

PERSONAL AND CONFIDENTIAL

February 16, 1965

Mr. L. A. Pechstein, Assistant Secretary
The Philip Carey Manufacturing Company
Lockland, Cincinnati 15, Ohio

Dear Mr. Pechstein:

Dr. Thomas Mancuso was in my office Saturday morning on a visit to Columbus.

He brought with him the final report and recommendation on "Asbestos and Cancer" to be released for publication as a result of the meeting sponsored by New York University in New York in October of 1964.

A copy of the report is enclosed for your information, but it should be kept confidential for months because it probably will not be released for publication until about June.

We will continue to keep you informed of material that comes to our attention on the subject of the exposures of asbestos.

Yours very truly,

JOHN T. CANTLON & ASSOCIATES, INC.

John T. Cantlon, President

JTC/kb
Enclosure

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REPORT & RECOMMENDATIONS OF THE WORKING GROUP ON

ASBESTOS & CANCER

Convened under the auspices of Geographical Pathology of the
INTERNATIONAL UNION AGAINST CANCER (UICC)

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List of Delegates

Place of Meeting: Barbizon Plaza Hotel

Dates: October 22, 23, 1964

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*Invited, unable to attend, sent apologies.

The Group met and then divided into three Working Parties: Epidemiology (E); Pathology (P); and Physics and Chemistry (C); with the terms of reference suggested by the Planning Group which met in London in February 1964.

The Working Parties agreed on their reports which were then discussed and modified at the final Plenary Session (Chairman - Dr. H. Stewart).

Terms of Reference

I. Epidemiology

- A. To investigate the incidence of mesothelial tumors of the pleura and peritoneum in groups and/or regions where exposure to only one type of asbestos fiber has occurred.
- B. To investigate the risk of bronchial carcinoma in populations exposed to asbestos dusts where the incidence of asbestosis is known or believed to be low.
- C. To investigate the incidence of other tumors.

II. Pathology and Experimental Pathology

- A. Establish criteria for diagnosis of mesothelial tumors, assemble material to assist in standardization of diagnosis, and form consultative panels.
- B. Develop a standard of grading of fibrosis of the lung due to asbestosis.
- C. Develop a standard method of assessing semi-quantitatively the amount of asbestos fibers and/or bodies in sputum, fresh lungs, and fixed tissue.
- D. Collate work in various countries.

III. Physics and Chemistry

- A. Investigate the usefulness of providing in one center a set of standards of the main types of asbestos fibers and their extracted organic matter for distribution to centers investigating the biological, physical and chemical properties of this material; if it is regarded as useful and practical suggest a center.
- B. Suggest a minimum list of characteristics by which differences between these samples should be identified.

Physics & Chemistry (Cont'd)-6-

- C. Suggest standard methods for identification of types of asbestos in the lung in (a) large samples, and (b) tissue sections.

THE ASSOCIATION OF EXPOSURE TO ASBESTOS DUST AND CANCER

The main types of asbestos of commercial interest are amosite, anthophyllite, chrysotile, crocidolite, and tremolite. There is evidence of an association between exposure to asbestos and malignant neoplasia. This has been established mainly on information from Germany, Italy, South Africa, the United Kingdom, and the United States of America.

The types of tumors which have been shown to be associated with exposure to asbestos dust are:

1. Carcinoma of Lung
2. Diffuse mesothelioma of the Pleura and Peritoneum.

There is some suggestion of an association also with gastrointestinal carcinoma, and possible ovarian tumors.

The latent period between first exposure to the dust and detection of the related tumors is many years, usually 20 or more. Instances up to 60 years have been reported. For this reason, further cases of these associated tumors will be expected to occur for many years to come, even if dust exposures are now greatly reduced.

Present evidence indicates that the associated carcinomas of the lung are not limited to exposure to any one type of asbestos fiber. However, further investigations are urgently needed to establish whether the degree of risk is important related to the type of fiber inhaled.

In the case of mesotheliomas evidence from several countries suggests that exposure to crocidolite may be of particular importance, but it cannot be concluded that only this type of fiber is concerned with these tumors, and further investigation of this problem is needed.

Certain types of asbestos fibers in the virgin state have been found to contain oils, waxes, and other organic matter. In addition, asbestos fibers readily absorb hydrocarbons subsequent to mining. Small or trace amounts of various elements such as nickel, chromium, are also found associated with some types of fiber. The possible role of such associated materials in the development of tumors, following exposure to asbestos dust is not yet clear.

These findings, when considered in relation to the great increase in the use of asbestos for many purposes in all countries, suggest that a more serious and widespread hazard from exposure to asbestos dust may exist than is widely appreciated.

RECOMMENDATIONS ON PROBLEMS REQUIRING EPIDEMIOLOGICAL STUDY

1. That the importance of fiber type on the risk of developing asbestosis, carcinoma of the lung, and mesothelial and other tumors be investigated.

International and intranational comparative studies of mining and other populations exposed to only one type of fiber are recommended. Among the countries in which and between which studies should, if possible, be made are:

Australia	-	Crocidolite
Canada	-	Chrysotile
Cyprus	-	Chrysotile
Finland	-	Anthophyllite
Italy	-	Chrysotile
South Africa	-	Amosite Chrysotile Crocidolite
U.S.A.	-	Chrysotile Tremolite
U.S.S.R.	-	Chrysotile

The studies of the effect of exposure to different types of fiber within a country are likely to be of special value, but studies of groups exposed to apparently similar fibers in different parts of the same country are also likely to be informative.

2. That the relationship of dust dosage (including concentration and duration of exposure), and the composition and physical state of the dust to the incidence of asbestosis, carcinoma of the lung, mesotheliomas, and other cancers be studied.

Comparative studies in factory populations in the asbestos textile and other manufacturing processes using asbestos are likely to be useful, especially when there are records of past dust measurements. In any prospective studies of new entrants, the measurement of dust by a standardized method should be regarded as an essential part of the investigation.

3. That the effects of removal from further exposure to asbestos dust be investigated.

It is important to establish the subsequent morbidity and mortality from asbestosis, and the mortality from cancers associated with exposure to asbestos in population groups no longer exposed to the dust.

4. That further investigations be made of past and all future cases of diffuse mesothelial tumors of the pleura and peritoneum to establish any association with asbestos and other factors.

These tumors should be diagnosed on the criteria suggested by the Panel on Pathology (see below) and reviewed by a Panel of Pathologists with experience of these rare tumors. The tumor and lungs should be investigated for the presence of asbestos by physical and chemical methods. (See below)

5. That studies of morbidity and mortality be extended to asbestos-exposed populations that have not so far been widely investigated.

1. It is recommended that special attention be directed to surveys in:
 - a. The insulating industry including that in ships
 - b. The asbestos cement industry
 - c. Asbestos products industry
 - d. Other plants in which asbestos is regularly used, such as certain paper, paint, and plastic factories.
2. It is also recommended that, since incidental exposure to asbestos dust may occur in certain trades and occupations, attention be directed to:
 - a. Handling and transporting asbestos
 - b. The building industry
 - c. Pipe fitting
 - d. Ship building and breaking
3. It is recommended that surveys be made to study environmental and community exposures, including populations near mines and factories and elsewhere.
4. It is recommended that general population surveys be made nationally and internationally to establish by standardize methods, in areas of presumed high and low exposure to asbestos dust the prevalence of asbestos bodies and fibers.
5. It is recommended that surveys of asbestosis in domestic and wild animals be extended to areas of high and low exposure.

6. Epidemiological methods

1. General
 - a. In addition to the usual information about the individual collected in such surveys, special attention should be directed to a detailed social (including work)

habits), occupational, environmental, and medical history from early childhood to elucidate any possible exposure to or association with any type of asbestos or other dusts. A study of the family unit or household may be of interest in view of the occasional reports of significant neighborhood and household exposures.

b. In view of the association between exposure to asbestos dust and pulmonary fibrosis and its complications, it is important to obtain as much information as possible about morbidity and mortality from all causes with particular attention to:

Asbestosis	Chronic bronchitis and emphysema
Bronchiectasis	Diffuse interstitial fibrosis
Pneumonia	Tuberculosis
Cor pulmonale	Carcinoma of the lung
Diffuse mesothelial tumors of the pleura and peritoneum	
Gastro-intestinal tumors	Ovarian tumors

c. The principal epidemiological surveys likely to be used are:

(a) Retrospective (b) Cross sectional (c) Prospective, or a combination of these. In most of the surveys one or preferably more control groups will be needed. It is strongly urged that early and full consultation with statisticians be made at all stages from planning to analysis of the findings.

2. Clinical criteria

The need to establish the minimal clinical information to be obtained in surveys of workers exposed to asbestos was agreed. A small panel met informally after the main working party and their recommendations are as follows:

a. Symptoms

It was agreed that in all surveys the presence or absence of cough, sputum, dyspnea, and chest pain should be recorded as a minimum. This should be recorded using a standardized questionnaire.

The British Medical Research Council Questionnaire on Respiratory Symptoms (1960)¹, with the additional questions relating to chest pains in the W.H.O. Questionnaire on Cardio-vascular Disease (1962)² is suitable for this purpose, and these questionnaires have been widely used internationally for interview surveys. The Cancer Society Questionnaire in the Cancer Prevention Study has been widely applied in the U.S.A. It contains questions about a wide range of symptoms and diseases and was designed for self-completion.

b. Signs

It was agreed that when physical examination was possible, the minimal observations should include the presence or absence of clubbing of fingers, cyanosis, and basal râles in the chest.

It was agreed that the measurement of sputum volume (first hour on rising) and degree of purulence recorded in a standard way (Miller)³ was useful in association with the questionnaires for assessing the prevalence of bronchitis, and this may be relevant to the disability caused by asbestosis.

The epidemiological usefulness of examinations of sputum for asbestos bodies and fibers is at present uncertain, but needs investigation.

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1. Medical Research Council. Brit. med. J. 1960, ii, 1665.
 2. Rose G. A. Bull. Wld. Hlth. Org. 1962, 27, 645.
 3. Miller D. L. Amer. Rev. resp. Dis. 1963, 88, 473.

3. Classification of chest radiographs of asbestos-exposed individuals

There is no international or national standardized classification of the radiological appearances of asbestosis. It is recommended that a scheme based if possible on an extension of the I.L.O. Classification (1953)⁴ be developed.

Possible means of doing this were presented at the New York Academy of Sciences Conference on the Biological Effects of Asbestos (1965)⁵ by Finnish, German, South African, and British contributors. The aim should be to specify separately and record semi-quantitatively the principal radiological features seen in asbestos-exposed groups, but exposure to mixed types of dust is not uncommon and the appearances may, therefore, include those caused in part by other pneumoconioses.

The classification should be purely descriptive of the radiological features and not imply pathological change or extent of disability. It is probable that the type and severity of alterations in radiological features such as pleural plaques etc., vary with the type of dust exposure and other factors so that a classification based on the principles in the I.L.O. Classification for Pneumoconiosis, in which there is a semi-quantitative assessment of several qualitatively different types of abnormality, may be expected to be useful.

It is recommended that a working group be set up to develop and test a new international classification.

4. Lung function assessment

The preferred lists of lung functions to be used will vary according to the type of survey and the facilities available. A

4. International Labor Office. Occup. Safety Hlth. 1959, 9,2.

5. Proceedings of the New York Academy of Sciences Symposium on "The Biological Effects of Asbestos", 1964. (1965, in the press.)

list of minimal and additional tests likely to be of use is as follow

Minimal

- Forced vital capacity (F.V.C.)
- Forced expiratory volume over 1 sec. (F.E.V._{1.0})

Additional

- Transfer factor (Diffusing Capacity) of lung for carbon monoxide - single breath method
- Lung compliance
- Standard exercise test
- Peak expiratory flow
- Airways resistance

RECOMMENDATIONS ON PATHOLOGY AND EXPERIMENTAL PATHOLOGY

1. Diagnosis of Asbestosis

(a) Macroscopic examination:

(1) At necropsy the parietal pleura should be stripped if possible with the thoracic contents. It is desirable that at least one lung be inflated with fixative and whole lung sections be prepared.

(2) It is recommended that special note be made of the following:

Pleura for thickening and plaques (defined as localized areas of stiff hornlike material)⁶. The site and size of all pleural lesions should be recorded.

Lungs: The presence of interstitial fibrosis, bronchiectasis, cystic change, tuberculosis, pneumonic consolidation and tumors. The site of any tumor should be recorded as precisely as possible.

6. Gloyne, S.R. Tubercle 1933, 14, 445

Mediastinal tissues should be examined for evidence of neoplastic infiltration and for tuberculosis.

Peritoneum parietal and visceral fibrosis in the peritoneum such as parietal plaques and "sugar-icing" of the spleen should be recorded.

(b) Microscopic examination:

It is recommended that at least six sections be examined from the lungs before the degree of asbestosis is decided and these blocks should be taken from specific sites and identified in the following standard manner:

- (1) Apex of right upper lobe, pleural surface.
- (2) Right middle lobe, lateral pleural surface.
- (3) Right lower lobe, middle of, basal surface.
- (4) Left upper lobe, central section.
- (5) Lingula, central section.
- (6) Left lower lobe, central basal section.

In addition, sections be taken from the bronchi and peritracheal and peri-bronchial lymph glands.

It is presumed that all examining pathologists will take further sections of any suspicious or abnormal tissue.

(c) Assessment of the severity of asbestosis

The need to assess this in some standard way was agreed. It was recommended that the assessment should be based on the severity of interstitial fibrosis and the amount of tissue involved. The proposed scheme is as follows:

<u>EXTENT OF LUNG INVOLVEMENT</u>	<u>DEGREE OF ASBESTOSIS</u>	<u>DEGREE OF INTERSTITIAL FIBROSIS</u>
SLIGHT	SLIGHT	SLIGHT
MODERATE	MODERATE	MODERATE
MARKED	MARKED	MARKED

A category of MINIMAL asbestosis is also proposed to describe slight focal fibrosis in the region of the respiratory bronchioles associated with the presence of asbestos bodies; such changes are commonly confined to sections taken from the bases of the lower lobes.

This scheme puts more emphasis on the extent of the lesions than the degree of fibrosis (which should be averaged for the six sections). Thus, a lung with moderately extensive disease but only slight severity of fibrosis is graded as 'moderate asbestosis'. The use of an average assessment of the six sections makes it impossible to have a grading of slight involvement and moderate or marked degree of fibrosis.

(d) The detection and significance of asbestos bodies and fibers

(1) Sputum

The presence of asbestos bodies and fibers is an indication of the exposure to asbestos dust and not evidence of asbestosis. It is therefore suggested that the bodies should be referred to as 'asbestos bodies' and not by the previously used term 'asbestosis bodies'. Because of their sporadic appearance and in the case of fibers their relation to recent dust exposure, the Group were of the opinion that quantitative assessment of bodies and fibers in sputum was not, in the light of present knowledge, a very useful procedure but might become more useful when further investigated.

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In sputum detection by direct examination under a coverglass and the use of phase contrast, oblique illumination or narrowed condenser diaphragms is proposed. If sputum concentration is used antiformin or "Eusol" treatment is recommended. If this technique is used for large scale comparative epidemiological surveys, the exact method should be standardized.

(2) Lungs

From fresh lungs, the highest positive results are obtained from smears from the base of a lower lobe (of the thickness of a thick blood smear as for malarial parasites), air dried and mounted in balsam. A rough quantitative examination by low power magnification is easy and practicable. For fixed lungs, the smear technique is of limited value and unstained sections 30 microns thick are recommended.

(3) For investigation of asbestos fibers in lung sections, microincineration, acid treatment, and examination by phase-contrast are suggested. Morphologically the fibers appear as straight rods of varying lengths and thickness, but with longitudinal shredding and in the thicker fibers smaller fibrils may be recognized. While there are other structures described as pseudo-asbestos bodies, or curious bodies, it was the view of the Group that in practice, little difficulty is found in distinguishing the genuine from the others. These other types usually have a carbon-black center, a shape which is other than linear, but the body that can mimic an asbestos body completely is the small one found in talcosis, which may be tremolite, a form of

(e) Chemical analysis of lung tissue

Where possible, blocks of tissue from these six areas chosen for histology should be taken and the amount of collagen relative to total proteins estimated by the standard hydroxyproline methods). The results of collagen estimations should be expressed in absolute amounts and as percentages of defatted dried lung tissue.⁷

2. The diagnosis of diffuse mesothelial tumors

(a) Macroscopic

(1) The salient characteristic of the diffuse mesothelioma is its predilection to spread along the serosal membrane in which it occurs. In the pleural cavity, the entire surface may become replaced by a continuous layer of tumor due to symphysis of the pleural surfaces. This is uncommon in the peritoneum where the surfaces often remain separate but covered by isolated plaques and nodules or diffuse infiltration. Only those mesothelial tumors in which serosal spread is unequivocal should be termed "diffuse". A few benign diffuse mesotheliomas have been described. Almost all diffuse mesothelial tumors show evidence of malignancy by direct infiltration of adjacent tissues and organs and metastases to regional lymph nodes.

(2) The differentiation from metastatic tumor is the main problem in diagnosis and can only be made with complete certainty by the exclusion of all other sources of tumor at necropsy. Because of the tendency of the tumor to

7. Harington, J.S. and Kilroe-Smith, T.A. Archives of Environmental Health (1964) 9, 395

surround and infiltrate subserosal organs, these organs most commonly come under suspicion as points of origin. The possibility that the primary growth may have been surgically removed must be borne in mind.

(b) Microscopic ,

Diagnosis is possible because the growth commonly shows histologic patterns which are infrequent in other tumors and especially those which might metastasize to serosal membranes. Particularly helpful is the presence in some diffuse mesotheliomas of a mixed structure of malignant elements of both epithelial and mesenchymal character, or other diverse combinations. Growths in which only one type of cytoarchitecture is present may also have a highly distinctive pattern. For example, 1) a tubular or tubulo-papillary pattern in which the tumor cells show marked uniformity and are cubical or flattened; 2) masses of collagen in which there are either a) clef-like spaces lined or occupied by tumor cells, or b) fine hyaline strands which form complex meshworks and laminated bundles. Other examples of these tumors may have an entirely non-specific structure presenting the appearance of a spindle cell sarcoma or of an anaplastic tumor.

Because of the difficulty in clearly distinguishing on histologic grounds alone, some of these growths from metastatic tumors, cases diagnosed from biopsy material should be called "probable mesotheliomas". In epidemiological studies precise details of the pathological evidence on which the diagnosis was based should be stated.

(c) Histochemistry

(1) Most of the mucoid material within mesotheliomas, although often intimately associated with the surfaces of the tumor cells, is extra cellular. Intracellular or intra-tubular mucin of an adenocarcinoma will usually take mucicarmin or PAS stains. The absence of these reactions in a tumor which contains mucoid material can when taken with the other features, be useful additional evidence for a mesothelial tumor.

(2) Hyaluronic acid is often present in mesothelial tumors. The demonstration of its removal from the tissue sections by specific hyaluronidase preparations is a useful histochemical test, but since the acid is soluble in water, the test requires that the tissues be fixed in a special precipitating fixative, such as formol alcohol acetic acid (Wagner et al, 1962).⁸ It also appears likely that the quantitative measurement of hyaluronic acid in effusions, where this polysaccharide has been isolated and chemically characterized, may well become a reliable method for assisting in the diagnosis of mesothelioma, but it is emphasized that positive results for both the histochemical and chemical tests for hyaluronic acid are found in only a proportion of cases of mesothelioma and they should not be used as sole diagnostic criteria.

Wagner, J. C., Munday D. E., and Harington, J.S.
J. Path. Bact. 1962, 84, 73

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(d) Exfoliative cytology

The contribution to the diagnosis of diffuse malignant mesothelioma by exfoliative cytology of serous fluids requires that: 1) the exfoliated neoplastic mesothelial cells sufficiently resemble exfoliated non-neoplastic mesothelial cells for them to be recognized as having a mesothelial origin, and they also, 2) possess the generally accepted features of malignancy. The latter are not often present so a definite diagnosis of mesothelioma is then difficult.

Usually mesothelioma cells, while atypical, do not appear malignant but still show evidence of mesothelial origin. From cytology of the serous fluid it is possible to be strongly suspicious and in a few cases confident of the diagnosis of mesothelioma, but usually its presence can only be reported as possible.

The diagnosis of malignant mesothelioma by exfoliative cytology requires a familiarity with the non-neoplastic mesothelial cell. Mesothelial hyperplasia and hypertrophy can easily be mistaken for malignant mesothelioma and vice versa. It may be imprudent to do more than suggest malignant mesothelioma if there is not good supporting clinical, radiological, or biochemical evidence. The exfoliated malignant mesothelioma cell must also be distinguished from the adenocarcinoma cell, by far the commonest malignant cell recovered from serous fluids. This cell is well described in standard texts of exfoliative cytology.

(e) The use of tissue-culture in the diagnosis

The various manifestations of mesotheliomas in some cases cause difficulty in distinguishing such tumors from bronchogenic carcinoma, metastatic ovarian carcinoma, fibrosarcoma, etc. It is therefore recommended that where possible biopsy specimens of pleural and peritoneal neoplasms be studied after short-term passage, a) in vivo, and b) in vitro, to determine whether the rate of growth and/or morphology after transplantation can provide useful criteria for a differential diagnosis of mesotheliomas. To achieve this it is recommended that clinical pathologists seeing these tumors cooperate with workers in experimental carcinogenesis who are using tissue-culture methods.

3. The contribution of experimental pathology

It was agreed that various types of asbestos induce in many species of animals lesions similar to those seen in human cases of asbestosis, mesothelioma, and carcinoma of the lung, but the need for more precisely planned experiments was emphasized.

The desirability of using healthy animals of known response to asbestos under quantitative conditions of exposure by inhalation, feeding, and parenteral injection at several sites was agreed. Experiments in great variety are being undertaken in many parts of the world, particularly in Canada, Great Britain, South Africa, and U.S.A. The prospect of providing standardized samples of chrysotile, amosite, crocidolite, tremolite, and anthophyllite asbestos for experimental work was welcomed.

It was agreed that there was a need for improving methods of identifying the type of asbestos in submicroscopic fibers in tissues. Further studies of the rate of formation and resistance to destruction

of asbestos bodies in different species of animals may be useful.

It is recommended that there be closer collaboration between clinical and experimental pathologists and other scientists in the field of experimental pathology, biochemistry, and biophysics, as applied to the problems of the biological action of asbestos.

4. Proposal for Pathology Reference Panels

It is recommended that central consultation and reference panels be set up on regional, national, and international levels. These panels will:

- (1) Assist in establishing standards for pathological classification of asbestosis.
- (2) Serve as consultation centers for diagnosis of mesotheliomas and other tumors associated with exposure to asbestos.
- (3) Serve as general exchange of pathological material related to asbestosis and its associated tumors.
- (4) It is suggested that a comprehensive atlas on the pathology of mesotheliomas should be prepared.

RECOMMENDATIONS RELATING TO PHYSICS AND CHEMISTRY

1. Reference samples of asbestos for experimental work

It is anticipated that there will be an expansion of demand for asbestos of various types for biological and other studies. It is at present impossible to predict the biological effects of differences in mineral composition, size distribution, associated organic matter and trace metals in different samples of asbestos. Also fibers from a particular mine may vary in composition and in the amount of absorbed material.

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It is therefore recommended that:

(a) Standard reference samples, of respirable size, of amosite chrysotile, crocidolite, tremolite and anthophyllite be prepared from as pure parent material as possible and held at the Pneumoconiosis Research Unit (P.R.U.) in Johannesburg for distribution to centers requiring them. (These standards should also serve as references for comparison with larger amounts of material - such as may be required for inhalation or chemical extraction).

It is proposed that samples of chrysotile from different countries - for example, from Arizona, Havelock (Swaziland), Quebec, and Shabani (S. Rhodesia) be included in the reference collection.

(b) The standard samples be analyzed and characterized quantitatively by:

- (1) Chemical and spectrographic analysis
- (2) Optical and electron microscopy, for determination of shape, size distribution, and optical properties such as refractive indices, extinction angle, etc.
- (3) X-ray diffraction analysis by the powder technique.
- (4) Specific surface measurement by low temperature gas adsorption.
- (5) Determination of amount and type of organic matter present.

(c) Oils and waxes isolated from asbestos be prepared and also distributed through the P.R.U., Johannesburg.

(d) When the standards have been collected and prepared, the P.R.U., Johannesburg, will notify workers of their existence through the U.I.C.C. Bulletin and any other appropriate channels.

1. Identification and quantitative assessment of asbestos in tissues

In tissue sections it may be only possible to identify the type of asbestos present. For quantitative studies of the amount present in any organ such as the lung, it is necessary to analyze representative samples of the organs.

It is recommended that:

- (a) Tissue sections should be treated to remove organic material, for instance, by ashing or treatment with active oxygen; but this will not remove the asbestos bodies completely, and chemical treatment may be necessary to free the fibers. The best method for this is not known, but treatment with acetic acid may be useful. Identification of the type of fiber can only be made on free asbestos fibers and not on fibers inside asbestos bodies. It is recommended that methods of distinguishing asbestos from other fibers which may be present be further investigated. This can probably best be done by phase contrast or polarized light microscopy. It is also recommended that the treated sections be examined by electron microscopy for recognition of sub-microscopic fibers.
- (b) For large samples of tissue, acetic acid, hydrogen peroxide and formamide methods be tried as they appear to be superior to ashing, but the best method of extraction of mineral matter from tissue is not yet known and needs further study. However, the quantitative determination of asbestos in the residue obtained in this way is difficult but could be based on chemical analysis, x-ray diffraction, or fiber counts. It is recommended that methods for concentrating such asbestos and separating different types of asbestos from each other be further investigated.