Bull. Environ. Contam. Toxicol. (1987) 38:1–8

Bulletin of Environmental Contamination and Toxicology

Occurrence and Biological Activity Testing of Particulates in Drinking Water

James R. Millette,* Patrick J. Clark, Richard L. Boone, and Motiel T. Rosenthal

*McCrone Environmental Services, Inc., 5500-200 Oakbrook Parkway, Norcross, GA 30093, and Health Effects Research Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH 45268

In general the actual suspended particle constituent of a drinking water source is not considered when a water plant is designed. Suspended solids and turbidity measures are used for design and monitoring plant processes. In the last few years considerable interest has developed in examining the particulate fraction more clearly. The Safe Drinking Water Committee of the National Academy of Science concluded that a survey of suspended particulate matter in raw and treated drinking water supplies in several typical communities was urgently needed as background information (NAS, 1977).

Shipley and Fitzpatrick (1977) list over twenty instruments used for optical measurements of suspended material in surface water supplies. Our laboratory has been involved in using one of the most specific of those methods, electron microscopy. Although the analysis of water for asbestos was our primary concern, we also examined, analyzed, and recorded a number of other particles which are part of the water turbidity. We report here a number of observations we made in our studies.

MATERIAL AND METHODS

Samples of drinking water were collected by local water utility or water supply regulatory personnel and sent to the USEPA in Cincinnati. Most were involved in a water quality survey, epidemiology study, or field investigation research project. The samples were collected in 946-ml (1-qt) cubitainers and shipped first class mail.

Aliquots of 10 to 250 ml from each sample were filtered by suction through a 0.1 um pore size Nuclepore filter backed by a 0.45 um pore size Millipore filter. A backing filter was used to insure uniformity of deposition of the particulate material on the filter surface. A quarter of the wet filter was cut, attached to a glass slide, dried, and carbon coated in a vacuum evaporator. A portion (approximately 2 mm^2) of the carbon coated filter was placed on a 200-mesh copper electron microscopy grid. A few drops of chloroform were placed on the filter with a 50 ul microsyringe to

Send reprint requests to J. R. Millette at above address.

affix the filter to the copper grid. The filter was dissolved using a modified Jaffe wick apparatus. Further details on the asbestos analysis technique can be found in Anderson and Long (1980).

After the filter was dissolved, the grid was placed in a carbon specimen holder in the transmission electron microscope (TEM), operated at an accelerating voltage of 80 KV and 70 uA beam current, and viewed at a magnification of 17,000 times. At least 15 grid openings from each of two grids were examined for each sample. The morphology (size, shape, appearance), crystal structure, and elemental composition of the particulates found in the water sample were investigated using magnifications up to 500,000 times, selected area diffraction (SAED), and energy dispersive x-ray analysis (EDAX). Mineral standards, glass wool, diatomaceous earth and biological organisms were prepared in a manner similar to that described above to be used for microscopic comparison.

RESULTS AND DISCUSSION

The concentration and size of asbestos fibers found in water supplies has been reported previously (Millette et al., 1983). The majority of water supplies analyzed did not contain asbestos concentrations over 1 x 10^6 fibers per liter. There were, however, some waterborne asbestos concentrations over 10 x 10^6 fibers per liter caused by natural erosion, mine processing wastes, waste pile erosion or corrosion of asbestos-cement materials.

Chrysotile was the asbestos type most frequently found in drinking water. Some amphibole fibers identified as crocodolite were found in systems with deteriorating asbestos-cement pipe. Amphiboles from the actinolite-tremolite series have been found in some water supplies in the Pacific Northwest and California. The amphibole fibers found in Lake Superior, a source of drinking water for several towns, have been identified as cummingtonite-grunerite (Cook and Olsen, 1979). Anthophyllite asbestos has not been found in drinking water. In several thousand water samples from all over the U.S., only one fiberglass particle was found. Fiberglass from water filter units has been reported (Cook et al., 1978). Glass fibers were reported in sewage sludge (Bishop et al., 1985).

Significant numbers (over 10 million fibers per liter) of attapulgite fibers (palygorskite clay) have been found in well waters on islands off the coast of Georgia and in two Florida water systems, both using ground water supplies. The palygorskite fibers found in these systems occurred mostly in short (less than 2 um long) bundles and clumps (see Figure 1a) with individual fibrils less than 200 Å in diameter. Halloysite clay fibers have been found in some California waters and in one water system in Virginia. Previous work (Millette et al., 1979) investigated the similar appearance of the two fibrous clays, palygorskite and halloysite found in water to that of chrysotile asbestos.

Trace concentrations of some titanium fibers, possibly rutile, have been found in water supplies such as Buffalo, NY, and Montgomery, AL. In Fort Wayne, IN, fibers visible by optical microscopy were found on one occasion in a water distribution system. Optical and electron microscope examinations led to the conclusion that these were cotton fibers (Figure 1b). The origin of the fibers was not determined, but suspected to be the remnants of a cloth accidentally left in the distribution system by workers making pipe repairs.

Some fibers of biological origin, pieces of diatoma, algae scales and other fiber-like fragments of organisms have been found in water samples, sometimes in high concentrations. As indicated by Moestrup (1974), as many as a hundred of these silica scales, each consisting of a slender spine on a flat disc, may be attached to an algae cell (Spumella vestiti). (Figure 1c). The spines are about 4-5 um in length and less than 0.1 um in diameter. Since 1955 when the first electron microscope images of these scales were seen, over 60 kinds of scales have been reported (Takahashi, 1975). Most are the more rounded shape as shown in Figure 1d. In a number of drinking water samples, iron bacteria were present. These bacteria arise in iron-rich groundwater sources and in the distribution systems with rusty pipes (McMillan and Stout, 1977). Individual strands of Gallionella (Figure 1e) are composed of ferric hydroxide and are less than 0.1 um in diameter.

Because clay minerals are commonly the products of weathering, they are in surface waters which are used for drinking sources. There are few data available, however, on the clay composition in drinking waters. Of the five principle clay mineral groups (kaolinite, illite, smectite, vermiculite and palygorskite), the kaolinite and palygorskite groups are of present interest because they include minerals with fiber-like morphology. Other clay minerals are added to water supplies by erosion or other mechanisms but proper concentration and analysis techniques have not been applied to identify them. Thin silica and calcium particles are found in drinking waters of Idaho (Figure 1f). There is little definitive evidence that the ingestion of particulates commonly found in drinking water adversely affects health. However, the Safe Drinking Water Committee of the National Academy of Sciences after reviewing the available data concluded that small particles of some materials, such as asbestos minerals, may have the potential to affect human health directly when they are ingested, and many kinds of particles, though apparently harmless in themselves, many indirectly affect the quality of water of other pollutants (NAS, 1977). Suspended sediment plays a major role in the pollutant transport process (Karickhoff et al., 1979). Most of the mercury and other heavy metals transported to the sea by the rivers are fixed to suspended particles less than 16 um in diameter (De Groot et al., 1971).

There is some evidence that ingested solid particles can penetrate the digestive tract. In their mini-review of intestinal absorption of particulate matter, Le Fevre and Joel (1979) list

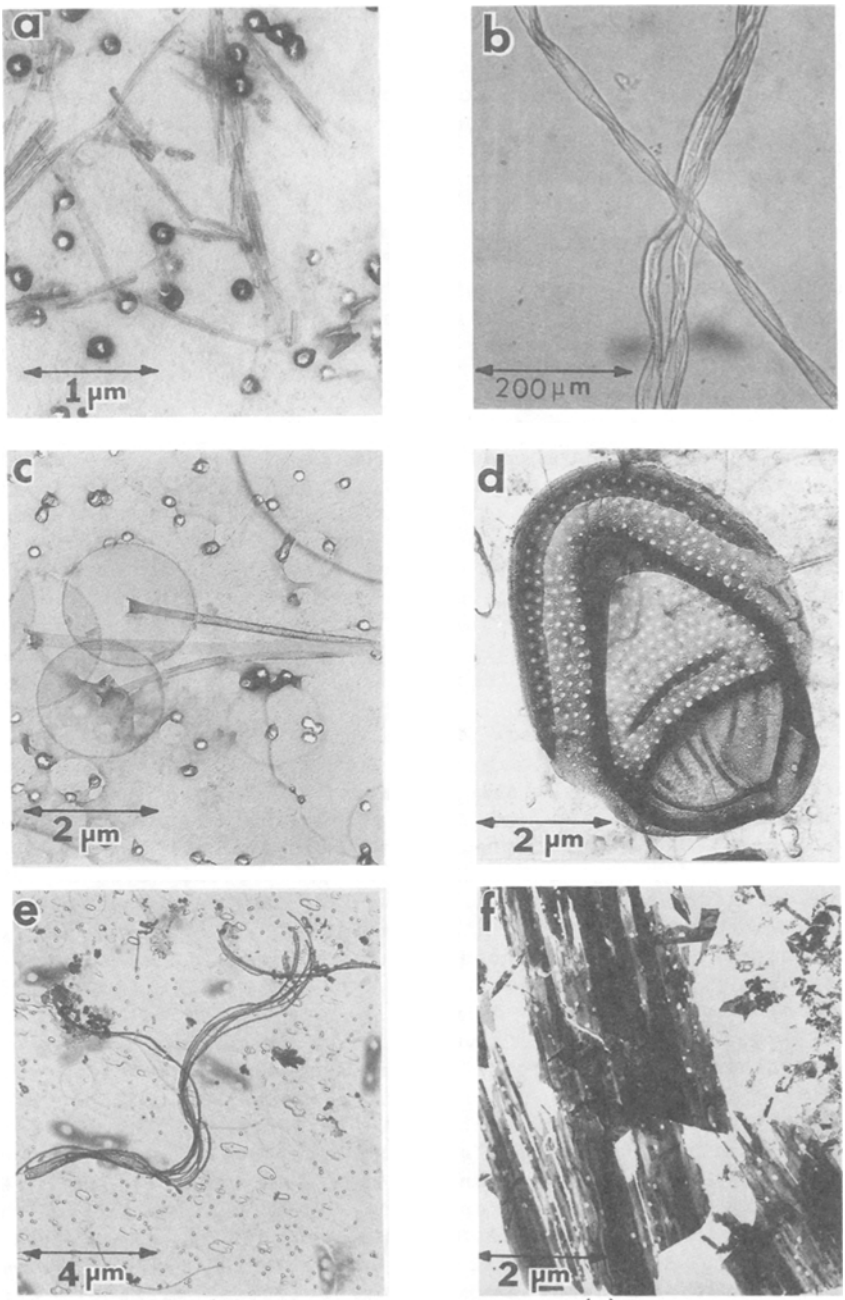

Figure 1. Particulates in drinking water: (a) Electronmicrograph (EM) of palygorskite fibers from a well in Georgia. (b) Optical micrograph of cotton fibers from a distribution system in Indiana. (c) EM of algal scales (Spumella vestita), water system in New York. (d) EM of algal scales, water system in North Carolina. (e) Iron bacteria strands, distribution system, Arizona. (f) EM of particles in water from Idaho.

colloidal metals, latex spheres, polyvinyl chloride pellets, and iron filings. Cook and Olsen (1979) reported finding nonfibrous particles of silica, diatom fragments, fibers of iron, titanium and glass of probable manmade origin in human urine. Attapulgite clay fibers have been reported in the urine of a person ingesting large doses for medical purposes (Bignon et al., 1979).

The question of the biological activity of suspended particulates is complex because of the interactions between particles. Samples of concentrated particulates representing the entire suspended fraction were collected from 3 drinking water systems and were tested for biological activity in cell culture assay by two independent laboratories. Concentrated samples of particulates were collected from three drinking water supplies in Duluth, MN, San Francisco, CA, and Seattle, WA. The Duluth sample was collected from the drinking water before the filtration plant was on-line. The sample from San Francisco was collected at the chlorination/fluorination station on the outflow from Crystal Springs reservoir, San Mateo County. There was no further treatment of the water from that point. The sample from Seattle was collected after the final treatment of the water before it was distributed to consumers. The samples were collected using a Nuclepore Quick Rinse (QR) type cartridge. The internal construction of the QR consisted of polycarbonate membranes (0.2 um pore size) layered between polypropylene screens. Together they were pleated so that 3.7 square meters of membrane were contained in a 50.2 cm long cartridge. The cartridge housing unit was attached at the water plant after final treatment and the water was allowed to flow through the filter until it was essentially plugged. Typically a thousand gallons were filtered. The exact amount was dependent on the water turbidity. After filtration the cartridge units were plugged and sent to the EPA in Cincinnati. Sections of the membrane and screens were rinsed repeatedly with prefiltered distilled water in 4 liter flasks until the materials were clean. The resulting suspension (about 10-12 liters for each sample) was reduced to a powder by freeze drying. Each cartridge yielded several grams of dried, previously suspended material.

Two additional samples of particulate representative of those found in water supplies were obtained. A sample of the less than 2 um-size fraction taconite tailings was prepared by a sedimentation separation procedure. A sample of attapulgite (palygorskite) clay, a non-asbestos mineral was obtained in a relatively pure form from a mine in Attapulgas, GA. The attalpulgite fibers in the test sample were found to be quite similar to those found in the drinking water samples. The fibers were generally less than 2 um in length and tended to clump and form small bundles when in water.

Small portions of dried particulate samples were dispersed in prefiltered distilled water, stirred over an hour by magnetic stirring, and shaken vigorously before an aliquot was taken and filtered through a 47 mm diameter Nuclepore filter. The filter

was prepared in the way described previously. Random photographs were taken at 5000 times magnification. A rectangular grid was inscribed over each photo and random areas selected for particle shape analysis. At least 300 particles were examined and classified as fibers, equants, aggregates or accircular shapes. Only those particles which appeared to be crystalline in nature were considered. The results of these analysis are shown in Table 1. As might expected most of the particles found had less than the 3:1 length:width measurement used to define a particle as a fiber.

Table 1. Analysis by Electron Microscopy of the Crystalline Fraction of the Samples of Particulates

Sample	Fibers[a] (%)	Equants[b] (%)	Aggregates (%)	Accirculars (%)
Duluth	4.2	61.1	9.3	0.9
San Francisco	7.7	37.4	18.1	0.0
Seattle	2.0	80.9	1.5	0.0
Attalpulgite	100.0	Trace		

[a]Fibers are elongated particles with a length at least three times the width.
[b]Single crystal shapes and aggregate classification as described in the Bureau of Mines Information Circular/1977 IC-8751.

Portions of the particulate matter were coded and sent to the Naylor Dana Institute for Disease Prevention, American Health Foundation New York to test the effect of the particulates on the inhibition of colony-forming efficiency of cultured human embryonic intestine-derived epithelial (I-407) cells. This toxicity assay had been developed by researchers at the Institute to compare the toxicities of the different forms of asbestos. A sample of amosite asbestos was included in the set of coded with materials that had been studied more thoroughly in other tests. The results of the assay indicated that the most toxic of the samples tested blindly was amosite asbestos which was equal in toxicity to the amosite used, by chance, as a positive control. The toxicities of the particulates concentrated from drinking water were approximately 100-fold less than the amosite asbestos of these, the order of toxicity of the samples was San Francisco > Seattle > Duluth. The samples of attalpulgite clay and taconite tailings displayed approximately half the toxicity of the drinking water particulates (Reiss et al., 1980).

Portions of the samples were coded and also sent to the Chemical Biomedical Environmental Research Group at the Ohio State

University, Columbus, Ohio. The coded samples were tested in cultured cell assays employing normal human fibroblast cells and primary Syrian hamster embryonic cell cultures. These assay systems had been previously developed to investigate the effect of different cytotoxicity, virally-directed cellular and biochemical parameters of transformation, cell membrane composition, and cyclic nucleotide concentrations (Hart, 1979). As with the assay test done by the American Health Foundation, the cytotoxicity of the concentrated drinking water particulates was much less than that of commercial asbestos. In this case, the order of toxicity of the samples was Duluth > Seattle > San Francisco. This is reverse of the toxicity ranking done by the American Health Foundation assay. The reason for the different response is not known at this time. The particulates collected from the drinking waters were more cytotoxic than the samples or attapulgite of taconite mine tailing (less than 2 um). The attapulgite and taconite tailings, however, had a greater effect on cellular components than did the water particulates. (Hart et al., 1979).

An analyses of over 2000 drinking water samples from many parts of the United States suggest that most water consumers do not drink water containing large numbers of elongated mineral particulates which have lengths three times the diameter. Some drinking waters do contain high amounts of particulate. While it is still to be determined how predictive cell assay tests are of potential health hazards, it is apparent the the suspended particulate fractions of 3 drinking waters have some effect on biological systems. It is important to note the the results of two independent assays show the drinking water particulate to be much less biologically active than commercial asbestos fibers but further work on the assay systems is necessary before the interpretation of the relative toxicity rankings is clear.

Acknowledgments: The authors would like to thank P. Cook, USEPA, Duluth, MN, for the tailings samples. The authors would also like to thank P. Underwood, J. Riano and M. Wendel for preparation of the many drafts of this manuscript. The Health Effects Research Laboratory has reviewed and approved this article for publication. Mention of trade names does not constitute endorsement.

REFERENCES

Anderson CH, Long JM (1980) Interim method for determining asbestos in water. EPA Res Report, EPA-600/4-80-005, pp 34.

Bishop K, Ring SJ, Zoltai T, Manos CG, Ahrens VD, Lisk DJ (1985) Identification of asbestos and glass fibers in municipal sewage sludges. Bull Environ Contm Toxicol 34:301-308.

Bignon J, Sebastian P, Gaudichet A, Jaurand MC (1979) Biological effects of attapulgite. In Proc Symp on Asbestos, UICC, WHO, Lyon France.

Cook PM, Olson GF (1979) Ingested mineral fibers: elimination in human urine. Science 204:195-198.

De Grott AJ, De Goey JJM, Zegers C (1971) Contents and behavior of mercury as compared with other heavy metals in sediments from the River Rhine. Ems Geologie en Mijnbouw 50, 393-398.

Hart RW, Fertel R, Neuman HAI, Daniel FB, Blakeslee JR (1979) The effects of selected asbestos fibers on cellular and molecular parameters, Research Report EPA-600-1-79-021, pp 35. Avail. from NTIS, Springfield, VA, Order #PB85127496.

Karickhoff SW, Brown DS, Scott TA (9179) Sorption of hydrophobic pollutants on natural sediments. Water Res 13, 241-248.

LeFevre ME, Joel DD (1979) Minireview: intestinal absorption of particulate matter. Life Science 21, 1403-1408.

McMilliam L, Stout R (1977) Occurrence of Spaerotilus, Caulobacter, and Gallionella in raw and treated water. J. Amer Water Works Assoc 69(3):171-173.

Millette JR, Twyman JD, Hansen EC, Clark PJ, Pansing MF (1979) Chrysotile, paylsorskite and halloyskite in drinking water. Scanning Electron Microscopy/1979/I 579-586.

Millette JR, Clark PJ, Stober J, Rosenthal M (1983) Asbestos in Water Supplies of the United States. Environ Health Persp 53, 45-48.

Moestrup O (1974) Scale formation: a special function of the golgi apparatus in algae. Eight Inter Cong on Electron Microscopy, Canberra, Vol II 564-565.

National Academy of Science (1977) Drinking Water and Health, Part I, pp IV, Natl Res Council, Washington, DC.

Reiss B, Millette JR, Williams GM (1980) The activity of environmental samples in a cell culture test for asbestos toxicity. Environ Res 22, 315-321.

Shipley GL, Fitzpatrick JA (1977) Measurements of suspended material in surface water supplies. Proc Amer Water Works Assoc Conf, May 8-13 paper 16-3 pp 1-12.

Takahashi E (1975) The fine structure of the scales and flagella of the Crysophyta. In: Advance of Phycology in Japen. (Tokida J and Hirose H, eds) Dr. W. Junk b.v. Publ The Hague, The Netherlands pp 68-97.

Received July 12, 1985; accepted September 18, 1986.