DOE/ER/60654-T3

FINAL TECHNICAL REPORT

DE-FG05-88ER60654

**MORPHOMETRY FOR ALPHA PARTICLE HITS
OF CRITICAL TARGETS IN THE LUNGS**

ROBERT R. MERCER¹

OVERVIEW	Page 2
BACKGROUND	Page 2
RESULTS	Page 2
The size of airway cell nuclei	Page 4
The position of airway nuclei	Page 4
Frequency of critical target cells	Page 6
Total number of critical target cells	Page 8
Mucous lining layers	Page 8
Alpha Particle Tracks	Page 10
SIGNIFICANT SCIENTIFIC ACCOMPLISHMENTS	Page 13
PUBLICATIONS RESULTING FROM PROJECT	Page 14
REFERENCES	Page 15
APPENDIX	Page 20

¹ Current Address:
Robert R. Mercer, Ph.D.
NIOSH
1095 Willowdale Road
Morgantown, WV 26505

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

MASTER

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

**Portions of this document may be illegible
electronic image products. Images are
produced from the best available original
document.**

OVERVIEW

The objective of this study is to provide detailed data on the number, location and type of critical target cells in the airspaces and to use these data in order to make risk assessments of the health effects of radon and radon progeny in the lungs. This will be done by quantitative morphometric study of the distribution of the various cell types and mucous lining layers in the lungs. Our results provide anatomically correct models for dosimetry in the rat and human airways which significantly improve the ability to do risk assessment for radon exposures by providing quantitative data for specific cell types and provide a basis for mechanism based comparison between data available in animal exposures and human epidemiology.

BACKGROUND

Since the recognition of human health risks from radon exposure at high levels, significant progress has been made toward the goals of evaluating the risks from low level exposures and in understanding the mechanisms for radon induced lung cancers. These have include the formation of extensive epidemiological data bases describing lung cancer risks versus exposure in mine workers (Ellett, 1987), postmortem analysis of the distribution and types of lung cancers present in this population (Archer et al, 1974), exposures of radon and radon progeny to laboratory rats (Chameaud et al, 1974, 1984, Cross et al, 1984), hamsters (Cross et al, 1981) and beagle dogs (Cross et al, 1982), empirical and analytic analysis of exposure and deposition resulting from exposure to radon and its progeny (Cohen et al, 1980, Cohen, 1987, Cohen et al, 1988) (Hofmann et al 1990), morphologic analysis of airways specifically directed to define the location and number of critical targets for radon (Gastineau et al, 1972, Mercer et al, 1991), development of models to determine dose and cancer risks as a function of the level of exposure (Simmons and Richards, 1984, James, 1988, Hui et al, 1990, Hofmann et al, 1990), implant analysis of expression of neoplastic and preneoplastic cells (Gillett et al, 1989), development of technologies to produce specific numbers and types of alpha particle traversals of individual cell nuclei and analysis of the genetic alterations, neoplastic transformations and cell death in tissue cultures following alpha particle irradiation (Raju et al, 1991).

RESULTS

In our study, the central objective was to provide detailed data on airway cell structure necessary for assessing the effects of radon and radon progeny in the lungs. To attain this goal, we have determined the diameter of all major types of airway cell nuclei, the position of airway cell nuclei, airway surface area, the frequency and total number of airway epithelial cells, the distribution and thickness of mucous lining layers and the size and number of alveoli by precise, state of the art morphometric techniques. These provide a rigorous anatomic basis by which the dose to critical cell nuclei of human and rat lungs may be determined.

1. **The size of different airway cell nuclei has been accurately determined.** Previous dosimetry models for radon have not included the differences between nuclear size of different cells types. In some cases even the assumed value of nuclear diameter differs significantly from those obtained by direct measurements (2 fold). Our studies have determined that nuclei of human bronchi are larger and more densely packed than rat airway cell nuclei. Cell deaths and transformations from alpha particle irradiation are more likely in human bronchi because nuclei of human airway cells are larger targets for alpha particle tracks.

2. **Morphometric determination of cell nuclear position in human and rat airway epithelium.** These data demonstrate that basal and secretory cell nuclei in human bronchi are within range of alpha particle emissions from radon and radon progeny and account for a significant percentage of the interactions which alpha particle have with airway cell nuclei. In human bronchi, the dose to secretory cell nuclei is two (P^{214}) to six (P^{218}) times that of basal cell nuclei.

3. **Development and use of methods to determine the frequency and total number of epithelial cells in human and rat airways.** Prior to this study, the frequency and total cell number were only available for the alveolar region of the lungs. We have determined both the frequency and total number of epithelial cells. While secretory cells have a higher dose, secretory cells are rare in human bronchi. Basal cells are ten times more numerous than secretory cells in human bronchi. The arrangement, thickness and cell types populating the airways of the rat differ significantly from human airways. Secretory cells other than goblet cells are a dominate cell type in all airways of the rat, but account for less than 3 percent in human intrapulmonary bronchi.

4. **The first direct measurement of airway surface area in human and rat lungs.** Prior estimates of airway surface area have been based on indirect calculations from incomplete evaluations of airway casts models of the lungs. In this study we accurately determined airway surface area by state of the art morphometric analysis. The results indicate that prior estimates have significantly over-estimated airway surface area. Airway surface area is a critical factor in determination of cell number and dose from deposition. These measurements will significantly improve the uncertainties in assessment of health risks from exposure to radon and radon progeny.

5. **Preservation and analysis of the distribution and thickness of mucous lining layers in human and rat airways.** In this study, we have developed methods to preserve the mucous lining layers of the lungs and applied morphometric analysis to determine the distribution and thickness. The measured values of mucous thickness (approximately 8 microns for human bronchi and 3 microns for rat bronchi) are significantly lower than the values (about 15 microns) which have been assumed in previous models.

6. **The arrangement of cells within the lungs has been determined.** To carry out accurate microdosimetric calculations for critical cell targets in the lungs, a detailed description of the arrangement and number of cells has been made. This analysis of airway cells has allowed us to calculate the number of cell nuclei which will be hit by one, two or more alpha particle tracks and plays an important factor in understanding the mechanisms which may be responsible for tumor generation by radionuclides. A similar series of data analysis has been carried out for the alveolar region of the lungs. The alveolus of the human lung has a diameter which is 2.5 fold larger than that of the rat and contains 4 fold more cells. Accurate prediction of dose based on the configuration of alveoli and cell nuclei in lung parenchyma is important as alveolar type II cell may be a progenitor for pulmonary tumors (Shen et al, 1989).

In the following a brief overview of these studies is given.

The size of airway cell nuclei

Figure 1 Volume of cell nuclei in the human lung.

The diameter of human airway cell nuclei is approximately 30% larger than those of the rat, i.e., the nuclei of human airway cells are larger targets for alpha particle tracks. In addition we note that these results differ considerably from the values "assumed" by previous investigators. For instance, Hui et al (1990) used a diameter of 4.6 μm for the nuclei of secretory and progenitor cells in a microdosimetric model study of radon decay products in the respiratory tract. This is significantly less than the average diameter of 8.4 μm which we have found for airway epithelial cell nuclei in human bronchi.

The position of airway nuclei

The depth of the cell and the cell nucleus within the epithelium is critically important in determining how the differences in cell composition influence the cell type(s) which are most sensitive to radon induced damage in the airway generations of the lung. To allow this depth-dose calculation to be accurately made for the various cell types and cell nuclei, we have broken the airway epithelium into a series of layers of increasing distance from the surface of the airway epithelium. A stereologic procedure, described in Appendix A was devised to determine the relative proportions of the cell body and cell nucleus for each cell type as a function of depth from the luminal surface of the airway epithelium. By these measurements the effects of depth in the dose calculations may be included and cell specific sensitivities to radon induced damage determined.

In addition we have carried out three dimensional reconstructions of cells and cell nuclei to determine the variability in the position of individual cell nuclei. A histogram of the average depth of the center of the cell nucleus from the epithelial surface for various airway cell nuclei is given in the following figure.

Figure 2 Histograms of percent of cells centered at different depths from epithelial surface.

As given in Figure 2, the average depths of 19.7, 24.9 and 32.6 μm for ciliated, secretory-goblet and basal cells are all within the range of alpha particle emissions from radon and its progeny. It should also be noted that 90% of the basal cell nuclei are within 40 μm of the epithelial surface. Given this measurement and the relatively thin mucous lining layer present on the epithelial surface, essentially all basal cell nuclei are within range of radon and its progeny. Data in Figure 2 is currently being incorporated into microdosimetric calculations by Dr. Hofmann.

Based on the studies of Lloyd et al (1979) it appears that 60 rad is the mean dose to the cell nucleus necessary to kill a mammalian cell. From the depths for ciliated, secretory-goblet and basal cell nuclei and data on nuclear diameter given in the figure, it can be calculated that a single alpha particle emission (^{214}Po or ^{218}Po) which traverses the entire nucleus will exceed the 60 rad mean lethal dose. In the proposed study we will determine the fraction of alpha particle tracks which traverse a sufficient distance of the nucleus to exceed the 60 rad mean lethal dose using calculations similar to those described by Rossi and Rosenzweig (1955). When these computations are complete we will be able to determine the significance of cell death at different levels of exposure to radon and radon progeny. These results would also be useful as a guide to the appropriate survival fraction in alpha particle irradiation of cell cultures which seek to emulate the in-vivo conditions of the epithelium.

Collectively these data on the position and size of cell nuclei allow a precise determination of the average dose to specific cell types as given for basal and secretory cells of human and rat bronchi in the following table.

PREDICTED ABSORBED DOSE FOR BRONCHI*			
		HUMAN	RAT
BASAL	p ²¹⁴	78	200
	p ²¹⁸	22	222
SECRETORY	p ²¹⁴	151	237
	p ²¹⁸	149	232

* Dose in nGy for a 1 dpm per cm² source.

These dosimetry calculations based on the thickness of the epithelium and mucous lining layer as well as the measurements of the position of the cell nuclei indicate that secretory cells receive a high absorbed dose in proximal bronchi of humans and all intrapulmonary airways of rats. However, from data on the frequency of airway cells, we know that secretory cells are rare in the proximal bronchi of human airways while they represent approximately 50% of the population in rat bronchioles. It is unlikely that comparable lifetime lung tumor risks could be attributable to one cell type with such disparate differences in the types and number of cells at risk in the critical sites.

Frequency of critical target cells

Morphometric procedures were used to determine the density of airway cell nuclei in human and rat lungs. The number of cells per unit area of epithelial basement membrane are given for the rat and human airways in the following two figures. The stratified nature of the human epithelium is evident in the data for bronchi as it demonstrates a significantly higher number of total cells than the simple, single cell layer present in the bronchioles. Bronchi and bronchioles of the rat are of the simple, single cell layer.

Figure 3 The number of human airway cell nuclei per mm^2 of basement membrane.

Figure 4 The number of rat airway cell nuclei per mm^2 of epithelial basement membrane.

Secretory cells other than goblet cells are a dominate cell type in all airways of the rat but account for less than 3 percent of the cells in intrapulmonary bronchi of the human lung. On the other hand, cell types populating bronchioles are similar in both species.

Total number of critical target cells

From the measurements of cell density (number per unit area) and airway surface area the total number of airway cells in bronchi and bronchioles of human and rat lungs were determined (Figure 5). The results given in the following figure demonstrate significant differences in the number of basal and secretory cells in rat and human airways.

Figure 5 Log plot of airway cell number in human and rat lungs.

In determination of the number of cells in the airways of human and rat lungs we have found that there are 250 times more airway cells in the human. The total number of airway epithelial cells were 20.8×10^9 for humans and 0.11×10^9 for rat lungs. In comparison to the alveolar region there are 10 times more alveolar cells than bronchial epithelial cells (Stone et al, 1992). Of these cells approximately 35% are basal and secretory cells. As these are the progenitors for other airway cells they constitute the critical target cells for cancer induction. Thus, the airways of the human lung contain approximately 250 fold more critical targets for cancer induction by radon and radon progeny. In the first four airway generations distal to the trachea, basal cells account for 30 % of the cells in human airway epithelium and 2% of the cells in rat airway epithelium. The smaller nuclei of human bronchioles are comparable to those of alveolar cells. In the pseudostratified epithelium of human bronchi, basal cells have a large surface area in contact with the basement membrane ($51.3 \pm 4.6 \mu\text{m}^2$) when compared to ciliated ($1.1 \pm 0.1 \mu\text{m}^2$), goblet ($7.6 \pm 1.2 \mu\text{m}^2$) or other secretory cells ($12.0 \pm 2.1 \mu\text{m}^2$).

Mucous lining layers

It has been long known that the lung clears or removes inhaled particulate matter by means of a muco-ciliary escalator mechanism. Perhaps the first clearly definitive work on this was that of Lucas and Douglas (1934), who proposed a 2-layer model of the material lining the airways. In such a model there is an underlying layer in which the cilia beat (sol layer) with a second viscous layer (gel layer) forming a blanket over the sol layer. Whether there is a continuous gel layer throughout

the airways is a matter of debate. For instance, Luchtel (1978), Ressig et al (1978) and Yoneda (1978) all found evidence for a continuous layer in nasal epithelium and upper airways while studies focusing on more distal airways have not (Gil and Weibel, 1971, Van As 1974).

Methods for preservation of the mucous lining layer have included: direct visual observation on dissected airway specimens (Adler et al, 1973, Iravani and Van As, 1971), fixation by immersion (Sturgess, 1977, Ressig et al, 1978), vascular perfusion fixation (Gil and Weibel, 1971, Luchtel, 1978), quick freezing (Van As and Webster, 1974, Yager et al, 1991) and osmium tetroxide vapor fixation (Yoneda, 1976). Of these different methods of preservation the use of vascular perfusion of fixative is the most generally applicable as it eliminates mechanical disruption due to immersion or airway instillation of fixative by using the extensive capillary bed of the lungs to place the fixative in the immediate vicinity of the fluid lining layers and has been shown to significantly improve the preservation of mucous lining layers in the airways (Gil and Weibel, 1971) and the surface active film of the alveolar region (Weibel and Gil, 1968).

In spite of the large number of studies cited, a quantitative description of the depth, extent and composition of the mucous lining layers throughout the lungs has not been available for any of the fixation techniques. This information is critical for understanding normal airways function, evaluation of the results of bronchio-alveolar lavage and in risk assessment of health effects resulting from inhalation of radon and radon progeny. In this study we have applied the improved vascular perfusion fixation technique in a quantitative analysis of the thickness and distribution of mucous lining layers throughout the airways of rat and human lungs. Results of these studies are described in detail in Appendix F.

Alpha Particle Tracks

Previous radon dosimetry models of bronchial cells have assumed that each alpha particle traversing the airway epithelium will hit at most one cell nucleus. A further assumption has been that only single traversal of cell nuclei are significant at low levels of exposure. Based on this reasoning it has been concluded that multiple cell interactions, such as cell killing, are not important factors in lung cancer development from low levels of exposure. Our results indicate that neither of these assumptions is correct.

The effect of cell stratification on the number of nuclei traversed by each alpha particle through the epithelium is illustrated in the schematic of alpha particle tracks through a human bronchus given in the following figure.

Figure 6 Diagram of different kinds of multi-cell traversals typical of alpha particle passage through a human bronchus.

A single alpha particle which makes a complete passage through the nucleus may deposit enough energy to kill the cell. When multiple cells are traversed from a single alpha particle, it is likely that some of cells will be killed as a result of complete passage, while other neighboring cells will receive a partial hit and survive the injury. Thus even at low levels of exposure, cell killing and other interactions between injured cells will be frequent events.

These differences between the stratified, multi-cell layer of the human epithelium and the rat airways or other simplified models of the airway epithelium have not been taken into consideration in evaluating the health consequences for radon inhalation. For instance, in human bronchi our preliminary analysis of alpha particle tracks in three dimensionally reconstructed cells indicates that 55% of the alpha particle paths traverse 2 or more nuclei (on average 1.6 cell nuclei are hit by an alpha particle which traverses the epithelium). Less than 8% of the alpha particle paths traverse 2 or more nuclei in the rat (on average 0.8 cell nuclei are hit by an alpha particle which traverses the epithelium).

A three dimensional histogram of this preliminary data is given in Figure 7. This figure demonstrates the distribution of nuclei which the alpha particle first encounters on one axis (e.g. the nuclei which are traversed first) and the distribution of the second nuclei encountered on the other

axis. The height of each box corresponds to the joint frequency of the pair of events. As might be expected from the arrangement of cells in bronchi, the most common event is for an alpha particle to traverse the nucleus of a ciliated cell (which are common and have nuclei close to the surface) and a second traversal of a basal cell nucleus (which are also common and have a more deeply located nucleus). Secretory cells other than goblet cells were too infrequent to be included in this analysis but will be measured in the more extensive proposed study.

FREQUENCY OF NUCLEI HIT BY AN α PARTICLE TRACK

Figure 7 Frequency of multiple nuclear traversals by a single alpha particle in human bronchi.

Combining these results with the previous data on cell killing suggests that half of the alpha particle tracks through the airway epithelium of the humans lungs will generate a pair of injuries in the nuclei of adjacent cells. For a significant fraction of these, one of the nuclei will receive a relatively low dose (less than 60 rad) while the other will receive a cytotoxic dose. Since cytogenetic effects have been reported for doses as low as 0.5 rad (Lloyd et al, 1979, Hieber et al, 1987, Nagasawa et al, 1990), it is predicted that one of the injuries will be a non-lethal nuclear transformation and the other will result in cell death. These results suggest a spatial association between cell killing and preneoplastic transformation at low levels of exposure. It is important to note that this spatial association is principally due to the stratified nature of the human airway epithelium and does not apply to rat airways.

Are there a significant number of critical cell nuclei traversed by two or more alpha particles at low levels of exposure to radon and its progeny?

In the following we will provide an answer to this question based on our measurements of the total number of cells, airway surface area and the probability that an alpha particle will traverse a critical cell nucleus. The specific methods section contains a more rigorous derivations of these calculations and describes the necessary morphometric measurements which will be undertaken in the proposed study.

Based on our measurements approximately 60% of the alpha particle tracks in a bronchus will traverse a basal cell. For a 1 WLM exposure there will be approximately 30,000 alpha particle emissions per cm^2 of bronchus (Harley, 1988). Given that the surface area of human bronchi is $1,300 \text{ cm}^2$, there will be a total of 39×10^6 emissions. Thus approximately 1% of the basal cells in human bronchi (23.4×10^6) will be traversed by an alpha particle emission for a 1 WLM exposure.

Making the assumption that the first and second hits are independent, approximately 0.01% of the basal cells will be traversed by two alpha particles or 234,000 basal cells. Thus the number of double hit cell nuclei per human subject exposed to 1 WLM is on the order of 200,000. This is approximately the same as the number of cells are typically used in cell culture studies of neoplastic transformation (Raju et al, 1991). If we assume that the transition probability for each traversal is 2×10^{-5} then approximately 1 in 10,000 exposed subjects would produce a cancerous lesion. The cancer risk based on the number of nuclei traversed by two alpha particles and a transition probability similar to that measured in cell culture systems gives estimates of cancer risk comparable to those of underground miners and exposures of experimental animals. While these calculations do not address all the issues of this complex subject, they do demonstrate that **a significant number of cell nuclei are traversed by two or more alpha particles at low levels of exposure to radon and radon progeny.**

SIGNIFICANT SCIENTIFIC ACCOMPLISHMENTS

From our studies we have determined the number of cell nuclei which are the critical targets for radon induced lung cancer. The human lung contains approximately 11 billion airway cell nuclei which is 170 fold more than present in rat airways. In both human and rat airways, approximately 40 percent of the cells are possible targets for radon induced lung cancer.

From our studies of the paths taken by α particles released by decay of radon and radon progeny in airways, we have concluded that there are fundamental differences between alpha particle traversals of nuclei in large and small airways of the human lung. The densely packed cells of large airways result in several neighboring airway cell nuclei being hit by each alpha particle emission. In small airways α particle tracks on average traverse only one nucleus. The possibility for interactions between neighboring airways cells hit by alpha particle emissions in large airways may account for the prevalence of large airway cancer induction found in humans.

The majority of the radiation delivered to the lungs is by radon progeny attached to submicron particles. In order to determine the specific sites where the radiation is delivered we have studied the pattern of deposition and clearance of submicron particles in the lungs. These studies have demonstrated that the particles deposit in dense concentrations at the ridge of airway bifurcations. In addition, the deposited particles are slowly removed from these sites. From these results we have concluded that airway cells located on the ridge of airway bifurcations receive a dose which is several orders of magnitude higher than in other regions of the airways.

PUBLICATIONS RESULTING FROM PROJECT

Mercer RR, ML Russell and JD Crapo. Radon dosimetry based on the depth distribution of nuclei in human and rat airways. *Health Physics* 61:117-130, 1991.

Mercer RR, S Anjilvel, FJ Miller and JD Crapo. Inhomogeneity of ventilatory unit volume and its effects on reactive gas uptake. *J Appl Physiol* 70:2193-2205, 1991.

Stone KC, RR Mercer, BA Freeman and JD Crapo. Distribution of lung cell numbers and volumes between alveolar and nonalveolar tissues. *Am Rev Respir Dis* 146:454-456, 1992.

Mercer RR and JD Crapo. Three-dimensional analysis of lung structure and its application to pulmonary dosimetry models. In: *Toxicology of the Lung*, 2nd ed., DE Gardner, JD Crapo and EJ Massaro, eds. Raven Press, New York, 1993.

Pinkerton KE, JT Gallen, RR Mercer, VC Wong, CG Plopper and BK Tarkington. Aerosolized fluorescent microspheres detected in the lung using confocal scanning laser microscopy. *Micro Res Tech* 26:437-443, 1993.

Mercer RR, ML Russell, VL Roggli and JD Crapo. Cell number and distribution in human and rat airways. *Am J Respir Cell Mol Biol* 10:613-624, 1994.

Mercer RR, ML Russell and JD Crapo. Alveolar septal structure in different species. *J Appl Physiol* 77:1060-1066, 1994.

Koblinger L, W Hofmann, RC Graham and RR Mercer. Aerosol inhalation in the rat lung. *J Aerosol Med* 8:7-19, 1995.

Mercer RR, DL Costa and JD Crapo. Effects of prolonged exposure to low doses of nitric oxide or nitrogen dioxide on alveolar septa of the adult rat lung. *Lab Invest* 73:20-28, 1995.

REFERENCES

- Adler KB, O Wooten and MJ Dulfano. Mammalian respiratory mucociliary clearance. *Arch Environ Health* 27:364-369, 1973.
- Altshuler, B., N. Nelson, and M. Kuschner. Estimation of lung tissue dose from the inhalation of radon and daughters. *Health Phys.* 10:1137-1161, 1964.
- Archer, VE, G Saccomanno and JH Jones. Frequency of different types of bronchogenic carcinoma as related to radiation exposure. *Cancer* 34:2056-2060, 1974.
- Chameaud J, R Perraud, J LaFuma, R Masse and J Pradel. Lesions and lung cancers induced in rats by inhaled radon 222 at various equilibriums with radon daughters. Chapter in: Experimental Lung Cancer: Carcinogenesis and Bioassays. Karbe E and JF Park eds. Springer-Verlag, New York pp 411-421, 1974.
- Chameaud, J, R Masse and J Lafuma. Influence of radon daughter exposure at low doses on occurrence of lung cancer in rats. *Radiat Prot Dos* 7:385-388, 1984.
- Chang, L-Y, RR Mercer, and JD Crapo. Differential distribution of brush cells in rat lung. *Anat. Rec.* 216:49-54; 1986.
- Cohen, BS, Eisenbud, M and Harley NH. Measurement of the α -radioactivity on the mucosal surface of the human bronchial tree. *Health Phys* 39:619-632, 1980.
- Cohen BS. Deposition of ultrafine particles in the human tracheobronchial tree: a determinant of the dose from radon daughters. In: *Radon and its Decay Products, Occurrence, Properties and Health Effects*. PK Hope (ed) pp. 475-486. American Chemical Society, Washington, D.C., 1987.
- Cohen, BS, Harley NH, Schlesinger RB and M Lippmann. Nonuniform particle deposition on tracheobronchial airways: Implications for lung dosimetry *Ann Occup Hyp* 32:1045-1053, 1988.
- Crapo, JD, SL Young, EK Fram, KE Pinkerton, BE Barry, and RO Crapo. 1983. Morphometric characteristics of cells in the alveolar region of mammalian lungs. *Am. Rev. Respir. Dis.* 128:S42-S46.
- Crawford-Brown, DJ Dosimetry. In: Cotheran, C.R. and J.E. Smith, J.E., eds. *Environmental radon*. New York: Plenum Press; 1987:173-213.
- Crawford-Brown, DJ and W Hofmann. A generalized state-vector model for radiation-induced cellular transformation. *Int. J. Radiat. Biol.* 57:407-423, 1990.

Cross FT, RF Palmer, RH Busch, RE Filipy and BO Stuart. Development of lesions in syrian golden hamsters following exposure to radon daughters and uranium ore dust. *Health Phys* 41:135-153, 1981.

Cross FT, Palmer RF, RE Filipy, GE Dagle and BO Stuart. Carcinogenic effects of radon daughters, uranium ore dust and cigarette smoke in beagle dogs. *Health Phys* 42:33-52, 1982.

Cross FF, RF Palmer, GE Dagle, RH Busch and RL Buschbom. Influence of radon daughter exposure rate, unattachment fraction, and disequilibrium on occurrence of lung tumors. *Radiat Prot Dos* 7:381-384, 1984.

Ellett, WH Epidemiology and risk assessment: Testing models for radon-induced lung cancer. In: Gammage, RB and SV Kaye, eds. *Indoor air and human health*. Maine: Lewis Publishers, Inc.; 1987:79-107.

Gastineau, RM, Walsh PJ and N Underwood. Thickness of bronchial epithelium with relation to exposure to radon. *Health Phys* 23:857-860, 1972.

Gil J and ER Weibel. Extracellular lining of bronchioles after perfusion fixation of rat lungs for electron microscopy. *Anat Rec* 169:185-200, 1971.

Gillett, NA, DG Thomassen, A Buchholz and G Kelly. Cell-line-dependent inhibition of phenotypic expression of preneoplastic and neoplastic tracheal epithelial cells by normal tracheal cells in denuded tracheal transplants. *Carcinogenesis* 10:1147-1154, 1989.

Harley, NH and BS Pasternack. Alpha absorption measurements applied to lung dose from radon daughters. *Health Phys.* 23:771-782; 1972.

Harley, NH. Interaction of α particles with bronchial cells. *Health Phys.* 55:665-669; 1988.

Hieber, L, G Ponsel, H Ross, S Fenn, E Fromke, and A M Kellerer. Absence of a dose-rate effect in the transformation of C3H 10T1/2 cells by α -particles. *Int. J. Radiat. Biol.* 52:859-869, 1987.

Hofmann, W Cellular lung dosimetry for inhaled radon decay products as a base for radiation-induced lung cancer risk assessment. II. microdosimetric calculations. *Radiat. Environ. Biophys.* 20:113-122; 1982.

Hofmann W, TB Martonen and MG Menache. A dosimetric model for localized radon progeny accumulations at tracheobronchial bifurcations. *Rad. Prot. Dosim.* 30:245-259, 1990.

Hui, TE, JW Poston, and DR Fisher. The microdosimetry of radon decay products in the respiratory tract. *Radiat. Prot. Dosi.* 31:405-411; 1990.

Iravani J and A Van As. Mucus transport in the tracheobronchial tree of normal and bronchitic rats. *J Path* 106:81-93, 1972.

Jacobi, W. The dose to the human respiratory tract by inhalation of short-lived ^{222}Rn and ^{220}Rn -decay products. *Health Phys.* 10:1163-1174; 1964.

James, AC. Lung Dosimetry. In: Nazaroff, WW and AV Nero Jr. eds. *Radon and Its Decay Products in Indoor Air*. New York: John Wiley and Sons Inc., pp. 259-309, 1988.

Kalina M and DC Pease. The preservation of ultrastructure in saturated phosphatidyl cholines by tannic acid in model systems and type II pneumocytes. *J Cell Biol* 74:726-741, 1977.

Lloyd, EL, MA Gemmell, CB Henning, DS Gemmell and BJ Zabransky. Cell survival following multi-track alpha particle irradiation. *Int J Radiat Biol* 35:23-31, 1979.

Lloyd, EL, MA Gemmell, CB Henning, DS Gemmell and BJ Zabransky. Transformation of mammalian cells by alpha particles. *Int. J. Radiat. Biol.* 36:467-478, 1979.

Lucas AM and LC Douglas. Principles underlying ciliary activity in the respiratory tract: II. A comparison of nasal clearance in man, monkey and other Mammals. *Arch Otolaryng* 20:518-541, 1934.

Luchtel DL. Ultrastructural observations on the mucous layer in pulmonary airways. *J Cell Biol* 70:350A, 1976.

Luchtel DL. The mucous layer of the trachea and major bronchi in the rat. *SEM* 2:1089-1095, 1978.

Mercer, RR, JM Laco, and JD Crapo. Three-dimensional reconstruction of alveoli in the rat lung for determination of pressure-volume relationships. *J. Appl. Physiol.* 62:1480-1487, 1987.

Mercer, RR and JD Crapo. Structure of the gas exchange region of the lungs determined by three-dimensional reconstructions. Chapter in **Toxicology of the Lung**, D.E. Gardner, J.D. Crapo and E.J. Massaro eds. Raven Press, New York, 1988.

Mercer RR, SH Randell and SL Young. Measurement of structures using a digitizer tablet to enter boundaries. *J Microscopy*, 160:97-105, 1990a.

Mercer RR, GM McCauley and S Anjilvel. Approximation of surfaces in quantitative 3-D Reconstructions. *IEEE Trans BME* 37:1136-1146, 1990b.

Mercer RR and JD Crapo. The spatial distribution of collagen and elastin fibers in the lungs. *J Appl Physiol*, 69:756-765, 1990.

Mercer, RR, SH Randell, and SL Young. Measurement of structures using a digitizer tablet to enter boundaries, *J. Microscopy*. 160:97-105, 1990.

Mercer, RR, ML Russell, and JD Crapo. Radon dosimetry based on the depth distribution of nuclei in human and rat lungs, *Health Physics*. 61:117-130, 1991.

Nagasawa, H, JB Little, WC Inkret, S Carpenter, K Thompson, MR Raju, DJ Chen and GF Strniste. *Mutation Res.*, 244:233,238, 1990.

Nakamura, H, A Tonosaki, H Washioka, J Takahashi and S Yasui. Monomolecular surface film and tubular myelin figures of the pulmonary surfactant in hamster lung. *Cell Tissue Res*. 241:523-528, 1985.

Raju MR, Y Eisen, S Carpenter and WC Inkret. Radiobiology of α particles. III. Cell inactivation by α -particle traversals of the cell nucleus. *Radiat Res* 128:204-209, 1991.

Ressig M, BG Bang and FB Bang. Ultrastructure of the mucociliary interface in the nasal mucosa of the chicken. *Am Rev Resp Dis* 117:327-341, 1978.

Rossi HH and W Rosenzweig. A device for the measurement of dose as a function of specific ionization. *Radiology* 64:404-411, 1955.

Shen Z., C. Ye and D. Wu. Microdosimetry of rat alveolar type II cells irradiated with α particles from $^{239}\text{PuO}_2$. *Radiat Res* 120:234-250, 1989.

Simmons JA and SR Richards. Microdosimetry of alpha-irradiated lung. *Health Phys* 46:607-616, 1984.

Stone KC, RR Mercer, P Gehr, B Stockstill and JD Crapo. Allometric relationships of cell numbers and size in the mammalian lung. *Am J Respir Cell Mol Biol* 6:235-243, 1992.

Sturgess J. The mucous lining of major bronchi in the rabbit lung. *Am Rev Resp Dis* 115:819-827, 1977.

Thomas DC. A model for dose rate and duration of exposure effects in radiation carcinogenesis. *Environ Health Perspec* 87:163-171, 1990.

Thomassen DG, FA Seiler, LJ Shyr and WC Griffith. Alpha-particles induce preneoplastic transformation of rat tracheal epithelial cells in culture. *Int. J. Radiat. Biol.* 57:395-405, 1990.

Van As A and I Webster. The morphology of mucus in mammalian pulmonary airways. *Environ Res* 7:1-12, 1974.

Van As A. Pulmonary airway clearance mechanisms: a reappraisal. *Am Rev Resp Dis* 115:721-726, 1977.

Weibel ER and J Gil. Electron microscopic demonstration of an extracellular duplex lining layer of alveoli. *Respir Physiol* 4:42-57, 1968.

Weibel, ER *Morphometry of the human lung*. Academic Press, New York, 1963.

Yoneda K. Mucous blanket of rat bronchus: an ultrastructural study. *Am Rev Resp Dis* 114:837-842, 1976.

APPENDIX
Final Technical Report
DE-FG05-88ER60654

- A. Mercer RR, ML Russell and JD Crapo. Radon dosimetry based on the depth distribution of nuclei in human and rat airways. *Health Physics* 61:117-130, 1991.
- B. Mercer RR, S Anjilvel, FJ Miller and JD Crapo. Inhomogeneity of ventilatory unit volume and its effects on reactive gas uptake. *J Appl Physiol* 70:2193-2205, 1991.
- C. Stone KC, RR Mercer, BA Freeman and JD Crapo. Distribution of lung cell numbers and volumes between alveolar and nonalveolar tissues. *Am Rev Respir Dis* 146:454-456, 1992.
- D. Mercer RR and JD Crapo. Three-dimensional analysis of lung structure and its application to pulmonary dosimetry models. In: *Toxicology of the Lung*, 2nd ed., DE Gardner, JD Crapo and EJ Massaro, eds. Raven Press, New York, 1993.
- E. Pinkerton KE, JT Gallen, RR Mercer, VC Wong, CG Plopper and BK Tarkington. Aerosolized fluorescent microspheres detected in the lung using confocal scanning laser microscopy. *Micro Res Tech* 26:437-443, 1993.
- F. Mercer RR, ML Russell, VL Roggli and JD Crapo. Cell number and distribution in human and rat airways. *Am J Respir Cell Mol Biol* 10:613-624, 1994.
- G. Mercer RR, ML Russell and JD Crapo. Alveolar septal structure in different species. *J Appl Physiol* 77:1060-1066, 1994.
- H. Koblinger L, W Hofmann, R.C Graham and RR Mercer. Aerosol inhalation in the rat lung. *J Aerosol Med* 8:7-19, 1995.
- I. Mercer RR, DL Costa and JD Crapo. Effects of prolonged exposure to low doses of nitric oxide or nitrogen dioxide on alveolar septa of the adult rat lung. *Lab Invest* 73:20-28, 1995.

APPENDIX
Final Technical Report
DE-FG05-88ER60654

- A. Mercer RR, ML Russell and JD Crapo. Radon dosimetry based on the depth distribution of nuclei in human and rat airways. *Health Physics* 61:117-130, 1991.
- B. Mercer RR, S Anjilvel, FJ Miller and JD Crapo. Inhomogeneity of ventilatory unit volume and its effects on reactive gas uptake. *J Appl Physiol* 70:2193-2205, 1991.
- C. Stone KC, RR Mercer, BA Freeman and JD Crapo. Distribution of lung cell numbers and volumes between alveolar and nonalveolar tissues. *Am Rev Respir Dis* 146:454-456, 1992.
- D. Mercer RR and JD Crapo. Three-dimensional analysis of lung structure and its application to pulmonary dosimetry models. In: *Toxicology of the Lung*, 2nd ed., DE Gardner, JD Crapo and EJ Massaro, eds. Raven Press, New York, 1993.
- E. Pinkerton KE, JT Gallen, FR Mercer, VC Wong, CG Plopper and BK Tarkington. Aerosolized fluorescent microspheres detected in the lung using confocal scanning laser microscopy. *Micro Res Tech* 26:437-443, 1993.
- F. Mercer RR, ML Russell, VL Roggli and JD Crapo. Cell number and distribution in human and rat airways. *Am J Respir Cell Mol Biol* 10:613-624, 1994.
- G. Mercer RR, ML Russell and JD Crapo. Alveolar septal structure in different species. *J Appl Physiol* 77:1060-1066, 1994.
- H. Koblinger L, W Hofmann, RC Graham and RR Mercer. Aerosol inhalation in the rat lung. *J Aerosol Med* 8:7-19, 1995.
- I. Mercer RR, DL Costa and JD Crapo. Effects of prolonged exposure to low doses of nitric oxide or nitrogen dioxide on alveolar septa of the adult rat lung. *Lab Invest* 73:20-28, 1995.

Reprints pulled for separate
processing