PARAMETERS OF IMPORTANCE IN ASSESSING BIOLOGICAL ACTIVITY OF FIBROUS MATERIALS

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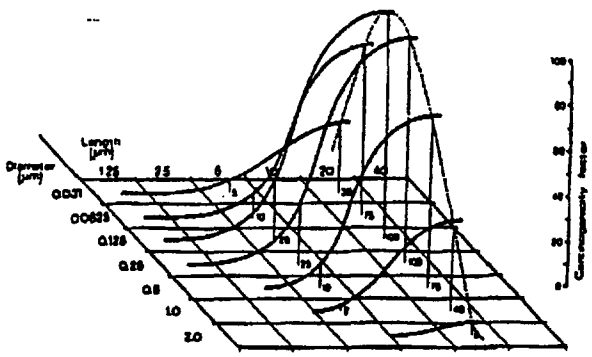
More than 10 years have past since the benchmark publication by Stanton and Layard, which has become known as «the Stanton Hypothesis». In their 1978 publication «carcinogenicity of natural and man-made mineral fibers», the authors indicated that in relation with the carcinogenic potential of fibrous materials, «...The strongest correlation was found with fibers that measured ≥0.25 micron in diameter and 8 microns in length». And thus, fiber size was identified as the first, most important parameter of pathogenicity of fibrous materials. But this was construed to a point where almost all other possible parameters were excluded for consideration.

In the past decade however, it became increasingly difficult to relate exclusively all the known effects observed experimentally or following epidemiological investigation to the sole parameter of size, and clearly, other parameters had to be considered in addition to size to account for some peculiar differences observed in the potential between mineral fibers.

One of the first indications that the «Stanton's Hypothesis» could not explain everything appeared in 1983 in the British Journal of Cancer. In an investigation carried out on fibrous erionite from Oregon, USA, British researchers of the MRC Pneumoconiosis Unit of the Llandough Hospital in Penarth found that when comparing Oregon erionite with UICC crocidolite, the fibrous erionite was shown to be far more potent than the amphibole asbestos, even when a much smaller number of fibers (~1000 times less) of erionite fibers where used. The British authors commented: «Either the fiber size hypothesis is incorrect, or there is some other property of the zeolite fiber which

[redacted]



(Pott, 1978)

This model shows that long, thin fibers are more carcinogenic than short, thick fibers.

With regard to short fibers (L ≤5 μ), a consensus was reached in 1987 at the IARC/WHO Conference in Lyon. Sir Richard Doll summed up the evidence:

> «...there is increasing evidence that short fibers (properly described as elongated particles) are much less carcinogenic, if they are carcinogenic at all. It is now 15 years since Stanton and Wrench (1972) and Pott and Friedrichs (1972) independently found that the physical dimensions of fibers were a major factor in determining their ability to cause cancer when injected intrapleurally or intraperitoneally; however, the difficulty in obtaining sufficient numbers of fibers of defined sizes made it difficult to be sure that the same was true when fibers were inhaled. The data that Davis reported now make it highly probable that the physical dimensions of the fibers are equally important in these circumstances and, taken in conjunction with the many studies of the effect of fibers on intrapleural, intraperitoneal, or intratracheal injection, they indicate that, to quote Davis, «fibers <5 μm in length may be innocuous in lung tissue» (Richard Doll).

is responsible for its activities, or which augments the activities of the few fibers in the active size range».

Since this first challenge of the Stanton's Hypothesis, much research has been published, pointing to other physico-chemical parameters of importance in assessing the biological potency of fibrous materials.

1- SIZE

Surely, size is important, and the contribution of Merv Stanton was capital in determining what size range was biologically pertinent. With regard to respiratory diseases, the first obvious requirement is that fibrous materials must have size characteristics that enable penetration into the deep recesses of the lung: the alveoli. In other words, the fibrous materials must be «respirable». This requirement will automatically exclude any particle whose diameter is approximately ≥ 3 µ; for fibrous materials, this brings about the first concept of importance with regard to respirability: the diameter. Put in other words, it is the THIN fiber which is of concern, and practically speaking, any fiber with a diameter of less than 3 microns should be considered suspect.

Next is the parameter of length. It should be realized that thin fibers with lengths up to 200 microns have been observed in human lung samples. Surely, such long, thin fibers cannot be fully engulfed by macrophages, which rupture in attempting phagocytosis, and release cytoplasmic enzymes and other factors leading to fibrogenesis. On the other hand, shorter fibers which can be fully phagocytosed are less likely to damage macrophages, which may explain their relative innocuity.

In 1978, German experimentalist Friedrich Pott published his well-known three-dimensional model concerning the carcinogenic potency of fibers as a function of their size.

This is particularly significant for those who are concerned with the presence of fibers in the general environment, since it is known that more than 95% of asbestos fibers found are shorter than 3 microns. Indeed, quoting again from the Concluding Remarks of the Lyon Symposium by Sir Richard Doll:

> «It seems, therefore, that for practical purposes the best we can now do is to work on the assumption that all fibers that meet the criteria of the International Agency for Research on Cancer (IARC, 1988) for proven carcinogenicity in animals should be regarded as potentially carcinogenic to humans, but that we should base our estimate of potential risk on both the chemical constitution of the fibers and their size, counting only those fibers that are respirable and more than 5 µm long» (Richard Doll).

2- PHYSICO-CHEMISTRY

The results of the British group of researchers comparing the potency of fibrous erionite and crocidolite were among the first indications that physico-chemical parameters, in addition to size, were certainly operating in producing adverse biological effects.

Such difference in potency between asbestos fiber types became abundantly clear in the late seventies and in the eighties when a fairly large number of epidemiological studies were published from studies in workers in a variety of workplace settings. At the same time, the use of relatively recent analytical techniques for measuring the mineral lung burden was used, and reports also supported the concept of a much different potential between chrysotile asbestos and the amphibole varieties, typically crocidolite and amosite. I have tried to summarize these reports in the following 4 figures:

DIFFERENCES IN ASBESTOS FIBER TYPES

EPIDEMIOLOGICAL STUDIES (I)

TYPES OF EXPOSURE	FIBER TYPE	MESOTHELIOMA
MILLBOARD AND PAPER MANUFACTURING(1)	CHRYSOTILE ONLY	NO CASE
YARNS, CLOTHS, PACKINGS AND GASKETS MANUFACTURING(2)	A) «ALMOST» EXCLUSIVELY CHRYSOTILE	1 CASE/2,341 WORKERS
	B) MIXED FIBER TYPES	18 CASES/1,429 WORKERS
TEXTILES(3)	«ALMOST» EXCLUSIVELY CHRYSOTILE	1 CASE/798 WORKERS

1- Weiss, W. (1977) J. Occup. Med., Vol. 19, No. 11, pp. 737-740

2- McDonald, A.D. and Fry, J. (1982) Scand. J. Work Environ. Health, Vol. 8, Suppl. 1, pp. 53-58

3- Dement, J.M. et al. (1982) Ann. Occup. Hyg., Vol. 26, Nos 1-4, pp. 869-887

DIFFERENCES IN ASBESTOS FIBER TYPES

EPIDEMIOLOGICAL STUDIES (II)

TYPES OF EXPOSURE	FIBER TYPE	MESOTHELIOMA
FRICTION MATERIALS(4) (13,460 WORKERS)	A) CHRYSOTILE ONLY	NO CASE
	B) CHRYSOTILE + CROCIDOLITE	10 CASES
ASBESTOS-CEMENT FACTORY(5) (1,970 WORKERS)	A) CHRYSOTILE ONLY (40 YEARS)	NO CASE
	B) CHRYSOTILE + CROCIDOLITE FOR A BRIEF PERIOD (2 YEARS)	2 CASES
GAS MASKS(6)	CHRYSOTILE	NO CASE/570 WORKERS
	CROCIDOLITE	5 CASES/757 WORKERS

4- Newhouse, M.L. et al. (1982) Ann. Occup. Hyg., Vol. 26, Nos 1-4, pp. 899-909

5- Thomas, H.F. et al. (1982) Brit. J. Ind. Med., Vol. 39, pp. 273-276

6- Acheson, E.D. et al. (1982) Brit. J. Ind. Med., Vol. 39, pp. 344-348

DIFFERENCES IN ASBESTOS FIBER TYPES

MINERAL ANALYSES OF LUNG TISSUE (1)

GROUPS STUDIED	OBSERVATIONS	
WORKERS WITH ASBESTOS-RELATED DISEASES REPORTED TO THE U.K. PNEUMOCONIOSIS MEDICAL PANELS IN 1977(1)	AMPHIBOLES:	100-FOLD INCREASE IN CASES OVER CONTROLS
	CHRYSOTILE:	EQUAL AMOUNTS IN CASES AND CONTROLS
WORKERS WITH ASBESTOSIS IN ROYAL NAVY DOCKYARDS FOR THE PERIOD 1966-1982(2)	AMPHIBOLES:	AMOUNTS INCREASE WITH SEVERITY OF ASBESTOSIS
	CHRYSOTILE:	AMOUNTS REMAIN EQUAL
WORKERS WITH MESOTHELIOMA REPORTED IN BRITISH COLUMBIA IN 1982(3)	AMPHIBOLES:	300-FOLD INCREASE COMPARED TO GENERAL POPULATION
	CHRYSOTILE:	NO DIFFERENCE

1- Wagner, J.C. et al. (1982) Ann. Occup. Hyg., Vol. 26, Nos 1-4, pp. 423-431

2- Wagner, J.C. et al. (1986) Brit. J. Ind. Med., Vol. 43, No. 6, pp. 391-395

3- Churg, A. (1985) Cancer, Vol. 55, No. 3, pp. 672-674

DIFFERENCES IN ASBESTOS FIBER TYPES

MINERAL ANALYSES OF LUNG TISSUE (11)

GROUPS STUDIED	OBSERVATIONS
PATIENTS WITH PLEURAL PLAQUES IN BRITISH COLUMBIA(4)	50-FOLD INCREASE OF AMPHIBOLES COMPARED TO CHRYSOTILE
PATIENTS WITH MESOTHELIOMA IN U.K. IN 1976(5)	FAR GREATER NUMBER OF AMPHIBOLE FIBRES, BUT SAME NUMBER OF CHRYSOTILE IN CASES COMPARED TO CONTROLS
PATIENTS WITH MESOTHELIOMA IN NORTH AMERICA IN 1972(6)	FAR GREATER NUMBER OF AMPHIBOLE FIBRES, BUT SAME NUMBER OF CHRYSOTILE IN CASES COMPARED TO CONTROLS

4- Churg, A. (1982) Amer. J. Pathol., Vol. 109, No. 1, pp. 88-96

5- Jones, J.S.P. et al. (1980) WHO/IARC, Pub. No. 30, Vol. 1, pp. 187-199

6- McDonald, A.D. (1980) WHO/IARC, Pub. No. 30, Vol. 2, pp. 681-685

3- DURABILITY

It is interesting to note that the mineral analysis of lung tissue had also provided precious information regarding a possible pertinent parameter of importance in biological potency of fibrous materials: durability, or persistence in lung tissues.

It should be noted that this difference in potency applies not only to mesothelioma and lung cancer, but also applies to asbestosis. Thus, Dr. J.C. Wagner showed that the proportional counts of the amphibole fibers crocidolite and amosite increase considerably with the severity of asbestosis whereas the proportional count of chrysotile appears to be virtually unchanged, as shown in this figure.

ASBESTOS CONTENT BY FIBER TYPE IN LUNG TISSUE:
CORRELATION WITH SEVERITY OF ASBESTOSIS

Slide V

GRADE OF ASBESTOSIS	TOTAL NUMBER OF CASES	MEAN FIBRE COUNT (10^6)	FIBRE NUMBER (16^6) per g dry lung tissue		
			AMOSITE	CROCIDOLITE	CHRYSOTILE
NONE	19	22.94	1.34	2.44	9.62
MINIMAL	36	42.13	2.44	14.47	12.89
SLIGHT	69	59.47	14.94	21.30	12.25
MODERATE	49	343.15	94.98	73.88	13.75
SEVERE/MARKED	16	2550.29	266.21	2224.04	17.34
ALL GRADES	189	336.9	53.21	218.21	12.93

(from Wagner J.C. et al (1986) Brit. J. Ind. Med. 43: 391-395)

These and other observations led J.C. Wagner and his colleagues to state in 1987, at the Lyon IARC symposium:

«...We believe, therefore, that chrysotile is the least harmful form of asbestos in every respect and that greater emphasis should be placed on the different biological effects of the various amphibole fibers» (J.C. Wagner).

4- DOSIMETRY

So far, we have delt with the parameters of size and fiber types, and we can conclude that for fibrous materials, those which are of concern are fibers which are long, thin, and durable. But there is yet another parameter which must be delt with: the dose.

In fact, we must return to the sets of data of difference in potency according to fiber type to appreciate the importance of dose. One of the nagging problems which has puzzled many experimentalists for years was the apparent inconsistencies between the effects reported from experimentation and those from epidemiology.

Careful examination of the great majority of IN VITRO and IN VIVO experimental protocols to assess the biological effects of mineral fibers reveals that comparison of the effects (IN VITRO cytotoxicity, fibrogenicity and tumour yield) has traditionally been based on a gravimetric basis, i.e., comparing the effects produced by equal mass of tested minerals (for example: 50 $\mu g/10^5$ cells; 20 mg dose by intraperitoneal injection; 20 mg/M^3 by inhalation, etc.

As I mentioned, the reported effects were often not consistent with epidemiological observations. In the recent years, development of sophisticated techniques for tissue mineral analysis

has led to possible explanations of the inconsistencies between animal experimental data and epidemiological evidence, pointing to the different durability of mineral fibers, and their different relative lifespan persitence between laboratory animals and man.

But there is yet another explanation, which resides in the basis for reporting the observed effects, i.e., per equal mass vs equal number of fibers.

Contrary to experimental (animal) data, human experience of exposure to different asbestos fiber types and man-made mineral fibers shows:

a) an impressive difference in pathological potency between asbestos fiber types, chrysotile being much less fibrogenic and carcinogenic, and having very little mesothelioma-producing potential (if at all), compared to amphiboles.

b) Man-made mineral fibers, especially Rock/Slag wools, have been positively correlated with excess incidence of lung cancer in production workers AT LOW EXPOSURE LEVELS, indeed at exposure levels (~1 f/ml) where chrysotile has been shown to produce no excess lung cancer (Copenhagen WHO 1986). It should be mentioned that there is some uncertainty about the estimates of exposure in the early production phase of rock/slag, and that there may have been additional contributing factors (PAHs, arsenic, etc.) to the reported excess lung cancer mortality; all these sources may have contributed to the observed hazard, but as Sir Richard Doll mentioned in his overview and conclusions of the 1986 Copenhagen meeting «...none has produced a quantitatively similar hazard elsewhere, unless exposure was both intense and prolonged.».(2)

The problem is that in spite of the human data, some regulatory agencies maintain that chrysotile has the same potential as the other asbestos fiber types, and that man-made mineral fibers have lower pathogenic potential, BASED ON ANIMAL EXPERIMENTS USING EQUAL MASS DOSES.

Limited attempts to transform retrospectively gravimetric doses into fiber number-doses have indicated that if based on fiber number, the pathogenicity would show that «fiber for fiber», chrysotile would be seen as less pathogenic than the other asbestos fiber types, and possibly some other man-made mineral fibers as well.

For instance, in a 1978 publication (Brit. J. Cancer, 37: 673-688) by Davis et al., the authors report that rats submitted to dust clouds of chrysotile, crocidolite and amosite showed more lung fibrosis and tumours after inhalation of chrysotile, than with either amphiboles.

But, they state: «...it was found that the chrysotile dust clouds used in this study contained many more fibers longer than 20 microns than either of the amphibole dust clouds».

In 1986, J. Peto, using Wagner's 1984 animal data, made an analysis of effects per fiber rather than per unit mass relevant to the comparison of asbestos and man-made mineral fibers in the induction of pulmonary tumours by inhalation. The following table will illustrate this.

TABLE: LUNG TUMOUR DISTRIBUTION RELATED TO CUMULATIVE DOSAGE
(After Wagner et al., 1984)

MATERIAL (NO. OF RATS)	BAH[1] (a)	ADENOMA[2] (b)	ADENOMA[3] (c)	ADENO-CARCINOMA (d)	ALL NEOPLASMS (b+c+d)	AVERAGE[4] CUMULATIVE DOSE PER RAT AT 12 MTHS	TUMOURS PER 100 FIBRES/cm^3 X HRS
UICC CHRYSOTILE (48)	5	0	1	11	12	656	1.8
GLASS MICROFIBRE (48)	3	0	0	1	1	223	0.4
ROCK WOOL (48)	1	1	1	0	2	39	5.1
GLASS WOOL WITH RESIN (48)	3	0	0	1	1	55	1.8
GLASS WOOL RESIN FREE (47)	1	1	0	0	1	41	2.4
CONTROLS (48)	1	0	0	0	0	-	-

① = bronchoalveolar hyperplasia.

② = benign.

③ = some features of malignancy.

④ = fibers/cm^3/hours.

NOTE: This table is reproduced from «Request from HSE for an opinion on the carcinogenic potential of glass fiber, glass wool, slag wool and rock wool. A resume of the revelant biological properties» (reference cc/86/2), which was prepared for the Committee on Carcinogenicity.

The results shown indicate that a similar mass of chrysotile produced a greater tumour incidence than man-made mineral fibers, but that the risk per fiber may have been similar for chrysotile and glass wool, and possibly greater for rock wool. (All groups had been exposed to 10 mg/M^3, but the fiber count was more than 10 times higher for chrysotile than for rock wool or glass wool). While there is some scepticism in the scientific community about Peto's analysis, it would be most desirable to have more relevant experimental data.

Also in 1986, Goldsmith, J.R. indicated that «based on fiber or particle counts, man-made mineral fibers appear to be more potent than asbestos with regard to chronic pulmonary disease».

In a more recently published IN VITRO study on the comparison of mass vs number of fibers in the cytotoxic response of Chinese hamster lung V79 cells to erionite, crocidolite and chrysotile, especially for fibers L >8 microns, W <0.25 microns, the UICC chrysotile fibers emerge as the least potent. Their data show that both samples of erionite required fewer fibers than UICC crocidolite, and that UICC chrysotile required a significantly higher number of fibers than the other three tested materials to produce similar cytotoxicity. For instance, the LD_{50} values (in fiber number) for V79 cytotoxicity shows the following: erionite samples: ~1.1 X 10^5; crocidolite: 6.28 X 10^5; chrysotile: 348.8 X 10^5. For fiber lenghts equal to or smaller than 5 microns, the difference is even more pronounced. The following table will illustrate this.

TABLE 2

ESTIMATED LD_{50} VALUES FOR V79 CYTOTOXICITY OF MINERAL FIBERS IN TERMS OF MASS AND NUMBER OF FIBERS

Mineral samples	Mass (μg)	Number of fibers × 10^5		
		$A \geq 3$	$L \geq 8$ μm, $W \leq 0.25$ μm	$L \leq 5$ μm, $W \leq 0.1$ μm
		LD_{50} values/ml		
Erionite (w)	35	297.5	1.16	53.20
Erionite (c)	52	561.6	1.09	202.80
Crocidolite (UICC)	73	2452.8	6.28	717.59
Chrysotile (UICC)	32	6720.0	348.80	3616.00

From: Palekar, L.D., Most, B.M. and Coffin, D.L. (1988)
Environ. Res. 46: 142-152

The next 3 tables will illustrate that equal masses of different materials may contain vastly different numbers of fibers; and this means that comparison of potency between different materials should be made on the basis of fiber number, not on mass of material. An illustration of the importance of this is the comparison of experimental effects between aramid fibers and chrysotile fibers.

Table 1. Analysis of mineral fiber parameters by means of transmission electron microscopy[a]

Parameter	Erionite (W)	Erionite (C)	Crocidolite (UICC)	Chrysotile (UICC)
Number of Fibers x 10^9/mg, $A \geq 3$[b]	850	1080	3360	21,000
Number of Fibers x 10^9/mg, $L \geq 8\ \mu m$ $W \leq 0.25\ \mu m$[c]	3.3	2.1	8.6	1,090
Number of Fibers x 10^9/mg, $L \geq 5\ \mu m$, $W \leq 0.1\ \mu m$	152	390	983	13,300
Mean Length (μm)	2.22	1.41	1.30	2.38
Median Length (μm)	1.60	0.99	0.82	1.08
Mean Width (μm)	0.25	0.17	0.16	0.10
Median Width (μm)	0.28	0.13	0.14	0.08
Mean Aspect Ratio (L/W)	10.76	10.27	7.95	29.55
Median Aspect Ratio (L/W)	8.00	7.31	5.94	11.53

[a]Analyzed by Dr. Philip Cook
[b]A = aspect ratio (L/W)
[c]L = length, W = width

COFFIN, D.L. et al. (1988) Proc. Int. Symp. in Biological Interaction of Inhaled Mineral Fibers and Cigarette Smoke. Ed. A.P. Wehner and D.L. Felton, Battelle Press, Columbus, Ohio, pp. 347-354

ARAMID FIBRES (1)	UICC CHRYSOTILE (2)
18 mg/M³ = 2,000 f/ml	1 mg = 13,300 x 10^6 fibres(*) 13,300 x 10^6 f/M³ 13,300 f/ml 18 mg = 239,400 f/ml

18 mg/M³ = 2000 f/ml **18 mg/M³ = 239,400 f/ml**

(1) Industry data

(2) EPA data
(*) L ≥ 5 µ

KEVLAR (10 <L <30 µM) INHALATION
(ILO WORKING DOCUMENT, APRIL 17-25, 1989)

2 WEEKS: 18 MG/M³ (2,000 F/CC) ⟶ FIBROSIS

2 YEARS: 100 F/CC ⟶ FIBROSIS, TUMOURS

KEVLAR	CHRYSOTILE
18 MG/M³ = 2,000 F/CC	18 MG/M³ = 239,400 F/CC (EPA DATA)

KEVLAR	CHRYSOTILE
100 F/CC	IF 239,400 F/CC = 18 MG/M³ 100 F/CC = 0,007 MG/M³
FIBROSIS	NO FIBROSIS
TUMOURS	NO TUMOURS

Of course, these observations do not rule out other important factors in possible reasons for a gradient in fiber potency, such as chemical differences in fiber types, durability (persistence in tissues), and possibly others.

Presently, TLVs for asbestos fiber types are in fiber number almost everywhere, whereas gravimetric standards (from ~5 to 10 mg/M^3) are the current rule for man-made mineral fibers, although some man-made mineral fiber companies have recently cautioned their customers to observe low levels in terms of fiber numbers.

In the meantime, carefully planned animal experimentation, where doses are measured in fiber numbers, and where results are expressed in effects/fiber number, would certainly help to support the contention that if chrysotile must be controlled to such low levels as 1 or 0.5 f/ml, so must the man-made fibers be controlled at the same levels (and possibly lower levels in some cases). These man-made fibers are used/proposed as asbestos substitutes in a large number of applications: friction materials, thermal insulation, fiber-reinforced plastics and resins, gaskets and joints, etc.

In conclusion, I think we can now say with a fair degree of confidence that:

1- For the purpose of biological assessment, fiber size, fiber types (physico-chemical parameters) and durability should always be taken into consideration.

2- For the purpose of experimental comparison of biological potency of different fibrous materials, dose applied to in vitro and in vivo models should be in fiber numbers rather than in mass.

3- For the purpose of regulatory actions, all long, thin and durable fibers should be controlled whether they are natural or synthetic, whether they are mineral or organic.