Ann. occup. Hyg., Vol. 46, No. 2, pp. 135–142, 2002

Published by Oxford University Press
DOI: 10.1093/annhyg/mf034

Carcinogenicity Studies after Intraperitoneal Injection of Two Types of Stone Wool Fibres in Rats

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Received 17 August 2001; in final form 1 November 2001

A summary is given of the pathology results after intraperitoneal (i.p.) injection in rats of insulation wool HT, representing the new biosoluble types. The pathology results are compared with a previously conducted i.p. study with traditional stone wool D6 (with similar chemical composition to MMVF21). The HT fibre is characterized by a relatively high content of aluminium and a relatively low content of silica compared to MMVF21. HT has a high *in vitro* dissolution rate at pH 4.5, a relatively low dissolution rate at pH 7.5 and is less biopersistent than the MMVF21 fibre. Female Wistar rats received a dose of 2×10^9 WHO HT fibres by i.p. injection. The fibres had been size-selected to be largely rat respirable. The negative control group was exposed to saline. Following exposure, the animals were maintained until survival in one group fell below 20%. At this time, all animals were killed. All animals were subjected to a necropsy examination; any gross abnormalities observed at necropsy were subjected to histopathological examination. In addition, histopathology was carried out on a predefined list of tissues. The incidences of lesions and survival in the control and fibre dosed animals were compared using appropriate statistical methods to determine whether the dosed animals showed adverse effects on survival or a positive carcinogenic response. The main protocol for the previously conducted study with D6 (MMVF21) was similar, but the animals were maintained as long as they survived, and the WHO fibre dose was lower. The results of the comparative study showed a marked difference in the i.p. pathogenicity of D6 (MMVF21) and HT in terms of their carcinogenic potential. D6 (MMVF21) caused a statistically significant increase of mesotheliomas in the peritoneal cavity compared to the negative control, but the HT fibre did not cause any mesotheliomas or any increase in other tumour types.

Keywords: man-made vitreous fibres; HT stone wool; intraperitoneal injection; pathogenicity; rat

INTRODUCTION

Man-made vitreous fibres (MMVF)—synonymous with synthetic vitreous fibres (SVF) and manufactured vitreous fibres (MVF)—with alkaline oxide and alkali earth oxide (Na_2O + K_2O + CaO + MgO + BaO) content >18 % by weight are classified within the European Union as 'carcinogenic category 3' (possibly carcinogenic) and as 'irritant' (irritating to skin) (European Commission, 1997b). According to Nota Q in this directive, the classification as a carcinogen need not apply if certain conditions are fulfilled. Among these conditions are absence of relevant pathogenicity or neoplastic changes in a suitable long-term inhalation test or no excess of carcinogenicity after an appropriate intraperitoneal (i.p.) test.

It is generally accepted that results from well-designed, long-term inhalation tests are most relevant for predicting health effects in humans; however, the German authorities have a regulatory requirement for chronic i.p. studies (TRGS, 1994).

There has been considerable debate on the appropriateness of using results from i.p. injection studies to predict the potential carcinogenicity of airborne fibres following inhalation (McConnell, 1995; NRC, 2000). The main criticism of the inhalation methodology is its alleged low sensitivity compared to the i.p. test. When fibres are injected into the abdominal cavity, naturally occurring mechanisms are unlikely to be similar to those occurring in the lungs after inhalation and this calls into question the validity of using i.p. tests to assess potential inhalation hazards.

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The i.p. test appears, however, to be very sensitive and able to detect a low carcinogenic potential of fibres, but generally, any increase in sensitivity of a test will decrease the specificity.

In the following, a summary is given of the pathology results from an i.p. study of a newly developed stone wool fibre with increased biosolubility, HT (high temperature) and the results compared to the previously tested traditional stone wool (D6 with similar chemical composition to MMVF21).

The objective of the Rockwool International (RI) sponsored study with the HT fibre finalized in 2001 was to assess the potential carcinogenic effects after i.p. injection of stone wool fibres in rats from a detailed pathological examination on selected tissues of each animal. A statistical analysis for differences in the number of abnormalities and in survival or disease incidence between fibre dosed group and control animals was made.

D6 (MMVF21) has previously been tested in a similar study at the same laboratory; the study was finalized in 1994, but only the preliminary findings from that study have been published (Collier, 1995). The data from that study are used in the present paper. In addition, MMVF21 has also been tested by i.p. injection at other laboratories and the results reported (Roller *et al.*, 1996; Miller *et al.*, 1999). In these studies the MMVF21 material was a size-selected preparation produced by the North American Insulation Manufacturers Association (NAIMA).

MATERIALS AND METHODS

Design of studies

The animal experimental work and pathomorphological examinations in both studies were conducted at AEA Technology, Biomedical Research, Harwell, UK. The study with HT fibre was carried out in compliance with guidelines for good laboratory practice. The fibre size selection in the studies was done by the Johns Manville Technical Centre (JMTC), Littleton, CO, USA (D6 and HT) and by the Fraunhofer Institute of Toxicology and Aerosol Research (Fh-ITA), Hannover, Germany (HT).

Laboratory rats were exposed by i.p. injection to well-characterized fibre suspensions that had been selected to be largely rat respirable. The rats were randomly assigned to the individual exposure groups.

In both studies, rats were exposed at one nominal exposure level aimed at ~0.5×10^9 WHO fibres administered as a single injection. A WHO fibre is defined as a fibre (length/width ≥3) with a length >5 μm and a diameter <3 μm. The negative control group was exposed to saline. The animals were given one injection each. Following exposure, the animals were maintained until survival in one group fell below 20% (HT study), or the animals were maintained as long as they survived (D6 study). The findings of the studies were considered in terms of possible adverse effects, the necropsy and histopathological findings. The evaluations included tumour incidence, survival analysis and comparison of body weights.

Test substances

The i.p. studies included two different stone wool fibre types: the HT fibre, CAS registry number 287922-11-6—a newly developed commercial insulation wool product (Roxul®)—and traditional stone wool. In the studies, the HT fibre was identified as RIF39001 and the traditional stone wool fibre as D6, but they had similar chemical compositions to MMVF34 and MMVF21, respectively, used in the previously conducted chronic inhalation studies with these fibres, although they had different dimensions (specifically shorter mean length). The negative controls in both studies were saline.

The chemical compositions of the two MMVFs are given in Table 1. The specific gravity of the two fibres are very similar (2.8 g/cm^3). The new biosoluble HT stone wool fibres are characterized by a higher amount of alumina and a lower amount of silica than the traditional stone wool fibres. Due to analytical variation and minor differences in the different batches used, variations may occur in published chemical compositions of these fibres.

The bulk products were produced without the addition of binder or oil. Bulk fibres were size-separated, using a water-based process (D6) or in combination with an air-based dry process (HT), to be largely rat respirable.

Prior to administration to animals, an initial assessment of the test materials was made. The bivariate size distributions were measured by phase contrast optical microscopy (PCOM) for D6 and scanning electron microscopy (SEM) for HT. The fibre numbers per milligram of material were determined. The measurements on the HT stock fibre were done by Fh-ITA and on the D6 stock fibres by AEA.

Based on these measurements, trial suspensions of the fibres, identical to those to be used for the injec-

Table 1. Chemical composition of fibers by weight %

Weight %	D6	HT
SiO_2	45.9	39.6
Al_2O_3	13.8	21.5
TiO_2	3.0	2.0
FeO	6.2	6.4
CaO	17.0	15.4
MgO	9.5	10.7
Na_2O	2.5	0.1
K_2O	1.3	0.8
Other oxides	0.4	0.0
Sum	99.4	96.5

Table 2. Total animal numbers per group killed because of clinical signs, found dead or killed at the termination of study

Animals dosed with	No. of animals dosed	Animals died/killed within 16 days of injection	Animals entering study	Killed as a result of clinical signs	Found dead	Killed at end of study
D6	60	3	57	51	6	0
Control (D6)	96	4	92	87	5	0
HT	50	0	50	30	7	13
Control (HT)	50	0	50	40	1	9

tions, were prepared. Samples were taken from these suspensions in an identical way to that used for the injections and the measurements of fibre dimensions and numbers were made. Based on data from these samples, the numbers and sizes of fibres injected into the rats were determined for D6 and for HT. In the case of the HT fibre, discrepancies were found between the numbers of fibres in the samples and the expected numbers of fibres/mg measured in the stock fibres using the measurements carried out by Fh-ITA. In order to ensure a sufficiently high dose and a valid study, it was therefore decided to base the amounts used for actual dosing on the measurements of the stock fibres.

The possible effects of the size-selection process on the chemical and surface characteristics of these fibres have been found to be insignificant. This is based on a comparison of the morphology of the fibre surfaces of the bulk fibres and the size-separated fibres using SEM, the chemical composition and the *in vitro* dissolution rates at pHs 4.5 and 7.5. The HT fibre is characterized as having a high dissolution rate at pH 4.5 (Knudsen *et al.*, 1996).

Test system

Rats were selected for appropriate comparison of results from these studies with those from previous i.p. injection studies performed under similar conditions with other MMVFs. Female Wistar rats from two different suppliers were used for the two studies (Harlan UK, Oxon and Charles River UK, Kent). The rats were 10–12 weeks old at injection and weighed ~200–230 g. The animals in the D6 study were somewhat lighter than those in the HT study. A total of 50 HT-group and 57 D6-group rats entered the studies. The HT control group comprised 50 animals, while the D6 control group comprised 96 animals. Following the exposure, the HT exposed animals were held for lifetime observation (until ~20% survived), which occurred at ~26.5 months. In the D6 study, the animals were maintained as long as they survived. An account of all HT and D6 exposed animals in the studies is given in Table 2.

Unique numbers identified the rats. When not being exposed, the rats were housed with a maximum of three animals per cage in plastic cages. The studies were conducted under optimum hygienic conditions behind a barrier system.

The rooms were air-conditioned and had ~20 air changes per hour, with an approximate temperature of 17–25°C and a relative humidity of 40–70%. The animals are maintained on a 12:12 h light/dark cycle. All environmental conditions were monitored and recorded. Pelleted standard rat maintenance diet and chlorinated community tap water were supplied *ad libitum* during the study.

Exposure

Intraperitoneal injection was used as the method of administration. The nominal dosage of 0.5×10^9 WHO fibres was selected to be comparable to the other study on D6 done previously. In the D6 study, the dosage of 0.5×10^9 fibres was selected based on another fibre definition, 'critical fibres'. A 'critical fibre' was defined as a fibre (length/width >5) with a length >5 μm and a diameter <2 μm.

The draft EU guideline ECB/TM/18(97) rev. 1 (European Commission, 1997a) 'Carcinogenicity of synthetic mineral fibres after intra-peritoneal injection in rats', uses the number of WHO fibres as the basis of the choice of exposure condition. The guideline specifies a single dose of 1×10^9 WHO fibres as the dose and optionally defines two additional dosage groups with 1×10^7 and 1×10^8 WHO fibres. In addition, at least 20% of the WHO fibres in suspension should have a length >20 μm.

The reported studies were conducted before these guidelines were available, and to reduce cost, only one dosage group was included in each of the studies.

All apparatus used in preparation and injection of the suspensions was purchased either sterile or sterilized prior to use. The fibre samples were suspended in saline (0.9 % NaCl in water). Pre-weighed fibre samples were suspended in the saline and sonicated.

Prior to administration, each animal was examined to ensure that it was fit to be included in the study. Any that were not were excluded from the study and replaced with animals from a spare group. Each rat was lightly anaesthetized with halothane. The animal was placed on its back and a 14G intravenous catheter inserted into the peritoneal cavity through the abdominal wall. The needle was inserted so it was just inside the abdomen.

The needle was withdrawn from the catheter and discarded. The instillation suspension was manually shaken and vortex mixed for ~30 s. Two millilitres of suspension were drawn into a 2 ml sterile polypropylene syringe. The syringe was connected to the catheter and the contents of the syringe injected into the animal. The catheter was withdrawn carefully and any bleeding or loss of suspension from the injection site was recorded. The peritoneal cavity was gently massaged to disperse the fibres whilst the animal regained consciousness.

In the D6 study administration was similar, but a 2.5 ml syringe connected to a 25G sterile needle was used for injection.

In the HT study, each animal in a group was dosed in an order randomized by cage number. The randomization was achieved by having a random list of cage numbers generated for each day. In the D6 study, no formal randomization scheme was employed. The date and time of dosing were recorded for each animal. The animals were given a single injection each.

Observations, examinations and measurements

The rats were examined once daily for clinical signs, morbidity and mortality. A detailed clinical observation was given once per week during initial quarantine and acclimatization, at allocation to individual groups, twice per day on administration days, once daily during the first 2 weeks after the end of the administration phase, once weekly thereafter and immediately prior to the animals being killed.

The rats were weighed on the first day of administration, prior to administration start, once weekly thereafter through to week 13 and once monthly thereafter.

Following exposure, in the HT study the animals were maintained until survival in one group fell below 20%; in the D6 study the animals were maintained as long as they survived.

Any moribund animals or those showing palpable abdominal masses >2 cm in diameter were killed. As mesothelioma metastasizes rapidly throughout the peritoneal cavity, ascites resulting in distension of the abdomen is frequently the first detectable symptom. Any animals showing signs of abdominal pain or distension of the abdomen were also killed. The animals in the HT study were killed by CO_2 inhalation followed by exsanguination of the brachial artery. In the D6 study, animals *in extremis* were killed by CO_2 inhalation. The date and time of death was recorded in the study records.

A detailed necropsy examination was conducted on all animals. All gross necropsy findings were recorded on a necropsy data sheet for the individual animal. The necropsy included a detailed examination of all peritoneal organs, and the thoracic cavity. At necropsy, the cause of death/moribundity was recorded to indicate whether any observed lesions were thought to be incidental or causative.

From all animals the following tissues were sampled for histology: diaphragm including the falciform ligament (if present); omentum; intestinal mesenteries including a segment of gut, liver, spleen, pancreas; and any lesions observed at necropsy. The tissues were removed and fixed in formalin. The remaining carcass was stored in formalin until the end of the study to allow examination of other organs if requested by the pathologist. The falciform ligament atrophies with age; the region where the ligament is normally found was examined, although the ligament may not have been present.

The sampled tissues were mounted in paraffin, sectioned and stained with haematoxylin and eosin prior to examination by the pathologist.

Data analysis

For each animal, survival in days from i.p. administration was calculated. A Kaplan–Meier analysis was conducted to determine whether any statistical differences in survival existed between the groups.

The effect of exposure on weight was made by monitoring the change in weight over different periods (~50 days) during the growth period (up to 300 days). This approach took into account the differences in original starting weights. A quadratic regression was fitted to the growth data, and the intercepts, curvature and slopes of these functions between groups were compared.

For each group, the incidence of pathology findings (including specific neoplasms) was calculated. Tumour incidences in the exposed and the control animals were compared using χ^2 tests.

RESULTS

Fibre suspensions

The injection mass, mean number of fibres, WHO fibres, fibres with a length >20 μm and the mean dimensions of the fibres in the injection suspensions measured by AEA are presented in Table 3.

Differences in fibre measurements between PCOM and SEM are known to exist, since the resolution of the two methods is not the same. In consequence, comparative assessment of fibre sizes and numbers should be considered with caution.

As can be seen from Table 3, the HT fibres were thinner and longer than the D6 fibres. The number of fibres injected in the HT group was approximately double that of the D6 group. The different size-selection methods used may have contributed to the differences seen in the mass injected.

Clinical signs and mortality

There was no indication that the i.p. injection of HT fibre had any effect on the health and survival

Table 3. Total injection mass (mg) per animal, mean fibre numbers ($\times 10^9$) and dimensions (μm) in injection suspensions

Study	Injection mass (mg)	Fibres ($\times 10^9$)	WHO ($\times 10^9$)	Length > 20 μm ($\times 10^9$)	Fraction WHO length > 20 μm (%)	Geometric mean (μm		Median (μm)	
						Diameter	Length	Diameter	Length
D6	36	1.2	0.9	0.2	18	0.80	8.5	0.87	7.8
HT	9	2.6	2.1	0.6	28	0.65	10.7	0.67	9.9

Table 4. Number of fibre and saline injected animals dying, by survival time

Survival interval (days)	HT	Control (HT)	D6	Control (D6)
1–100	0	0	0	1
101–200	0	0	1	3
201–300	0	0	2	0
301–400	6	2	11	6
401–500	2	4	18	20
501–600	6	9	23	37
601–700	16	7	1	18
701–800	20	28	1	7
n	50	50	57	92

Table 5. Results of Kaplan–Meier survival analysis on all animals, point estimate (days)

All animals	HT	Control (HT)	D6	Control (D6)
Mean survival	646	672	474	536
25% quartile	564	573	424	468
50% quartile	691	717	480	553
75% quartile	n.c.	776	547	607

n.c. = not calculated.

compared to the control group. Survival of D6 injected animals was significantly less than that of their comparative controls ($P < 0.0001$; Table 4). The difference seems to be caused by a significant drop in survival at ~350 days.

The survival in days of each animal was used to conduct a Kaplan–Meier analysis. The mean, median and 25 and 75% quartile values are given in Table 5 and the survival curves plotted in Fig. 1.

The life expectancy of mesothelioma bearing animals in the D6 group was significantly lower compared with those animals not dying of mesothelioma.

There is no apparent reason for the differences in survival between the two control groups, but two different suppliers of rats were used in the two studies.

Body weights

In the study with HT, body weight gains were comparable to the control group. In the study with D6, the animals showed some reduction of growth compared to the control.

Pathology

At necropsy, numerous macroscopic nodules in the peritoneal cavity were observed in the majority of D6 (88%) exposed animals and further smaller nodules were found associated with the abdominal organs during microscopic examination. These were found to be clumps of fibres, either adherent to the surface of the viscera or free in the abdominal cavity. There was evidence of a foreign body response with granuloma formation. These long standing granulomata become fibrotic and sclerosed, effectively isolating mineral fibres from the surrounding environment. At post-mortem examination of HT exposed animals and control animals, a low incidence of macroscopic nodules was found (three animals and one animal, respectively). Pathological examination showed these to be cellular in nature, having no fibres associated with them. Whilst no macroscopic fibrous nodules were observed in the HT or control animals, at microscopic examination one animal injected with HT fibre was found to have a nodule of fibres (1×3 mm). This had an incomplete connective tissue capsule containing a mass of fibres and cellular debris. There was no evidence of an ongoing cellular reaction.

There was also evidence of fibrosis in the peritoneal cavity of some of the animals, with adhesions formed between some organs. This occurred almost exclusively in the fibre injected animals. A principal site for this type of finding was between the liver and diaphragm, which frequently involved the complete fusion of the liver to the diaphragm. Other sites for adhesion included the loops of the small intestine, between the stomach and other organs, and between the adipose tissue and other organs.

The individual incidences of macroscopic nodules and adhesions are given in Table 6.

Three tumour types showed significantly different incidences in fibre injected animals compared with concurrent controls (Table 7). The highest tumour incidences in control animals were for benign pituitary adenomas (42–54%) and mammary adenocarcinoma (40–46%). These are very common tumours in this strain of rat and the finding is not unexpected. In the D6 injected animals, mesothelioma was the most common tumour type (56%). This tumour was not found in any other group (control or HT injected) and as such was a highly significant finding. The

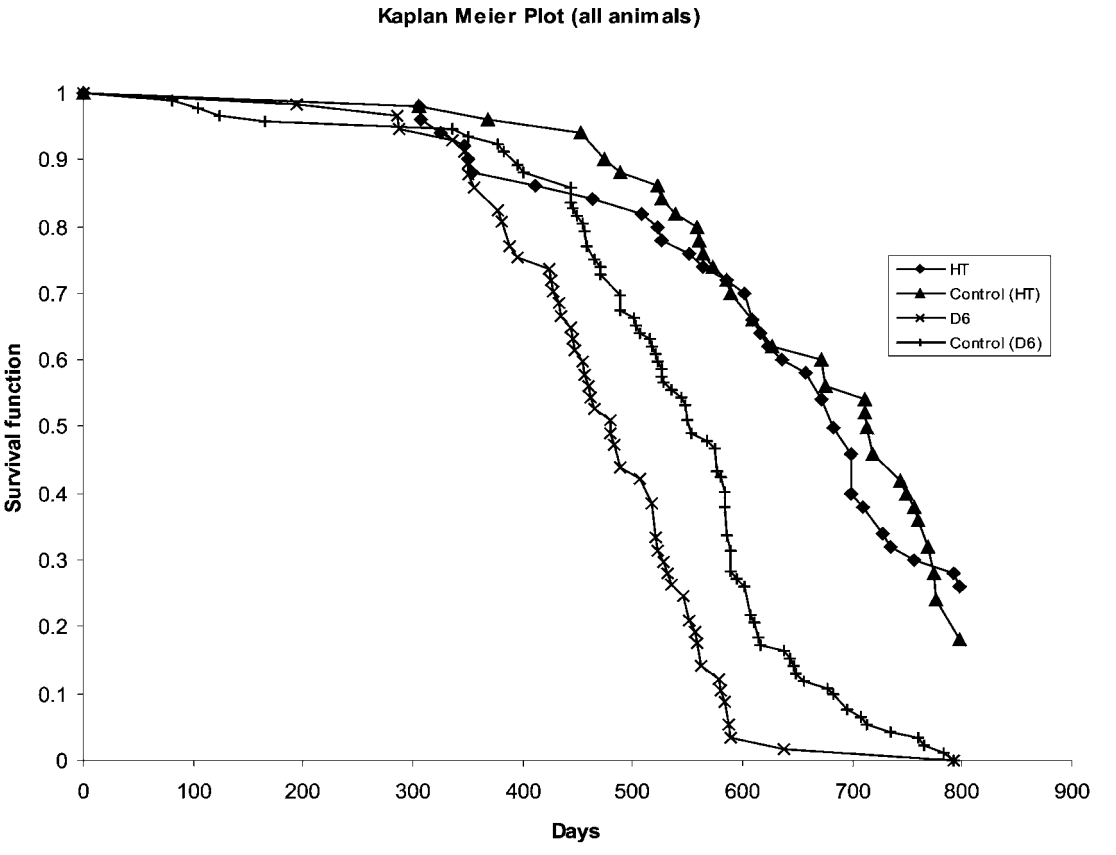

Fig. 1. Survival of all animals (Kaplan–Meier plot).

Table 6. Occurrence of macroscopic nodules and adhesions

Group	*n*	Fibrous nodules		Adhesions	
		n	%	*n*	%
D6	57	50	88	53	93
Control (D6)	91	1	1	5	5
HT	50	3	6	29	58
Control (HT)	50	1	2	1	2

Table 7. Summary incidence of tumours showing significant differences from concurrent controls

Group	*n*	Mesothelioma		Pituitary		Mammary tumour	
		n	%	*n*	%	*n*	%
D6	57	32[a]	56	9 [b]	16	12[b]	21
Control (D6)	91	0	0	38	42	36	40
HT	50	0	0	22	44	20	40
Control (HT)	50	0	0	27	54	23	46

[a]Statistically significant compared to concurrent control, χ^2 tests ($P < 0.05$).
[b]Lower incidence attributed to the premature death of the mesothelioma bearing animals.

mesotheliomas produced by the injections of D6 showed the usual histological patterns that have been well documented from studies involving i.p. injection of mineral fibres in rats (Roller *et al.*, 1996; Miller *et al.*, 1999). Significantly lower incidences of pituitary and mammary tumours were found in the D6 injected animals compared with controls. This was as a result of mesothelioma acting as a competing cause of death.

Various other tumours were observed; however, all were at low incidences and showed no significant differences between treatment groups.

DISCUSSION

The primary objective of the present study was to assess the pathological effects of the HT fibre in an i.p. carcinogenicity assay. The HT fibre is a newly developed stone wool with increased biosolubility. Long-term inhalation studies have been done with both the HT fibre and the traditional stone wool fibre MMVF21 and have shown no excess of tumours compared to control; however, some fibrosis was seen with MMVF21 (Kamstrup *et al.*, 2001). There has been considerable debate on the appropriateness of using results from i.p. injection studies to predict the potential carcinogenicity of airborne fibres following inhalation. The long-term inhalation model has the advantage of simulating the situation that occurs

following actual workplace exposure. The main criticism of the inhalation methodology is its alleged low sensitivity, which requires prolonged administration of high doses of fibres to give a significant number of tumours. When fibres are injected into the peritoneal cavity, naturally occurring mechanisms are unlikely to be similar to those occurring in the lungs after inhalation. However, the i.p. test appears to be very sensitive and therefore able to detect a low carcinogenic potential of fibres. This means that negative results obtained using this assay can be interpreted as reflecting an absence of carcinogenic potential.

The nominal dosage of 0.5×10^9 WHO fibres was selected in order to be comparable to the other AEA study done previously with the traditional stone wool (D6). The draft EU guideline (European Commission, 1997a) on i.p. carcinogenicity studies with mineral fibres in rats specifies that dosages to which the animals are exposed in the highest dose should be at least 1×10^9 WHO fibres. The nominal fibre dosage in the study with the HT fibre was lower, with a dosage of 0.5×10^9 WHO fibres. However, based on the measurements by the laboratory conducting the study on the fibre suspensions used for actual dosing, the achieved dosage was $\sim 2 \times 10^9$ WHO fibres for the HT fibre. The D6 dosage, measured by the same laboratory, was 0.9×10^9 WHO fibres. The dose proposed by the German authorities for i.p. injection of fibres for regulatory testing is 5×10^9 WHO fibres (TRGS, 1994).

While the failure to calculate the dose of fibres to be injected more accurately was unfortunate, the error did in fact make the testing of HT fibres by i.p. injection much more rigorous. The present testing of HT fibres was in fact undertaken at more than twice the EU recommended dose, which makes the negative findings particularly remarkable. An additional factor was that the injected HT fibres were longer and thinner than the D6 used as comparison and this should have made them more carcinogenic, if the biopersistence of the two fibre types had been equal. HT fibres are, however, much more biosoluble than D6 and thinner fibres would be expected to dissolve faster than thicker ones.

The HT fibre was developed recognizing that the potential pathogenicity of a given fibre type is mainly dependent on the extent to which the fibres can be inhaled and persist in the lung (Davis, 1991). A short-term (5 day) inhalation biopersistence study with the HT and MMVF21 fibres showed elimination half-times for the HT fibre, calculated from the decrease of WHO fibres and long (>20 μm) fibres, of 25 and 6 days, respectively. The elimination of the traditional stone wool (MMVF 21) was much slower, with elimination half-times of 65 days for WHO fibres and 92 days for long fibres (Kamstrup *et al.*, 1998). In long-term inhalation studies in rats, the lung burdens of long fibres during the study also demonstrated the higher biosolubility of the HT fibre compared to traditional stone wool. Much lower maximum lung burdens were found with the HT fibre at comparable exposure levels. In addition, the significance of biopersistence was also confirmed, as the pathology after 3, 6, 12, 18 and 24 months exposure showed minor histopathological changes with HT compared to MMVF21. The HT showed minimal collagen deposition, similar to what would be expected for any biologically inert dust at this exposure level, while MMVF21 resulted in fibrosis. For both fibre types, there was no evidence of carcinogenic activity in either the lungs or pleura (Kamstrup *et al.*, 2001).

In a previously conducted comparative study between HT and MMVF21 (Collier, 1997), biopersistence was determined up to 6 months after administration by either intratracheal instillation or i.p. injection. For both fibre types, significant differences in the biopersistence of fibres in the lung and peritoneal cavity were found. For MMVF21, no fibre clearance from the diaphragm occurred following i.p. injection, compared with measurable clearance from the lung following intratracheal instillation. For HT fibres, long fibres cleared twice as quickly from the diaphragm as from the lung. This suggests differences in dissolution behaviour in the peritoneal cavity when compared with biosolubility in the lung. Another study (Dörger *et al.*, 2001) found marked differences in the physiology of alveolar and peritoneal macrophages in their response to inflammatory stimuli and mineral fibres, but the mechanisms behind the observed differences in the biopersistence in the peritoneal cavity and the lungs are not presently known. An important mechanism for the clearance of the HT fibres from the lung is thought to be intracellular and extracellular digestion and fragmentation by the pulmonary macrophages (Baier *et al.*, 2000).

The differences in biopersistence of fibres between the lung and peritoneal cavity call into question the validity of using i.p. injection to assess potential inhalation hazards from MMVFs. However, the findings in this study indicate the significance of the higher biosolubility of the HT fibre compared to traditional stone wool (MMVF21), as the results of the comparative study showed a marked difference in the i.p. pathogenicity of the traditional stone wool and HT in terms of their carcinogenic potential. D6 caused a statistically significant increase of mesotheliomas, the tumour type most commonly associated with the response of the peritoneal cavity to fibres, compared to the concurrent saline control. The HT fibre did not cause any mesotheliomas or any increase in other tumour types. The significance of biopersistence for the carcinogenicity of fibres after i.p. injection has previously been recognized (Roller *et al.*, 1996) and confirmed for the new generation of

fibres with increased biosolubility (Lambré *et al.*, 1998).

The concept, that all respirable mineral fibres will be carcinogenic if they are long, thin and durable enough to survive in the lung for long periods, is now supported by a considerable amount of experimental evidence. Very durable fibres such as asbestos, ceramic fibres or silicon carbide have all proven carcinogenic in rat inhalation studies as well as in the more sensitive i.p. injection studies (Wagner *et al.*, 1973; Davis *et al.*, 1986, 1996; Mast *et al.*, 1994). Traditional insulation fibres such as stone wool, slag wool and glass wool have proved insufficiently durable to cause tumours in rat inhalation studies, but do survive long enough in the rat peritoneal cavity to produce mesotheliomas following injection (McConnell *et al.*, 1996; Miller *et al.*, 1999). Fibres with specially reduced durability, such as the new HT fibre, have now proved unable to cause tumours following both inhalation and injection. It is plausible that organic fibres will obey the same rules as inorganic ones and that long, thin, durable fibres will be carcinogenic. Here, however, relatively little experimental work has been done. One organic fibre, cellulose, has been found to be durable in rat lungs (Muhle *et al.*, 1997) and cellulose has produced tumours in rats following i.p. injection (Cullen *et al.*, 2000); long-term inhalation studies with cellulose and other organic fibres are urgently needed.

The results of the current studies suggest that the HT fibre has little or no adverse biological activity when injected into the peritoneal cavity and confirms that the introduction of fibres with higher biosolubility has increased the safety margins in manufacturing and use of fibrous insulation material.

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