

## • Cigarette Smoke and Plutonium

Principal Investigator: R. E. Filipy

Other Investigators: W. J. Bair and R. L. Buschbom

Technical Assistance: R. F. Flores, K. E. Lauhala, and B. G. Moore

Autoradiographic techniques with liquid photographic emulsion and cellulose nitrate track-etch film are being used to investigate the spatial distribution of inhaled plutonium in the lungs of beagle dogs exposed to cigarette smoke or to the plutonium aerosol only. More plutonium than expected was detected on the inner surfaces of bronchi, and particles were observed beneath the bronchial mucosa.

The major objective of this project is to obtain experimental data on whether cigarette smokers are at a greater risk than nonsmokers to potential health effects of inhaled plutonium. Research efforts during the past year have been directed toward determining the effect of cigarette-smoke exposure on the spatial distribution of inhaled plutonium in the lungs of beagle dogs. Differences in deposition and/or retention sites of plutonium particles could result in irradiation of various cell populations within the lung, some of which are more susceptible to malignant transformation than others.

Beagle dogs were exposed to cigarette smoke and/or a plutonium aerosol, as shown in Table 1. The dogs of Groups 1 and 2 were exposed to cigarette smoke daily for approximately 6 months before Group 2 dogs received a single inhalation exposure to plutonium. Cigarette-smoke exposure of Groups 1 and 2 continued for approximately 400 days thereafter. Dogs of Group 3 were also exposed to the plutonium aerosol but received no smoke exposure either before or after the plutonium. On the last day of smoke exposure, the dogs were killed by exsanguination under deep anesthesia, and the lobes of their lungs were fixed individually by vascular perfusion with 1.5% buffered glutaraldehyde.

An autoradiographic technique, developed as part of this program, was used to determine the distribution of plutonium particles in the major pulmonary airways of two lung lobes (left apical and cardiac) from five of the dogs, one dog from Group 1 and two dogs each from Group 2 and 3. The technique was described in a previous report (1983). Briefly, it involved stripping the parenchyma from the airways to the approximate level of the tertiary bronchi, splitting segments of the airways, and pressing the inside surface of each segment against cellulose nitrate track-etch film for 6 weeks. At the end of that time, the pieces of film were

TABLE 1. Protocol to Determine the Effect of Cigarette-Smoke Exposure on Clearance of Plutonium from the Lungs of Beagle Dogs.

| Group | Number of Dogs | Exposure                     |
|-------|----------------|------------------------------|
| 1     | 6              | Smoke Only                   |
| 2     | 6              | $^{239}\text{PuO}_2$ + Smoke |
| 3     | 6              | $^{239}\text{PuO}_2$ Only    |

etched in a 4N NaOH solution at 60°C for 150 minutes and mounted on a glass microscope slide. An example of the exposed and etched film is shown in Figure 1. The number of films (usually, approximately 2 x 2 cm) per bronchial tree varied between 20 and 35, depending on the length of airway that could be isolated from parenchyma.

As a first approach toward quantifying the amount of plutonium detected on the inside surfaces of the pulmonary airways, the films were subjectively graded according to the density of alpha-particle-induced etched pores (tracks) observed by low-power microscopy. A grade of "1" was assigned if only a few alpha tracks were observed; a grade of "5" was assigned to films containing the greatest concentration of tracks. In order to compare the spatial distribution of plutonium within the lung lobes of the five dogs, the airway segments were categorized as follows:

1. primary bronchus only,
2. main lobar bronchi (except the terminal segment) after the first branch,
3. segments of bronchial branches between the terminal segment and the main bronchus,
4. terminal segments of the main bronchi and of the bronchial branches.



FIGURE 1. Photomicrograph of Track-Etch Film to Which a Plutonium Concentration Grade of "2" was Assigned.

Terminal segments were approximately 2 mm in diameter, the smallest size that could be isolated from the parenchyma and split for compression against film.

Average grades assigned to categories of bronchial-tree segments from the left apical and cardiac lung lobes of four plutonium-exposed dogs are listed in Table 2. The data indicate that the smallest bronchi contained the greatest concentrations of plutonium particles, and the main bronchi contained only small concentrations. There is no apparent relationship between particle concentration and lung clearance or exposure to cigarette smoke. The values for Dog #1700 are artificially low, especially for Category 4, because the entire cardiac lobe had very low bronchial concentrations of plutonium. If only the apical lobe was considered,

the values in Category 4 and the average grade would be very similar to the corresponding values for the other three dogs. The bronchi of Dog #1736 (cigarette smoke only) contained no detectable radioactivity.

Liquid-emulsion, cross-sectional autoradiographs were made from small portions of each bronchial segment removed before the track-etch film technique was applied. Figure 2, a photomicrograph of one of those sections, shows plutonium particles detected within the lamina propria of a bronchus. Several such instances have been observed in preliminary examination of these autoradiographs. Frequently, the particles were close enough to the epithelial layer so that basal epithelial cells were within range of the alpha radiation. The phenomenon of insoluble particles beneath the mucosa of pulmonary airways has been reported previously in the scientific literature. Speculations about their entry range from penetration through the mucosa to penetration from the parenchymal side of the wall. We cannot deduce the mode of entry from the autoradiographs, nor even whether the particles were contained within macrophages.

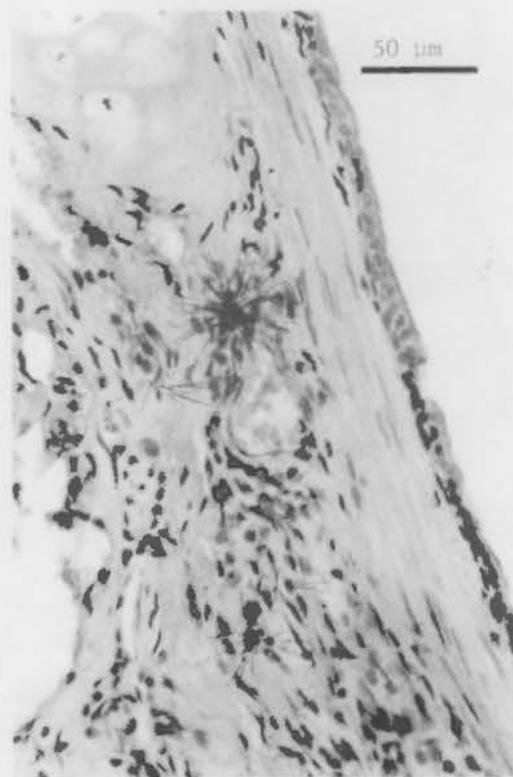
Two aspects of the autoradiographic results are of radiological interest: 1) the high concentrations of plutonium particles observed on bronchial surfaces 400 days after a single exposure and 2) the presence of plutonium particles beneath the bronchial mucosa in the lamina propria. These factors are of interest because the basal, dividing cells of the mucosa, which are highly susceptible to malignant transformation, are within range of alpha radiation from particles in both locations.

Future research efforts will be directed toward quantifying of the plutonium contained in the pulmonary airways. One approach will be to make standard track-etch autoradiograms from surfaces, such as filters, containing known amounts of plutonium particles of respirable size. An effort will then be made to compare the bronchial autoradiograms with the standards, using an image-analyzing computer. Also, lungs from the remaining four dogs per group will be processed for track-etch autoradiography.

**TABLE 2.** Average of the Subjective Grades of Alpha Activity Assigned to Track-Etch Film Pressed Against the Inner Surfaces of Bronchi of Plutonium-Exposed Beagle Dogs.

| Dog No. | Group | Plutonium Lung Burden |             | Subjective Grade (0-5) |     |     |     | Average of All Segments |
|---------|-------|-----------------------|-------------|------------------------|-----|-----|-----|-------------------------|
|         |       | Initial, nCi          | At Death, % | Segment of Bronchus    |     |     |     |                         |
|         |       |                       |             | 1                      | 2   | 3   | 4   |                         |
| 1736    | 1     | --                    | --          | 0                      | 0   | 0   | 0   | 0                       |
| 1700    | 2     | 915                   | 80.3        | 0.5                    | 0.7 | 1.8 | 1.2 | 1.0                     |
| 1722    | 2     | 1150                  | 86.5        | 0                      | 0.3 | 1.8 | 2.2 | 1.4                     |
| 1668    | 3     | 1340                  | 67.2        | 0                      | 0.9 | 1.5 | 1.9 | 1.4                     |
| 1733    | 3     | 1460                  | 68.5        | -(a)                   | 1.1 | 2.6 | 2.6 | 2.2                     |

(a) No sample of primary bronchus



**FIGURE 2.** Photomicrograph of a Liquid Photographic Emulsion Autoradiograph of Bronchial Wall from a Beagle Dog Exposed to  $^{239}\text{PuO}_2$ .