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DOW CHEMICAL U.S.A.

August 29, 1973

MIDLAND, MICHIGAN 48640

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PLAINTIFF'S EXHIBIT
DOW-1376

ASBESTOS IN WATER - ANALYTICAL METHODOLOGY

Asbestos suspended in water is receiving much attention as a potential carcinogen. It will be necessary for us to investigate the levels of asbestos in our water effluent in the near future. However, at the present time good analytical techniques for this analysis do not exist.

The Midland Division Analytical Laboratories (Pat Traylor, Don Beaman) have a sizable program underway for development of the necessary analytical methodology. The final version will probably involve the transmission electron microscope to determine morphology, electron diffraction to determine crystallinity, and some method for determining chemical composition, possibly the scanning electron microscope.

We may have an "established" method by about mid-October. It will probably involve use of the Midland facilities initially at a cost of about $100 per sample. More will follow after the next progress report scheduled for October 2.

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dmw

THE DOW CHEMICAL COMPANY

MIDLAND, MICHIGAN
January 3, 1972

cc - Jim Eichorst
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ASBESTOS IN WATER

1. Standards - Through Jim Teal's efforts contacts were made with EPA and the State of Illinois, and current literature was consulted from other states with regard to standards for asbestos in water. No such standards or recommendations exist. Asbestos would only be included under regulations related to suspended particulates in water.

2. Hazards - No information on the effects of asbestos in water on aquatic life was found. However, the enclosed article from Science describes an epidemiologic study on the high incidence of stomach cancer in Japan. Asbestos as a contaminant in talc used to dust rice has been suggested as the carcinogen. Thus ingestion of asbestos, e.g. in drinking water, could be quite hazardous. I expect we will see considerable activity in this area in the future, both in the setting of standards and in laboratory studies. Asbestos standards in effluent water and food grade NaOH should be considered within Dow.

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R. I. BRUBAKER, JR.
JAN 1 3 1972

tract appears to be a macromolecule with a mass of approximately 30,000 to 50,000 daltons; it is thermolabile and destroyed by trypsin. It is apparently not stable on long-term storage, even in the cold.

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SANFORD LEIKIN
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8. Supported in part by a contract from the Medicine and Dentistry Division of the Office of Naval Research.

24 May 1971

Talc-Treated Rice and Japanese Stomach Cancer

Abstract. *The Japanese prefer talc-dusted rice in their diet. The incidence of stomach cancer in Japan is unusually high. Most talc has some asbestos contaminants. Epidemiologic evidence is presented that the asbestos-contaminated talc on rice in the diet is the carcinogen or cocarcinogen responsible for the high incidence of Japanese stomach cancer.*

The high incidence of stomach cancer in Japanese men represents a challenging problem in cancer epidemiology. Study of age-adjusted rates of mortality from stomach cancer in 24 countries showed that from 1962 to 1963 Japanese men had the highest rate for all nations; the rate, 67.96 per 100,000 in the male population, was seven times that for men in the United States (*1*). Since talc frequently contains asbestos (*2*), and since workmen exposed to asbestos show a marked increase in gastrointestinal cancer, especially stomach cancer (*3*), the hypothesis that the increased rate of cancer of the stomach in Japan is due to ingestion of rice contaminated with asbestos seems attractive and has been recently suggested in a brief note (*4*). This report presents evidence for this relationship at somewhat greater length.

Rice grown in California and meant for the American consumer is milled mechanically. Rice is prepared differently for the Japanese consumer. It is milled and treated with glucose, and then talc is added. The talc is held to the surface of the grain and is said to preserve its flavor better. Japanese consider this rice more tasty, both here and in Japan. Two percent of all talc produced in California from 1959 to 1963 was used as a rice additive, primarily for export to Japan (*5*). Rice containers in Los Angeles markets intended for the Japanese customer usually bear the legend: "Coated with glucose and talc." Optical microscopy of the ash of such store-bought rice showed 3.7×10^6 asbestos-form fibers per gram (Fig. 1).

Talc, the mineral, is a hydrous silicate of magnesium. So are some forms of asbestos. The difference between these two minerals is not in their chemical compositions, but rather in their structure, asbestos being fibrous and mineral, talc being flaky or granular. However, commercial talc is composed not only of the pure mineral talc, but also of fibrous silicates, which are in part classifiable as asbestos. There is no firm mineralogic definition of commercial talc, and in most areas the mineral talc forms much less than half of commercial talc. The composition of commercial talc varies with the deposit. Vermont mines produce a platy or granular type of mineral relatively free of asbestos minerals (*6*). New York and California talcs contain more fibrous silicates, including anthophyllite and tremolite (*2*, *5*).

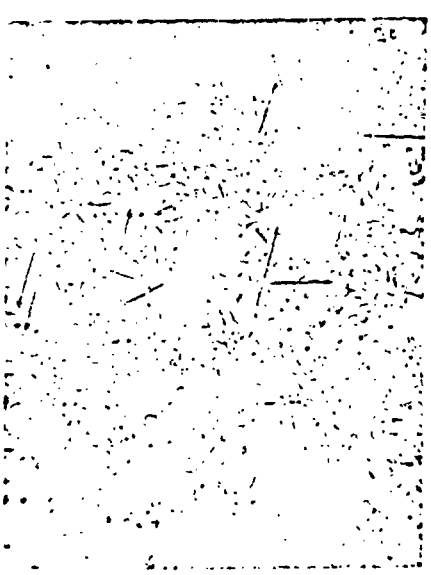


Fig. 1. Photomicrograph of the ash of a specimen of rice intended for Japanese consumption and purchased in a Los Angeles market. Arrows indicate asbestos-form fibers. The specimen contained 3.7×10^6 such fibers per gram.

Respired asbestos and talc dust cause similar disorders in workmen. Older talc workers show more than four times the expected incidence of lung and pleural cancer (*7*). Talc or talcose minerals, including contaminating asbestos, are apparently the responsible carcinogens since, where confirmatory evidence could be obtained, all those talc workers with pleural and lung cancer also had talc pneumoconiosis. Autopsies on six talc workers with talc pneumoconiosis showed the presence of the characteristic "asbestos body" in the lungs of every one (*8*). When talc is examined by phase contrast microscopy, from 8 to 30 percent of the particles are fibrous silicates. Identifiable tremolite, anthophyllite, and chrysotile are also seen (*9*). The large talc deposits of New York and California regularly contain the amphibole asbestos minerals, tremolite and anthophyllite. These are normal ingredients of commercial talcs from these areas. Chrysotile asbestos occurs in veins in talcose mineral deposits and may be then mined with the talc and create a contaminant (*9*).

Asbestos is an established carcinogen. Not only does it cause lung cancer, but it is associated with a substantial increase of morbidity from stomach cancer (*3*). Although chrysotile is the most important commercial fiber of asbestos, there is no reason to regard the amphibole asbestos minerals as safe. To the contrary in one study an amphibole asbestos mineral predominated in asbestos-bodies in the sputum of a group of men exposed to asbestos and having a high incidence of mesotheliomata (*10*). The fact that talc workers exposed to fibrous talc, which contains amphibole asbestos, develop more pulmonary injury than do workers exposed only to talcs relatively free of fibrous silicates or amphiboles (*11*), indicates the pathogenicity of amphibole asbestos.

The Japanese preference for talc-coated rice means that rice eaters of that nation are exposed to the hazard of ingestion of amphibole asbestos, as

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well as the unpredictable ingestion of chrysotile in areas where talc contains these carcinogenic materials.

Examination of the known epidemiologic characteristics of stomach cancer in Japanese strongly suggests that the causative agent is an additive to rice. Not only is the incidence of stomach cancer high in rice-eating Japanese, but inside Japan stomach cancer is most prevalent in those prefectures where rice is the main staple; it is less so in prefectures where rice is supplemented with cereal (*12*) and where the population eats heavily of the soybean preparations shoyo and miso (*13*). The morbidity from stomach tumors is greater among Japanese who eat rice with every meal than it is among Japanese who eat it less often; among Japanese men who like milk and vegetables there is a lesser morbidity from stomach neoplasms (*14*). Experimental consumption of polished rice will cause ulceropapillomata of the forestomach of rats (*15*).

These observations suggest a relationship between rice consumption and stomach cancer. However, the agent is not the rice itself, since in other oriental countries where talc-coated rice is not popular the incidence of stomach cancer is not unduly high.

A final indication of the environmental causes of stomach cancer in Japanese is provided by statistics concerning its prevalence in Japanese immigrants to western countries. The incidence in Japanese males in Japan in 1962 to 1964 was 77.9 per 100,000 (*16*). Japanese men in Hawaii had an incidence of only 45 per 100,000. Even this lowered rate was high compared to that for Chinese and Caucasian men of Hawaii who had incidences of 9.2 and 8.3, respectively. Native-born Japanese have a higher incidence of stomach cancer than Japanese born in Hawaii or continental North America (*17*). Dietetic studies on immigrant Japanese show that from 50 to 70 percent of their diet is Japanese-type food (*18*). The longer the immigrant has been in the West the more western his diet becomes; this increase in western food in the diet coincides with a decrease in the incidence of stomach cancer.

The hypothesis that the carcinogenic agent causing the high incidence of Japanese stomach cancer is asbestos-containing talc on rice appears therefore to satisfy the characteristics of the known epidemiology of that form of cancer in Japan. However, standardized mortality rates are also high for Chile, Finland, and Iceland, where rice is not a major dietary staple (*12*, *19*). In view of the evidence for the Japanese, the presence of asbestos-contaminated talc as an additive to some nationally popular food should be suspected in these countries. Further studies of the total contamination of the common diet by talc and asbestos in these nations with high incidences of stomach cancer are needed.

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7 June 1971

Cyclic Adenosine Monophosphate in Brain Areas: Microwave Irradiation as a Means of Tissue Fixation

Abstract. *Amounts of cyclic adenosine monophosphate in discrete regions of the brain were estimated after exposure of rats to microwave irradiation. Amounts were highest in the cerebellum and brainstem, intermediate in the hypothalamus and midbrain, and lowest in the hippocampus and cortex. Decapitation increased the concentration of cyclic adenosine monophosphate in all brain areas, although the increase in the cerebellum was three to four times greater than that in other areas. Microwave irradiation may provide a means of rapidly fixing brain tissue in situ while permitting easy dissection of the brain. In this way artifacts produced by decapitation can be eliminated, and concentrations of heat-stable compounds in the brain can be estimated under conditions which more closely approximate those in vivo.*

Rapid fixation of cerebral tissue in situ is required to obtain valid estimates of many brain components. Decapitation and delayed fixation lead to significant changes in brain glycogen (*1*) and in glucose, lactic acid (*2*), and other substrates and cofactors of the Embden-Meyerhof pathway (*3*). At present fixation of the brain in situ is carried out by freezing whole animals in liquid nitrogen or cooled isopentane. Even under such conditions, deep brain structures can require up to 75 seconds to reach 0°C (*4*). These freezing techniques also require restraint of the animal and produce considerable stress before it dies. Dissection of the frozen brain is almost impossible, and therefore experiments must be done on whole brains. These problems have prevented the measurement of cyclic adenosine monophosphate (AMP) in discrete brain regions. Drug-induced changes in concentrations of cyclic AMP in specific brain areas might easily be masked by massive amounts of unresponding tissue when nucleotide concentrations are measured in the frozen whole brain. Furthermore, the concentration of cyclic AMP in the whole brain increases severalfold within seconds after decapitation thereby prohibiting rapid dissection after death (*5*). Stavinoha (*6*) reported that amounts of acetylcholine in the brains of rats that had been killed by being exposed to microwave irradiation were significantly higher than previously reported

Table 1. Amounts of cyclic AMP in brain areas after microwave irradiation. Values represent the mean and standard error of the mean from six separate determinations.

Area	Cyclic AMP (nmole/g)
Cerebellum	1.86 ± 0.06
Brainstem	1.87 ± 0.06
Hypothalamus	1.60 ± 0.04
Midbrain	1.43 ± 0.11
Hippocampus	0.84 ± 0.09
Cortex	0.74 ± 0.06

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ELECTRON MICROSCOPE WORK

MEASUREMENT OF
ASBESTOS IN WATER SAMPLE

ST0513784

Submitted to

Mr. C. R. Young
Dow Chemical Co. of Canada Ltd.
Sarnia, Ontario

Prepared & Approved by _____

Dr. Eric J. Chatfield
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Electron Optical Laboratory
Department of Physics
December 6, 1972

File No: 88.3.2

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1. Introduction

A water sample was examined by electron microscopical techniques for the presence of chrysotile asbestos fibres. The number of such fibres per litre of water was determined and an estimate of their mass was made.

2. Experimental Technique

The amounts of asbestos in the water sample were expected to be very small; some method of concentration was therefore necessary. Much of the solid content of water samples is often organic, e.g., plant debris, bacteria, etc., and unfortunately this is also concentrated along with the asbestos. An ashing stage was therefore introduced into the process to destroy this organic material, which was of no interest in the analysis. Two methods of processing such specimens have already been described[1,2], both of which have some undesirable features. The method selected for this sample combined the best features of the published techniques. The water sample was thoroughly shaken to redisperse any solid material which may have settled out during storage. A 100ml sample of this was filtered through a 1000Å pore size membrane filter. This pore

size was adequately small, since fibres even shorter than this are unlikely to follow the tortuous path through such a membrane. It was necessary to assist this filtration by applying a vacuum to the clean side of the filtration equipment. The membrane filter was then placed in a silica crucible, covered with a silica lid and this was heated to 500°C for a period of more than three hours. The filter residue after ashing was dispersed in 4ml of freshly distilled water, and agitated in an ultrasonic vibrator for 10 minutes. The very heavy material was allowed to settle, and one ml of this suspension was added to a special centrifuge tube which contained a carbon coated electron microscope grid. The suspension was deposited uniformly on the electron microscope grid by operating the centrifuge at 8300g for a period of 30 minutes. The grid was then ready for inspection in the electron microscope.

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The grid was examined at a direct screen magnification of 25000 times at an accelerating voltage of 80kv. Using a series of calibration circles scribed on the microscope fluorescent screen, asbestos fibres were placed into size categories according to their lengths. A minimum of 10 grid squares (200 mesh size) were examined, and all asbestos fibres in this area were counted. One problem in fibre counting was caused by the presence of large amounts of other material. At a magnification of 25000 the area of sample examined

amounts to some 40 square metres, on which fibres perhaps as small as 3mm are being looked for among other material. This illustrates the main difficulty of the technique - that of manual counting of very widely spaced fibres; a method for which there is no substitute on samples such as these. However, counting times of about 2 hours were adequate to size the 10 grid squares required. During the search a typical fibre was photographed.

Identification of chrysotile asbestos fibres gave rise to little difficulty. The fibre itself has a characteristic tubular appearance, and in most cases (depending on previous history) such fibres give an equally characteristic electron diffraction pattern. This pattern can be compared with that obtained from specimens of known chrysotile.

The fibre counts obtained on the sample were processed by a computer programme, and results in terms of fibres/litre were obtained, in addition to estimates of the mass concentrations. A blank sample of distilled water was processed, in order to demonstrate that asbestos was not being introduced as a result of the analytical procedures. It is very easy to think of possible sources, such as airborne material, or contamination from furnace insulation, etc. In this blank sample 200ml of distilled water was filtered and

the sample processed in the same way as in the case of the other specimen. No fibres were detected in this sample.

In a further standardisation experiment, a known suspension of fibres in the size range of interest was added to a membrane filter prior to ashing. This sample indicated that the method was satisfactory, giving confidence in the results obtained on the actual sample.

3. Results

The results are shown in the computer printout (Figure 1). The concentration of fibres was 4.8×10^5 fibres per litre, which is of the same order as that in many beverages and natural sources of water.[1] The sensitivity of the method can be judged by a mass calculation, assuming a density of 2.4g/cc for chrysotile asbestos. The observed fibres correspond to one part in 3.2×10^{12} parts of water. The length distribution of the fibres is shown in Figure 2. Figure 3 shows a typical asbestos fibre found in this specimen. Figures 4 and 5 show reference chrysotile asbestos fibres and the electron diffraction pattern obtained from one of these fibres.

4. Conclusions

The concentration of chrysotile asbestos in the water sample was 4.8×10^{5} fibres/litre, corresponding to an estimated 3.1×10^{-4} µg/litre. This is of the same order as that in many beverages and natural sources of water.

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Figure 1

ASBESTOS FIBRE COUNT ANALYSIS

SAMPLE: WATER SAMPLE, DOW CHEMICALS

SIZE CATEGORY	PARTICLE SIZE, UM	NUMBER COUNTED	CUM NUMBER	CUM NO PERCENT	CUM MASS PERCENT
1	0.040	0	0.0	0.00	0.00
2	0.057	2	1.0	2.56	0.10
3	0.080	7	5.5	14.10	0.66
4	0.113	7	12.5	32.05	1.81
5	0.160	5	18.5	47.44	3.68
6	0.226	5	23.5	60.26	6.58
7	0.320	7	29.5	75.64	15.85
8	0.453	1	33.5	85.90	26.14
9	0.640	3	35.5	91.03	40.39
10	0.905	0	37.0	94.87	51.93
11	1.280	2	38.0	97.44	75.97
12	1.810	0	39.0	100.00	100.00

TOTAL NUMBER OF PARTICLES COUNTED = 39

CONCENTRATION OF ASBESTOS IN SAMPLE = 4.83×10^{5} FIBRES/LITRE

ESTIMATED MASS CONCENTRATION = 3.11×10^{-4} MICROGRAMS/LITRE

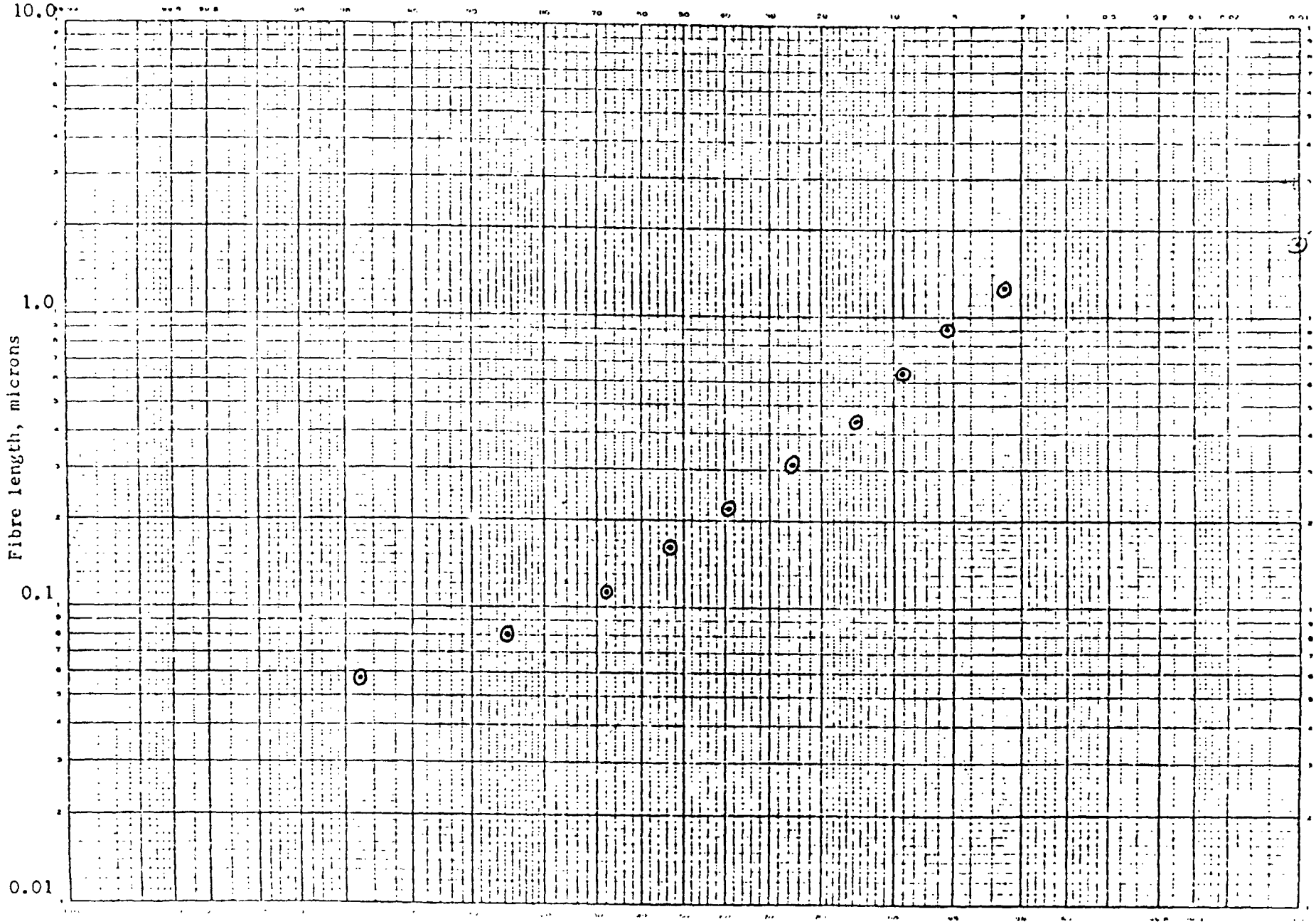
THIS MASS CONCENTRATION CORRESPONDS TO ONE PART OF

ASBESTOS IN 3.22×10^{12} PARTS OF WATER

DATA SHEET No. 107 LOGARITHMIC PROBABILITY (3 CYCLE)

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Figure 2. Fibre Length Distribution: Dow Chemical's Water Sample

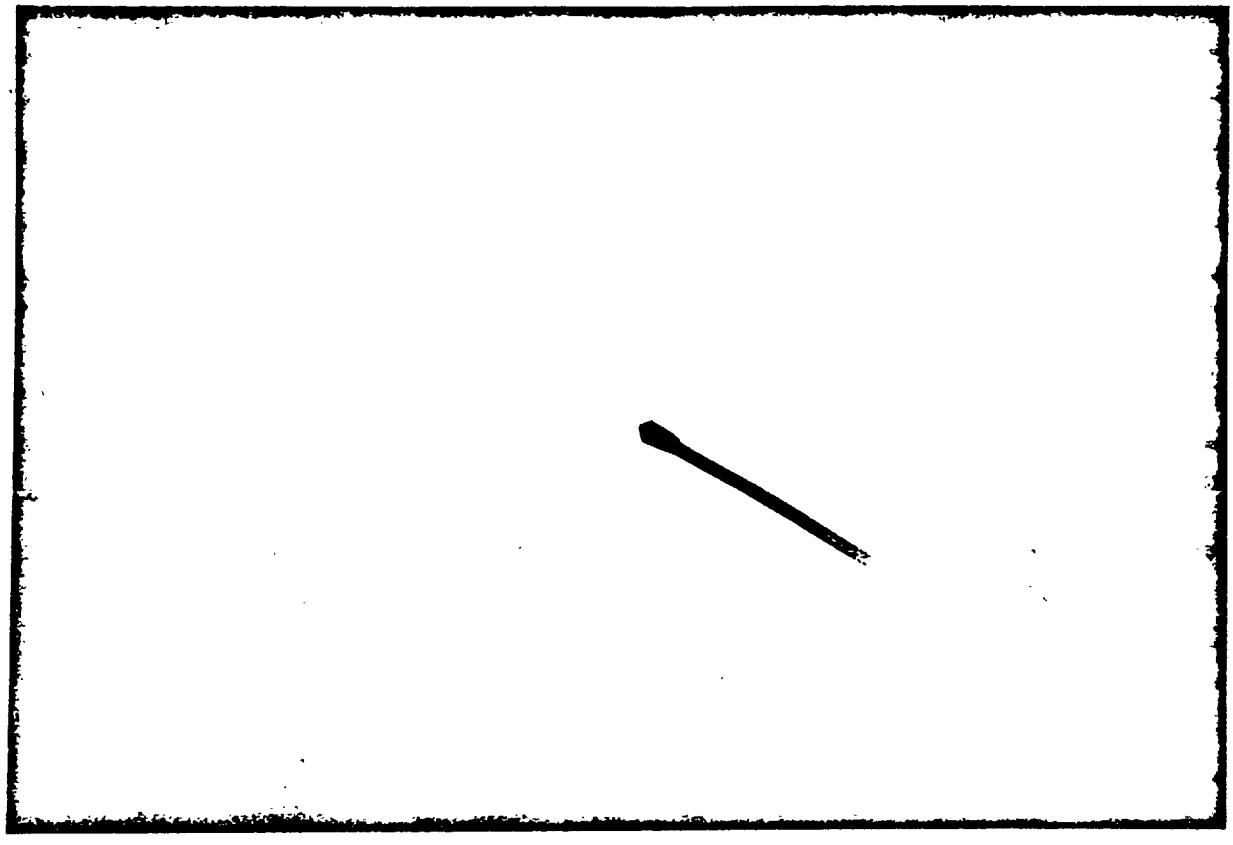


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Figure 3. Typical Chrysotile Fibre in Water Sample, X53000



Figure 4. Reference Chrysotile Fibres,
X53000



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Figure 5. Chrysotile Electron Diffraction Pattern