7 Potassium Octatitanate**

599 The intraperitoneal study carried out by Adachi *et al*. (2001) described above, also investigated

600 potassium octatitanate under the same conditions of exposure. In the dose-range finding study, a

601 77% incidence of mesothelioma was present, indicating that a reduced dose of 5mg/m^3 was

602 appropriate for the follow-on study. In the second study, exposure to potassium octatitanate

603 resulted in an incidence of 20% mesotheliomas (Adachi *et al*., 2001).

604

605 Yamato *et al*. (2003) conducted a low exposure (2.2 ± 0.7 mg/m^3 or 111 ± 34 fibres/ml) long term

606 inhalation study, exposing male Wistar rats to potassium octatitanate for one year for 6 h per day, 5

607 days per week. Histopathology showed the presence of mild fibrotic changes around macrophages

608 that had engulfed the fibres at 3 days, 6 and 12 months. No malignant pulmonary tumours were

609 observed, although adenomas were found in 2 rats (3.4%; 2/59) at 6 months post exposure and in 1

610 rat (1.7%; 1/59) at 12 months. Squamous metaplasia was also found in 1 rat (1.7%; 1/59) at the 12

611 month period. In a chronic inhalation study, Ikegami *et al*., (2004) reported toxicological findings

612 following exposure of male Fischer 344 rats via whole-body inhalation to 0, 20, 60, or 200 WHO

613 fibres/cc of potassium octatitanate for 6 h/day, 5 days/w for 24 months. At the mid dose, alveolar

614 wall thickening and minimal alveolar fibrosis were noted following 18 and 24 months of exposure. At

615 200 fibres/cc exposure, slight alveolar wall thickening was apparent after 12 months of exposure and

616 slight alveolar fibrosis after 18 and 24 months of exposure. No exposure-related pulmonary

617 neoplasms or mesotheliomas were observed.

618

619   An intratracheal instillation study investigating lung burden and biopersistence was carried out by

620   Oyabu et al. (2006) utilising data from the inhalation study reported by Yamato et al. (2003). The

621   authors interpreted that the data showed a threshold and that the dose would lie between 1.5 and

622   2.4 mg; one of four doses (0.5, 1, 2 and 5mg) were therefore instilled into male Kud:Wistar rats,

623   which were sacrificed at different time points for up to one year. Dose-related fibrotic changes and

624   thickening of the alveolar wall were observed (not included in Table 3 as no quantitative data). Thus

625   inhalation and intratracheal exposure to potassium octatitanate gave rise to no malignant tumours,

626   whilst the intraperitoneal study produced a high tumour yield.

627

628   **6.8 Quartz**

629   Quartz (a known macrophage toxin) was one of the dusts included in two of the intraperitoneal

630   studies carried out in the large study by Pott *et al*. (1987). The two doses used (10 mg and 40 mg)

631   induced tumours (sarcoma, mesothelioma or carcinoma in the abdominal cavity) at rates of 5.9%

632   and 22% respectively. These results can be compared to those from intratracheal instillation

633   experiments carried out using quartz. In a short-term (1 month) study, Luchtel *et al*. (1989) used

634   quartz as a positive control at a single dose of 5mg, which was associated with fibrotic lesions and

635   increased numbers of macrophages in the alveoli (study not included in Table 3 due to short

636   duration). In 2005, Pott and Roller conducted the "19 dust study" to test the carcinogenicity of a

637   number of dusts using intratracheal instillation. Quartz was used as a positive control due to its

638   known toxicity. Exposure to single instillation doses of 5 and 10 mg resulted in total tumour

639   (adenoma, adenocarcinoma or squamous mixed cell carcinoma in the lung) incidences of 65.7% and

640   71.4% respectively. Instillation of a higher dose of 20 mg, delivered in two doses of 10 mg each,

641   resulted in a 77.8% incidence of total lung tumours (Pott and Roller, 2005).

642

643   Although intraperitoneal and intratracheal studies confirm a tumorigenic response in the lungs

644   following exposure to quartz, a difference in the degree of tumour development is evident. At the

645 10 mg dose level, intraperitoneal injection resulted in an incidence of 5.9% tumours (Pott *et al.*,

646 1987) while the intratracheal study gave rise to 57.1% tumours (Pott and Roller, 2005).

647

648 **6.9 Kevlar**

649 The aramid fibre 'Kevlar' was assessed for carcinogenic potential by Pott et al. (1987) using an

650 intraperitoneal model, and by Warheit et al. (1994) in a 3 month inhalation study. In the

651 intraperitoneal study, 5.8% of rats administered 20 mg of Kevlar fibres (5 x 4mg) showed tumour

652 development. In the inhalation study, Crl:CD BR rats were exposed for 5 days to aerosols of Kevlar

653 fibrils (900-1344 f/cc; 9-11 mg/m^3). No pulmonary lesions were observed, which was considered to

654 be due to the rapid clearance of the Kevlar fibres. One chronic inhalation study on Kevlar has been

655 reported by Lee et al. (1988). Rats (male and female) were exposed to Kevlar fibrils at concentrations

656 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

657 exposed to 400 fibrils/cc for 1 year and allowed to recover for 1 year. Lung tumours were observed

658 in treated animals, however, the authors considered these to be a unique type of experimentally

659 induced tumour (cystic keratinizing squamous cell carcinoma) and not of relevance to the human

660 situation.

661

662 Thus both intraperitoneal and inhalation experiments appear to indicate low carcinogenic potential

663 for Kevlar.

664
665 **6.10 Polypropylene**

666 Polypropylene fibres have been assessed for carcinogenic potential using inhalation and

667 intraperitoneal exposure models. Hesterberg et al. (1992) administered polypropylene fibres at 15,

668 30, or 60mg/m^3 (actual doses achieved were 13.03, 28.07 and 59.61 mg/m^3) by nose-only inhalation

669 to male Fischer rats for 6 h per day, 5 days per week for 90 days. A dose-dependent increase in

670 pulmonary macrophages and reversible increase in mild cellularity were noted. In an intraperitoneal

671 study carried out by Pott et al. (1987) as part of a large carcinogenic study of around 50 dusts,

30

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 concent-ration[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	-	-	-	-	-

32

					Dose							
	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 x 10^6 fibres)^{SD} (single dose)	7.8	7.8	-	-	-	-	-
		32.5			0.05 mg (18.9 x 10^6 fibres)^{SD} (single dose)	15.7	19.6	-	-	-	-	-
		32.5			0.5 mg (188.6 x 10^6 fibres)^{SD} (single dose)	39.2	49.0	-	-	-	-	-
Grimm *et al.,* 2002	Crocidolite IP	31	>5, <15	<3	27 mg (0.5 x 10^6 WHO fibres)^{SD} (single dose)	52.9	NS	-	-	-	-	-
		31			45 mg (5.0 x 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)	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	5.6×10^8 fibres/kg bw SD	90	-	-	-	-	-	-
		Assessed for life	20.1 (± 1.01)	<1	1.2×10^7 fibres/kg bw SD	87	-	-	-	-	-	-
		Assessed for life	6.6 (± 0.40)	<1	8×10^8 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	granular		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)	-	Aerodynamic 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	Polypropylene 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.

689

690

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.

715   •   Potassium octatitanate

716

717   For silicon carbide, marked differences were noted in the results for whiskers and the granular form

718   in the intraperitoneal studies. Whilst silicon carbide whiskers showed positive findings, the non-

719   fibrous granular form was negative.

720

721   A number of important caveats apply in considering the results presented here. The majority of

722   experiments reviewed here were on fibres rather than dusts, and tumour type/location is often

723   different for dusts and fibres. Where the same fibre type has been used in more than one study,

724   there is not always consistency of manufacturer/producer. This could mean that fibres with slightly

725   different chemical compositions are being compared. Even with the same manufacturer, differences

726   may still occur due to inter-batch variations. This issue was highlighted by Guldberg *et al*. (2002) who

727   suggested that stone wool fibres cannot have a defined chemical composition, as variations will

728   necessarily occur during the processing of raw materials. This reasoning would apply to the majority

729   of fibres that are produced from natural raw materials of variable composition. Due to the lack of

730   definitive chemical compositions, it is difficult to confidently compare the study results on the same

731   fibre types, which in turn makes it problematic to definitively compare the results from studies using

732   different exposure methods of the same fibres. Also, not all of the papers reviewed here included

733   sufficient information on the chemical composition of the fibre test materials to allow a truly robust

734   comparison of results. The same is true of fibre/particle size distribution data. There is also a

735   problem with fibre nomenclature. For example, MMVF10 and 11 are glass fibres, but some studies

736   refer to glass wool or different types of glass fibre, which may or may not be the same; it is difficult

737   to confidently compare these studies without the specific chemical composition data to determine if

738   they are indeed the same fibre type.

739

740   Finally, the issue of dose is problematic in an exercise such as this. The majority of intraperitoneal

741   studies and some intratracheal studies use one single large dose (or a limited series of smaller

742   doses), while in the inhalation studies exposure is to a low concentration extended over a longer

743   period of time. As a result, doses are difficult to directly compare. In addition, lung overload can

744   occur and can lead to false positives, as shown for example in the study reported by Lee et al. (1986)

745   and implied for the RCF experiment by Mast (1995a). This underlines the importance of determining

746   a relevant and appropriate dose when designing studies, in order to be confident in the validity of

747   the findings. The same argument no doubt applies to intrapleural/intraperitoneal testing where the

748   basis and validity of the amount of material injected is subject to even greater uncertainty. In all

749   examples, the question of relevance to human exposures remains a source of uncertainty.

750

751   To summarise, for some of the dusts and fibres reviewed, there is conformity between the results of

752   intraperitoneal and inhalation such that they are either consistently positive (a few only) or

753   consistently negative. For the remaining dusts and fibres reviewed, intraperitoneal and inhalation

754   tests give different results, with positive results in the intraperitoneal test not being reflected by

755   positive inhalation test results. In no circumstances was a positive inhalation study reflected by a

756   negative intraperitoneal study.

757

758   Intraperitoneal studies appear to be more 'sensitive' to the carcinogenic potential of injected

759   materials, but as noted earlier this is a highly non-physiological route of exposure and false positive

760   results cannot be discounted. As shown in this paper, positive IP/IPl study results for carcinogenicity

761   are not consistently reflected by positive results in inhalation studies.

762

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

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

786 positive in either inhalation or intratracheal tests)[6] but it should not be used to determine that a

787 dust or fibre would be carcinogenic by inhalation (Bernstein et al., 2001b). We would argue against

788 the use of intraperitoneal tests for human health risk assessment except perhaps for the purpose of

789 exoneration of a material from classification as a carcinogen.

790

791 **Conflict of Interest Statement**
792
793 The named authors all contributed to this paper. Gail Drummond is a PhD student at the University

794 of Hertfordshire with research interests in this area. Paul Harrison and Ruth Bevan are independent

795 toxicology/risk assessment consultants; they are not involved in legal testimony related to the

796 materials and products discussed and do not have any form of commercial interest in them. Paul

797 Harrison acts as an advisor to ECFIA (an association representing the high temperature insulation

798 wool industry) in matters relating to health and safety.

799

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801
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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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963

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.