

Original Research

Diagnostic limitations of lung fiber counts in asbestos-related diseases

Joachim Schneider¹, Rolf Arhelger¹, Bernd Brückel², Xaver Baur³

¹ Institut und Poliklinik für Arbeits- und Sozialmedizin,, ² Institut und Poliklinik für Arbeits- und Sozialmedizin,, ³ European Society for Environmental and Occupational Medicine,

Keywords: Lung dust analysis, asbestos diseases, ferruginous (asbestos) bodies, chrysotile, crocidolite

<https://doi.org/10.35122/001c.70352>

Background

Lung dust fibre analyses have been used by some pathologists to estimate past asbestos exposure in the workplace and its related health risks. Asbestos, however, especially the predominately applied chrysotile asbestos type, undergoes translocation, clearance and degradation in the lungs.

Objectives

We quantified the asbestos fibre and ferruginous (asbestos) body (FB) content in human tissue with respect to the German asbestos ban in 1993 and the interim period of more than 20 years in order to evaluate the diagnostic evidence of these analyses for asbestos-related diseases (ARD).

Methods

Lung dust analyses have been used in empirical assessments of ARD since 1982. Tissue samples of about 2 cm³ were used and processed in standardized manner. FB was analysed by light microscopy and asbestos fibres by scanning transmission electron microscopy (STEM).

Results

Chrysotile and amphibole fibre concentrations in the lung tissue depend roughly on the cumulative asbestos exposure levels in the workplace.

However, the concentration of lung asbestos fibre and FB depends on the year of examination and especially on the interim period. As the interim period increases, the asbestos fibre burden decreases. There is no relationship between FB and chrysotile asbestos fibre concentrations and only a weak correlation between FB and crocidolite fibre concentrations.

There was no significant difference in chrysotile and amphibole fibre concentrations as well as in FB counts between the different ARD.

Conclusions

Due to the length of interim periods, a past exposure to chrysotile or amphibole asbestos can no longer be detected with FB or asbestos fibre measurement in lung tissue. This means that negative results of such measurements cannot disprove a qualified occupational case history of asbestos exposures and the related health risks due to the fibrogenic and carcinogenic potential of asbestos.

INTRODUCTION

Asbestos-related diseases (ARD), i.e. asbestosis, asbestos-related changes of the pleura, asbestos-related lung, larynx or ovarian cancer and malignant mesothelioma have gained particular importance as occupational diseases worldwide.

The diagnosis of ARD is based on a detailed personal interview of the patient, occupational data on asbestos exposure in the work history, and radiological findings, i.e., results of chest X-ray and, if necessary and available, high-resolution chest computed tomography.¹ Malignant tumors

such as lung cancer, larynx cancer, ovarian cancer or mesothelioma have to be confirmed by histopathological examination. The diagnosis of asbestosis and asbestos-related pleural changes can be made if radiological findings correspond to a work history compatible with significant asbestos exposure.²

However, the exposure to asbestos fibre dust from previous decades in the workplaces cannot always be adequately reconstructed due to lack of fibre measurements and/or incomplete information in work biographies. An alternative attempt was therefore made to correlate the asbestos fi-

bre and/or asbestos body concentrations in the lung tissue with potentially ARD to estimate past occupational asbestos exposure in general³⁻⁵ or amphibole asbestos exposure in particular.²

With limitations (see Discussion section below), such analyses of lung tissue for asbestos fibres and FB may provide data to supplement occupational history.

With regard to analytical methods, scanning electron microscopy (SEM) identifies fibre geometry in addition to the microstructure of the fibres. Energy dispersive X-ray spectroscopy (EDX) determines elementary composition. The crystallinity of fibres was additionally investigated with transmission electron microscopy (TEM) and electron diffraction. Using this method, it is possible to identify the different types of asbestos fibres as well as other mineral fibres. For clinical purposes, ferruginous (asbestos) bodies (FB) can be counted in lung tissue specimen as well as in ashed lung using light microscopy (Leitz Diaplan, Wetzlar, Germany) by use of 500x magnification.

Manke et al.⁶ evaluated the application of a plasma ashing method for STEM fibre analysis in human lung tissue. In order to carry out a complete check of the preparation method, a suspension of standard crocidolite fibres was used. No changes in length, diameter or aspect ratio distribution occurred during low temperature ashing method. Up to 78% of the fibres were recovered.

Asbestos and other inorganic mineral fibres in human lung tissue analyzed by an analytical scanning transmission electron microscopic method (STEM) at the Institute and Outpatient clinic for Occupational and Social Medicine at the University of Giessen in Germany, headed by Prof. Weitowitz, showed that the correlation between the estimated cumulative workplace exposure in fibre-years and the biomonitoring of asbestos content in the lungs is weak.^{7,8} This is caused by chrysotile asbestos – around 95% of asbestos used in Germany and also in many other countries – which is not biopersistent in the lungs and rarely form FB.^{7,9} SEM (SEM (Hitachi S-2300) was used with magnification between 10,000 and 100,000. This allows to detect particles until 20 nm diameter. Chrysotile asbestos fibres can splice lengthwise. All fibres of a length > 5 µm were counted, also fibres with extremely small diameters (BIA_Arbeitsmappe). Crocidolite asbestos fibres were counted when they meet the WHO fibre criteria (WHO fibre definition of length (L) > 5 µm, diameter (D) < 3 µm, ratio of length to diameter (L/D) >3:1). These fibres have been thought to be especially relevant for carcinogenicity and fibrogenicity. In addition to fibres of L > 5 µm fibres of all length (L>1 µm) can also be taken into account; for instance broken crocidolite fibres. Fibres between 1 and 5 µm length were detected and analysed separately in the last 20 years. Scanning electron microscopy (SEM; Hitachi S-2700; Hitachi, Ltd., Tokyo, Japan) was used to identify particle geometry in addition to the microstructure of the fibers. The element analysis resulted from energy dispersive X-rays (EDX). Transmission electron microscopy analysis combined with electron diffraction (detection of crystallinity) was performed using a transmission electron microscope (H-7100; Hitachi, Ltd.).

Given the German asbestos ban in 1993, the question arises as to whether and what concentrations of asbestos fibres can still be detected in the human lung tissue after an interim period of more than 20 years. In this study, we quantified lung asbestos fibre and FB counts as well as cumulative exposure data from medical expert opinion procedures (fibre-years) in patients with ARD. We did not aim to reevaluate the histopathology diagnosis of the study group.

MATERIAL AND METHODS

Since 1982 lung dust fibre analyzes have been carried out by the Institute and the Outpatient Clinic for Occupational and Social Medicine. 257 such lung dust fibre analyzes were done in potentially asbestos-related occupational diseases for diagnostic medical expert opinions on behalf of German social courts and statutory accident insurance institutions. An in-depth occupational history was taken from all patients examined by experts and a standardized cumulative asbestos dose estimate was carried out based on the BK report's "fibre-years".¹⁰ Detailed clinical investigations according to the guidelines on diagnosis and compensation of ARD,¹ combined with the occupational history data, had shown that 28 patients suffered from asbestosis as diagnosed by case history indicating asbestos exposure, computed tomography and/or chest X-ray, lung function testing. 105 suffered from primary lung cancer (59.2 ± 10.0 years) and 44 from diffuse malignant pleural mesothelioma (59.8 ± 10.0 years). 46 were controls without previous asbestos exposure undergoing lung surgery due to lung cancer associated with smoking, emphysema bullae or suspected hypersensitivity pneumonitis, and 34 other controls not exposed to asbestos had diseases not involving the lungs.

Approximately 2 cm³ of the formalin-fixed lung tissue obtained surgically or postmortem was cut into 3-5 mm pieces and mixed. About half of the material was freeze-dried for electron microscopy. A wet/dry factor (mean value 10) was determined from the ratio of the wet and dry weights to convert the concentrations from g/wet to g/dry (gdry) lung tissue. The light microscopic preparation for counting FB was obtained from the other half of the tissue using a modified NaClO method.⁸

When the starting material was paraffin blocks from the histological preparation of the patient's lungs, the lung tissue was excised from the block and completely deparaffinized by heat treatment and subsequent washing in xylene.

Further sample preparation included plasma ashing of the tissue and subsequent quantitative filtration of the remaining lung ash. Transmission preparations were made from the filter for SEM (Hitachi S-2300; Hitachi, Ltd., Tokyo, Japan) and TEM was used to identify fibre geometry in addition to the microstructure of the fibres. To determine the elemental composition Energy Dispersive X-ray spectroscopy (EDX) was applied. The crystallinity of fibres was additionally investigated with transmission electron microscopy (TEM) and selected area electron diffraction

(SAED). To increase the conductivity, all samples were sputtered with a fine layer of Au.

Fibre counting was done at 10,000x magnification in grid fields with an area of 0.01 mm². The searched area is up to 0.25 mm² for fibres of all lengths and up to 0.5 mm² for fibres with a length $\geq 5 \mu\text{m}$. The detection limit of 80,000 fibres per gram of dried lung tissue was determined for fibres of all lengths and 40,000 fibres per gram of dried lung tissue for fibres with a length $\geq 5 \mu\text{m}$ was achieved. Chrysotile and amphibole fibres were evaluated, which correspond to the WHO fibre definition of length (L) $> 5 \mu\text{m}$, diameter (D) $< 3 \mu\text{m}$, ratio of length to diameter (L/D) $> 3:1$. These fibres have been thought to be especially relevant for carcinogenicity and fibrogenicity.

Tremolite asbestos has not been used commercially in Germany but minimal contaminants by tremolite in some asbestos products cannot be excluded.

The correlation between the asbestos fibres observed in the lung dust fibre analysis using STEM and the FB concentrations were calculated with SPSS 20.0 according to Spearman's rank correlation test.

In the following section, the asbestos fibre or FB concentrations per gram of dry lung tissue (FB/g_{dry}) are presented.

To illustrate, with a section thickness of a histological preparation of 5 μm , a tissue cube with an edge length of 1x1x1 cm would result in 2000 histological sections. The detection of an asbestos body in the section corresponds to a concentration of 2,000 FB per cm³ of lung tissue.

RESULTS

In a previous study of our institute asbestos fibres were identified as follows: 28 % chrysotile asbestos, 4 % amosite asbestos, 4 % antophyllite asbestos and 68 % crocidolite asbestos.¹¹ Figures 1 and 2 represent characterization of IICC crocidolite and chrysotile fibres.

Figures 3 a, b, c, d show individual asbestos fibre types identified in lung dust analyses of this study.

Chrysotile and amphibole fibre concentrations as well as FB counts in the lung tissue are roughly associated with the cumulative asbestos exposure levels in the workplace. On average, about 50,000 chrysotile fibres/g_{dry} were found with asbestos dust exposure below 20 fibre-years, and about 190,000 chrysotile fibres/g_{dry} with asbestos dust exposure above 20 fibre-years. The corresponding concentrations were 300,000 and 9,200,000 F/g_{dry} for amphibole asbestos and 12,000 and 41,000 FB/g_{dry} for FB. The difference between the groups with high or low asbestos exposure was significant for chrysotile asbestos ($p = 0.011$), and crocidolite asbestos ($p = 0.004$), and FB ($p = 0.021$).

In the lungs of 17 control subjects without any history of asbestos exposure (so-called "normal population"), upper limits of 180,000 fibres F/g_{dry} with a length $> 5 \mu\text{m}$ for chrysotile fibres and 140,000 F/g_{dry} for amphibole fibres (length $> 5 \mu\text{m}$) were determined; 78 FB per cm³/g dried lung tissue were counted in controls.

According to the diagnoses "asbestosis and/or asbestos-related pleural changes", "asbestos-related lung cancer" or "diffuse malignant mesothelioma", a large range of fibre

concentrations were measured without any significant differences between these asbestos-related diseases (Fig.4). Mean chrysotile fibre concentrations were 152,000 in patients suffering from asbestosis, 46,000 in those with lung cancer, and 67,000 F/g_{dry} in patients with mesothelioma. Mean amphibole fibre concentrations were 850,000 in patients with asbestosis, 2,200,000 in lung cancer cases, and 532,000 F/g_{dry} in patients with mesothelioma. Mean FB concentrations were 5,100 FB/g_{dry} in patients with asbestosis, 8,800 FB/g_{dry} in those with lung cancer, and 1,840 FB/g_{dry} in those with mesothelioma.

For asbestos fibres with a length $> 5 \mu\text{m}$ determined in the lung tissue using STEM and the FB concentrations per gram of dry lung tissue, there was neither a correlation between chrysotile and amphibole asbestos fibre concentrations (Fig. 5) nor between chrysotile fibre and FB concentrations ($R^2 = 0.0$) (Fig. 6). There was a weak correlation ($R^2 = 0.26$) between amphibole fibre and FB concentrations (Fig. 7), which is obviously due to the known longer biopersistence and the higher FB rate of amphibole fibres compared to chrysotile fibres.

The asbestos fibre concentrations depended in particular on the time lung tissues was taken and the interim period. The highest asbestos fibre concentrations and FB were measured in the 1980s for interim periods of less than 1 year but decreased significantly with increasing interim periods (Figs. 8-10). After an interim period of more than 20 years, hardly any chrysotile or amphibole fibres were detectable. After the year 2000, i.e. 7 years after the German asbestos ban and decreasing asbestos levels in the work-sites over the 3 decades before,^{12,13} it was only possible to detect occasionally asbestos fibres in the lung tissues.

DISCUSSION

Asbestos fibres are mostly resilient in the environment. However, they undergo elimination kinetics in the lungs. In the lung environment, chrysotile fibres leach magnesium and fan out into fibrils with a diameter in the range of 0.02 – 0.2 μm .¹⁴ Chrysotile fibres are rapidly cleared, especially those short in length are transferred to the pleura, peritoneum, and pericardium. Suzuki et al. found 30 times more chrysotile than amphibole fibres in mesothelial tissue.¹⁵ Such fibres were also detected in neighboring organs.¹⁶⁻²³ Low biopersistence of chrysotile in lung tissue has been demonstrated by various authors in animal experiments and in previously exposed workers.^{15,20,24-28} Bernstein et al.^{29,30} report half-lives of only a few days in rats; however, these experiments have been criticized for significant bias in the pretreatment of the fibres and the failure to take account of fibre translocation to the pleura and to neighboring organs.^{31,32} In animal studies it was shown that the clearance half-life of Canadian chrysotile asbestos depends on the fibre length and is mostly in the range of days, but half-life of long chrysotile fibres in human lung was shown to be several years. Corresponding findings were reported in Quebec miners undergoing autopsy.³³

Accordingly, Churg wrote:

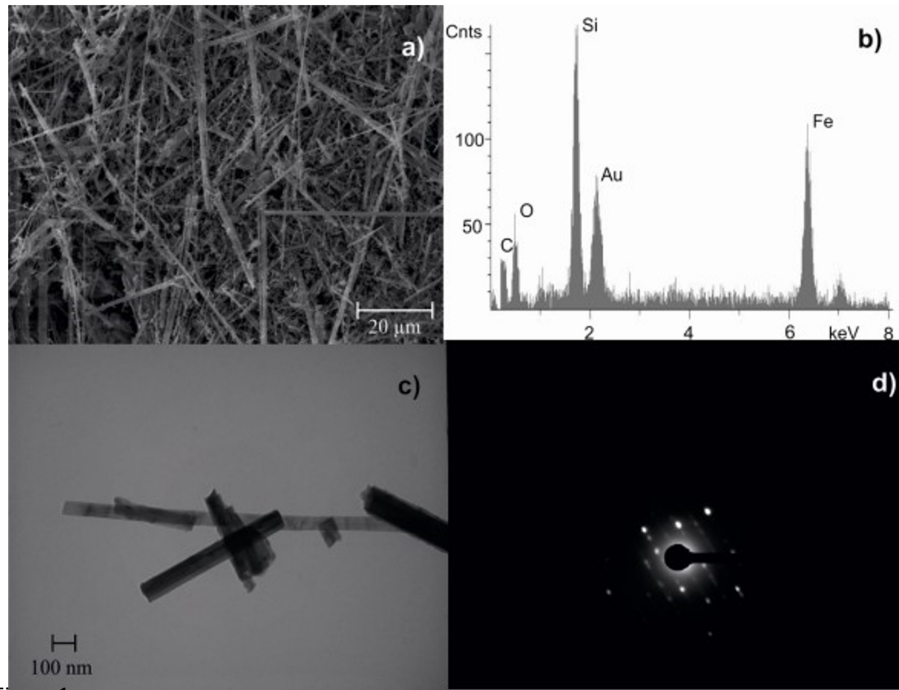


Figure 1

Figure 1. Union Internationale Contre le Cancer (UICC) crocidolite asbestos fibres.

- (a) Scanning electron microscopy (SEM) (1,000x magnification),
- (b) energy dispersive X-ray (EDX) analysis,
- (c) transmission electron microscopy (TEM) (40,000x magnification) and
- (d) electron diffraction pattern.

To optimize the conductivity (electron beam), all samples were deposited with a very fine gold (Au) layer using a sputtering technique.

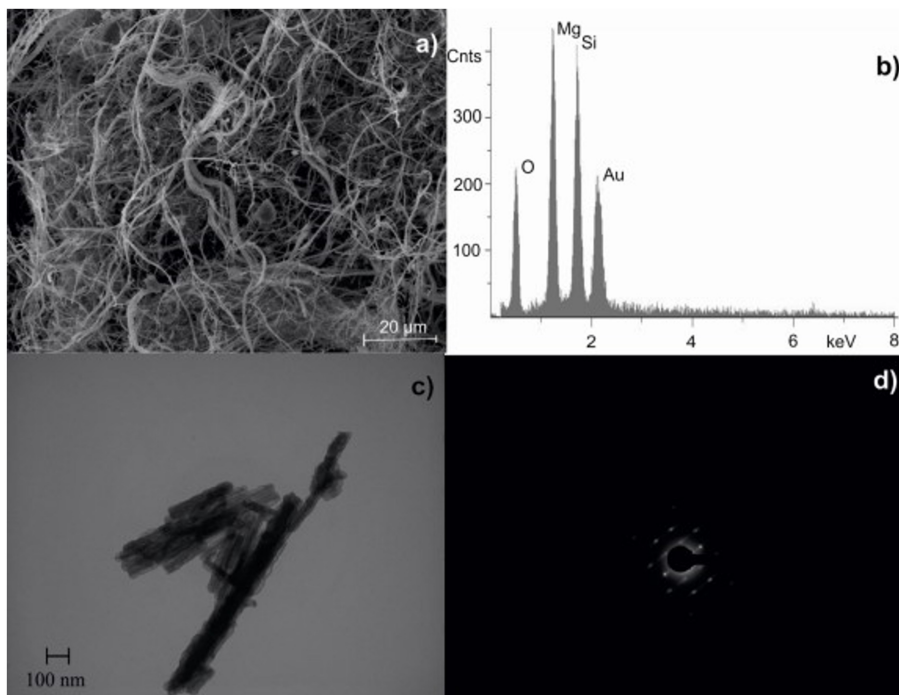
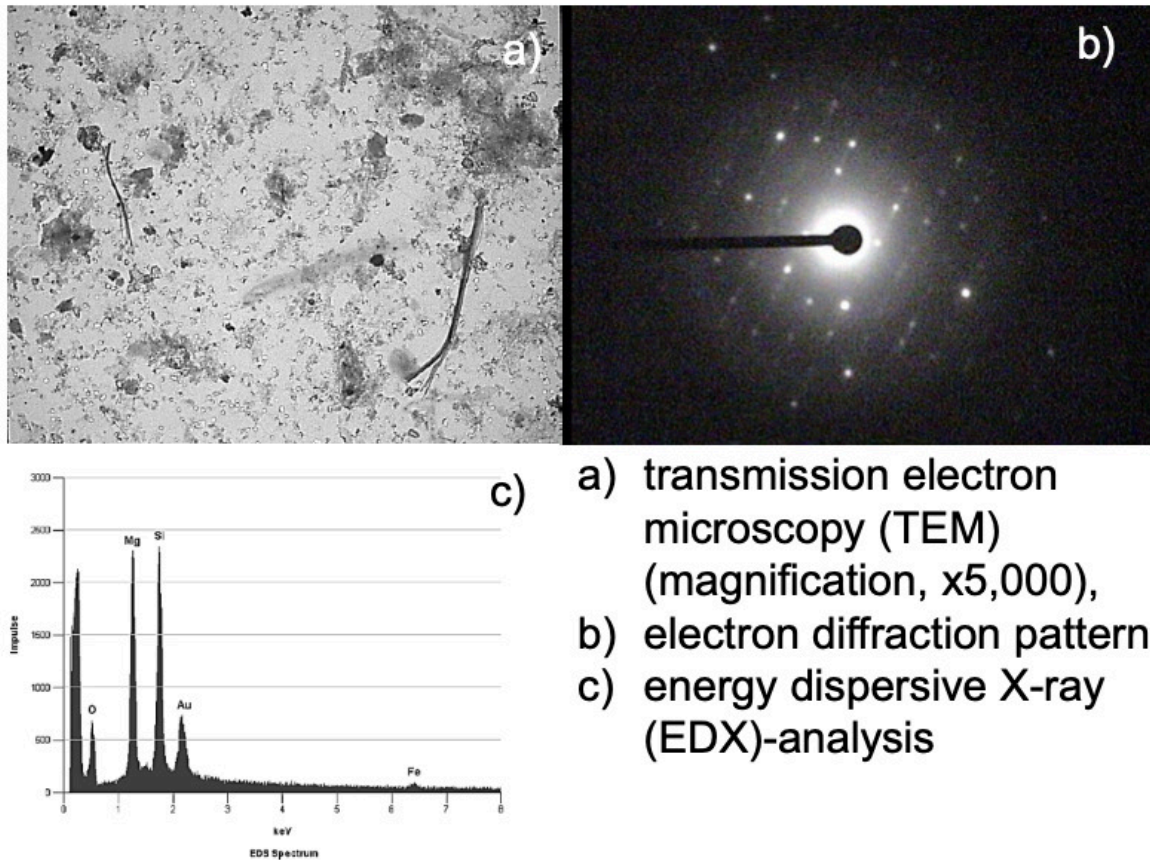


Figure 2. Union Internationale Contre le Cancer (UICC) chrysotile asbestos fibres.

- (a) Scanning electron microscopy (SEM) (1,000x magnification),
- (b) energy dispersive X-ray (EDX) analysis,
- (c) transmission electron microscopy (TEM) (40,000x magnification) and
- (d) electron diffraction pattern.

To optimize the conductivity (electron beam), all samples were deposited with a very fine gold (Au) layer using a sputtering technique.



a) transmission electron microscopy (TEM) (magnification, x5,000),
 b) electron diffraction pattern,
 c) energy dispersive X-ray (EDX)-analysis

Lung dust analysis of Chrysotile Asbestos

Figure 3a. Characterization of different asbestos fibres in lung dust analyses

“The available data suggest that chrysotile is deposited in the parenchyma but is cleared extremely rapidly, with the vast bulk of fibres removed from human lungs within weeks to months after inhalation; by comparison, amphibole clearance half-lives are of the order of years to decades”.

Churg and Wright 1994²⁶ added

*“...although some fibres may be sequestered and very slowly cleared. Overall, these studies suggest that the differences between amphibole and chrysotile fibre burdens in man reflect much faster clearance of chrysotile fibres, rather than a failure of chrysotile deposition”.*³⁴

Similarly, Neumann, Theile et al. formulated *“Thus chrysotile is removed from the lungs very quickly”*.³⁵

Everatt et al. were able to detect chrysotile in the lung tissue in only 18 of 302 cases (298 lung cancer, 4 mesothelioma) of whom 22.2 % had a cumulative asbestos dose of at least 5 fibre-years.³⁶ Friedrichs, Dykers and Otto examined an employee who had worked in a (chrysotile) asbestos spinning mill for 5 years and after an interim period of 60 years they could identify chrysotile in only 10.3% of the lung fibres.³⁷ Churg and dePaoli found

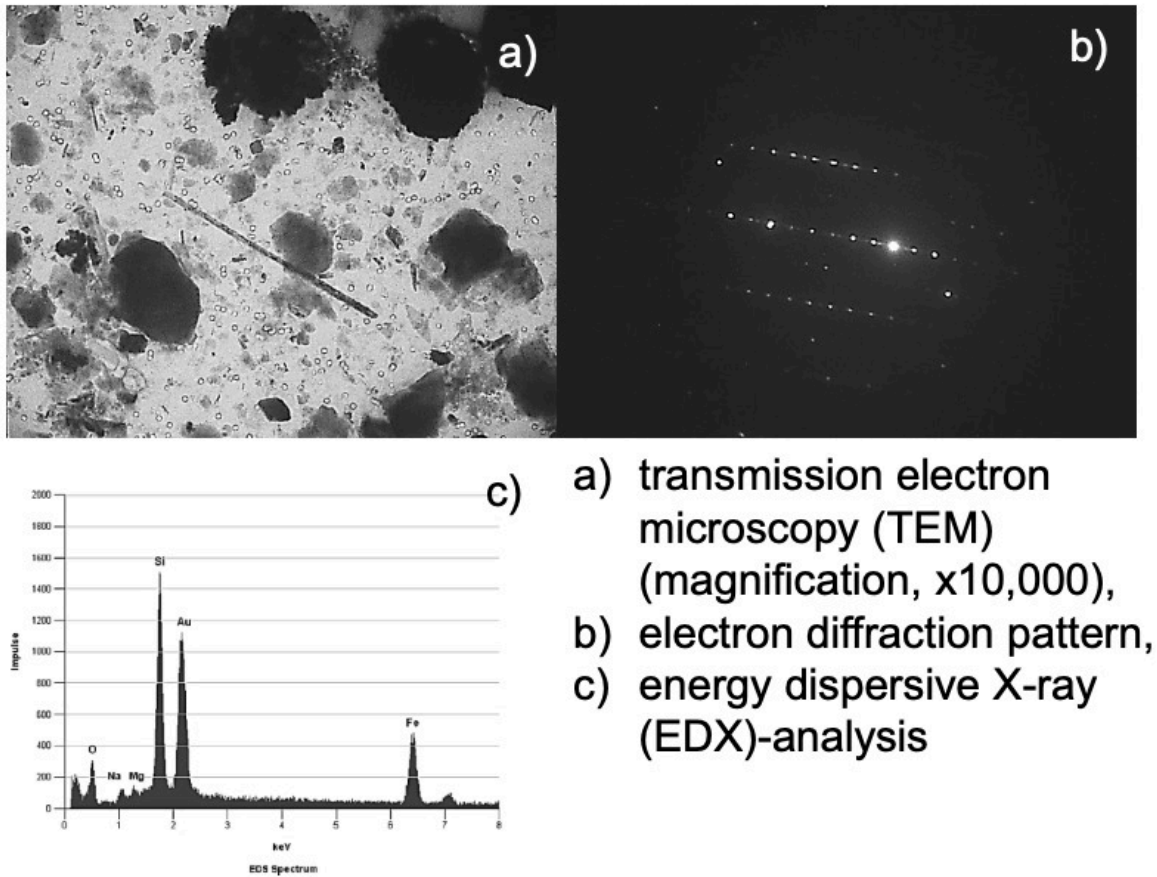
“...that the failure of chrysotile to accumulate in human lungs reflects events that occur early after exposure rather than long-term clearance mechanisms”.

noting no significant difference in chrysotile content after interim periods of less than 2 and greater than 12 years.³⁸

Short fibres do not undergo ferruginous coating. There exist interindividual variation of ferruginous coating of long asbestos fibres with a subgroup of proficient “coaters”.

As opposed to chrysotile, amphibole asbestos types were shown to be much more biopersistent in the lungs, with half-lives of several decades.^{39,40}

In accordance with the literature, our STEM lung dust fibre analysis shows that there was no correlation between chrysotile asbestos fibres and amphibole asbestos fibre concentrations each with lengths > 5 µm (Fig. 5) nor was there a correlation between fibre and FB concentrations for chrysotile asbestos ($R^2 = 0.0$) (Fig. 6). Because of the longer biopersistence of amphibole asbestos fibres than of chrysotile asbestos fibres, and the predominance of short chrysotile fibres in the workplace long amphibole asbestos fibres in particular are converted to FB. Thus, there is some correlation between amphibole asbestos fibre and FB con-



Lung dust analysis of Crocidolite Asbestos

Figure 3b. Characterization of different asbestos fibres in lung dust analyses

centrations with a correlation coefficient of $R^2 = 0.26$ (Fig. 7).

Chrysotile fibre, amphibole fibre, and FB concentrations in the lung tissue depend only roughly on the cumulative asbestos exposure levels in the workplace.⁷⁻⁹

For amphibole asbestos, the elimination kinetic in human lung is slower than that of chrysotile.

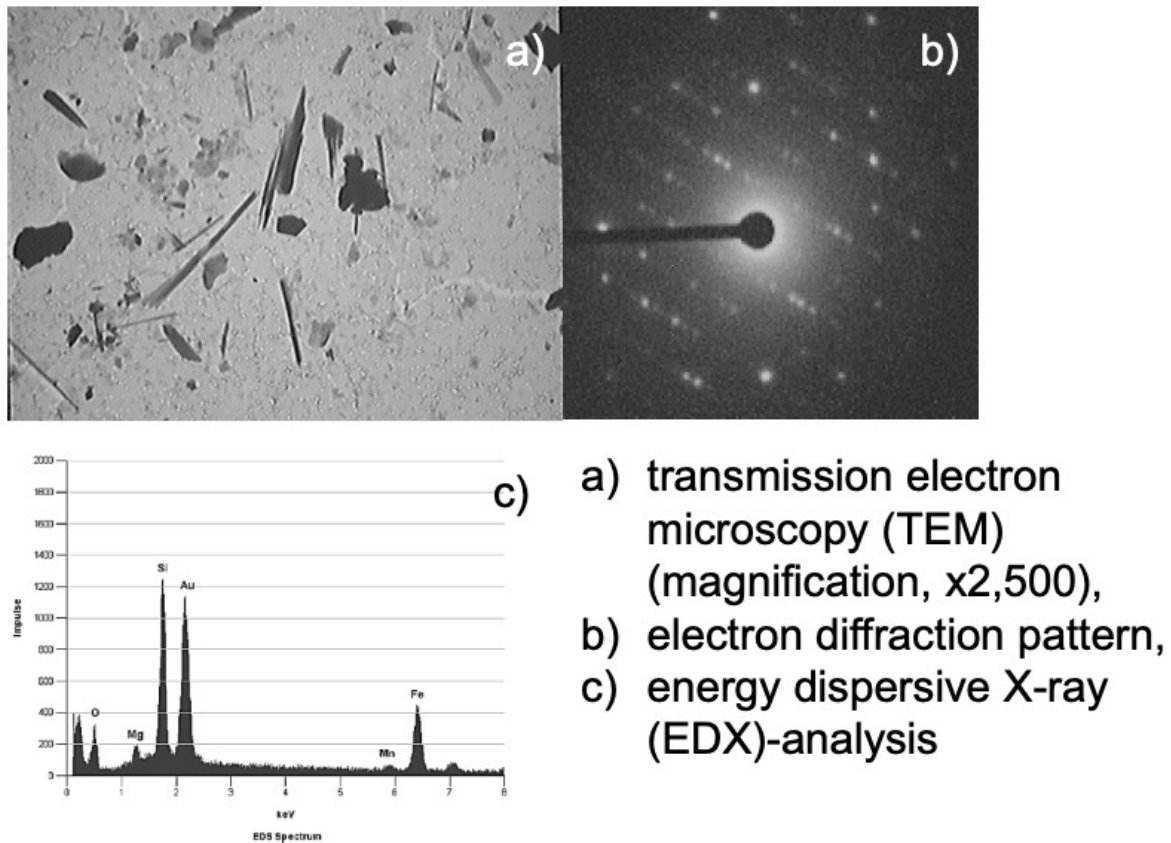
Furthermore, in our investigations no significant differences could be found between the lung asbestos fibre concentrations and the type of the asbestos disease, such as asbestosis/asbestos-related pleural lesions, asbestos-related lung cancer or mesothelioma; there was always a large range of the measured fibre concentrations (Fig. 4).

Correspondingly, the CAP/NIOSH definition of asbestosis does not consider quantification of asbestos fibres or FB for diagnostic purpose.⁴¹ However the CAP/NIOSH definition published more than 40 years ago was based on findings while asbestos exposure was high in many workplaces does not seem to represent the situation after introduction of an asbestos ban in the past, i.e. after a long interim period. There may be extensive lung fibrosis but since no ferruginous bodies, even with large amount of short chrysotile in lung tissue present, the pathologist may not attribute the

fibrosis to asbestos or call the pathological observation by term asbestosis.

We recommend to re-evaluate the CAP/NIOSH asbestosis definition in light of present workplace situations.

With regard to fibre analysis, counting FB by light microscopy can only detect fibres with a diameter of up to $0.2 \mu\text{m}$, and only fibres with a diameter of more than $1 \mu\text{m}$ can be classified on the bases of their optical properties.⁴² It is therefore not possible to detect and identify thin chrysotile fibres using a light microscope. In contrast, elementary fibrils of chrysotile can be identified with the analytical scanning transmission electron microscope (STEM). In our institute, Rödelsperger et al. coordinating a multicenter study found a total of 438 chrysotile fibres of all lengths and 163 chrysotile fibres with a length greater than $5 \mu\text{m}$ in a STEM lung dust analysis of 66 mesothelioma patients⁴³; in controls not exposed to asbestos, respective figures were 812 chrysotile fibres of all lengths and 374 chrysotile fibres with a length greater than $5 \mu\text{m}$. The definition of the so-called WHO asbestos fibres (length $\geq 5 \mu\text{m}$, diameter $< 3 \mu\text{m}$; ratio of length to diameter greater than 3:1) is methodically determined. It aims to identify the carcinogenicity of long and thin fibres.⁴⁴



Lung dust analysis of Amosite Asbestos

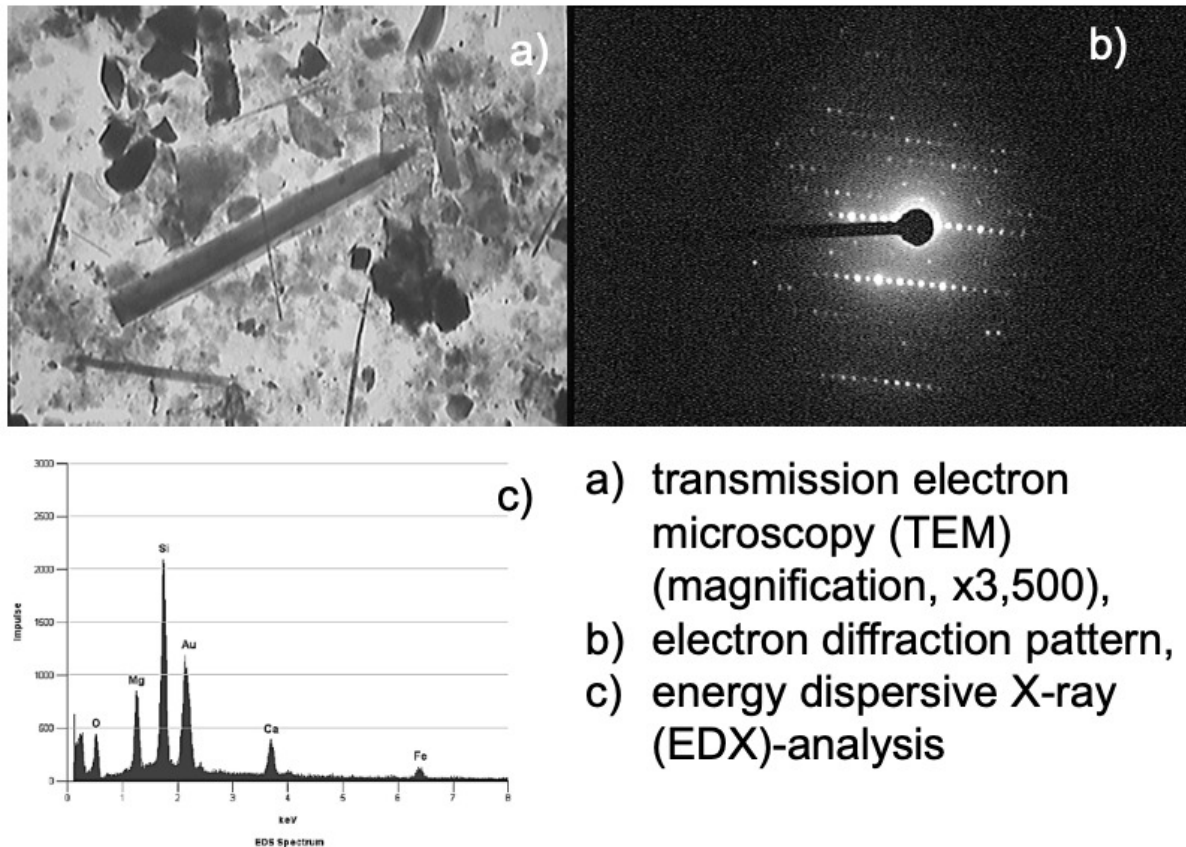
Figure 3c. Characterization of different asbestos fibres in lung dust analyses

Furthermore, previous transmission electron investigations of 134 FB from deceased patients who were predominantly exposed to chrysotile fibre dust in the workplace showed that only about 2.2% of them were attributable to chrysotile, almost 89.5% to amphibole, and 8.2% to other mineral fibres.⁴³ From this it can be concluded that chrysotile hardly forms any FB, and therefore their measurement is only useful for histopathological diagnostics when FB can be detected. As a result, the proportion of histopathologically diagnosed asbestosis grade 1 (“minimal asbestosis cases”), which has been primarily based on the detection of FB, is very low in workers previously exposed to chrysotile. In the Mesothelioma Register in Bochum was a proportion of about 6–10% of asbestosis grade 1 within about 1,000 examined cases per year reported.⁴ Obviously, the real asbestosis figure is much higher, when generally accepted less restrictive diagnostic definitions^{41,45} would be applied. It has to be mentioned that the Mesothelioma Register has applied the non-substantiated restrictive asbestosis definition of Roggli et al.⁴² combined with the requirement of FB in the neighbourhood of fibrotic areas and a concentration of 2 AF/cm² (Feder et al.^{3,4}) (for details see

below). There were severe criticism to this diagnostic practice which is not based on scientific data.^{46–48}

It has to be mentioned that our work is limited insofar as patients’ numbers of subgroups with different ages, diagnoses and interim periods were too small for statistical analyses and we only considered fibres with a length of ≥ 5 μm . Fibre counts in pleural tissues are not customary. Nevertheless, our data shows that the asbestos fibre content in human lungs depends only roughly on the cumulative exposure in the workplace, i.e. the duration and intensity of exposure, whereas its dependency on the interim periods is obvious. This means that hardly any generally applicable diagnostic data on asbestos fibres in human lung can be expected; this is especially due to the facts that

- there is no worldwide asbestos ban and interim periods are therefore heterogenous,
- in different countries various amphibole types - which, in contrast to chrysotile asbestos, are more biopersistent and lead to the formation of FB in a significantly higher proportion - were also used,
- fibres are heterogeneously distributed in the lungs; so, individual probes may not be representative,



Lung dust analysis of Tremolite Asbestos

Figure 3d. Characterization of different asbestos fibres in lung dust analyses

- applied methodology of fibre analysis varies considerably and many associated analytical problems still exist,^{49,50}
- there exists only a limited number of respective qualified studies,
- the fibrogenic and carcinogenic properties of short asbestos fibres (<5 μm) have only rarely been analyzed.⁶ Only a few publications report fibres of all lengths (greater than 1 μm), while many publications only report concentrations of WHO fibres with a length $\geq 5 \mu\text{m}$. However, short fibres with a length of 1 to 5 μm also have adverse health effects. There is strong evidence that these short fibres are also carcinogenic and especially cause pulmonary fibrosis.^{18,51–54}

All of the aforementioned facts demonstrate that from the diagnostic point of view the lung asbestos fibre and FB content in lung tissue is of very limited value. They indicate that a long past exposure to asbestos can usually no longer be confirmed by lung fibre analysis. This means in practice that a negative light or electron microscopic lung fibre analysis cannot overturn a qualified occupational his-

tory. The latter combined with a detailed industrial hygienist recording of occupational exposure remains the cornerstone of the diagnosis of asbestos-related occupational diseases.⁵⁵

The aforementioned limitations of lung fibre counting are also relevant for pathohistological diagnostics. They imply that when diagnostic considerations are limited to counting FB (which mostly disregards chrysotile exposure) and to the long WHO fibres ($\geq 5 \mu\text{m}$ in length), methodological problems arise in that chrysotile exposure, and exposure to short asbestos fibres in general, which obviously also cause pulmonary fibrosis and are carcinogenic,^{56,57} are not properly considered.

Pathohistological definitions of asbestosis are based on the detection of FB, although FB have no causal significance for asbestosis and other ARD, their numbers decrease with the interim period, and they are hardly reproducible and not generally applicable. If positive, they are merely an indicator that asbestos exposure has taken place. The pathohistological CAP/NIOSH definition of asbestosis grades 1 - 4, which is still favored by leading pathologists⁴⁵ and also recommended by the American Thoracic Society (ATS),⁵⁸ was developed by a large group of experts, sub-

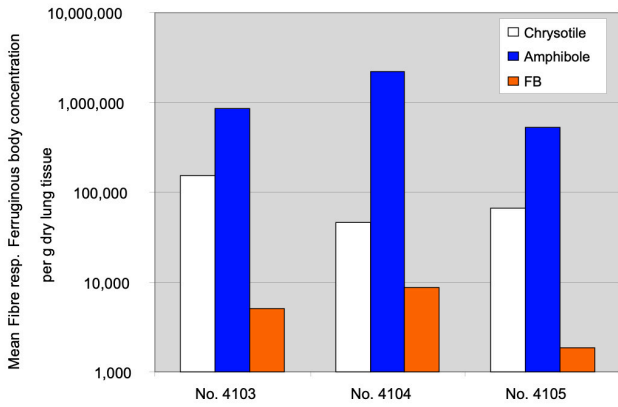


Figure 4. Mean concentrations of chrysotile asbestos fibres, amphibole asbestos fibres, and FB in the lungs depending on the occupational diseases asbestosis / asbestos-related pleural lesions (occupational disease no. 4103), asbestos-related lung cancer (occupational disease no. 4104) and mesothelioma (occupational disease no. 4105).

There were no significant differences between the ARD and identified chrysotile asbestos fibre (L >5 µm), amphibole asbestos fibre (L >5 µm), and FB concentrations.

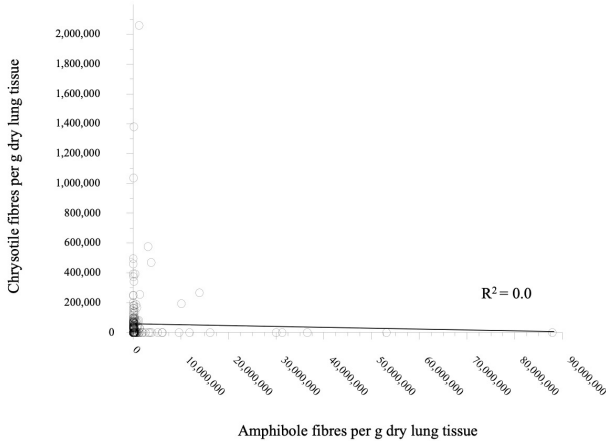


Figure 5. Correlation of concentrations of amphibole and chrysotile fibres in the lung tissue determined by STEM.

The Spearman correlation coefficient (R) is given

jected to a thorough, transparent review process and eventually received NIOSH approval.⁴⁵ In contrast, the modified definition by Roggli et al. requires that in asbestosis grade 1 (so-called “minimal asbestosis”) the fibrosis affects not only the bronchiolus wall but also the first layer of adjacent alveoli.⁴² At the same time, for the normal finding (asbestosis grade 0), the determination according to CAP/NIOSH (“no fibrosis of the bronchioles”) is converted by Roggli et al. into: “no significant peribronchiolar fibrosis, fibrosis limited to the bronchioles”. This means that early asbestos-related changes, which are assigned to asbestosis grade 1 according to CAP/NIOSH, are classified as “normal” without scientifically proven justification.

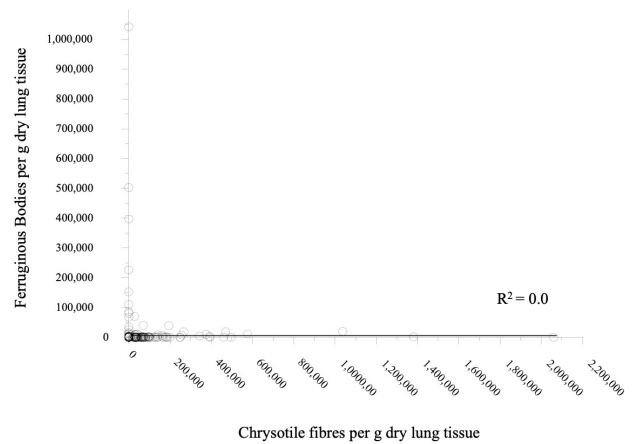


Figure 6. Correlation of the concentrations of lung FB determined by light microscopy and lung chrysotile fibres determined by STEM.

The Spearman correlation coefficient (R) is given.

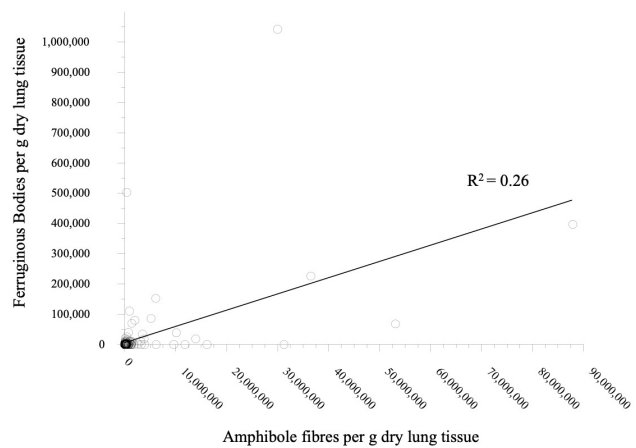


Figure 7. Correlation of concentrations of lung FB determined by light microscopy and lung amphibole fibres determined by STEM.

The Spearman correlation coefficient (R) is given

In addition to the aforementioned histopathologically defined asbestosis grading by CAP/NIOSH, the definition of asbestosis by Roggli et al. additionally presupposes the detection of asbestos fibres in the fibrosis area and a minimum concentration of an average of 2 FB per cm² of lung tissue. This average value according to Roggli et al. is not consistent with the experience of other leading pathologists.^{45,55,59–61} As mentioned, CAP/NIOSH does not require a minimum concentration of asbestos fibres or FB; only the histopathological findings of asbestosis and an association of individual FB with fibrosis are required.⁴¹

The CAP/NIOSH definition states: “Presently, the minimal features that permit the diagnosis are the demonstration of discrete foci of fibrosis in the walls of respiratory bronchioles associated with accumulations of asbestos bodies.... When only a single asbestos body is found in a histologic section, it is necessary to demonstrate additional asbestos bodies (either

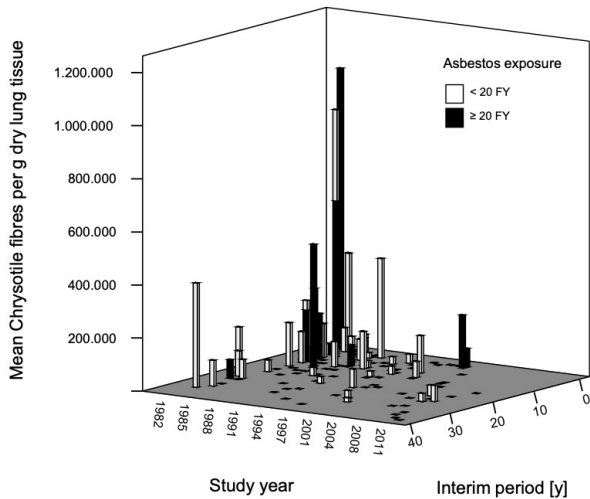


Figure 8. Bivariate histogram of mean chrysotile asbestos fibre concentrations (WHO) in the lung as a function of study year and interim period.

Low asbestos fibre dust exposure (< 20 fibre-years; Fy), high asbestos fibre dust exposure (≥ 20 fibre-years, Fy). After 2012 asbestos fibres are rarely detectable in only single lung specimen not presentable in this figure.

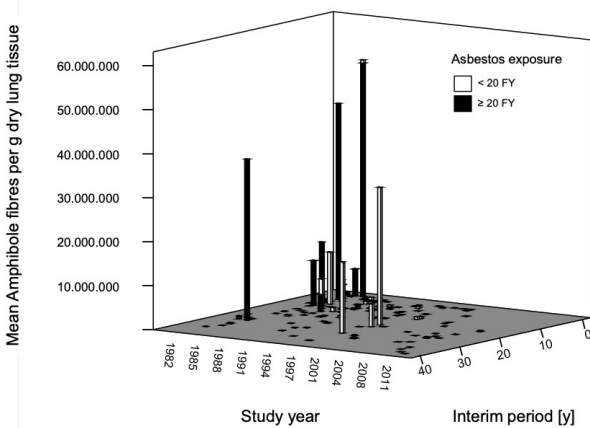


Figure 9. Bivariate histogram of mean amphibole asbestos fibre (WHO) concentrations in the lungs as a function of study year and interim time.

Low asbestos fibre dust exposure (< 20 fibre-years), high asbestos fibre dust exposure (≥ 20 fibre-years, Fy). After 2012 asbestos fibres are rarely detectable in only single lung specimen not presentable in this figure.

in deeper sections of the same block or in other sections of the same block or in other samples of tissue) to establish the diagnosis of asbestosis". It must be taken into account that, as explained above, under the current conditions in Germany and correspondingly in other countries with asbestos bans enforced decades ago there is generally no longer any evidence of an increased concentration of FB and asbestos fibres in the lungs of previous asbestos workers.

Roggli had recommended the aforementioned modified CAP/NIOSH system in the Helsinki criteria in 1997 and 2014.^{62–64} In 1997, the board of directors of the German

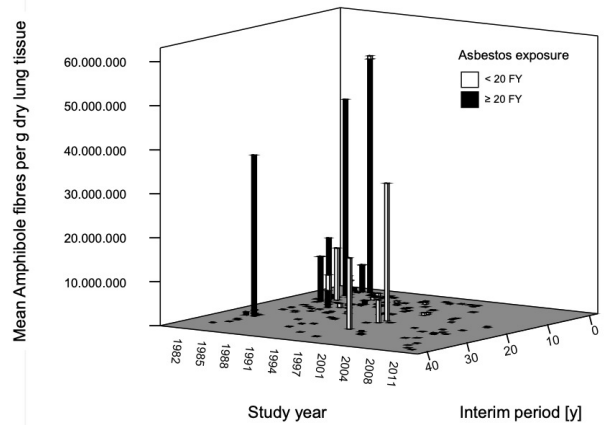


Figure 10. Bivariate histogram of mean FB concentrations in the lungs as a function of study year and interim time.

Low asbestos fibre dust exposure (< 20 fibre-years, Fy), high asbestos fibre dust exposure (≥ 20 fibre-years, Fy). After 2012 asbestos fibres are rarely detectable in only single lung specimen not presentable in this figure.

Society for Pathology essentially followed this definition of minimal asbestosis⁶⁵: "The internationally valid definition of minimal asbestosis (asbestosis grade I according to the Anglo-American nomenclature) includes the light microscopic evidence of minimal foci of fibrosis in the area of the bronchioli respiratorii and the accompanying vessels with maximum radiation into the directly adjacent alveolar septa and asbestos bodies stored in these areas. The random (one-off) detection of asbestos bodies is not sufficient to diagnose minimal asbestosis. A dust analysis limit value for minimal asbestosis has not been defined".⁶⁵ Remarkably, this text also mentions the involvement of accompanying vessels, which is neither mentioned in the Roggli publication nor in the Helsinki criteria, without citing a specific source or original data.

The pathologists Hammar and Abraham state with regard to the aforementioned discrepant diagnostic criteria of Roggli et al.: "As a historical note, the criterion of the 1982 CAP/NIOSH criteria⁴¹ for requiring "more than one" asbestos body to be found in a single lung tissue section was based on the exclusion of the "chance" finding of a single asbestos body in the lungs of the general background population in which one asbestos body might be expected to be found in approximately 50–100 or more lung tissue sections of average area and thickness (each with a volume of approx. 10⁻³ cm³)".⁴⁵ Furthermore, these pathologists state: "If one considers that this recommended concentration of 2 asbestos bodies/cm² of lung tissue section corresponds to approximately 4,000 asbestos bodies per cm³ or 4,000 asbestos bodies per gram of wet lung, one sees that this is at least 200 times higher than the upper limit of background asbestos body concentrations (20 asbestos bodies per gram of wet lung) relied upon by Roggli".⁶⁶

CONCLUSIONS

This work is limited insofar as the number of subjects in subgroups are too small for statistical analyses. Only num-

bers of WHO fibres ($L > 5 \mu\text{m}$, $D < 3 \mu\text{m}$, ratio of length to diameter ($L/D > 3:1$) were reported because these fibres have been thought to be especially relevant for carcinogenicity and fibrogenicity.

Our results demonstrate a clear decrease in identified chrysotile and amphibole concentrations as well as in FB in human lung tissue with increasing interim period. After an interim period of about 30 years, elevated chrysotile asbestos fibre concentrations cannot be detected in the lung tissue of formerly chrysotile-exposed workers. Also, as we could show for the first time, the more biologically stable amphibole asbestos fibres, such as crocidolite, are subject to elimination kinetics in the lungs. Therefore, false-negative results of lung dust fibre analysis must be expected not only for chrysotile asbestos, but also for crocidolite asbestos. This means that a negative light or electron microscopic lung dust analysis is not capable of overturning a reliable occupational history of asbestos exposure.^{28,67} Only in cases of questionable exposure to asbestos dust in the workplace or the environment lung dust fibre analyses may provide - in case of a detectable elevated fibre concentration - a supplementary exposure evidence.

Although historic histopathological definitions have required the detection of asbestos fibres and/or FB, nowadays these requirements which are based on findings at times with high asbestos exposures and a short or absent interim period, are of very limited diagnostic significance for ARD. We suggest a reevaluation of the CAP/NIOSH asbestosis definition for the current situation in many Western countries with asbestos bans and the consideration of exposure

to predominant short chrysotile fibres. This is even more relevant for the more restrictive and not scientifically based diagnostic recommendations including the detection of FB or increased asbestos fibre concentrations in lungs as published⁴² and also introduced in the Helsinki Reports⁶²⁻⁶⁴ under the chair of the pathologist V. Roggli.⁶⁸

DISCLOSURE STATEMENT

The authors declare that no competing interests exist.

Two of the authors have testified in occupational diseases litigation on behalf of plaintiffs, provide independent expert opinion for social courts or statutory insurances.

FUNDING

No funding supported this work.

ETHICS APPROVAL

Ethics approval is not applicable.

ACKNOWLEDGEMENTS

The authors thank Mrs. Natalia Haibel, graduate engineer, for her technical support during the fibres analysis.

Submitted: April 11, 2022 EDT, Accepted: February 10, 2023 EDT



This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International License (CCBY-4.0). View this license's legal deed at <http://creativecommons.org/licenses/by/4.0> and legal code at <http://creativecommons.org/licenses/by/4.0/legalcode> for more information.

REFERENCES

1. Baur X, Clasen M, Fisseler-Eckhoff A, et al. Diagnostics and expert opinion of asbestos-induced occupational diseases. *Pneumologie*. 2011;65(3):e1-e47. doi:10.1055/s-0030-1255992
2. Wolff H, Vehmas T, Oksa P, Rantanen J, Vainio H. Asbestos, asbestosis, and cancer, the Helsinki criteria for diagnosis and attribution 2014: recommendations. *Scand J Work Environ Health*. 2014;41(1):5-15. doi:10.5271/sjweh.3462
3. Feder IS, Tischoff I, Theile A, Schmitz I, Merget R, Tannapfel A. The asbestos fibre burden in human lungs: new insights into the chrysotile debate. *Eur Respir J*. 2017;49(6). doi:10.1183/13993003.02534-2016
4. Feder IS, Theile A, Tannapfel A. Histological findings and lung dust analysis as the basis for occupational disease compensation in asbestos-related lung cancer in Germany. *Int J Occup Med Environ Health*. 2018;31(3):293-305. doi:10.13075/ijomeh.1896.01148
5. Otto H, Fragstein Gv. Zur Häufigkeit von Asbestnadeln in menschlichen Lungen. *Int Arch Gewerbepath Gewerbehyg*. 1969;25(3):193-201. doi:10.1007/bf00678309
6. Manke J, Rödelberger K, Brückel B, Weitowitz HJ. Evaluation and application of a plasma ashing method for STEM fiber analysis in human lung tissue. *Am Ind Hyg Assoc J*. 1987;48(8):730-738. doi:10.1080/15298668791385480
7. Weitowitz HJ, Rödelberger K, Bödeker H, Brückel B, Gosch V. Biomonitoring nach Asbestfaserstaub-Einwirkung: Lichtmikroskopie versus Elektronenmikroskopie. *Arbeitsmed Sozialmed Präventivmed*. 1991;26:219-224.
8. Weitowitz HJ, Manke J, Breit S, Brückel B, Rödelberger K. Asbestos and other mineral fibers in the human lung. *Pathologie Sep*. 1986;7(5):248-257.
9. Weitowitz HJ, Manke J, Brückel B, Rödelberger K. Asbestkörperchen als Beweismittel einer beruflichen Gefährdung durch Weißasbest Chrysotil? Ferruginous bodies as evidence of occupational endangering by chrysotile asbestos? *Zbl Arbeitsmed*. 1986;36:354-364.
10. Deutsche Gesetzliche Unfallversicherung, ed. *BK-Report 1/2013 Faserjahre*. DGUV; 2013. http://publikationen.dguv.de/dguv/pdf/10002/bk_rep0113.pdf
11. Weitowitz HJ, Hillerdal G, Berghäuser KH, Rödelberger K, Jöckel KH. *Risiko- und Einflussfaktoren des diffusen malignen Mesothelioms (DMM)* Wirtschaftsverl. NW, Verl. für Neue Wissenschaft; 1994.
12. Baur X. Asbestos-Related Disorders in Germany: Background, Politics, Incidence, Diagnostics and Compensation. *Int J Environ Res Public Health*. 2018;15(1):143. doi:10.3390/ijerph15010143
13. Rösler JA, Weitowitz HJ, Lange HJ, Ulm K, Weitowitz RH, Rödelberger K. *Forschungsbericht Asbest IV. Asbesteinwirkung am Arbeitsplatz und Sterblichkeit an bösartigen Tumoren in der BRD*. Hauptverbandes der gewerblichen Berufsgenossenschaften e.V; 1993.
14. Riediger G, ed. *Messung von Gefahrstoffen - IFA-Arbeitsmappe 24- Bestimmung von anorganischen Fasern im menschlichen Gewebe*. (Transmissionselektronenmikroskopische Methode mit Präparation durch Kaltveraschung - TEM-Methode). Erich Schmidt; 2000.
15. Suzuki Y, Yuen SR. Asbestos fibers contributing to the induction of human malignant mesothelioma. *Ann N Y Acad Sci Dec*. 2002;982:160-176.
16. Sebastien P, Janson X, Gaudichet A, Hirsch A, Bignon J. Asbestos retention in human respiratory tissues: comparative measurements in lung parenchyma and in parietal pleura. *IARC scientific publications*. 1980;(30):237-246.
17. Suzuki Y, Yuen SR. Asbestos tissue burden study on human malignant mesothelioma. *Ind Health*. 2001;39(2):150-160.
18. Suzuki Y, Yuen SR, Ashley R. Short, thin asbestos fibers contribute to the development of human malignant mesothelioma: pathological evidence. *International Journal of Hygiene and Environmental Health*. 2005;208(3):201-210. doi:10.1016/j.ijheh.2005.01.015
19. Dodson RF, Williams MG Jr, Corn CJ, Brollo A, Bianchi C. Asbestos content of lung tissue, lymph nodes, and pleural plaques from former shipyard workers. *Am Rev Respir Dis*. 1990;142(4):843-847. doi:10.1164/ajrccm/142.4.843
20. Kohyama N, Suzuki Y. Analysis of asbestos fibers in lung parenchyma, pleural plaques, and mesothelioma tissues of North American insulation workers. *Ann N Y Acad Sci*. 1991;643:27-52.

21. Bignon J, Brochard P, Sebastien P. Asbestos pathology: clinical aspects epidemiological data and prevention. *Schweizerische medizinische Wochenschrift*. 1982;112(6):177-185.
22. Dodson RF, Atkinson MA. Measurements of asbestos burden in tissues. *Ann N Y Acad Sci*. 2006;1076(1):281-291. [doi:10.1196/annals.1371.015](https://doi.org/10.1196/annals.1371.015)
23. McDonald JC, Armstrong B, Case B, et al. Mesothelioma and asbestos fiber type. Evidence from lung tissue analyses. *Cancer*. 1989;63(8):1544-1547.
24. Wagner JC, Berry G, Pooley FD. Mesotheliomas and asbestos type in asbestos textile workers: a study of lung contents. *BMJ*. 1982;285(6342):603-606. [doi:10.1136/bmj.285.6342.603](https://doi.org/10.1136/bmj.285.6342.603)
25. Baker DB. Limitations in drawing etiologic inferences based on measurement of asbestos fibers from lung tissue. *Ann NY Acad Sci*. 1991;643:61-70. [doi:10.1111/j.1749-6632.1991.tb24444.x](https://doi.org/10.1111/j.1749-6632.1991.tb24444.x)
26. Churg A, Wright JL. Persistence of natural mineral fibers in human lungs: an overview. *Environ Health Perspect*. 1994;102(suppl 5):229-233. [doi:10.1289/ehp.94102s5229](https://doi.org/10.1289/ehp.94102s5229)
27. Roggli VL, Sharma A, Butnor KJ, Sporn T, Vollmer RT. Malignant mesothelioma and occupational exposure to asbestos: a clinicopathological correlation of 1445 cases. *Ultrastruct Pathol*. 2002;26(2):55-65. [doi:10.1080/01913120252959227](https://doi.org/10.1080/01913120252959227)
28. Schneider J, Arhelger R, Brückel B. Lungenstaubanalysen in der Begutachtung asbestverursachter Erkrankungen. *Zbl Arbeitsmed*. 2015;65(6):305-309. [doi:10.1007/s40664-015-0033-0](https://doi.org/10.1007/s40664-015-0033-0)
29. Bernstein DM, Donaldson K, Decker U, et al. A biopersistence study following exposure to chrysotile asbestos alone or in combination with fine particles. *Inhal Toxicol*. 2008;20(11):1009-1028. [doi:10.1080/08958370802259053](https://doi.org/10.1080/08958370802259053)
30. Bernstein D, Dunnigan J, Hesterberg T, et al. Health risk of chrysotile revisited. *Crit Rev Toxicol*. 2013;43(2):154-183. [doi:10.3109/10408444.2012.756454](https://doi.org/10.3109/10408444.2012.756454)
31. Pezerat H. Chrysotile biopersistence: the misuse of biased studies. *Int J Occup Environ Health*. 2009;15(1):102-106. [doi:10.1179/oeh.2009.15.1.102](https://doi.org/10.1179/oeh.2009.15.1.102)
32. Finkelstein MM. Letter to the Editor re Bernstein et al: Health risk of chrysotile revisited. *Crit Rev Toxicol*. 2013;43(8):707-708. [doi:10.3109/10408444.2013.825762](https://doi.org/10.3109/10408444.2013.825762)
33. Finkelstein MM, Dufresne A. Inferences on the kinetics of asbestos deposition and clearance among chrysotile miners and millers. *Am J Ind Med Apr*. 1999;35(4):401-412.
34. Churg A. Deposition and clearance of chrysotile asbestos. *Ann Occup Hyg Aug*. 1994;38(4):625-633, 424-425.
35. Neumann V, Theile A, Loseke S, Tannapfel A. Neue Aspekte zur Genese der Asbestose. *ASU*. 2011;46:569-679.
36. Everatt RP, Smolianskienė G, Tossavainen A, Cicėnas S, Jankauskas R. Occupational asbestos exposure among respiratory cancer patients in Lithuania. *Am J Ind Med*. 2007;50(6):455-463. [doi:10.1002/ajim.20467](https://doi.org/10.1002/ajim.20467)
37. Friedrichs K, Dykers A, Otto H. Materialstabilität von Asbestfasern im Lungengewebe. *Arbeitsmed Sozialmed Umweltmed*. 1995;30:18-20.
38. Churg A, DePaoli L. Clearance of chrysotile asbestos from human lung. *Experimental Lung Research*. 1988;14(5):567-574. [doi:10.3109/01902148809087829](https://doi.org/10.3109/01902148809087829)
39. Craighead JE, Gibbs AR. *Mineralogy of Asbestos. Asbestos and Its Diseases*. Oxford University Press; 2008. [doi:10.1093/acprof:oso/9780195178692.001.0001](https://doi.org/10.1093/acprof:oso/9780195178692.001.0001)
40. Churg A. *Non-Neoplastic Disease Caused by Asbestos. Pathology of Occupational Lung Disease*. Vol 2nd. Williams & Williams; 1998.
41. Craighead JE, Abraham JL, Churg A, et al. The pathology of asbestos-associated diseases of the lungs and pleural cavities: diagnostic criteria and proposed grading schema. *Report of the Pneumoconiosis Committee of the College of American Pathologists and the National Institute for Occupational Safety and Health Arch Pathol Lab Med*. 1982;106(11):544-596.
42. Roggli VL, Gibbs AR, Attanoos R, et al. Pathology of asbestosis- An update of the diagnostic criteria: Report of the asbestosis committee of the college of american pathologists and pulmonary pathology society. *Arch Pathol Lab Med Mar*. 2010;134(3):462-480. [doi:10.1043/1543-2165-134.3.462](https://doi.org/10.1043/1543-2165-134.3.462)
43. Rödelsperger K, Woitowitz H, Patzich R, Brückel B, Gosch V. Asbestfasern und Ferruginous bodies in der menschlichen Lunge. *Staub Reinh Luft*. 1990;55:99-105.

44. Stanton MF, Layard M, Tegeris A, et al. Relation of particle dimension to carcinogenicity in amphibole asbestoses and other fibrous minerals. *Journal of the National Cancer Institute* Nov. 1981;67(5):965-975.
45. Hammar SP, Abraham JL. Commentary on pathologic diagnosis of asbestosis and critique of the 2010 Asbestosis Committee of the College of American Pathologists (CAP) and Pulmonary Pathology Society's (PPS) update on the diagnostic criteria for pathologic asbestosis. *Am J Ind Med*. 2015;58(10):1034-1039. doi:10.1002/ajim.22512
46. Landrigan P, Lemen R, Collegium Ramazzini O. Letter to the Editor (April 4, 2018) concerning the paper "Histological findings and lung dust analysis as the basis for occupational disease compensation in asbestos-related lung cancer in Germany." *Int J Occup Med Environ Health*. 2018;31(6):845-847. doi:10.13075/ijom.1896.01345
47. Baur X, Belpoggi F, Budnik L, et al. Letter to the Editor (February 14, 2018) concerning the paper "Histological findings and lung dust analysis as the basis for occupational disease compensation in asbestos-related lung cancer in Germany." *Int J Occup Med Environ Health*. 2018;31(6):837-839. doi:10.13075/ijom.1896.01332
48. Oliver LC, Belpoggi F, Budnik LT, et al. Correspondence regarding the article "The asbestos fibre burden in human lungs: new insights into the chrysotile debate." *Eur Respir J*. 2017;50(6):1701644. doi:10.1183/13993003.01644-2017
49. Baur X, Woitowitz HJ, Budnik LT, et al. Asbestos, asbestosis, and cancer: The Helsinki criteria for diagnosis and attribution. Critical need for revision of the 2014 update. *Am J Ind Med*. 2017;60(5):411-421. doi:10.1002/ajim.22709
50. Egilman D, Baur X, Soskolne CL. Unreliable proposed 'new standard' for assessing asbestos exposure. *Occup Environ Med*. 2016;73(10):709. doi:10.1136/oemed-2016-103704
51. Egilman D, Bird T. Short fiber tremolite free chrysotile mesothelioma cohort revealed. *Am J Ind Med*. 2016;59(3):196-199. doi:10.1002/ajim.22552
52. Egilman D, Tran T. A commentary on Roggli's "The So-Called Short-Fiber Controversy." *Int J Occup Environ Health*. 2016;22(3):181-186. doi:10.1080/10773525.2016.1153866
53. Donaldson K, Brown GM, Brown DM, Bolton RE, Davis JM. Inflammation generating potential of long and short fibre amosite asbestos samples. *Br J Ind Med Apr*. 1989;46(4):271-276.
54. Zeng L, Zheng ZR, Li HY, Tan JS. Pathological study of bronchio-alveolitis induced by xinkang short-fiber chrysotile asbestos in rats. *Journal of West China University of Medical Science*. 1989;20(3):290-294.
55. Collegium-Ramazzini. Comments on the 2014 helsinki consensus report on asbestos. *J Occup Health*. 2016;58(2):224-227. doi:10.1539/joh.16-2004-It
56. Gao Z, Hiroshima K, Wu X, et al. Asbestos textile production linked to malignant peritoneal and pleural mesothelioma in women: Analysis of 28 cases in Southeast China. *Am J Ind Med*. 2015;58(10):1040-1049. doi:10.1002/ajim.22494
57. Baur X, Frank AL. Ongoing downplaying of the carcinogenicity of chrysotile asbestos by vested interests. *J Occup Med Toxicol*. 2021;16(1):6. doi:10.1186/s12995-021-00295-2
58. American Thoracic S. Diagnosis and initial management of nonmalignant diseases related to asbestos. *Am J Respir Crit Care Med*. 2004;170(6):691-715. doi:10.1164/rccm.200310-1436st
59. Dodson RF. Personal communication. Published online 2017.
60. Hammar SP, Dodson RF. Nonneoplastic Lung Disease. In: Tomaszefski JF, ed. *Dail and Hammar's Pulmonary Pathology*. Springer; 2008:950-1031. doi:10.1007/978-0-387-68792-6_27
61. Warnock ML, Isenberg W. Asbestos burden and the pathology of lung cancer. *Chest*. 1986;89(1):20-26.
62. Henderson D, Rantanen J, Barnhart S, et al. Asbestos, asbestosis and cancer: the Helsinki criteria for diagnosis and attribution. *Scand J Work Environ Health*. 1997;23:311-316.
63. FIOH. Asbestos, Asbestosis, and Cancer. Helsinki Criteria for Diagnosis and Attribution. *Finnish Institute of Occupational Health*. 2014:1-152. https://www.julkari.fi/bitstream/handle/10024/135068/TTL_978-952-261-459-9.pdf?sequence=7&isAllowed=y
64. FIOH. Asbestos hazard. Published online 2014.
65. Heitz P. Neue Definitionen der Minimalasbestose. *Dtsch Arztebl*. 1997;94:A975.
66. Roggli VL, Sanders LL. Asbestos content of lung tissue and carcinoma of the lung: a clinicopathologic correlation and mineral fiber analysis of 234 cases. *Ann Occup Hyg*. 2000;44(2):109-117.

67. Woitowitz RH, Rödelsberger K, Arhelger R, Giesen T. *Asbeststaubbelastung am Arbeitsplatz. Messwerte der internationalen Literatur. Nr 10 der Schriftenreihe gefährliche Arbeitsstoffe*. Bundesanstalt für Arbeitsschutz und Unfallforschung; 1983.

68. Baur X. Asbestos: Socio-legal and Scientific Controversies and Unsound Science in the Context of the Worldwide Asbestos Tragedy – Lessons to be Learned. *Pneumologie*. 2016;70(06):405-412. [doi:10.1055/s-0042-103580](https://doi.org/10.1055/s-0042-103580)