CORRESPONDENCE

Preliminary experimental findings using intraperitoneal assays to determine carcinogenic potential of man made mineral fibres: relevance to recent proposals for classification testing

Collier¹ criticises one of the rules established in the German TRGS 905 which states that inorganic fibre dusts are not to be classified in European Union category 2 or 3 as carcinogenic substances if an intraperitoneal injection test with 5×10^9 fibres (World Health Organisation (WHO) definition, length, and diameter distributions typical of those found in the workplace) is negative.²

The limit of 5×10^9 fibres of TRGS 905 is based on our intraperitoneal studies of Pott *et al.* and Roller *et al.*,³ which show that the carcinogenic potency of crocidolite and actinolite fibres as well as refractory ceramic fibres is roughly 1000-fold higher than that of the weakly biodurable B-01 glass fibres; 10×10^9 fibres of the tested B-01 sample showed no clear carcinogenic response in female Wistar rats and a relatively low tumour frequency in male rats. The B-01 glass fibre type was developed with the aim of creating a vitreous fibre with no or only a very weak carcinogenic potency which should not require classification in any category of carcinogenic substances. It has been concluded from the results of intraperitoneal studies that the aim was achieved and that the B-01 glass fibres should serve as a prototype for non-categorised vitreous fibres. This means that other vitreous fibre types can be exonerated from a justified suspicion of being carcinogenic if they show a similar weak or lower carcinogenic potency than the tested B-01 fibre sample.

Collier's objections are:

(1) the masses proposed for fibres to be classified as non-carcinogenic are unwork-

ably high; they would require dust masses in the range of 3400 to 5300 mg.

(2) the proposed test displays sensitivity to fibre length that could result in ambiguous results depending on the fibre length used.

(3) the classification in terms of WHO definitions of fibres with size distributions typical of those found occupationally are not suitable for describing the biological activity of the fibres.

(4) masses < 200 mg significantly reduced life spans in animals that did not develop tumours, suggesting that the maximum tolerated dose for intraperitoneal injection has been exceeded.

We reply to the four objections as follows:

(1) The two dust samples to which Collier refers differ significantly from those used in the recent inhalation studies performed in the RCC Laboratories, Geneva, which are considered to adequately mimic occupational exposure. Table 1 shows that RCF-1 as well as the four dust samples generated from glass, stone, and slag wool contain 5×10^9 fibres in less than 1000 mg. This also applies to the MMVF-11 and MMVF-21 intraperitoneal samples tested (table 2). Also, table 2 shows the data of experimental wools and microfibres. B-09-0-6 dust (300 mg) containing 6.1×10^9 glass microfibres with a relatively weak durability were associated with four mesothelioma cases from 39 rats; the WHO fibre number required for inducing a 25% tumour risk (TD_{25}) was calculated to be 18×10^9 fibres. However, the 95% confidence interval (95% CI) is large, but a dust sample with longer and thicker fibres of the same chemical composition (B-09-2-0) was roughly 10 times more potent ($TD_{25} = 1.7 \times 10^9$), and a different sample with a more durable composition and similar dimensions (B-20-2-0) was again eight

times more potent ($TD_{25} = 0.22 \times 10^9$).

The dust samples of Collier were longer (median lengths 20 and 25 μ m) and thicker (median diameter 1.5 and 1.4 μ m) than the samples used in the RCC studies. Moreover, the mass of particles that did not fulfil the WHO fibre definition and which could be called "impurities of the fibre dusts" may have been much higher in the samples of Collier than in the RCC samples and ours. Altogether, the data in tables 1 and 2 show that it is possible to generate vitreous fibre dusts from insulation wools which contain 5×10^9 WHO fibres with median lengths and diameters in the range of 15 μ m and 1 μ m and relatively small ranges of size distribution in roughly 600 to 700 mg.

(2) It has been well known for many years that longer fibres have a higher carcinogenic potency than shorter ones. However, there is a lack of clear knowledge on the differences in carcinogenic potency of durable fibres with a length of 5, 10, 15, 20 μ m or more. Therefore, it is difficult to develop true correction factors when the carcinogenicity of fibre samples with different lengths has to be compared, particularly because diameter and aspect ratio also play a part. Irrespective of the workplace situation, it would be advantageous to compare the effect of fibre samples with the same length and diameter distributions, but this cannot be achieved because it is technically not feasible to generate samples with exactly the same fibre size distribution; small variations in size distribution have to be accepted for experiments and the consequences for the biological effects should be estimated. According to the data of the RCC studies it may be adequate to consider median lengths in the range of 15 μ m and median diameters in the range of 1 μ m to be "workplace like" in the insulation industry, although higher median values of 25 μ m (length) and 1.5 μ m (diameter) were measured. On the other hand, shorter and much thinner glass microfibres, so called "special purpose fibres", have also to be taken into consideration (table 2).

Table 1 Examples of fibre dusts which contain 5×10^9 WHO fibres in less than 1000mg and which were tested by inhalation in the RCC laboratories

Fibre type	Median length (μ m)	Median diameter (μ m)	Exposure concentration		Dust mass containing 5×10^9 f(mg)	Source
			10^9 f/m ³	mg/m		
RCF-1	16.8	0.85	0.187	29.1	778	Mast <i>et al</i> 1995 ⁴
MMVF-10	12.5	1.33	0.232	29.1	627	Hesterberg <i>et al</i> 1993 ⁵
MMVF-11	13.3	0.68	0.246	28.9	587	Hesterberg <i>et al</i> 1993 ⁵
MMVF-21	15.5	0.92	0.150	16	533	McConnell <i>et al</i> 1994 ⁶
MMVF-22	15.5	0.85	0.213	30	704	McConnell <i>et al</i> 1994 ⁶

Table 2 Example of fibre dusts which contain 5×10^9 WHO fibres in less than 1000mg and which were tested by intraperitoneal injection*

Fibre type	Median length (μ m)	Median diameter (μ m)	10^9 Fibres per mg dust	Dust mass containing 5×10^9 f(mg)	$TD \pm$	
					10^9 f	mg
Fibres from insulation wools:						
MMVF-11	13.5	0.94	6.6	758	0.32	49
MMVF-21	14.6	1.03	7.8	641	0.037	4.7
Data of Collier for comparison ¹ :						
MMVF-11	24.9	1.39	0.94	5300	—	—
D6 (=MMVF-21)	20.1	1.5	1.5	3400	—	—
Fibres from experimental wools:						
B-20-2-0	6.6	0.83	16	313	0.22	14
B-09-2-0	8.6	1.3	10	500	1.7	170
B-01-0-9	~ 7.5	~ 0.7	22	227	13	570
Microfibres:						
M-753-104	~ 3.3	0.22	57	88	0.20	3.5
B-20-0-6	3.1	0.33	120	41	0.38	3.1
B-09-0-6	2.8	0.49	22	227	18	800
Tremolite	2.4	0.27	18	278	0.049	2.7

*Roller *et al.*, 1996, including unpublished final results.³

†WHO fibre number required for inducing a 25% risk of tumour.

(3) It makes sense to test fibres with workplace like dimensions,—for example, because a significant difference may exist between the carcinogenic potency of dust from a glass microfibre type with a moderately soluble chemical composition and much thicker inhalable fibres from insulation wools of the same chemical composition. The thicker fibres persist longer because they need more time to dissolve; accordingly, the induced tumour rate is significantly higher (compare type B-09-0-6 and B-09-2-0 in table 2). But there is no significant difference between the carcinogenicity of different diameters when the glass composition is relatively durable (compare type B-20-0-6 and B-20-2-0 in table 2).

It should be borne in mind that, on the one hand, the capacity of the intraperitoneal test model is certainly much larger than that of other models; on the other hand, as far as we know, the capacity may be limited at about 1000 mg dust, because the contact between cells and dust particles cannot be enhanced infinitely by higher masses. We do not have experience with masses higher than 1000 mg. This means that long and thick inhalable fibres which may be relevant to some extent in the work area cannot be tested with the same fibre number as thinner and shorter fibres.

(4) Up to now, important data from Collier have not been published which would allow us to judge whether life spans of rats after intraperitoneal injection suggest that the maximum tolerated doses have been exceeded. We have strong doubts that Collier's interpretation of her results is correct because our comprehensive data base on survival times of controls and rats injected with fibres speaks unequivocally against this suggestion. Similar conclusions were drawn from our data by McConnell⁹ but they were refuted as unwarranted.¹⁰

The maximum tolerated dose for an intraperitoneal carcinogenicity study with fibres has been exceeded when the lifetime of a group was reduced because of fibrosis or adhesions in the abdominal cavity. The mass dose of this maximum tolerated dose depends on the inflammatory and fibrogenic activity of the respective dust sample tested. In any case, this maximum tolerated dose is much higher than the 1.5 mg discussed by Collier *et al.*¹¹ But this does not mean that the observed tumours are non-specifically induced. Asbestos workers with severe asbestosis have a reduced life expectancy too. Nevertheless, they can die with a lung tumour or mesothelioma from asbestos. There are no signs of non-specific carcinogenicity in rats by intraperitoneal overloading with high amounts of non-fibrous dusts. Non-fibrous silicon carbide (1000 mg) or 125 mg B-01 glass fibres did not enhance the carcinogenicity of 0.5 mg crocidolite. Maximum tolerated dose of fibres may be reached with 1000 mg.

Even Collier disproves, with the zero effect of 55 mg of short MMVF-11, her earlier interpretation of our intraperitoneal tumours as non-specific. It seemed to Collier *et al.* "that only the first 1.5 mg of fibres in each intraperitoneal injection reaches the cells at risk, the rest being responsible for an inflammatory reaction."¹¹ They think that doses higher than 1.5 mg tend to produce rapidly infiltrating sarcomas. If this interpretation were true, Collier should have found many sarcomas after injection of 55 mg of short MMVF-11.

Collier mentions that she is restricted to a

maximum of four weekly injections of < 45 mg; but this limit only applies to her current project licence. The restriction is not absolute but is due to her proposal; it can be higher or lower in a different proposal according to the study design.

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Occupational medicine: the way ahead

Editor—I have read with interest the series of articles on *Occupational medicine: the way ahead*. It is indeed an exciting period of transition for occupational medicine. With employers increasingly being made aware of their legal responsibilities, and both employees and the general public becoming better informed about health, safety, and environmental issues, there is a window of opportunity in which to ensure that future service provision keeps pace with change, and meets the needs not only of our own nation but the wider global community.

As experts in our field we need to ensure that we are able to provide the best service possible in what is a constantly evolving and complex discipline. Within this requirement is a need to realise the limits of our own competence and to be willing and able to seek advice from specialists in other disciplines. Even for occupational physicians working in a specific type of industry, there is a constant need to expand and update our knowledge base, with the introduction of new products and processes, and the challenge of new legislation. For many practitioners the need to generate income brings us into contact with a much wider range of occupational health and safety problems. We clearly have responsibility for both the physical and psychological wellbeing of those within the workplace, but are also required to consider the impact of psychosocial issues on health, and increasingly the environmental impact of industrial problems on the general population.

Disasters such as the Braer tanker, Bhopal, and Chernobyl illustrate the potential consequences of lack of vigilance in safety and health matters. There is always the danger of similar events occurring when we are ill informed of all the issues, or are not keeping pace with new developments within our field. We should also be striving to ensure that progress within the field of occupational medicine is not limited to our industry or locality, but that information is shared across a wider industrial community and translated into formulae which can be practically applied in the developing world.

Epidemiology has provided us with valuable information about the distribution and determinants of many occupational diseases, enabling at risk groups to be identified and risk limitation measures developed. Studies of occupational groups have often been limited by poor quantification of exposure, due to lack of assessment skills, or appropriate monitoring devices, or failure to recognise the need for exposure assessment within study design. Liaison with occupational hygienists, and other specialists such as mineralogists and chemists, can ensure that monitoring methods are appropriate, and where deficiencies exist our combined knowledge can be helpful in developing new techniques. Elements of good study design and interpretation of complex data can be just some of the beneficial outcomes of discussions with an expert statistician. The establishment of clear exposure-response relations by adequate data collection and analysis will assist future planning of services and prioritising of resources, by targeting risks appropriately.

The emphasis on individual exposures and susceptibility highlights the need for techniques and models which are better able to measure such variables. The development of biomarkers to detect the impact of exposure on individual people at a cellular level is an important way ahead, and tests which have direct relevance in the workplace must be the aim. Physiologically based pharmacokinetic models can allow us to explore the impact of toxic substances on human systems, rather than relying on extrapolation from animal studies in which routes of metabolism and tumour susceptibility often show great variation between species. Occupational hygienists and toxicologists are leading the way in this field, and we have a role to contribute in our knowledge of disease mechanisms, and workplace implications.