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16. Insertion of DNA fragments into the Hind III site of pMB9 reduces the level of tetracycline resistance of cells carrying such recombinant plasmids to varying degrees dependent on the sequences cloned into this site [R. L. Rodriguez, R. Tait, J. Shine, F. Bolivar, H. Heyneker, M. Betlach, H. W. Boyer in *Tenth Annual Miami Winter Symposium* (Academic Press, New York, in press)]. We have previously observed that the insertion of DNA molecules containing poly dA · dT regions allows the expression of tetracycline resistance at reduced levels (5 to 10 μg/ml). Screening for recombinant plasmids was therefore carried out at 5 μg of tetracycline per milliliter for transformation into the Hind III site, and 20 μg of tetracycline per milliliter for transformation into Eco R1 site.
17. Plasmid DNA cleaved by Hind III or Eco RI was treated with bacterial alkaline phosphatase (Worthington, BAPF, 0.1 unit per microgram of DNA) at 65°C in 25 m*M* tris-HCl, *p*H 8, for 30 minutes, followed by phenol extraction to remove the phosphatase. After precipitation by ethanol, the phosphatase-treated plasmid DNA was added to cDNA (containing Hind III or Eco RI cohesive terminals) at a molar ratio of 3 : 1 (plasmid : cDNA). The mixture was incubated in 66 m*M* tris, *p*H 7.6, 6.6 m*M* $MgCl_2$, 10 m*M* dithiothreitol, 1 m*M* adenosine triphosphate (ATP) for 1 hour at 14°C in the presence of 50 units of T4 DNA ligase per milliliter. The ligation mixture was added directly to the χ1776 cells for transformation.
18. A modification (W. Salser, personal communication) of the transformation procedure originally provided to all recipients of χ1776 by R. Curtiss III was used. The work with this strain was done in a P3 physical containment facility in the EK-2 host-vector system *E. coli* χ1776–pMB9 in compliance with the NIH guidelines for recombinant DNA research.
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Environmental Asbestos Pollution Related to Use of Quarried Serpentine Rock

Abstract. *Crushed serpentinite quarried in Montgomery County, Maryland, has been extensively used for paving roads and other surfaces. The mineral assemblage includes antigorite or lizardite as well as chrysotile and tremolite. Air samples taken in the vicinity of serpentine-paved roads show that chrysotile concentrations are about 10^3 times greater than those typically found in urban ambient air in the United States.*

A rock quarry located near Rockville, Maryland, is the major source of crushed stone in the area north of Washington, D.C., including Montgomery and Prince Georges counties in Maryland. An estimated 28 million cubic meters or more of rock has been quarried during some 20 years of operation. The crushed stone is used as road metal, base course, and for resurfacing of highways, parking lots, and driveways. It has also been used as concrete aggregate and for other purposes in the construction industry, and as filler-binder for asphalt in blacktop paving.

The quarry is located in the Hunting Hill pluton, which is about 1½ km wide and 6½ km long and consists of serpentinized dunite cut by gabbro dikes. The structure, petrography, and mineralogy were described by Larrabee (*1*), who concluded that the bulk of the serpentinite consisted of antigorite in both platy and fibrous forms. Veins or lenticular bodies of chrysotile, tremolite, deweylite, talc, anthophyllite, clinozoisite, penninite, and other silicate minerals were reported to occur in the serpentinite. The chrysotile veins are generally less than 1 mm wide. Since the fiber is the harsh variety and occurs in short lengths it is not a commercial source of asbestos. Chlorite is common in the serpentinite, occurring chiefly as a replacement of antigorite.

The widespread and large-scale use of such crushed serpentinite raised the possibility of environmental contamination by the asbestos and asbestiform minerals present—chrysotile, tremolite, and anthophyllite. These minerals have a known human disease potential (*2*). The biological potential of platy and fibrous antigorite has not been determined.

We have investigated the possibility of environmental contamination by asbestos mineral dusts from quarry operations such as blasting, crushing, and truck loading and by the dissemination of dust along the quarry truck delivery routes as well as during unloading and installation of the crushed rock. We have been mindful that, after emplacement, considerable dust may be continually created by abrasion and erosion of pavement surfaces, particularly where the crushed rock is not bound by asphalt base or topping. Braking, turning, and acceleration of vehicles on these surfaces produce visible dust clouds. An example is seen in Fig. 1. Vegetation along roads paved with crushed stone is often heavily coated with road dust, particularly in the vicinity of intersections.

Fig. 1. A cloud of dust follows vehicles traveling on a road surfaced with crushed serpentinite, a large proportion of which consists of fines and small friable rock fragments. Samples of suspended air particulates taken in the vicinity contained a large number of chrysotile asbestos fibers.

Samples of raw serpentine rock and stockpiled crushed stone from the quarry were analyzed for their mineralogical content. Analytical techniques included petrographic microscopy, x-ray diffraction, and transmission electron microscopy coupled with selected area electron diffraction. Platy serpentine minerals (antigorite or lizardite or both) were found to be the major constituents of the samples. Electron microscopic analysis of pulverized material showed that chrysotile occurs both as free fibers and frequently intergrown with the platy serpentine minerals (Fig. 2a). Chrysotile was not restricted to veins but was also present throughout the groundmass of the serpentinite. Thus the proportion of chrysotile in the rock is greater than the few tenths of a percent estimated by Larrabee (*1*) on the basis of visible veins, and may be several percent or more.

Samples of roadside vegetation, on which there was visible settled dust, were also studied. Examination by electron microscopy confirmed the presence of chrysotile in both fibril and fiber bundle forms. In addition, chrysotile was observed intergrown or interlayered with particles of platy serpentine. It is difficult to make a precise quantitative determination of the chrysotile content of the pluton, primarily because of the heterogeneity of the large rock mass and the limitations inherent in analyzing representative samples of the rock by electron microscopy. X-ray diffraction cannot be used for accurate quantitative determination of chrysotile in an antigorite or lizardite matrix because of the close similarities in their diffraction patterns. Moreover, the proportion of chrysotile present in rock and settled dust samples could vary considerably according to source location in the quarry ore body, as well as other factors. For example, crushing the serpentine rock tends to cause fracturing along zones of weakness produced by the veins. These surfaces afford a greater potential for fiber release. Tremolite was identified in samples taken from the quarry and in settled dust samples. Anthophyllite was not found in these samples.

Ambient air samples were taken at five locations in the vicinity of Rockville to measure chrysotile asbestos concentrations. Sampling sites included locations in residential and school areas and in the vicinity of serpentinite-paved roads under varying traffic conditions. The sampling times varied from ½ to 2 hours. The sampling equipment was placed 10 to 100 m from the roads with the sampling heads pendent so as to collect only suspended dust particles. The air samples were taken on membrane filters with a 0.8-μm effective pore size.

Two techniques were used to prepare the five air samples for electron microscopic analysis. Each of the techniques provides distinctive information. In the first, the filters were ashed in plasma oxygen to eliminate organic materials and the residue was prepared for electron microscopic examination by a rub-out method (*3*) which comminutes large inorganic particles and fiber bundles of chrysotile and homogeneously redistributes all particles, which can then be counted and measured. Table 1 shows the results of electron microscopic mass measurements of chrysotile in air samples.

The data in Table 1 show that a chrysotile content of 500 ng/m^3 was found in a sample taken near a road surfaced with crushed rock, when only an occasional vehicle passed during the sampling period. Under moderately heavy traffic conditions chrysotile concentrations increased up to 4700 ng/m^3 in the vicinity of an intersection, decreasing to 1000 ng/m^3 at a distance of 100 m from the intersection. Evaluation of the significance of the chrysotile asbestos concentrations measured may be facilitated by comparing them with concentrations measured in other circumstances. In one investigation in this laboratory, 200 air samples collected in 49 cities in the United States were analyzed for their chrysotile contents by using the preparation technique described above. The mean concentration in these cities was 4.3 ng/m^3 in 1969 and 2.1 ng/m^3 in 1970. Concentrations of 10 ng/m^3 or less were found in 187 samples. Thus, the average chrysotile concentration in the five Rockville samples was approximately 1000 times greater than the average values found in 49 cities in the United States. In another study, airborne asbestos levels were found to be 0.5 to 15 ng/m^3 at urban sites and 0.1 ng/m^3 at a nonurban site (*4*). Measurements of airborne chrysotile asbestos levels in New York City were made during a period of extensive use of asbestos-containing fibrous spray mixtures for fireproofing high-rise buildings (*3*). Levels of 15 to 180 ng/m^3 were found in the vicinity of construction sites where such asbestos insulation was being applied. Because of environmental asbestos contamination produced by spraying, this use of asbestos has been banned in the United States (*5*).

The airborne mass concentrations shown in Table 1 are based on measurements of free chrysotile fibers only. In addition, an unmeasured proportion of chrysotile occurs interlayered or intergrown with platy silicates (Fig. 2a). Thus, the total mass of chrysotile that may be released may be several times or even an order of magnitude higher than those shown in Table 1. Whether such particles would degrade in vivo and release chrysotile fiber is unknown.

In the rub-out technique particles are pulverized and distributed for electron microscopic scanning. To determine whether a significant fraction of the individual chrysotile fibers measured was separated and freed during laboratory processing, the air samples were also prepared for electron microscopic analysis by a direct transfer method in which segments of the filter are carbon-coated, mounted on electron microscopic grids, and then dissolved (*6*). The undisturbed state of collected particles and their size distributions may then be determined. The five samples prepared by the direct transfer technique gave results similar to those obtained with the rub-out technique. Fiber bundles of chrysotile were common (Fig. 2, c and d), but few fibers longer than 5 μm were seen. For comparison, Table 1 shows the fiber counts we made in accordance with the optical microscopic technique adopted by the National Institute for Occupational Safety and Health (NIOSH) for determining asbestos levels in workplaces (*7*). This procedure, using phase-contrast optical microscopy at a magnification of × 430, records only large fibers—that is, those longer than 5 μm—and visualizes only fibers thicker than 0.5 μm (5000 Å). The electron microscopic technique, using magnifications of × 25,000 or more, permits the visualization and identification of all fibers, including chrysotile at its smallest cleavage fragment dimensions—approximately 250 Å in diameter and 750 to 1000 Å in length. It should be further noted that the electron microscopic method will specifically identify chrysotile, whereas the NIOSH technique does not distinguish different kinds of fibers (fibers are defined as particles greater than 5 μm in length and with a length-to-width ratio of at least 3 : 1). Comparison of the data obtained by the electron and optical microscopic techniques indicates that the NIOSH technique is not capable of determining the actual levels of asbestos exposure. Similar observations have been made with regard to occupational exposure to asbestos (*8, 9*). In these situations it was demonstrated that the total asbestos exposures, in terms of fiber number, mass, and surface area, were much greater than those indicated by optical fiber-counting procedures.

Geologists have long been aware of the association between serpentinite belts and Alpine-type mountains (*10*). Typically, serpentinized peridotites are found in folded belts straddling their central axes (*11*). Such a serpentinite belt system occurs along the East Coast of North America, including the Appalachians, and extends from Labrador to Alabama (*11, 12*). Similar belts are associated with the Coast Ranges of California and are found elsewhere in the United States. Some of these deposits are commercial sources of chrysotile asbestos, soapstone, talc, tremolite, and other minerals. Other deposits, with asbestos present in less than commercial quantities or concentrations, may be quarried for materials such as crushed stone, as at the Rockville quarry. The extent to which this occurs on the East Coast and the West Coast has not been investigated.

While the data we have collected are limited to five single measurements, they suggest that a possible public health hazard exists associated with the distribution and use of quarried asbestos-containing serpentinite. Similarly, the exploitation of crushed amphibolite and a variety of ultramafic rocks also raises the possibility of contamination by actinolite, tremolite, crocidolite, asbestos, or by their asbestiform analogs. The use of such quarried rock for road surfacing and similar purposes may result in widespread asbestos contamination of community air. In some circumstances, this may yield high levels of airborne respirable asbestos and asbestiform minerals.

The evaluation of the possible health hazard that may be associated with this

Table 1. Concentrations of airborne chrysotile asbestos in the vicinity of roads surfaced by quarried serpentinite crushed rock in the Rockville, Maryland, area. The electron microscopic technique gave a magnification of × 25,000. With optical microscopy (phase-contrast; magnification, × 430) only fibers 5 μm or longer were recorded.

Sample	Sample location	Fiber concentration: Electron microscopy (ng/m^3)	Fiber concentration: Optical microscopy (fibers per milliliter)
1	10 m from road intersection; light traffic	500	0.0
2	10 m from road intersection; moderate traffic	4700	0.05
3	School parking lot; 100 m from site of sample 2	1000	0.0
4	Residential area; 70 m from site of sample 2	2000	0.01
5	10 m from roadside abutting the National Bureau of Standards; moderate traffic	3600	0.01

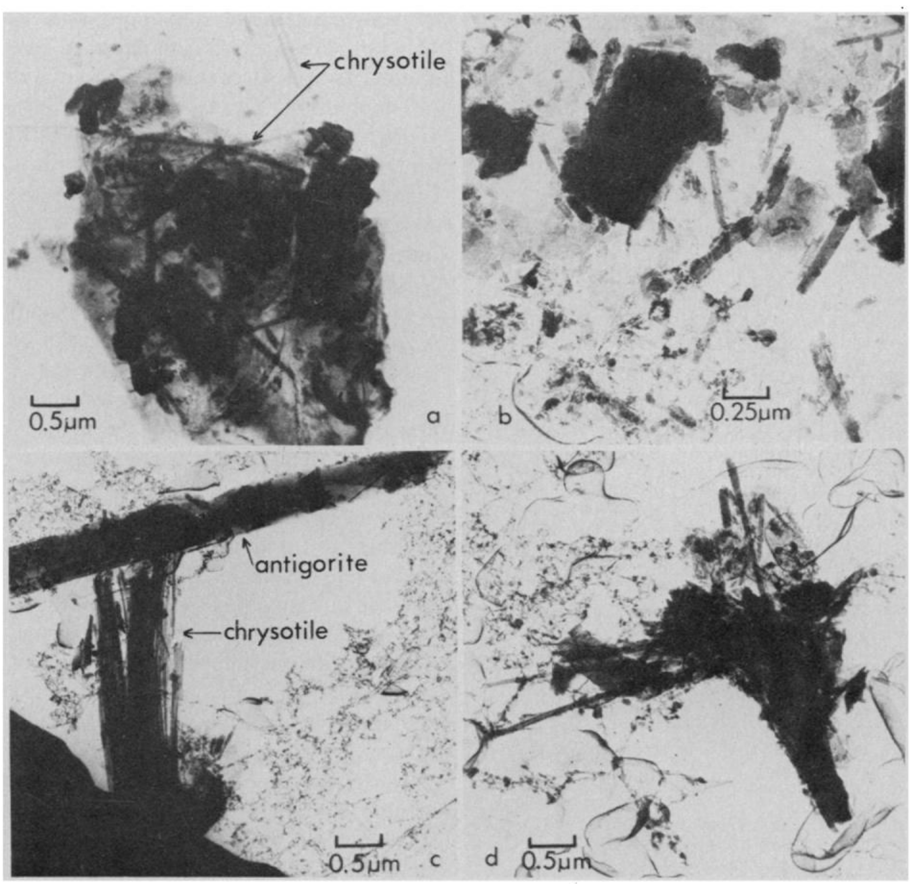

Fig. 2. Electron micrographs of air samples taken in the vicinity of Rockville, Maryland. (a) Fibrous serpentine (chrysotile asbestos) occurs both as free fibers and intergrown or interlayered with platy serpentine (antigorite or lizardite). (b) Air sample prepared by the direct transfer technique, leaving the original particle size distribution intact. Numerous submicrometer fibers and fibrils of chrysotile asbestos are visible. (c) Air sample prepared by the direct transfer technique. A large fiber bundle of chrysotile abuts a single fiber of antigorite. (d) Air sample prepared by direct transfer technique. Several intersecting bundles of chrysotile, splaying out and liberating individual fibrils, are seen.

exposure requires information that is not yet known in the scientific community: (i) the biological activity of short chrysotile fiber, (ii) the level of exposure to asbestos which is safe insofar as human cancers are concerned, if a safe level exists, and (iii) the biological activity of asbestiform silicates, not necessarily asbestos.

We urge that environmental analyses be made rapidly in areas where dusts may be anticipated from the use of quarried rock. Since public water supplies and crops may also be contaminated, they too should be monitored. In addition, a systematic inventory of quarry operations in the United States should be developed, with priority given to more heavily populated areas. Information should include the kind of rock worked, possible presence of asbestos, volume of production, types of uses, and levels of exposure.

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Meteorite Impact Crater Discovered in Central Alaska with Landsat Imagery

Abstract. *Several supporting observations indicate that Sithylemenkat Lake, Alaska; occupies a meteorite impact crater formed near the end of the Wisconsinan glaciation. The initial identification with Landsat imagery is attributed to the unique perspective provided by such imagery.*

Meteorite impact craters are important to geomorphology because they are produced by a process which occurs throughout the universe. The craters are unique on Earth, for they are created instantaneously. Most terrestrial landforms are slowly evolved by erosion or deposition from some preexisting situation. Therefore, meteorite impact craters provide a reference for local geologic chronologies and a representation of the intensity of geomorphic processes which have occurred since the time of impact.

A feature that appears to be an eroded meteorite impact crater has been discovered in central Alaska as a result of a systematic search for impact features of the entire state of Alaska using band 7 of available Landsat imagery. The crater is a bowl-shaped depression approximately 12.4 km across and 500 m deep. A lake about 3 km across occupies the center of the depression. The lake is named Sithylemenkat, which is a Koyukukian term for the "lake in the hills." Sithylemenkat Lake is located 90 km south of Bettles, Alaska, at latitude 66°07′N, and longitude 151°23′W, in the northern Ray Mountains.

The search was conducted by visually scanning complete orbital tracks of Landsat imagery that cross the state. One track from a single orbit was viewed at a time. The resolution of the imagery dictated that recognizable features would probably be greater than 1 km^2. The imagery was scanned for geologic features that might be indicative of impact events. The features included: (i) circular outlines of rock types, lakes, or structures, (ii) bowl-shaped depressions, with or without rims, and (iii) radial or concentric structures as evidence by geomorphic features such as valleys, ridges, or stream channels.

The major problem with locating and identifying a meteorite impact feature in Alaska is that there are numerous circular features of nonmeteorite impact origin. Periglacial lakes, volcanic vents, and the structural features of a tectonically active area are often represented by circular expressions. A circular feature was considered as a possible impact feature only if it is located in terrain unsuited for the formation of periglacial lakes and unlikely to contain volcanic vents.

In spite of these restraints, the search produced one feature which appears to be a meteorite impact crater. A circular lake in a depression located within the margins of a bedrock upland area can be seen on the Landsat imagery (Fig. 1). A system of radial features can also be observed. Because the rock-walled depression and the associated radial features are located in an area unlikely to contain volcanic vents, a closer investigation of the Sithylemenkat Lake area was warranted.

Sithylemenkat Lake is not accessible by surface vehicles except in deep winter when the ground is covered with snow, and observation conditions are limited by the arctic night. Because of this inaccessibility, aerial photographs and maps that covered the area were acquired from government sources. A search was also made for published reports about the area.

Stereo viewing of the aerial photography indicated that there were no features such as end moraines or ice-scoured bedrock knobs in the vicinity of the depression. The photographs did not reveal any nearby features which would indicate that glacial processes might have excavated the basin. Faults and fractures in the bedrock can be seen on the aerial photographs. The bedrock exposed in the walls of the depression has extremely serrated physical outlines and the exposures are crossed by numerous long notches. Because the outlines and notches show preferential alignment and form a definite pattern they indicate that the bedrock is intensely fractured. There is one set of fractures which is parallel to the regional structural trend of N45°E (*1*). A second set of fractures can be seen in the bedrock radiating in nearly all directions from the lake in the center of the depression. These radial fractures are not to be confused with the strings of alluvial material that are also radial to the lake and lie in a direction parallel to the slope of the walls. Also, a less obvious set of fractures is seen in the bedrock of the walls concentric to the lake. The radial and concentric fractures that can be seen on the aerial photographs are similar in pattern to those associated with