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Identification and Localization of Toxic Elements in Normal Human Lung Macrophages (41145)

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Abstract. The elemental content of pulmonary macrophages obtained by bronchopulmonary lavage from 13 healthy adult volunteers was assessed. Subcellular localization of elements was accomplished using energy-dispersive X-ray analysis in the scanning transmission microscope. Silicon, aluminum, iron, and sulfur were identified and localized to subcellular particles. These observations indicate that the elemental content of alveolar macrophages lavaged from living individuals can be determined by microanalysis and that the alveolar macrophages of normal persons retain potentially toxic elements.

The alveolar macrophage is the first line of defense for the lung against microbial invasion and functions critically in clearing inhaled particles (1). Diseases due to inhalation of particulate matter encompass a wide spectrum of disorders including occupational lung diseases such as asbestosis and silicosis (2–5) as well as nonoccupational disease states due to inhaled pollutants (6, 7). Inhaled particles may contain elements with high carcinogenic potential such as nickel, cadmium, chromium, and fibrous silicates (8). Particles less than 1 μm in diameter are deposited predominantly in the alveolar spaces (9) where the absorption efficiency for many trace elements is estimated to be 50–80% (10). Therefore the lungs are the major pathway to the bloodstream for elements absorbed from airborne particles.

The effects of the various environmentally caused lung disorders may be measured as an end product pathologically and by quantitation of diminished pulmonary function. At present, however, there is no method for measuring inhaled pollutants *in situ* that have not been absorbed into the blood. Elemental analysis of lung tissue from biopsy and autopsy specimens has been done (11–13); however, there is relatively little information available regarding the chemical composition of material phagocytosed and sequestered by the alveolar macrophages of normal persons (14, 15). During diagnostic fiberoptic bronchoscopy alveolar macrophages may easily be recovered from living patients. We reasoned that analysis of the alveolar macrophages retrieved by bronchopulmonary lavage would give some approximation of the elemental content of particles retained in the alveolar spaces. We therefore undertook identification of the elemental content of alveolar macrophages obtained from normal smoking and nonsmoking persons and localization of these elements to subcellular structures.

Materials and methods. Alveolar macrophages were retrieved from appropriately informed normal volunteers by bronchopulmonary lavage as previously described (1, 16, 17). Briefly, an Olympus fiberoptic bronchoscope was passed transnasally and wedged in a right lower-lobe bronchus. The segment was lavaged with 300 ml of saline in 50-ml aliquots. Eight smokers and five nonsmokers were studied. Smoking and nonsmoking male and female volunteers were between the ages of 18 and 33. The average yield of macrophages per lavage in five nonsmokers was 2.7×10^7 ($1.1–4.4 \times 10^7$). In eight smokers the number of macrophages retrieved was 7.4×10^7 ($2.9–29.3 \times 10^7$). Volunteers smoked an average of 17.5 (4–45) pack years. Cells were collected in 0.9% sodium chloride and resuspended in Hanks' balanced salt solution containing 20% fetal calf serum. The macrophages were recovered by centrifugation and fixed in 2% glutaraldehyde with 0.1 *M* cacodylate buffer and 1% sucrose at pH 7.2.

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In initial studies we used energy-dispersive X-ray analysis of single cells or groups of cells viewed in the scanning electron microscope (SEM) (17). For this technique the cells were washed in distilled water after fixation and dried on carbon planchets. Cells were analyzed on an ETEC Autoscan scanning electron microscope at 45° tilt with beam current at 1 μA and at 20-kV accelerating voltage. The microscope was equipped with a KEVEX X-ray energy dispersive spectrometer with a lithium-drifted silicon detector. Working distance was 15 mm from the specimen. A Tracer Northern multichannel analyzer was used and each sample was analyzed for 500 sec; the area scanned was between 1 and 100 μm^2. In these studies seven smokers and four nonsmokers were studied.

Subsequently we employed the transmission electron microscope in the scanning mode (STEM) in conjunction with the emission spectrometer to determine the subcellular distribution of various elements. After fixation, cells were dehydrated through a graded series of ethanol and propylene oxide and embedded in Epon 812. Cells were not postfixed in osmium, and thick sections (3000 Å) were cut with glass knives and made to adhere to uncoated 3.05-mm 75-mesh carbon polymer grids. Sections were examined uncoated in the STEM mode using a JEOL 100 CX TEMSCAN microscope equipped with a special hard X-ray aperture system. All samples were examined at a 30° tilt and the total emission current was 110–120 μA, with 75-μA beam current at 60-kV accelerating voltage. The microscope was fitted with a KEVEX X-ray energy spectrometer with a lithium-drifted silicon detector 16 mm from the specimen. With this system, resolution was 15 Å in the STEM mode. Either a KEVEX 7000 or a Tracer Northern 880 multichannel analyzer was used. Each sample in the cell was analyzed for 100 sec and the average area scanned was 200 Å on a side. In each specimen the Epon background, nuclear area, and cytoplasm were separately analyzed, as well as were several areas within the granules. One nonsmoker and two smokers were studied and macrophages were selected at random.

Results. Energy-dispersive analysis of the alveolar macrophages with the SEM and the STEM gave similar results. Sulfur, silicon, and chlorine were consistently found and aluminum, calcium, iron, magnesium, and potassium were present less regularly. In general, macrophages from smokers had higher peaks of silicon, aluminum, sulfur, and iron. Localization and better resolution of these elements were obtained in the STEM. Therefore only the data from STEM are presented.

Although the sections were not stained for STEM, there was good visual resolution of subcellular structures (Fig. 1). Heterochromatic and euchromatic areas of the nucleus were easily distinguished and cytoplasmic inclusions that were identified as lysosomes or granules in conventional transmission electron micrographs could be clearly seen.

High phosphorus and calcium peaks were identified in areas of heterochromatin (Fig. 2). The phosphorus peak was expected because of the DNA-bonded phosphorus. When an Epon background blank was scanned, chlorine was identified throughout the sections and was partly due to the embedding material (Fig. 2c). A small amount of background silicon was observed and was partially attributable to the use of glassware in sample preparation and to sectioning with glass knives. However, in some granules, high silicon peaks were observed which occasionally were associated with a large aluminum peak, suggesting the presence of an aluminum silicate (Fig. 3). The silicates were not associated with visible crystalline structures. Significant iron peaks were often identified within the macrophage granules. Frequently a high iron peak was associated with high silicon peaks, although the presence of these elements was heterogeneous within granules of a single cell and between cells of the same sample (see Figs. 1b and 3b).

When the combined data from all the granules of the smokers are compared to those from nonsmokers it is seen that the greater amounts of aluminum, silicon, and iron are found in the smokers' (Fig. 4). The percentage relative mass fraction calculated tends to substantiate this (Fig. 5).

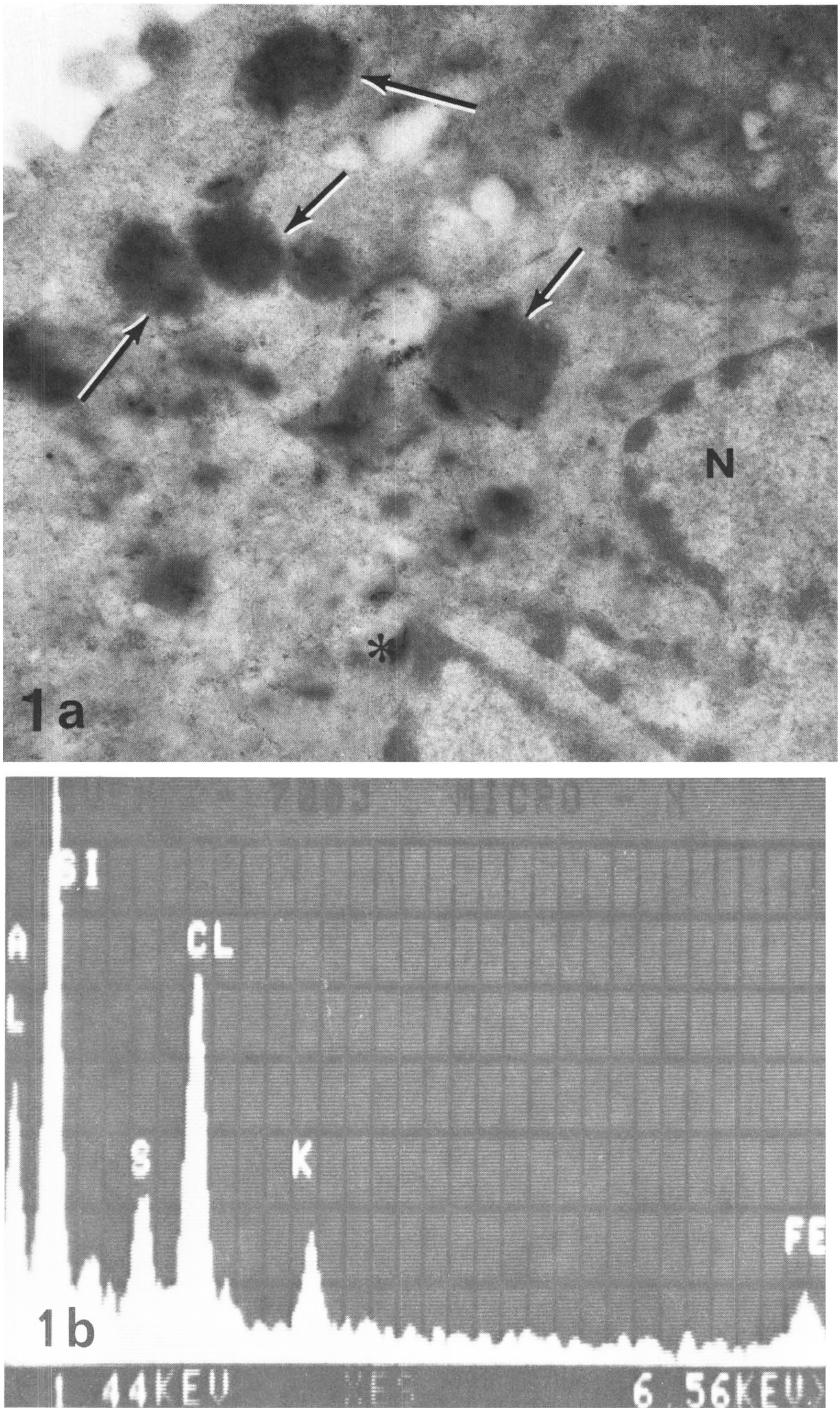
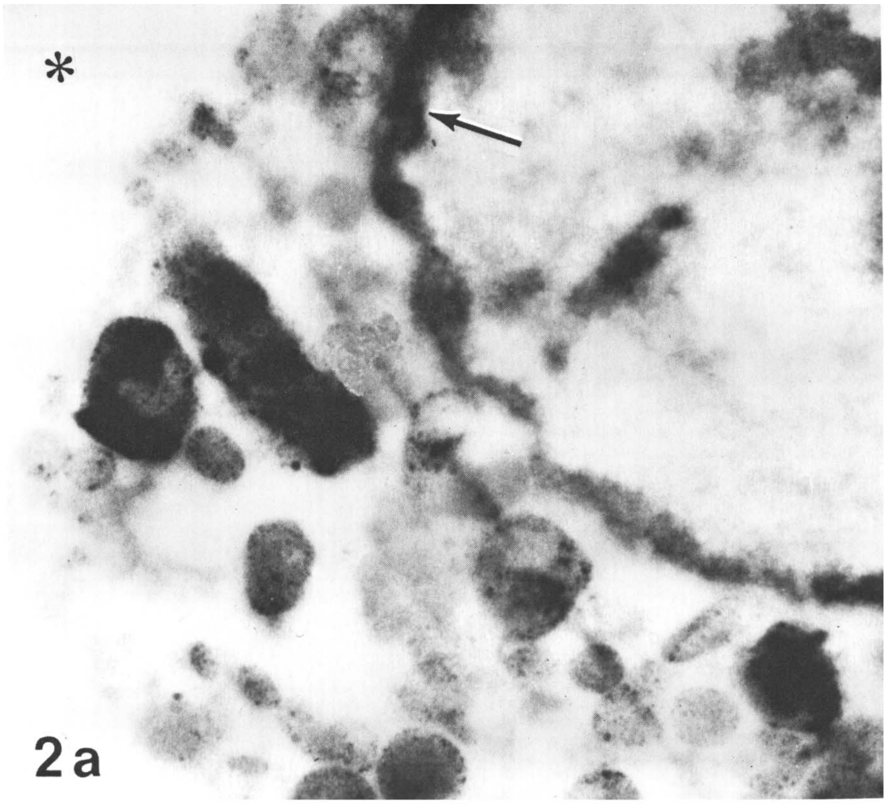


FIG. 1. STEM micrograph of a human alveolar macrophage from a cigarette smoker (a) (×10,000). The nucleus (N) and granules (arrows) in the cytoplasm are easily recognized in this thick and unstained section. The asterisk indicates the area probed and the X-ray elemental analysis data are shown in (b). Vertical scale is 256 counts.



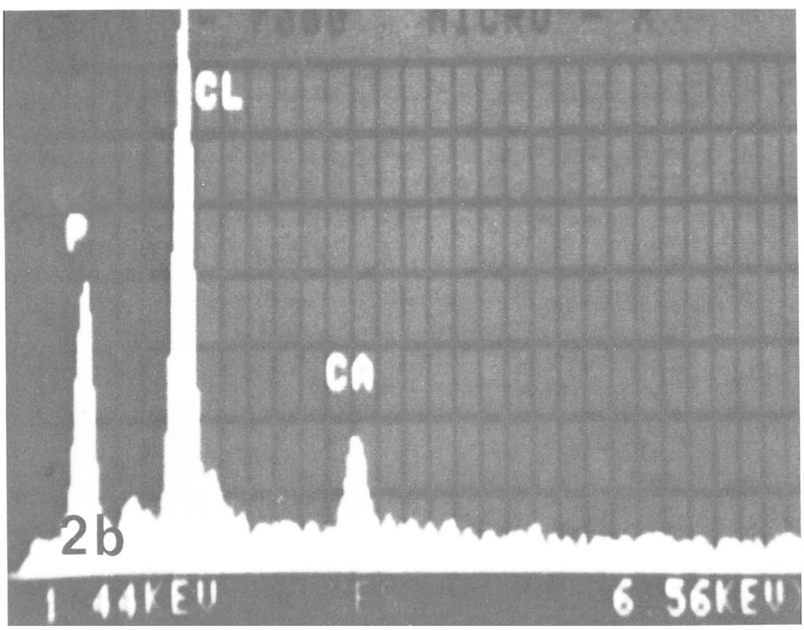


FIG. 2. STEM micrograph of macrophage from a smoker (a) (×5500). The arrow indicates the heterochromatic area probed and the resultant X-ray energy spectrum is shown in (b). The asterisk indicates the area analyzed for elemental background and the corresponding spectrum is shown in (c). Vertical scale is 512 counts.

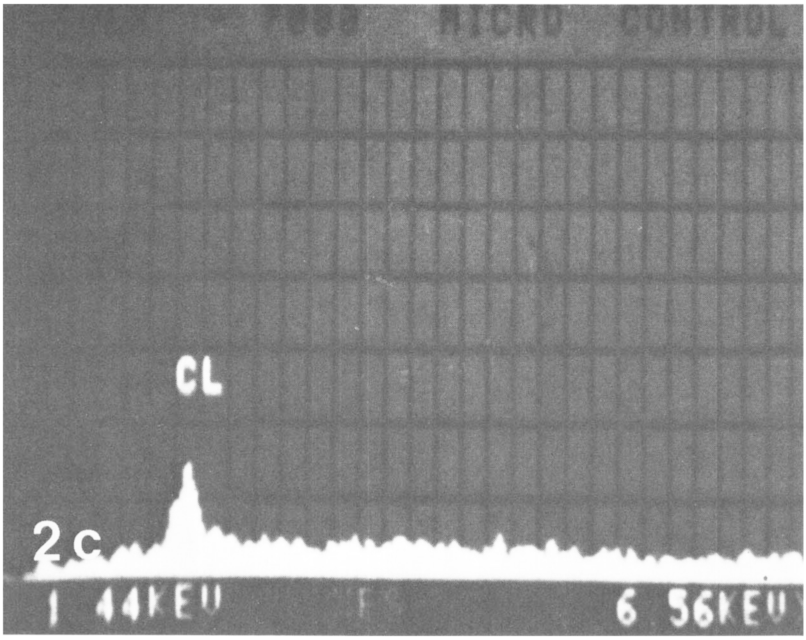


FIG. 2—*Continued.*

Discussion. We have used scanning electron microscopy and scanning transmission electron microscopy in conjunction with X-ray energy-dispersive spectrometry to identify and localize potentially toxic elements in human alveolar macrophages lavaged from healthy subjects. The origin of the elements identified was not determined although some may be derived from inhaled air pollutants and tobacco smoke (14, 18). The presence of measurable quantities of iron in these macrophages may be due to subclinical bleeding into the lung tissue and resultant erythrophagocytosis (19), or inhalation of particles containing iron. High sulfur peaks were noted in certain macrophage granules and these may relate to inhaled particles containing sulfur in insoluble forms (10). High levels of phosphorus and chlorine may relate to concentrations of these elements in the culture medium used. We found no evidence for the presence of nickel, cadmium, or chromium in any of our normal volunteers. Substantial accumulation of these elements may require special occupational exposure or lung disease (20).

Although the method of tissue preparation in this study is not ideal for the preservation and immobilization of chemical elements (21, 22), it is the only one applicable for the correlation of chemical composition to cell organelle. The localization of the elemental spectra to a specific part of the cell or organelle in SEM and STEM delineates the heterogeneity in concentration and distribution of elements within the cell; such is not possible in point analysis (23). Our data for the presence of aluminum and silica in lysosomes or granules are comparable to those obtained by others (14, 23).

The limit of the detection system is about 10^{-18} g so the possibility that these elements were present at lower levels cannot be excluded. Also, elements present in a soluble form are not measured in this system and some elements such as cadmium may be lost during fixation (24).

Our studies indicate that the elemental content of the human alveolar macrophage may be semiquantitatively analyzed with modern microanalysis technology. The use of the scanning transmission electron microscope in conjunction with emission spectroscopy permits elemental analysis of various subcellular structures. We have

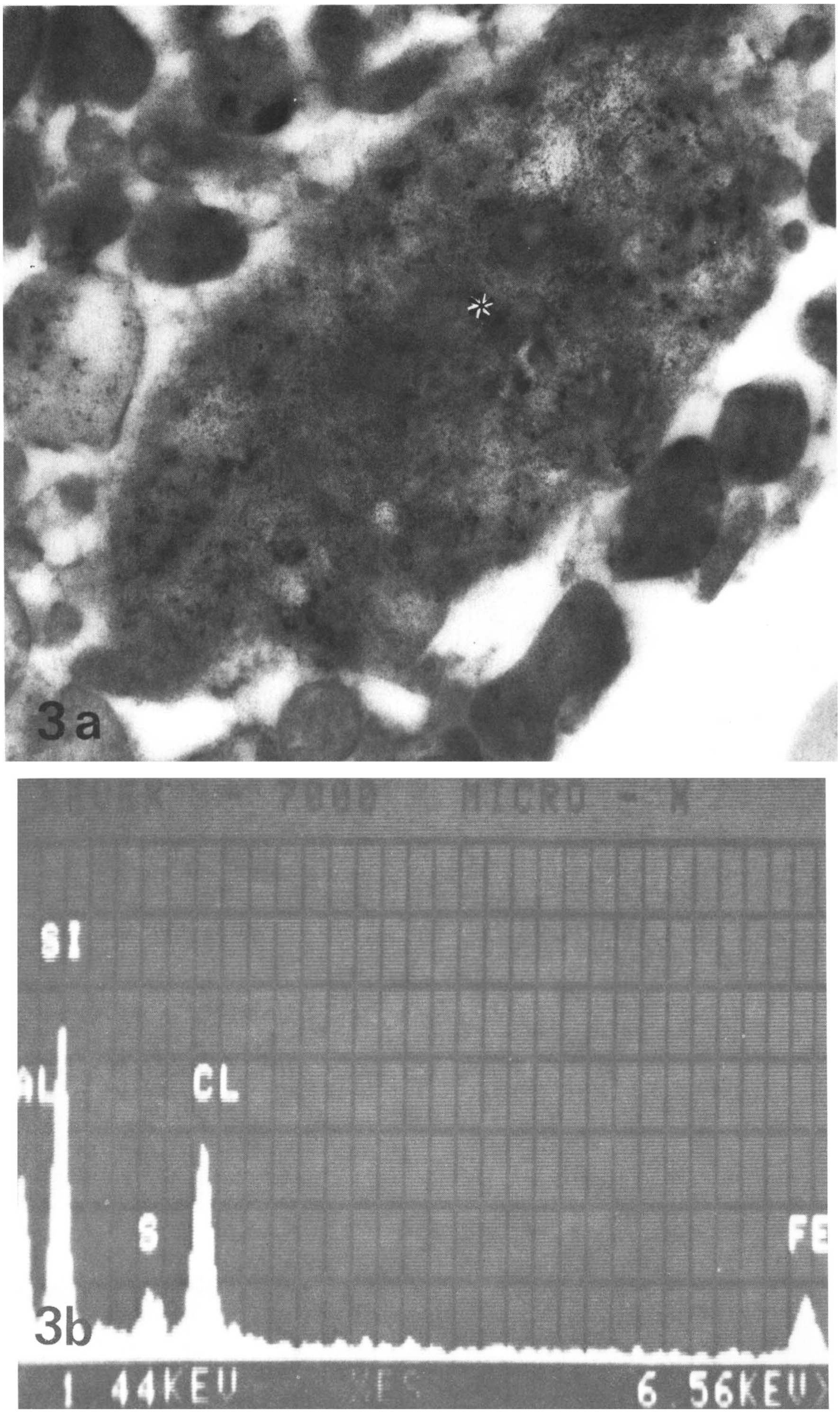


FIG. 3. STEM micrograph and X-ray energy spectrum of an alveolar macrophage granule from a smoker (a) (×38,000). Asterisk indicates area probed in the STEM and data are shown in (b). The full vertical scale is 512 counts.

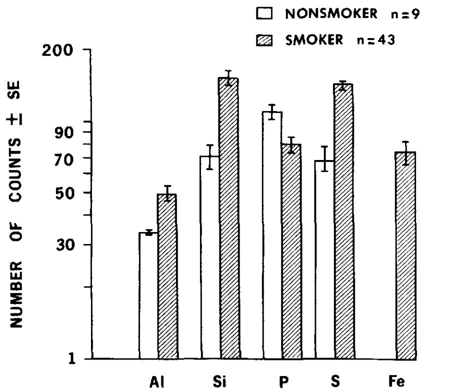


FIG. 4. Diagram of the difference in the number of counts between smokers and nonsmokers.

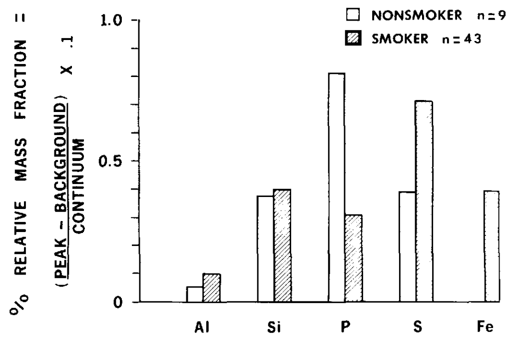


FIG. 5. Diagram of the percentage relative mass fraction of smokers and nonsmokers.

found significant quantities of silicon, iron, and sulfur in normal human alveolar macrophages and occasionally aluminum silicates were identified. The application of electron microprobe analysis in determining the elemental content of normal human alveolar macrophages may be a useful first step in quantitating industrial and nonindustrial exposure to environmental pollutants.

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