PLAINTIFF'S EXHIBIT

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Asbestos Measurement

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USES OF THE UICC SAMPLES

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INTRODUCTION

Since they were made in 1966, the UICC standard reference samples of asbestos have found numerous applications, the majority not envisaged when the Working Group on Asbestos and Cancer (1965a, b, c) recommended their preparation (Timbrell & Rendall, 1971/72; Timbrell et al., 1968a). Main reasons for this wide diversity of uses are:

- The five samples include all the more widely used types of asbestos and were derived from biologically important deposits: crocidolite, North West Cape Province, South Africa; amosite, Transvaal, South Africa; anthophyllite, Finland; chrysotile, Rhodesia; chrysotile, Canada.

- The preparation of 500 kg of each sample has made possible numerous large inhalation experiments and several hundred other studies.

- The fibres appear to be near optimum size for the induction of asbestos-related diseases in short-lived animals. Longer fibres would be more difficult to administer, while shorter fibres are less toxic and carcinogenic. In both inhalation and inoculation experiments, all five samples have proved capable of producing mesotheliomas, which take 30 years to develop in humans.

- The purity of the starting materials supplied by the mines has made the samples acceptable as analytical and identification standards.

- Between them, the samples exhibit all the sizes and shapes encountered in respirable fibres of asbestos and other fibrous substances, mineral and synthetic. These features have made the samples useful models in studies on the biological importance of these parameters and pertinent materials in development of fibre-handling and fibre-measuring equipment.

- Not least, the samples have facilitated rapid entry of researchers in many disciplines into the study of the biological effects of fibres, bringing with them different viewpoints leading to new applications for the materials.

INHALATION EXPERIMENTS

Since inhalation experiments are the type of biological study most closely related to human exposure, they play a major role in the investigation of asbestos-induced diseases. Although the UICC samples were intended to be references and not substitutes for other asbestos materials, in many inhalation experiments they have been the only fibres used. At the National Centre for Occupational Health, Johannesburg, converted rooms are used for long-term exposure of large primates to dust clouds generated from the samples (Rendell, 1974). Other laboratories, for instance the Battelle in Richland, Washington (Craig, 1974), have adapted chambers originally developed for exposure of small animals to gases, vapours or dusts. A degree of standardization has been effected through the adoption by laboratories, including the Institute of Occupational Medicine, Edinburgh, of the low-cost chamber developed at the MRC Pneumoconiosis Unit, Penarth (Timbrell et al., 1970b) to accommodate 48 SPF rats without need to remove them for servicing. Most short-duration studies are conducted using nose-only exposure. Craig (1974) has described a chamber for exposing a dog and Timbrell & Skidmore (1971) one for 12 rats. The latter is the basis of the chamber used at the Atomic Energy Research Establishment, Harwell, for exposing rats to neutron-irradiated UICC samples (Evans et al., 1973; Morgan, 1974).

Because most dust-feed mechanisms designed for dispensing particles of compact shape function badly with fibrous particles, the UICC samples demanded new dispensers. For dispensing the relatively large amount of UICC fibre (20 g per hour) required by the Johannesburg chambers, Rendall (1974) uses a cutter, spun by a fan driven by compressed air on the same shaft, to stir up the packed fibres, which are then sucked into the chamber by a venturi tube; feed is regulated by water dripping into the tank in which the fibre container floats. Initially, at Penarth, a modified coffee grinder was employed in which a rotor shaved individual fibres from a plug advanced in a motorized syringe at a feed rate of 1-3 g per hour (Timbrell et al., 1968b). Availability of a commercial version incorporating several improvements has achieved useful standardization of aerosol generation in many laboratories and economized use of the samples. Workers in Edinburgh have added a second air jet to disintegrate aggregates that may accumulate in the bowl, particularly in the case of the chrysotiles

(Beckett, 1975). The Industrial Hygiene Group at Los Alamos have made a version in stainless steel for additional use with highly abrasive glass fibre (Ortiz et al., 1977). Craig (1974) also uses a motorized piston infuser with an air jet to agitate beads supported by the asbestos plug. To achieve total exposure to 10 mg of neutron-irradiated UICC samples, Hounam (1971) has devised the 'Konimiser' in which the asbestos is driven up a silica tube by a piston to be blown off the top by air from a hypodermic needle.

The many inhalation studies performed with the UICC samples include the following: Wagner et al. (1974) exposed SPF Wistar rats for periods of between one day and two years. All five samples produced asbestosis and lung tumours ranging in severity from adenoma to squamous carcinoma. Of the 11 mesotheliomas produced, four were with crocidolite, four with Canadian chrysotile and two occurred with only one day's exposure to asbestos. There was a positive association between asbestosis and tumours. Morgan et al. (1975) studied deposition and subsequent clearance in rats, using radioactive tracer techniques; autoradiographs of lung section showed that at the termination of exposure fibre was present in the conducting airways, particularly at bifurcations, and was also distributed uniformly throughout the alveoliated regions. In a study to investigate the mechanisms of the pathogenesis of asbestosis, Kagan & Miller (1979) exposed three-month-old rats to airborne UICC crocidolite and examined alveolar macrophages from sacrificed animals in lymphocyte-macrophage coculture experiments. They suggested that induction of cell-mediated immunity in the lung by dusted macrophages could initiate a chain reaction, involving the interplay of lymphokines, lysosomal hydrolytic enzymes and macrophage-related chemotactic substances, with pulmonary fibrosis as the ultimate end-result. In other inhalation experiments, this group has conducted scanning electron microscope studies on macrophages from rat lungs dusted with UICC crocidolite (Miller & Kagan, 1976) and on the ultrastructure of the rat lung following exposure to crocidolite and quartz (Miller et al., 1978). Davis et al. (1978) have conducted a study of the mass and number of fibres in rat lungs exposed for 12 months to UICC chrysotiles, crocidolite or amosite. Their results suggest that long asbestos fibres are more dangerous than short and that neither a single mass hygiene standard nor the present fibre number standards are satisfactory.

INGESTION EXPERIMENTS

Only a few ingestion experiments using the UICC samples have been reported, but quantities were recently issued for some large studies. Amacher et al. (1974) investigated the effects of ingested chrysotile on DNA synthesis in the gastrointestinal tract and liver of rats following administration of a suspension of fibre in saline by lavage on a single occasion; the suspensions were prepared by 30-minute treatment of UICC Rhodesian chrysotile in a Waring blendor. No mortality or significant weight loss occurred. This preliminary study indicated that transient early and delayed alterations in the mitotic activity of the gastrointestinal tract cells occur following chrysotile ingestion; that these effects are both dose-dependent and time-dependent; that asbestos penetrates the gastrointestinal mucosa and influences regulation of DNA synthesis in the gastrointestinal tract; and that differences in response of DNA synthesis in different segments of the alimentary tract reflect anatomical and other factors, such as variations in pH and motility, which may influence the interaction of chrysotile fibres with the epithelial lining.

INOCULATION EXPERIMENTS

The UICC samples were not intended for use in intratracheal inoculation studies and have been found unsuitable for this method of application; the chrysotiles tend to gel in liquid suspension and suffocate the animal. Burger & Engelbrecht (1970) first milled the individual UICC samples in an agate-ball mill to reduce the mean fibre length. The Canadian chrysotile proved to be exceptionally tough: its mean fibre length was only slightly reduced after 40 hours of grinding; it may be noted that in the preparation of the UICC samples the starting materials of both chrysotiles required a long milling period (Timbrell & Rendall, 1971/72). Each rat was injected intratracheally with 50 mg asbestos in 1 ml saline followed by 1 ml of air; however, those injected with the Canadian chrysotile received a dose of 25 mg/0.5 ml, into one lung, to prevent the high mortality rate due to the abundance of long fibres in this material. The results indicated that the various asbestos samples induced an initial foreign-body reaction, which appeared to be more severe in the long-fibre Canadian chrysotile group; the biological responses to the short-fibre materials ground from the other UICC samples were so similar that no significant histopathological differences could be detected.

The UICC samples were early criticized as being unusable in intrapleural inoculation studies, since the fibres, and particularly those of chrysotiles, blocked the needle. Personal demonstrations have overcome this objection (Wagner & Berry, 1973). The problem does not arise with the method of Stanton (1973), in which the sample is applied to 45 mg fibrous glass pledgets prior to insertion by open thoracotomy. Intrapleural inoculation is recognized to be an unphysiological means of applying fibre, since the defence mechanisms of the pulmonary tissues are circumvented (Wagner & Berry, 1973) as are the aerodynamic barriers of the respiratory tract (Timbrell, 1973a). One advantage, however, is that the dose administered is known with reasonable accuracy.

Some intrapleural inoculation experiments have involved use of the UICG samples as well as other fibrous materials. Wagner et al. (1973) found crocidolite to be the most carcinogenic of the UICC samples, and it has become common practice to employ this sample as a control in various biological studies. This exemplifies proper use of the samples, which were intended to be used as baselines for investigations with other materials. Stanton & Wrench (1972) found that UICC crocidolite caused fewer mesotheliomas (20-32%) when reduced to submicroscopic fibrils; this illustrates that if they are to act as standards the UICC samples should be used as received. Wagner et al. (1973) observed mesotheliomas in a considerable proportion of rats with all of the UICC samples; they also found that removal of the oils by benzene extraction did not alter the carcinogenicity. Carcinogenicity was not related to the trace metal content of the samples.

Kanazawa et al. (1970) used UICC crocidolite to study migration of asbestos from subcutaneous injection sites in mice. Wagner & Berry (1973) have pointed out biological disadvantages of this method.

IN VITRO EXPERIMENTS

Harington et al. (1971) included the UICC samples in their measurement of the haemolytic activity of asbestos. A marked haemolytic action of chrysotile and a relative inactivity of several other forms of asbestos, notably crocidolite and amosite, were observed. From their investigation on amphiboles, including the UICC amosite, anthophyllite and crocidolite, Morgan et al. (1977) concluded that haemolytic activity is related primarily to specific area and not to the magnesium content of the fibres, as suggested by Harington.

In experiments to examine the effects of the UICC samples on cultures of mouse cells, Allison (1971) found that, independent of the sample used, peritoneal macrophages quickly took up short fibres (< 5 um) but not longer fibres (> 30 um). In medium lacking serum, chrysotile was the most effective in early cytotoxicity, followed by crocidolite, amosite and anthophyllite, in that order. He considered that this rapid effect was probably due to direct interaction of the fibres with constituents of the cell membrane, analogous to haemolysis, but doubted whether in serum-free media it had much physiological relevance. However, even in the presence of serum, cells showed some cytotoxic reaction as well as other abnormalities. Some macrophages exposed to UICC chrysotile showed pyknotic cytoplasm and nuclei after two or three days and about 20% were no longer viable. Mesothelial cells, and to a lesser extent macrophages, developed brown pigmented, autofluorescent granules in the cytoplasm; this effect was greatly accentuated after ingestion of asbestos, and especially of crocidolite. Allison suggested that iron may favour lipid peroxidation.

Robock & Klosterkotter (1973) concluded from tests performed on peritoneal macrophages of guineapigs that the UICC amphiboles are significantly less cytotoxic than the UICC chrysotiles and that the Canadian chrysotile is more active than the Rhodesian. The same relation was found for fibrogenic behaviour in animals following intraperitoneal inoculation. Mossman et al. (1977) used organ cultures of hamster trachea to study the effects of UICC crocidolite on the respiratory epithelium. Necrosis and desquamation of surface cells were accompanied by basal-cell hyperplasia; these proliferating cells phagocytosed the dust and incorporated it into lysosomes.

Although previously the UICC samples were used only infrequently in *in vitro* studies, this has recently developed into a major field of application. Current research topics include mechanisms of interaction of asbestos with cellular membranes; absorption of cellular molecules on the surface of asbestos; mechanism of action of asbestos on DNA metabolism; cyclic nucleotides and viral transformation; and, most prominently, relative toxicity and carcinogenicity of mineral and synthetic fibres.

ANALYTICAL INNOVATIONS

Early results obtained by international laboratories from analysis of the UICC samples for chemical composition and fibre size by well-established methods, including X-ray diffraction and electron microscopy, were presented by Rendall (1970) and Timbrell (1970a), and data sheets giving further results have been prepared periodically. To use the UICC samples as standards, most investigators have rationally obtained reference data with their own instruments. Users for these purposes include universities, museums, public health authorities, hygiene inspectors and building contractors.

The samples have been applied extensively to investigate means of improving classical methods of asbestos analysis and to look for properties which could provide new ones. A technique so developed (Timbrell, 1975) enables fibres of any asbestos or other mineral to be aligned magnetically on membrane filters for improvement of analysis by X-ray diffraction or polarized infra-red spectrophotometry; the alignment modes exhibited are related to the fibres' geographic source. Another employs an electric grid to align chrysotile fibres on a glass slide for quantitative analysis in specially designed X-ray diffraction geometry (Birks et al., 1978).

In experiments on UICC amosite and Canadian chrysotile suspended in paraffin oil, Fleming & Rubin (1978) measured the frequency dispersion of the dielectric coefficient and of the dielectric loss. The chrysotile showed relatively large energy dissipation maxima, which were explained in terms of relaxation of transverse and longitudinal polarization with slightly different energies of activation. The amosite showed much less dielectric activity. Rubin[1] has proposed a feasibility study on a method for the continuous and automatic counting of airborne asbestos fibres in which they would be drawn through a charged parallel plate capacitor with an air gap comparable to the length of the fibres. Pulses produced whenever a fibre made contact with both plates simultaneously could then be displayed or fed to a counter.

The UICC samples have been measured in terms of several magnetic parameters, including coercive force (Timbrell, 1979); results obtained on magnetic susceptibility and magnetite content are presented by Rendall[2]. These reference data assist analysis of unknown samples for asbestos type and quantity.

SIZE SEPARATION

Fibres in narrow ranges of diameter and length would be useful for certain biological and physical experiments, and the Physics and Chemistry Panel of the 1972 conference held at the IARC in Lyon (Bogovski et al., 1973) recommended the development of preparation techniques. Microgram quantities of the UICC samples can be accurately separated into a continuous spectrum by fibre diameter using the gravitational aerosol spectrometer of Timbrell (1965, 1972) or the centrifugal spectrometer of Stober (1972). These devices achieve no separation by length, since this parameter has little influence on fibre sedimentation rate.

The transcript of the meeting convened by the Institute of Occupational and Environmental Health, Montreal (Pelnar, 1974) gives an extensive account of experiments to prepare fibres for biological studies. Bundles of uniform diameter glass yarn embedded in a removable matrix had been cut into discrete fibre lengths by Speil and Leinweber using a lathe and by Timbrell using a microtome. The technique was not successful with the UICC samples or with longer fibres. At considerable cost, Speil and Leinweber separated gram quantities of the very difficult chrysotile fibres, according to diameter by centrifugation and length by screening.

Using similar procedures, Spurny et al. (1979) separated fine fibres from UICC chrysotile, crocidolite and amosite and from Johns-Manville fine glass fibres in the quantities required for inoculation studies. The materials were ground in a special mill, screened in a vibrating-bed aerosol generator, suspended in liquids by ultrasonic treatment, and separated by slow sedimentation. Preparation of longer size-selected fibres required starting materials longer than the UICC fibres.

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1 Personal communication

2 See p.

The UICC samples were prepared from fine-grade commercial fibre to minimize rock content. Nevertheless, they contain particles of several minerals, some intimately bound in the fibre bundles: the anthophyllite, approximately 60% fibrous by mass, has a high proportion of talc. A simple magnet may be used to remove highly-magnetic magnetite particles from aqueous suspensions of the samples, but other contaminants are too magnetically weak for simple attraction to be effective. The fibres can, however, be captured preferentially from aqueous suspensions poured through a plug of steel wool, using a magnetic field to align the fibres and to prevent them from following the fluid streamlines (Timbrell, 1977). Their subsequent recovery is promoted by ultrasonication and precoating of the wool with a suitable medium.

When the amphibole UICC samples are ground, the fibre lengths decrease to a greater extent than do the diameters. Gram quantities of fibres which vary in length but much less so in diameter, prepared by timed residence in a simple laboratory mill, have been used to develop fibe sizing methods (Timbrell[1]) and for *in vitro* studies (Brown et al., 1978). Simple description of size differences between these ground samples by median fibre diameter and median fibre length is promoted by retention of the log-normal form of the distributions of the UICC samples: Other fibre samples that differ in both diameter and length have been prepared by timed centrifugation of the UICC samples (Timbrell, 1975).

FIBRE SIZING

Evidence that fibre shape, diameter and length are important in the etiology of fibre-induced diseases has stressed the need for means of measuring these parameters. The UICC samples exhibit pertinent ranges: the amphibole fibres are straight and the chrysotiles curved; diameters range from 0.015 μm upwards and lengths from about 200 μm downwards. Unlike glass fibres, for instance, the fibres are strong, and a deliberate effort is required to change the diameter and length distributions. These features make them suitable materials for the development and calibration of instruments for sizing respirable fibres. The need in such work for reference size data for the UICC samples prompted the development in collaboration with Coulter Electronics of the 'Shearicon', a television version, applicable to light microscopy and electron microscopy, of the double-image system described earlier (Barnett & Timbrell, 1962; Timbrell, 1952). Its use on fibres magnetically aligned on parallel electron microscope grids to eliminate masking by the bars (Timbrell, 1975) has shown that the amphibole UICC samples have log-normal distributions of diameter and length.

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[1] See p.

Fibre counting instruments, in the development of which the UICC samples were used, include the GCA Fibrous Aerosol Monitor devised by Lilienfield et al. (1979). This instrument automatically counts light pulses produced by fibres illuminated by a laser beam and subjected to a rotating high-intensity electric field. In the counter for airborne fibres developed by Detenbeck (1979), using UICC Rhodesian chrysotile, two detectors are employed to sense the intensities of light scattered in mutually perpendicular directions when a fibre intercepts a laser beam; efficiency is enhanced by aligning the fibres with an electric field to maximize the ratio of the intensities. Light scattering applied to magnetically aligned fibres has been employed for measurement of fibres in lung tissue (Timbrell[1]) and for counting airborne fibres after collection by the conventional membrane filter method (Gale & Timbrell[2]). To assist the development of light scattering methods, Bohren & Timbrell (1979) have written computer programs for calculating scattering and absorption by cylinders; values of statistical parameters describing the size distribution of a UICC sample can be inserted to calculate the integrated response of the whole material.

Jedwab & Losman[3] have employed the UICC samples in developing a technique promoting easy detection of asbestos fibres under the light microscope. Silvering solution, composed of reducing sugar, silver nitrate and ammonia, is applied in a tight container to the asbestos fibres affixed to a glass slide. Because absorption of sugar is confined to asbestos, the fibres become coated with a thin layer of silver, while the glass and any nonasbestos particles present are not affected. The method applies to all asbestos types.

A useful technique described by Hamilton & Phelps (1956) for transforming particles of coal or silica into transparent profiles to eliminate depth-of-focus problems in optical microscopy has been adapted for measurement of the UICC samples by transmission electron microscopy. The specimen is coated with silver in a vacuum plant, the fibres removed with water or an air jet, and the metal film then stripped from the supporting glass slide to make the profiles transparent to the electron beam (Timbrell, 1973b). The technique also has the advantage in light scattering studies of effectively eliminating fibre refractive index as a factor.

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[1] See p.

[2] See p.

[3] Personal communication

Results from the manual counting of fibres deposited onto membrane filters, currently the prime method of monitoring airborne asbestos, are highly subjective and variable. Using a liquid filtration technique, Ortiz et al. (1975) prepared multiple nominally-identical membrane filter samples from UICC Canadian chrysotile, ranging in concentration from 200-1500 fibres/mm^2 of filter area, to permit standardization of procedures by regulatory agencies. Samples prepared in this way were included in an international fibre counting trial (Walton et al., 1976).

The UICC samples have provided useful models in studies on size and shape characteristics of asbestos fibres. Size analysis by transmission electron microscopy showed that these samples differ in mean diameter: crocidolite < amosite < anthophyllite. This variation suggested the use of fibre diameter as an aid to identification of mining source of amphibole fibres encountered in human lung specimens or air samples (Timbrell et al., 1970a). Subsequent measurements on amphibole fibres in the North West Cape and Transvaal provided an early indication of importance of fibre size in induction of fibre-related mesotheliomas (Timbrell et al., 1971).

The UICC chrysotiles have promoted the development of quantitative means of describing the shape of curved fibres, and comparative studies on the chrysotiles and amphiboles have prompted suggestions (Timbrell, 1970b) that fibre shape has a role in effecting the observed gradient in health risks of these asbestos groups (Becklake, 1976; Harris & Timbrell, 1977; Wagner et al., 1971).

DISCUSSION

Some investigators, in reporting their experiments, have not made it clear that the UICC samples were modified before use - most importantly, that they had been further ground or separated into different fractions by elutriation. In some cases, other asbestos samples have been included in the studies, and there is doubt which data refer to these materials and which to the UICC samples of the same asbestos type. There have also been instances where the Rhodesian chrysotile (A) and the Canadian chrysotile (B) have not been distinguished, although they are known to differ in several important properties. These omissions have led to confusion and have prevented the UICC samples from fulfilling their prime function, namely, to allow different research groups to compare results from use of exactly the same materials.

There are advantages to be gained from modifications of samples by investigators in their own laboratories, particularly for physical studies; several instances are described in this paper. However, as these modifications are unlikely to be accurately reproducible and as there is always a risk of contamination, such materials should never be referred to as 'the UICC samples'; this term should be reserved for the UICC samples as received.

In general, reporting on the UICC samples has been very satisfactory.

SUMMARY

The UICC samples are now used in a wider variety of applications than was originally envisaged. They have been employed in inhalation, ingestion, inoculation and *in vitro* experiments. Other applications are in identification of asbestos type in aerosols and tissues; standardization of conventional fibre counting; development and calibration of techniques and instruments for fibre assessment; and studies on influence of fibre size and shape on inhalation and retention. The paper outlines applications and gives literature references.

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