FILE NAME: Welding (WELD)

DATE: 1968

DOC#: WELD008

DOCUMENT DESCRIPTION: Journal Article - Asbestos Bodies and Mesothelioma

Ann. Occup. Hyg. Vol. 11, pp. 283–293. Pergamon Press 1968. Printed in Great Britain

ASBESTOS BODIES AND MESOTHELIOMA*

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(*Received* 28 *February* 1968, *in revised form* 24 *April* 1968)

Abstract—The relation between exposure to asbestos and the occurrence of neoplasia is reviewed and it is concluded that the increasing use of asbestos will result in an increasing incidence of tumours. It is known that inhalation of asbestos fibres results in the formation of asbestos bodies in the lungs.

Typical asbestos initiated tumours—pleural and peritoneal mesothelioma—were found at an unusually high frequency in shipyard-workers. Only slight asbestos exposure had taken place in these cases. On the other hand the persons involved had been exposed to high concentrations of iron oxide (flame cutters and welders). Objects similar to asbestos bodies were found in their lungs. Originally it was thought that these were pseudo asbestos bodies, containing an iron oxide core, and it was suspected that pseudo asbestos bodies might play a part in the genesis of the tumours. Investigations carried out at TNO institutes† showed that the kernel of the bodies contained asbestos of the amphibole type thus proving that they were not pseudo but normal asbestos bodies. The simultaneous exposure to low concentrations of asbestos and high concentrations of iron oxide raises the suspicion that iron oxide might be an important cofactor in the genesis of mesothelioma found in the shipyard-workers. Experiments with different types of asbestos and iron oxide, on animals, are considered to be highly desirable in order to verify or disprove this hypothesis.

INTRODUCTION

IN RECENT years an increasing number of reports about exposure to asbestos and mesothelioma have appeared in medical literature. There is an alarming rise in the incidence of this rare tumour and, nearly always, exposure to asbestos is found.

Asbestos—an indestructable material—is mined in increasing quantities, for ever wider use inside and outside industry. Known as a dangerous material, leading to illness and death by asbestosis and lung cancer when inhaled in great quantities during work, it now seems to have a long-term effect when inhaled in minor quantities.

The last form may result in mesothelioma. Exposure to asbestos also results in the formation of asbestos bodies.

*Publication No. 297 of the Research Institute for Public Health Engineering TNO.

† The investigations using electron diffraction and electron microscopy were carried out by H. B. Zeedijk, and then with the X-ray microanalyzer by A. P. von Rosenstiel, both from the Metal Research Institute TNO. They will report in detail about their investigations in the journal *Micro Chimica Acta*.

(TNO is the Dutch abbreviation of "The Organisation for Applied Scientific Research in the Netherlands".)

The frequent finding all over the world of asbestos bodies at autopsy, without distinct asbestos exposure, perhaps indicates a widespread contamination with asbestos, unless there is a possibility of pseudo-asbestos bodies being formed.

The relatively sudden appearance of a great number of mesotheliomas in shipyard-workers without definite asbestos exposure, or any sign of asbestosis, together with a high incidence of asbestos bodies in the sputa of this group of people called for an examination of these asbestos bodies which is described in this paper.

ASBESTOS

Asbestos, the well-known mineral with the "thousand-and-one applications", also called "the magic mineral", consists of silicates having a fibrous crystal-structure.

The two most important groups are:

1. serpentine asbestos (chrysotile);
2. amphibole asbestos (amosite, crocidolite).

Serpentine asbestos is a silica–magnesium compound; amphiboles are composed of silica and iron.

Magnesium, aluminium, calcium and other elements are also found in amphiboles.

Asbestos may be described as a mineral that breaks down into fibres when crushed.

The molecular units are very strongly linked together in one direction; cleavage results in fibres which are sometimes of considerable length. This property, together with the many other well-known qualities, results in the widespread use of asbestos, in and outside industry, for textiles, heat and electrical insulation, friction-materials (brake linings), filters, packings, filling material for plastics, asbestos cement, etc.

Industrial use started about 1880, especially in the asbestos textile industry. Nowadays an enormous increase in applications can be seen. To meet the growing world consumption a 1000-fold increase in output has been required in the last 60 years. The greatest increase started after World War II. In 1945 the total production was about 0·6 million tons. The total yearly world production is now about 3·2 million tons: 90 per cent is chrysotile, 10 per cent amphiboles.

The fact that asbestos is practically indestructible means that an ever-growing quantity of asbestos is descending upon the human community, especially in the industrial parts of the world. Nowadays in many circumstances there is no other material that can replace asbestos.

ASBESTOSIS

A shadow was thrown over the famous qualities of asbestos when the first case of asbestosis was found (MURRAY, 1907).

People exposed to asbestos dust in asbestos textile works showed increasing deterioration of lung function; asbestosis became a well-known professional disease.

After long, or sometimes short, exposure progressive fibrosis of lung-tissue sets in, with increasing illness leading to death.

The development of safety measures diminished exposure in the asbestos textile industry, but the incidence of asbestosis was offset by the increasing use for other purposes such as heat insulation.

ASBESTOS BODIES

It was not until 1924, that Cooke described another case of asbestosis. This case is associated with the discovery of the "curious bodies" found in the lungs of a female asbestos textile worker. Since then much has been published about asbestosis, especially in Britain.

The "curious bodies" were found regularly and it was STEWART (1929) who described them as "asbestosis bodies". In later literature these bodies, together with asbestosis or signs of asbestos exposure, were found regularly. Because of this fact, the name was altered to asbestos bodies. However, for many years the finding of these bodies was frequently considered as pathognomic for asbestosis. The very fine needles in inhaled asbestos dust easily descend the respiratory tract and are deposited mainly in the lower lobes of the lungs. The organism seems to protect itself against this irritating material by enclosing the needle with layers of protein. The deposition of protein starts at both ends; later it is deposited on the shaft.

In this protein iron is precipitated. Under the microscope the bodies have a brownish-gold colour; with an iron-colouring they show up as pitch-black objects.

The asbestos bodies are easily recognised: they have a typical dumbell form.

Asbestos needles may be very thin: the thickness varies from 0·3–3 μm and many are invisible under a light-microscope. Since the asbestos bodies are very easily visible, relatively large quantities of protein-iron must be deposited.

Exposure to asbestos results in the formation of asbestos bodies, as has been proved in many animal-experiments. They may be found at post mortem or even in sputum when exposure was short and occurred a long time ago. In bodies of long standing disintegration occurs and the bodies then resemble a string of beads. It seems that this disintegration is not sufficient to enable the organism to get rid of the inhaled asbestos.

During the last decade a great number of reports have been made about finding asbestos bodies in the lungs at post-mortem, without known asbestos exposure.

THOMSON (1963) describes asbestos bodies in 30 per cent of the males and 20 per cent of the females in 500 consecutive autopsies over the age of 15 in Cape Town, South Africa. Similar findings with higher percentages were made in Miami, Florida; Pittsburgh, Pennsylvania; Montreal, Canada; and Milan, Italy.

In 1966 THOMSON *et al.* tried to evaluate the recognition of asbestos bodies in their article "Asbestos as an urban air contaminant".

The formation of asbestos bodies results from protein-iron being deposited on the thin asbestos fibres. It is possible that a similar process takes place with other inhaled materials and has been reported in connection with coal dust and graphite, pseudo-asbestos bodies being formed. Other articles describe similar ferruginous bodies formed around carbonaceous like materials (GROSS, 1966). There is a high incidence of pseudo-asbestos bodies in human beings, especially in cities and industrial regions. However, the increasing amount of indestructable asbestos, used in numerous circumstances, may lead to world-wide contamination of human inhaled air. Perhaps the first symptoms of this mass-contamination are beginning to show themselves and the so-called pseudo bodies are, in fact, normal asbestos bodies.

ASBESTOS AND NEOPLASIA

The first suggestion of an association between asbestosis and bronchial carcinoma was made about 1934 (LYNCH and SMITH, 1935; GLOYNE, 1935). Later, in reports from the British Ministry of Labour, more evidence was brought up.

BUCHANAN (1965) summed up the incidence of asbestosis and lung cancer in Britain. In the U.S.A., SELIKOFF *et al.* (1964) made a retrospective study of 632 insulation workers, with over 20 years' exposure; the follow-up was made with the help of the labour-union administration. Of these workers, 255 had died by 1964. Amongst these 255 the cause of death in 45 cases was a carcinoma of the lungs or mesothelioma. The expected incidence was 6·6. Carcinoma of the digestive tract had caused 29 deaths against an expected 9·4. For other carcinomas the incidence was normal. The incidence of mesothelioma of the pleura and peritoneum was found to be relatively high.

Later SELIKOFF *et al.* (1965) found that among 307 ex-insulators mesothelioma was the cause of death in 10 cases.

Mesothelioma was long considered an extremely rare tumour. Many pathologists denied its existence as a substantial tumour and looked upon it as metastasis of a primary tumour elsewhere. Nowadays this rare tumour is more and more widely reported. There is no longer any question as to whether this neoplasm is a metastasis or not: it is a very substantial tumour, typically located in the pleural and peritoneal membranes. Metastasis is rarely seen, except in the final stages. It grows fast, encasing the intestines and lungs, usually without infiltrating them. It is a polymorphous tumour, with many different structures, mostly fibrous alternating with tubular and papillar areas; typical clefts may be present. It resists any form of therapy. It became more and more obvious that there was a connection between asbestos and mesothelioma, particularly from the reports from WAGNER *et al.* (1960) and NEWHOUSE and THOMPSON (1965), who reported the occurrence of mesothelioma in patients with no known direct contact with asbestos. Asbestos mines or asbestos industries were located in the neighbourhood, but exposure in these neighbourhood-cases was slight. There are signs that minor crocidolite intake, especially, may result in mesothelioma. The incubation time may be about 30 years.

In conclusion, asbestos exposure may lead to asbestosis, to carcinoma of the lungs and digestive tract and to mesothelioma. In the latter case there may be no indication of definite exposure to asbestos.

FINDINGS IN SHIPYARD WORKERS

Asbestos bodies

Since 1875 there exists in Flushing, the Netherlands, a big shipyard and machine tool manufacturer the Royal "de Schelde" Ltd. This yard is the continuation of an old navy yard, that has been there for several centuries.

The shipyard is the only big industry in this little town. The production line consists mainly of new ships, heavy machinery and boilers. The number of people employed was end of 1967 more than 3100.

In March 1962 a 41 years old worker had an accident. He acquired a fracture of the 11th and 12th rib, without any further complications. He was sent home; 5 days later he was found dead.

The social insurance fund requested a post-mortem to ascertain whether there was any connection with the accident. The post-mortem revealed the two broken ribs and as the certain cause of death a pneumonia of the influenza type. The pathologist also found numerous asbestos bodies and amorphous iron oxides in the lungs, however without any trace of fibrosis. So no asbestosis could be diagnosed, nor any malignancy.

In his working-life the man had had only a very slight contact with asbestos.

In building new ships two kinds of insulation processes are used in the last stages of the construction process.

Chrysotile felt is used in the first one for the insulation of pipes in particular in the engine room.

The second process is used in particular between walls. A mixture of cement, chrysotile, amosite, crocidolite and water is mixed and injected in the indicated locations.

It should be noted that asbestos insulation has never been applied by employees of the shipyard and that the asbestos injection process is increasingly substituted by injection of polyurethane foam or by the use of glass fibers and mineral wool. In repair yards there is more asbestos exposure because of the breaking up pipeline and boiler insulations during repairs. The man had worked in the yard for 15 years, mostly in the first stages of shipbuilding—the construction of the hull where "clean" iron is used. He was a welder; so there had been a heavy exposure to iron oxides.

Investigation of the welding-rods used revealed that no asbestos containing welding-rod had ever been used in this yard.

Of course exposure to asbestos cannot be entirely denied; it is used in so many places and circumstances.

The fact that there was no trace of asbestosis indicated only slight asbestos exposure. The numerous asbestos bodies however called for further investigation.

In the following 4 years a great number of sputa of yard workers was screened during the period of coughing after the "common cold" when there is always some alveolar sputum.

After the sputa had been concentrated and stained, microscopic research revealed that out of 247 sputa, 147 contained asbestos bodies, mostly combined with amorphous iron. This total attained greater significance when split up over the various work-departments of the yard.

	Total sputa	Asbestos bodies	(%)
Shipbuilding	87	65	75
Engineering	88	52	59
Maintenance, etc.	40	22	55
Administration	32	8	25
Total	247	147	53.5

In a group of 24 insulators 22 (92 per cent) were found to have asbestos bodies in their sputum.

Especially in the shipbuilding department a lot of work is done with exposure to hot-iron (welding, burning, etc.). So it might be asked if there could be any connection between the asbestos bodies found and exposure to iron oxides.

Inhaled iron oxides may lead to harmless siderosis of the lungs; examination of 180 welders in the same yard revealed no significant decrease in lung function, nor any trace of asbestosis on the X-ray photographs. However there had sometimes been exposure to welding-smoke for 25 years or more.

The possibility that the asbestos bodies found could be pseudo-asbestos bodies or ferruginous bodies had to be investigated.

Mesothelioma

In 1964 the first case of pleural mesothelioma of a yard worker was reported. In the following three years the number of mesotheliomata rose to 21 in the whole province of Zeeland. From this number 17 had worked in the same shipyard in Flushing. Two more yard workers probably have mesotheliomata; these are not yet pathologically proved, but the clinical symptoms are unmistakable. All pathological work in Zeeland is done by one institute; all cases of mesothelioma of the pleura and peritoneum were correctly diagnosed in biopsies and post-mortems by this institute, sometimes controlled by other pathologists. All cases were concentrated in Flushing and the immediate neighbourhood. In nearly all cases a good occupational history could be obtained. Only 4 cases had never worked in the yard. Of these 4, one had had definite exposure to asbestos; 1 had worked with hot iron (foundry) and 1 in agriculture. For one no occupational history could be obtained.

All were people of 60 years or older, when they succumbed, and it was known that nearly all had had a risk exposure to oxides of iron.

In all post-mortems asbestos bodies were found in the lungs; no trace of fibrosis or asbestosis could be detected, even after thorough search. In three other cases asbestos bodies were found in sputum. The question of the nature of the asbestos bodies found became urgent. In this matter we received great help from the TNO institutes in Delft (the Netherlands).

The asbestos bodies were extracted from post-mortem lungs in considerable numbers and thoroughly washed to get rid of most of the organic materials. In this form they were presented to the TNO institutes with three questions.

1. The determination of the kernel of these bodies: asbestos or not?
2. If asbestos, what type could be detected in these bodies?
3. If not, what other elements could be found?

PROCEDURES USED FOR INVESTIGATING THE COMPOSITION OF THE KERNELS OF ASBESTOS BODIES

The electron microscope and the "X-Ray microanalyzer" were the main instruments used during the investigation. Isolation of the asbestos bodies from the samples, which proved to be necessary, was carried out with the aid of a micromanipulator.

Electron diffraction utilising an electron microscope

The electron microscope offers unique possibilities for investigating the structure of microscopically small objects. This is not only due to the much larger resolving power of the electron microscope compared to the light microscope (100–1000 times better) but also due to the possibility of applying diffraction methods on a small selected section of the specimen under investigation. By the use of diffraction methods, it is in principle possible to deduce the three-dimensional structure of atoms within the crystal lattice of the surface of the specimen (BEECHING, 1946; ULLMAN, 1961).

From the diffraction pattern, it was possible to calculate the dimensions of the elementary cell, which indicated the presence of asbestos. Subsequently the diffraction patterns of known asbestos types were compared in detail with the diffraction pattern to be identified.

The X-ray microanalyzer

In the classical microscope light is projected upon the object each point of which then functions as a light-emitter. By processing (with lenses) the electromagnetic waves generated by the emitters, a picture is constructed which is a magnified image of selected parts of the object. An alternative possibility consists of the scanning of specimen sections by a beam of light. After amplification, the light which is transmitted or reflected, is made visible on a display tube by use of television techniques. The degree of magnification can be controlled by causing a small beam movement in a horizontal direction over the object under investigation to produce a larger horizontal movement over the electron image display tube, see Fig. 1.

The resolving power of this type of instrument depends in the first place on the size of the cross-section of the beam which must possess sufficient intensity to be registered by the detection system employed.

The principle described above is applied in the X-ray microanalyzer. A beam of electron scans over the specimen's surface. The secondary electrons, which are

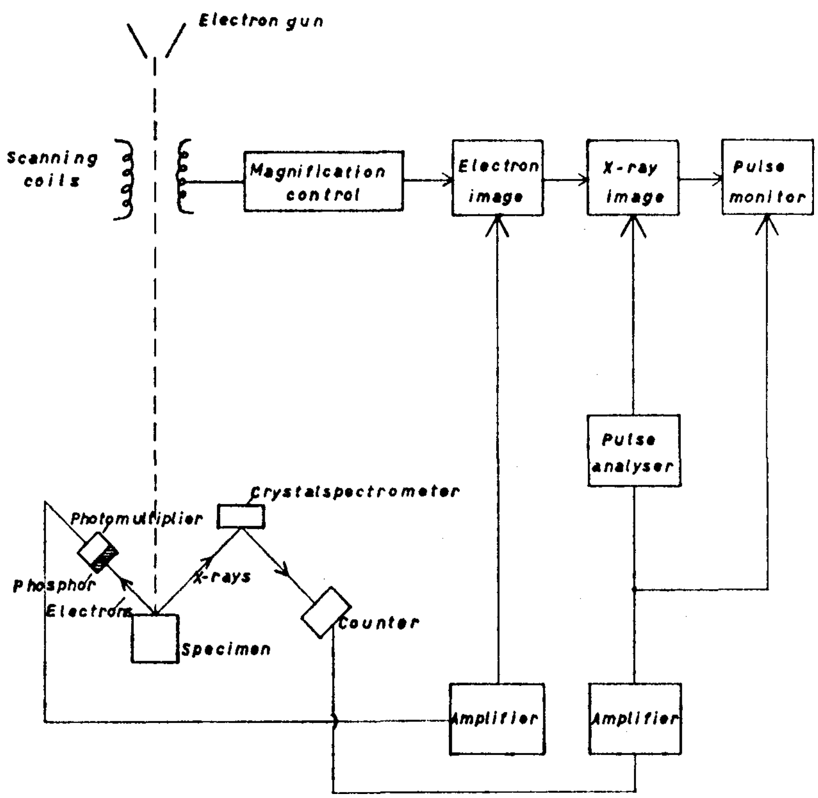

FIG. 1. Schematic diagram of microanalyzer.
Example of an electron image Fig. 4a
Example of a X-ray image Fig. 4b
Example of the recording of the pulse monitor Figs. 5, 6, 7.

emitted by the specimen's surface, are trapped in the phosphor of a photomultiplier tube. This results in a signal, which, after amplification, is made visible in a display tube forming the electron image, see Fig. 1.

When struck by the beam of electrons emitted by the electron gun, the specimen will emit not only secondary electrons but also X-ray fluorescence radiation. The wavelength of this radiation is characteristic for the elements which emit the radiation while the radiation intensity is related to the concentration of these elements. By spectroscopic analysis of the radiation emitted, it is possible to obtain a qualitative and semiquantitative composition of the selected part of the specimen (COSSLETT, 1966).

EXPERIMENTAL RESULTS AND THEIR DISCUSSION

Figure 2 shows a typical asbestos body as seen in the light microscope.

The identification of the kernel of the asbestos bodies by electron diffraction was hampered by the amorphous protein coat and was attempted by means of the X-ray microanalyzer. The first results gave the impression that the kernel consists mainly of iron and oxygen, while traces of silicon, phosphorous and calcium were also found.

At this point of the study it was deemed useful to continue study of the structure of the kernel of the asbestos bodies by means of electron diffraction. The asbestos bodies were isolated by the use of a micromanipulator and treated with a mixture of HCl and H_2O_2 in order to strip, at least, partly the coat from the kernel, Fig. 3.

During this stripping operation, it became clear that the coat is not homogeneous but consists of layers, varying in density which are deposited around the original fibre. The section seen as the kernel of the asbestos body, when using a light microscope, often consists of the kernel plus the first layers of the coating. The actual kernel is often thinner than originally presumed (a few tenths of a micron). As the resolving power of the X-ray microanalyzer is of the order of $1\,\mu m$, the X-ray fluorescence radiation must principally originate from the high iron content of the coat and for a very minor part from the asbestos fibre. The effect of the thickness of the coat is shown in Fig. 4, which illustrates an asbestos body for which the composition has been studied at sections where the coat varies in thickness. The results of this study are shown in Figs. 5, 6 and 7. Figure 5 gives the X-ray image of the same asbestos body produced by the iron fluorescence radiation, giving a qualitative picture of the distribution of the iron. Figures 6 and 7 give more accurate quantitative results. So in Fig. 6a the vertical axis gives an indication of the concentration of iron along the location on line *a* from Fig. 4, and Fig. 7a shows similar data for silicon. Figures 6b and 7b provide the same information as 6a and 7a, but now for line *b* of Fig. 4. Figures 6c and 7c provide this information for line *c* of Fig. 4.

Similar lines have been established for the elements calcium, phosphorous, oxygen and magnesium.

Electron diffraction studies carried out on practically stripped asbestos bodies showed that the kernel consists of an amphibole type of asbestos.

By this method it was possible to identify the core of eight asbestos bodies. In addition, investigation of the original sample, sent to TNO, was found to contain uncoated asbestos fibers. Out of 27 fibres investigated by electron diffraction 17 appeared to belong to the serpentine variety chrysotile and 10 were classified as belonging to the amphibole type (probably variety crocidolite).

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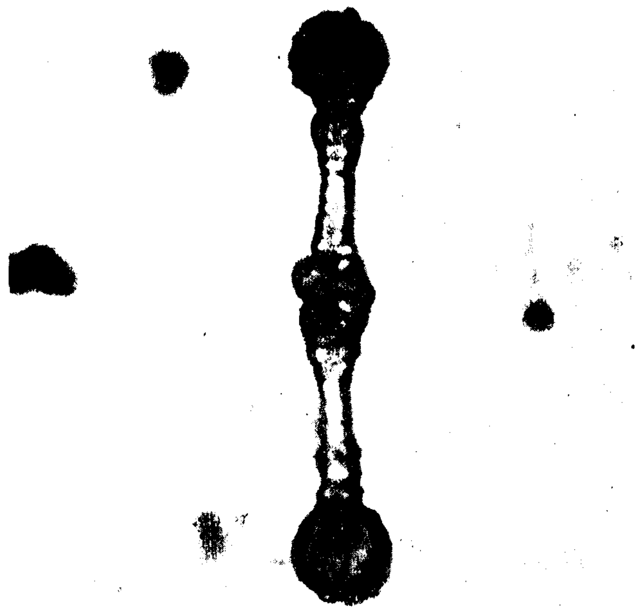

FIG. 2. Asbestos body as seen in the light microscope. Magnification 1500 ×.

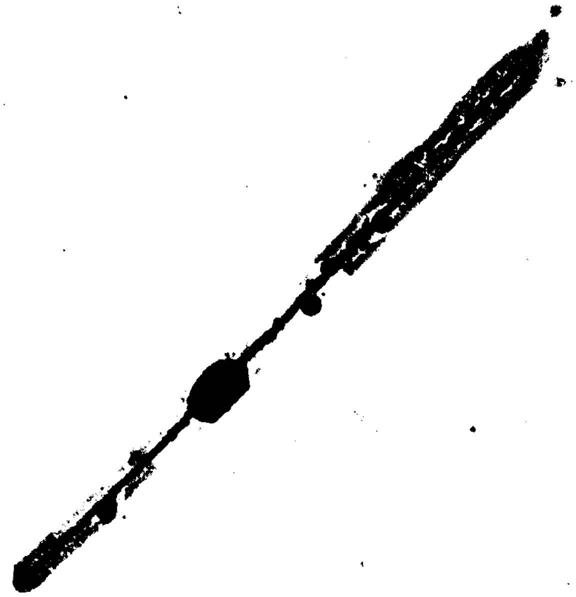

FIG. 3. An electron micrograph of a partly stripped asbestos body. Magnification 2200 ×.

(facing p. 290)

FIG. 4. Electron image of asbestos body produced by the microanalyzer. Magnification 2000 ×.

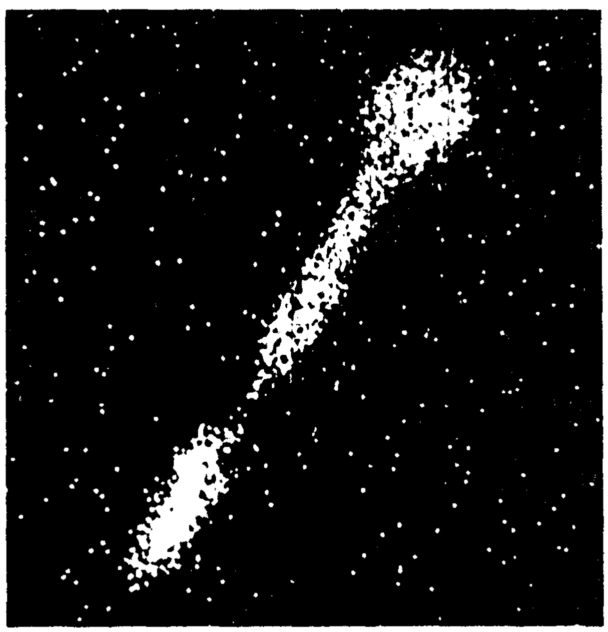

FIG. 5. X-ray image of asbestos body produced by the iron fluorescence radiation. Magnification 2000 ×.

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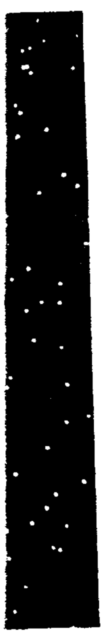

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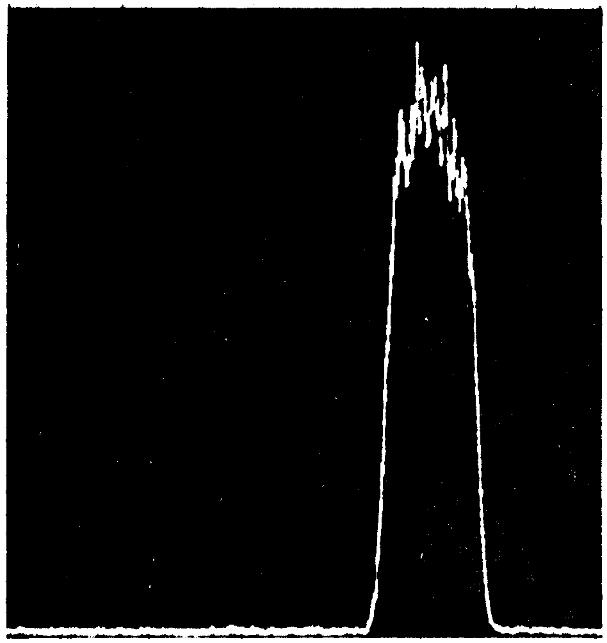

FIG. 6a. Pulse monitor display of the iron X-ray fluorescence radiation. The abscissa gives the location on line *a* of Fig. 4, the ordinate is proportional with the iron concentration.

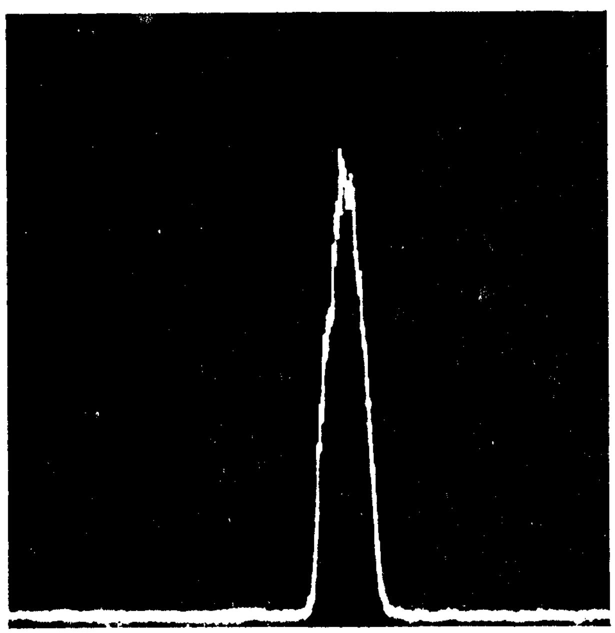

FIG. 6b. Same as 6a, but the abscissa and ordinate are now related to line *b*.

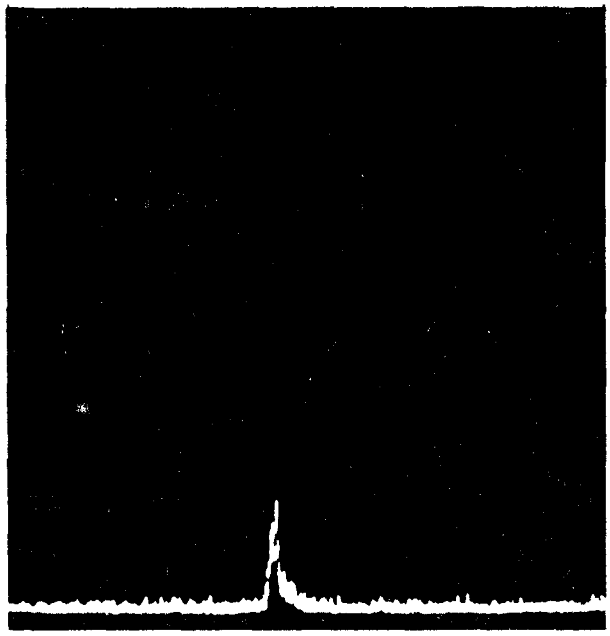

FIG. 6c. Same as 6a, but the abscissa and ordinate are now related to line *c*.

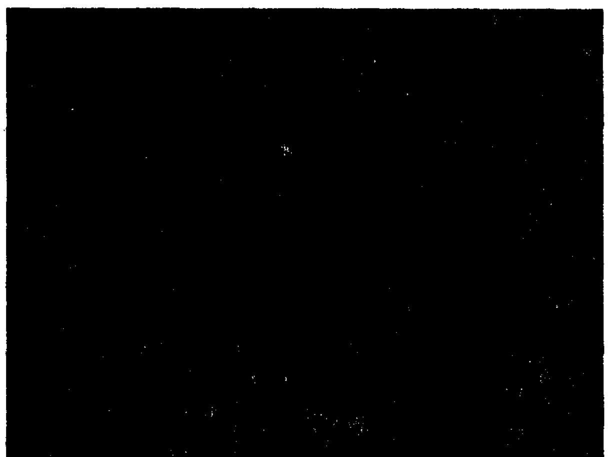

FIG. 7a. The same type of information as in 6a, but now for silicon instead of iron.

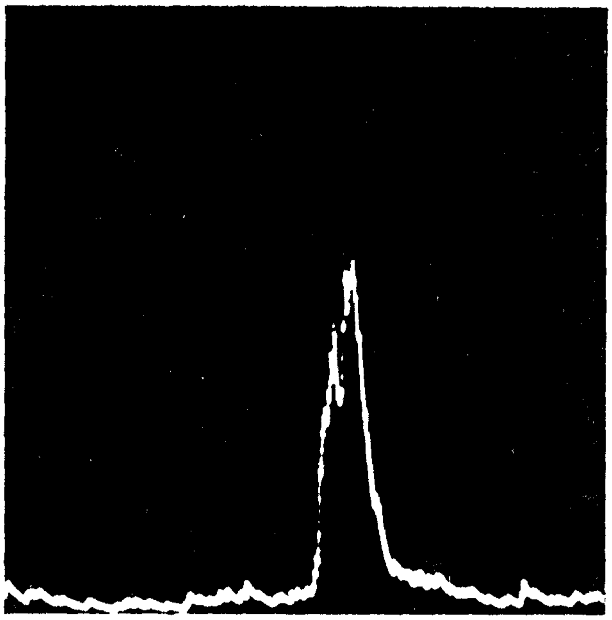

FIG. 7b. The same type of information as in 6a, but now for silicon instead of iron.

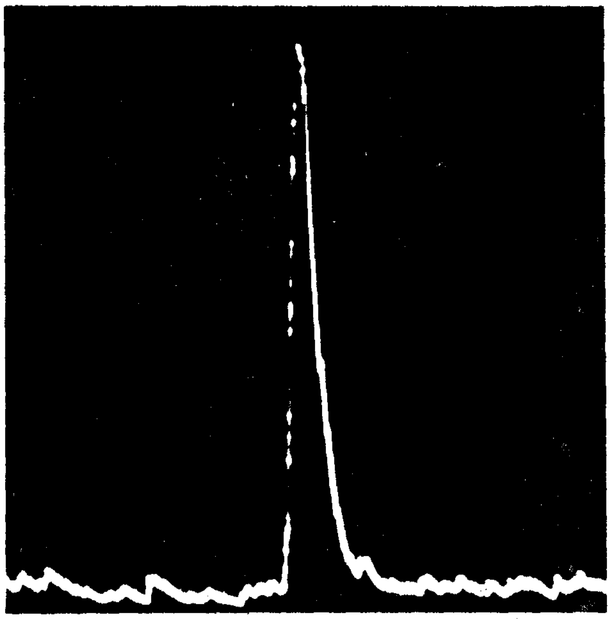

FIG. 7c. The same type of information as in 6c, but now for silicon instead of iron.

Although approximately ⅘ of the asbestos fibres collected from the original sample could be classified as being of the chrysotile type, while the rest were amphibole, it is noteworthy that all the cores of the asbestos bodies which have been investigated consist of an amphibole type of asbestos. Various explanations for this phenomenon can be offered, for instance:

1. since asbestos fibres of the amphibole type are generally thicker than those of other types the asbestos bodies are more noticeable and are therefore easier to isolate;
2. when both amphibole and chrysotile types of asbestos are present simultaneously in the lungs the amphibole type of asbestos is more likely to be used as a kernel for asbestos bodies.

It has been reported (BERKLEY, 1967) that the proportions of iron, silicon, oxygen and magnesium of the core of asbestos bodies, as determined with an X-ray microanalyzer, do not correspond to any known type of asbestos. BERKLEY (1967) suggested that this is due to attack of the asbestos in the pleura where atoms of silicon and magnesium are displaced by iron atoms. The investigations described above show that the lack of correspondence can be explained by the varying thickness of the protein coating containing iron, which is deposited on the asbestos fibre. This is illustrated by Fig. 7a, band *c* and *b* which show the increase of the silicon concentration due to the decrease of the iron containing protein coat of the asbestos fibre. Proof that the increase of the silicon concentration corresponds to in successive traverses, *a* to *c* a decrease in the thickness of the coating is evident in Fig. 4. The iron concentration, on the other hand, decreases with decreasing thickness of the coating as shown in Fig. 6a, b and c due to the fact that the iron content of the protein coat is higher than the iron content of the asbestos.

Apart from this, no indication of contamination or attack of the asbestos fibres forming the kernels of the asbestos bodies has been found. Concerning the existence of pseudo-asbestos bodies, it is to be noted that in all cases investigated by TNO, the core of the supposed pseudo-asbestos bodies proved to consist of asbestos. While it is premature to draw any definite conclusions on account of the limited number of determinations which have been carried out to date, definite doubt regarding the existence of pseudo-asbestos bodies appears justified. From experience acquired during the tests, the impression is gained that electron diffraction, supported by the X-ray microanalyzer, is the only method which is able to eliminate the influence of the coating or residuals of the coating on the analysis of the core.

Finally, it should be remembered that the role of asbestos bodies in the genesis of mesothelioma has not been dealt with. It is believed that experiments with animals are necessary to clarify this.

CONCLUSIONS

1. Though there had never been great asbestos exposure in the mesothelioma cases in Flushing, the amphiboles found in the asbestos bodies may have caused the tumour.

2. So far, there have been no cases of mesothelioma in the small community living in the immediate vicinity of the yard at Flushing. It should be realised that there may be other factors functioning as co-carcinogens. The iron oxides should be

taken into account since all mesothelioma cases from the yard had had heavy exposure to them.

3. It should be realised that the ever growing amount of asbestos in the human community, which cannot be neutralised in any way, may lead to a steep rise in mesothelioma. The amphiboles, in particular, may be a special risk.

4. There are strong indications that electron diffraction, supported by investigations with the X-ray microanalyzer, is the best way for examining the cores of asbestos bodies.

5. The existence of pseudo-asbestos bodies must be considered extremely doubtful; it is believed that the analytical method used to identify them was inadequate.

6. It is believed that the unusual composition of the cores of asbestos bodies, found by chemical analysis and with the X-ray microanalyzer, is caused by the protein coating which contains a high percentage of iron.

7. Research on the part played by the inhalation of amphiboles and iron oxides in the genesis of mesothelioma must be carried out with animals.

Acknowledgements—Figures 3–7 were made by A. P. VON ROSENSTIEL and H. B. ZEEDIJK of the Metal Research Institute TNO.

The isolation and stripping of asbestos bodies was carried out at the Microbiological Laboratory of Prof. Wikén, Technical University, Delft by Miss Vos and J. VAN DER TOORN.

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