1 of 1

Ivančica Trošić, PhD
Institute for Medical Research and Occupational Health,
Zagreb, Croatia

**REPORT OF WORK PERFORMED BY DR. IVANČICA TROŠIĆ IN RESPONSE
TO PROCUREMENT REQUEST # 4478H UNIVERSITY OF CALIFORNIA,
LOS ALAMOS NATIONAL LABORATORY, LOS ALAMOS, NEW MEXICO**

The Pulmonary Biology Program in the LS Division has been investigating a process which occurs in the lung following particle deposition known as "particle redistribution phenomenon". A hypothesis, emanating from Dr. Lehnert's studies, is that particle redistribution phenomenon may be more pronounced following deposition of particles which are more cytotoxic than the relatively benign polystyrene microspheres and titanium dioxide (1,2,3). This hypothesis has been experimentally examined by Dr. I. Trošić and her staff at the Institute for Medical Research and Occupational Health, Zagreb, Croatia. Statement of Work, Materials and Methods, Results and a diskette are attached.

Present Address: Institute for Medical Research and Occupational Health
Ksaverska cesta 2, 41001 Zagreb, Croatia
Fax No. (041) 274 572

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.

MASTER

STATEMENT OF WORK

The scope of work is as follows: One group of laboratory rats (n=30) were intratracheally instilled with 1×10^8 fluorescent polystyrene microspheres (2 micro-meter dia.) in phosphate buffered saline (PBS) as the carrier vehicle. A second group (n=30) of rats were intratracheally instilled with asbestos particles (0.5 mg) suspended in PBS. A third group (n=30) of rats were intratracheally instilled with a suspension of a co-mixture of polystyrene microspheres and asbestos. A fourth group of rats (n=30) were instilled with carrier vehicle only, i.e. PBS. Six animals from each group were dispatched on days 1, 7, 30, 80 and 150 days after their respective instillations. Their lungs were lavaged, and several parameters were assessed as described by Lehnert (4). These include: quantification of lavaged fluorescent microspheres, analyses of distributions of microspheres in lung phagocytes by type, quantification of unlavaged lung burdens of microspheres, quantification of tracheobronchial lymph node burdens of microspheres.

Data obtained in the experimental component of the project will be summarily presented to describe: lung retention kinetics of the microspheres, analyses of particle distributions in lung phagocytes over the course of alveolar phase clearance, analyses of changes in the sizes of the lung's population of free cells.

MATERIALS AND METHODS

Animals:

Healthy male Wistar rats were used from the Institute's outbreeding colony (Institute for Medical Research and Occupational Health, Zagreb). The animals were 12-14 weeks old with 325-355 g body weight. The animals (N=120) were randomly assigned to one of four experimental groups. Group 1 (N=30) was intrabronchially instilled with physiological buffered saline (0.4 ml); Group 2 (N=30) was instilled with 0.4 ml of 9.6×10^7 fluorescent polystyrene particles; Group 3 (N=30) was instilled with a co-mixture of polystyrene particles and asbestos, and Group 4 (N=30) received 0.4 ml suspension of 0.5 mg of asbestos fibers. After the instillations the animals were held in a vertical position for 30 s before being returned to their cages. Each group of animals was divided into five subgroups (N=6). All groups of animals were fed and watered ad libidum. At specific time intervals (1,7,30,80 and 150 post-instillation day) the animals (six in each group, except one animal in experimental group 2 on day 80, which died during the course of the experiment) were sacrificed.

Reagents

Polystyrene fluorescent microspheres - Fluoresbrite carboxil microspheres (2.5% solids-latex) dia.# 2.37 micron, SD=0.027 micron (Polysciences, INC., Warrington, PA. kindly donated by Dr. B. Lehnert) were used in this study. Before use the particles were washed 6 times with distilled water, 3 times with 75% ethanol and 3 more times with normal saline before final resuspension. The final suspension (2.4×10^8 particles/ml normal saline) was sonicated for 45 min. in a 0°C water bath. Prior to instillation the microspheres were kept in an ice bath and briefly (5 min.) sonicated by ultrasonic disintegrator (MSE, 100 W), in order to avoid agglomeration of microspheres.

The cleaning water and physiological saline were stripped of particulate contamination by filtering through a Millipore filter of 0.45 μ m pore size (Millipore Corp.,

Bedford, Mass., USA) and fluids were then sterilized for 30 min. at 1 Atm and 120°C by using an autoclav.

Crocidolite asbestos - Russian crocidolite blue asbestos, $\text{Na}_2\text{Fe}_3\text{Fe}_2\text{Si}_8\text{O}_{22}(\text{OH})_2$ consisted of 50.13% SiO_2 , 19.1% FeO , 16.3% Fe_2O_3 , 5.0% MnO , 0.8% CaO , 1.3% MgO , 5.7% Na_2O , 0.4% CO_2 and 1.9% H_2O (crist.) was used in this study. Asbestos was suspended in normal saline (pH=7) to make a solution of 1.25 mg asbestos/ml normal saline.

In an attempt to dissociate and fragment some bundles or aggregates of fibers which were too long for the biological experiment, aqueous suspension of crocidolite was subjected to ultrasonic treatment for 3 x 30 min. Before use, fibers were autoclaved and dispersed ultrasonically for 10 min.

Co-mixture of asbestos and microspheres

Suspension of the co-mixture was obtained by mixing 50 vol.% of the previously prepared fluorescent microspheres and 50 vol.% of the previously prepared asbestos suspension. The co-mixture consisted of 0.625 mg asbestos/ml PBS and 1.2×10^8 microspheres/ml PBS. Each animal from the group instilled with the co-mixture (N=30) received 0.4 ml of the co-mixture of asbestos fibers and fluorescent particles. Before use the co-mixture of microspheres and asbestos fibers was briefly (10 min.) sonicated to avoid clumps of fibers and agglomerations of microspheres.

Sacrifice of animals and bronchoalveolar lavage

Six animals from each group were sacrificed on days 1,7,30,80 and 150 days after their respective instillations, by intraperitoneal injections of 50 mg thiopental-sodium ("Lentia"-Hormon-Chemie Munchen GMBH). The animals were exsanguinated prior to apnea via carotid artery transection, and the trachea of each rat was cannulated with a blunt 18 ga. needle secured with ligature. After opening the abdomen, both lung halves were collapsed by incision of the diaphragm. The trachea and lungs were then excised en block and the esophagus, extraneous mediastinal tissue and heart were removed. The lungs were lavaged eight times with eight milliliters of phosphate buffered saline per wash, while they were gently massaged. Usually >93% of wash volumes instilled during the lavage was recovered. Fluids retrieved during lavage of each lung were pooled in centrifugation tubes

maintained in ice and centrifugation was performed by using Hettich Rotanta R centrifuge (250 g, 0°C).

Preparation of lung and lymph node tissue for analysis

After lung lavages were performed the tracheobronchial lymph nodes were separated from lung tissue. Both the lymph nodes and lung tissue were stored at -20°C in separate tubes. The nodal tissues from each animal were chemically dissolved by incubating them in 10 ml of 10% potassium hydroxide (KOH) for 48 h at 60°C. Lung tissue was solubilized by incubation in 15 ml of 10% KOH for 48 h at 60°C. Lung tissue and lymph node dissolution liquid were then diluted with 60 ml and 40 ml redistilled water respectively. Finally, aliquots from lung tissue and lymph node solution were F_D ratio (F_D represents dilution factor) of their original volume. The solutions were stored at +4°C until further examination.

Analysis of particles in the lavaged cell samples

Particle-cell relationship among the alveolar macrophages and polymorphonuclear leukocytes harvested from the animals instilled with microspheres and co-mixture of particles and asbestos fibers were assessed by light microscopy under oil immersion optics (640x). A minimum of 600 AM and 200 PMN were observed in random sequential field and the number of microspheres associated with each cell type was counted (5).

Lavaged alveolar macrophages were categorized as being associated with zero (O category), 1-2 (I), 3-4 (II), 5-6 (III), 7-8 (IV), 9-10 (V), 11-12 (VI), 13-14 (VII), 15-16 (VIII) and more than 16 (IX) particles per cell. Lavaged polymorphonuclear leukocytes were categorized as being associated with zero, 1,2,3,4 and 5 particles per cell. The various categories burden per alveolar macrophages and polymorphonuclear leukocytes were expressed as percentage of AM and PMN total number lavaged per group, respectively.

Quantification of lung free cells, viability and differentiation of cells by type

The total number of cells harvested in 8 consecutive bronchoalveolar lavages was determined by microscopic cell count, using a Burker-Turk hemocytometer. Cell viability

was measured using 0.4% trypan blue stain exclusion test. For cell differential analyses and particle-cell analyses three cytocentrifuged slide preparations (Shandon, Southern Cytospin) were prepared and stained with 10% Giemsa solution provided by KEMIKA Zagreb, Croatia. The cell type determination was assessed by light microscopy under oil immersion optics, counting 2x400 cells per lavage series. The lavaged cells were classified as alveolar macrophages (AM), polymorphonuclear leukocytes (PMN), lymphocytes (Ly), monocytes (Mo), and "other" cell types. The differential values of the cells obtained by pooling of eight consecutive lung lavages, were averaged. These values were used in conjunction with the total number of cells in the lung lavage fluid in order to calculate the total number of each cell type lavaged.

Analyses of lung and tracheobronchial lymph nodal particles

The solubilized lung and tracheobronchial lymph nodal fluids were quantitatively diluted with redistilled water, vortexed, and sonicated for 10 minutes. Tissue sample aliquots were passed through 25-diam., 1.0 μ m pore size Nucleopore polycarbonate membrane filters (Nucleopore Corp., Pleasanton, CA) (generously donated by Dr. Lehnert) to collect the microspheres. Two filters were prepared for each tissue samples and the particles on the filters were counted using a fluorescent microscope (Opton, magn. 640 x). Forty-five fields per filter were randomly examined and the average number of particles per field was calculated for the filter samples. The total number of particles on a filter was estimated by multiplying this number by a factor derived from the quotient of the total particle collection area on the filter and the area observed in one field of vision. The total number of particles in a given tissue sample was then calculated from the total number of particles counted from the filtered aliquots of known volumes and from the original volumes of solubilized tissue samples. (6). The total number of particles in a given tissue sample was calculated by multiplying the total number of particles per filter collection area by dilution factor (F_D).

The total number of microspheres from the lung was calculated according to the following formula:

$$T_{MI/L} = X_{FV} \times F_D \times F(R:r)$$

where $T_{MI/L}$ is the total number of microspheres per lung

F_D represents dilution factor

$F(R:r)$ represents the ratio of total particle collection area radius on the filter and field of vision radius. $F(R:r)$ was 13 024.

The total number of microspheres per lymph node was calculated according to the same formula.

Because of difficulties in determining asbestos fibers in the tissue samples, after lung lavage of asbestos instilled animals (Group 4) these lungs and lymph nodes were frozen at -20°C for eventual analytical analysis. However, it would be possible to gain access to insight of fiber redistribution in the lung and lymph node tissue by comparative analysis of results.

References:

1. Lehnert, B.E., Ortiz, J.B., Steinkamp, J.A., Tietjen G.L., Sebring, R.J. and Oberdorster, G.: Mechanisms underlying the redistribution of particles among the lung's alveolar macrophages during alveolar phase clearance. Paper No. S3.1. In press.
2. Lehnert, B.E., Valdez, Y.E., Bomalski, S.H.: Lung and pleural "Free-cell responses" to the intrapulmonary deposition of particles in the rat. J. of Toxicology and Environ. Health 16:823-839, 1985.
3. Oberdorster, G., and Lehnert, B.E.: Toxicological aspect of the pathogenesis of fiber-induced pulmonary effects. Mechanisms in Fibre Carcinogenesis. Eds. R.C. Brown et al. Plenum Press, New York, 157-179, 1991.
4. Lehnert, B.E.: Alveolar Macrophages in a Particle "Overload" Condition. J. of Aerosol Med 3:S-9 - S30, 1990.
5. Lehnert, B.E., Toevs, K.E., Sebring, R.J. and Valdez, Y.E.: Failure of a Simple Model of Alveolar Macrophage-Mediated Particle Clearance to Depict Particle-Alveolar

Relationship During Early Alveolar Phase Clearance. J Aerosol Med 4:41-56, 1991.

6. Lehnert, B.E., Valdez, Y.E., Tietjen, G.L.: Alveolar Macrophage-Particle Relationship during Lung Clearance. Am J Respir Cell Mol Biol 1:145-154, 1989.

Descriptive statistics and figure legends

- Table 1: Lavage free cell number and estimated number of alveolar macrophages (AM), polymorphonuclear leukocytes (PMN), lymphocytes (Ly), monocytes (Mono) and "other" cells (Oth) following instillation of phosphate buffered saline (PBS) in experimental group 1.
- Table 2: Lavaged free number and estimated number of alveolar macrophage (AM), polymorphonuclear leukocytes (PMN), lymphocytes (Ly), monocytes (Mono), and "other" (Oth) cells following instillation of microspheres (MI) in experimental group 2.
- Table 3: Lavaged free cell number and estimated number of alveolar macrophages (AM), polymorphonuclear leukocytes (PMN), monocytes (Mono) and "other" cells (Oth) following instillation of co-mixture of particles and asbestos fibers (MIX) in experimental group 3.
- Table 4: Lavaged free cell number and estimated number of alveolar macrophages (AM), polymorphonuclear leukocytes (PMN), lymphocytes (Ly), monocytes (Mono) and "other" cells (Oth) following instillation of asbestos fibers (ASB) in experimental group 4.
- Table 5: Number of alveolar macrophages (AM), polymorphonuclear leukocytes (PMN) containing microspheres (MI) and the number of particles per lung and lymph node tissue following instillation of microspheres in experimental group 2.
- Table 6: Number of alveolar macrophages (AM), polymorphonuclear leukocytes (PMN) containing microspheres (MI) and the number of particles per lung and lymph node tissue following instillation of co-mixture of particles and asbestos fibers in experimental group 3.

Table 7: Distribution of microspheres in alveolar macrophages (AM) in various categories burden following instillation of microspheres in experimental group 2.

Table 8: Distribution of microspheres in alveolar macrophages (AM) in various categories burden following instillation of co-mixture of microspheres and asbestos fibers in experimental group 3.

Table 9: Distribution of microspheres in polymorphonuclear leukocytes (PMN) in various categories burden following instillation of microspheres in experimental group 2.

Table 10: Distribution of microspheres in polymorphonuclear leukocytes (PMN) in various categories burden following instillation of co-mixture of microspheres and asbestos fibers in experimental group 3.

Fig. 1: Total number of free cell population lavaged following instillation of PBS, particles, co-mixture and asbestos fibers.

Fig. 2: Number of alveolar macrophages (AM) lavaged following instillation of PBS, particles, co-mixture and asbestos fibers.

Fig. 3: Number of polymorphonuclear leukocytes (PMN) lavaged following instillation of PBS, particles, co-mixture and asbestos fibers.

Fig. 4: Number of lymphocytes (Ly) lavaged following instillation of PBS, particles, co-mixture and asbestos fibers.

Fig. 5: Number of alveolar macrophages (AM) containing particles lavaged following instillation of particles and co-mixture of particles and asbestos fibers.

Fig. 6: Number of polymorphonuclear leukocytes (PMN) containing particles lavaged following instillation of particles and co-mixture of particles and asbestos fibers.

Fig. 7: Estimated percentages of alveolar macrophages (AM) containing particles in various burden categories following instillation of microspheres in experimental group

2.

Fig. 8: Estimated percentages of alveolar macrophages (AM) containing particles in various categories following instillation of co-mixture in experimental group 3.

Fig. 9: Estimated percentages of polymorphonuclear leukocytes (PMN) containing particles in various particle categories following instillation of microspheres in experimental group 2.

Fig. 10: Estimated percentages of polymorphonuclear leukocytes (PMN) containing particles in various burden categories following instillation of co-mixture in experimental group 3.

Fig. 11: Number of particles in the lung tissue following instillation of particles and co-mixture of particles and asbestos fibers.

Fig. 12: Number of particles in the lymph node tissue following instillation of particles and co-mixture of particles and asbestos fibers.

Description of variables: (diskette enclosed for Dr. B.E. Lehnert)

PRF	-	Particle redistribution phenomenon
T	-	Time
G	-	Group
1	-	Group 1 (PBS instilled rats)
2	-	Group 2 (microspheres - MI instilled rats)
3	-	Group 3 (co-mixture - MIX instilled rats)
4	-	Group 4 (asbestos - ASB instilled rats)
Total	-	Total cell number in BAL/lung $\times 10^4$
Viab	-	Total number of viable cells in BAL/lung $\times 10^4$
AMBAL 1	-	alveolar macrophage number /400 cells - counting 1
AMBAL 2	-	alveolar macrophage number/400 cells - counting 2
PMNBAL 1	-	polymorphonuclear cell number/400 cells - counting 1
PMNBAL 2	-	polymorphonuclear cell number/400 cells - counting 2
LYBAL 1	-	lymphocyte number/400 cells - counting 1

LYBAL 2 - lymphocyte number/400 cells - counting 2
 MONOBAL 1 - monocyte number/400 cells - counting 1
 MONOBAL 2 - monocyte number/400 cells - counting 2
 OTHBAL 1 - other cell type number/400 cells - counting 1
 OTHBAL 2 - other cell type number/400 cells - counting 2
 NAMBAL - mean of AMBAL 1 and AMBAL 2
 NPMNBAL - mean of PMNBAL 1 and PMNBAL 2
 NLYBAL - mean of LYBAL 1 and LYBAL 2
 NMONOBAL - mean of MONOBAL 1 and MONOBAL 2
 NOTHBAL - mean of OTHBAL 1 and OTHBAL 2
 MIAMBAL 0 - number of AM/600 AM not containing particles
 MIAMBAL 1, MIAMBAL 2, MIAMBAL 3MIAMBAL 16 - number of AM/600 AM
 containing 1,2,316 particles
 T0; T1; T2T5 Total number of PMN containing various categories
 particulate burden
 MIAMBAL 17- number of AM/600 AM containing 17 or more particles
 MIPMBAL 0 - number of PMN/200 PMN not containing particles
 MIPMNBAL 1, MIPMNBAL 2MIPMNBAL 5 - number of PMN/200 PMN
 containing 1,2,3,4 and 5 particles
 MIAMBAL I; II ...IX Number of AM/600 macrophagocytes containing various
 categories, particulate burden
 MIFVLT - mean of 45 countings of particles from aliquot of lung
 tissue samples observed in field of vision (n=45)
 MIFVLLNT - mean of 45 countings of particles from aliquot of lymph
 node samples observed in field of vision (n=45)
 F_D - dilution factor
 F_{R,r} - ratio of total particle collection area radius on the
 filter and field of vision radius, F_{R,r}=13 024.

TABLE 1 LAVAGED FREE CELL NUMBER, AND ESTIMATED NUMBER OF ALVEOLAR MACROPHAGES (AM), POLYMORPHONUCLEAR LEUKOCYTES (PMN), LYMPHOCYTES (LY), MONOCYTES (MONO) AND "OTHER" CELLS (OTH)* FOLLOWING INSTILLATION OF PHOSPHATE BUFFERED SALINE (PBS) IN EXPERIMENTAL GROUP 1.

Sacri- fice times** (days)		Total no. of free cells harvested (x10/4)	No. of AM-s (x10/4)	No. of PMN-s (x10/4)	No. of Ly-s (x10/4)	No. of Mono-s (x10/4)	No. of Oth-s (x10/4)	Viabil- ity of cells (x10/4)
1(6)	med.	420.0	367.5	20.7	12.8	0.75	0.0	333.0
	min.	220.0	194.4	11.2	4.8	0.46	0.0	174.0
	max.	535.0	481.0	100.9	43.4	1.7	0.0	465.0
7(6)	med.	927.5	774.2	42.9	61.2	1.0	0.0	619.0
	min.	742.0	644.5	19.0	38.0	0.0	0.0	619.0
	max.	1170.0	1000.3	184.8	102.3	2.2	0.0	759.0
30(6)	med.	845.0	719.6	28.6	93.7	4.2	0.0	722.0
	min.	810.0	583.0	15.7	53.9	1.1	0.0	647.0
	max.	1125.0	922.5	79.8	172.9	8.1	0.0	934.0
80(6)	med.	702.5	640.3	11.8	58.1	2.6	0.0	562.5
	min.	650.0	574.2	6.9	34.5	0.8	0.0	510.0
	max.	1150.0	1024.9	42.9	104.9	8.6	0.0	940.0
150(6)	med.	442.0	386.4	8.4	29.0	2.4	0.9	352.5
	min.	365.0	317.3	0.79	16.3	1.0	0.7	285.0
	max.	635.0	596.9	46.1	66.7	3.1	1.6	483.0

* Values represent median, maximum and minimum

** Number of animals studied is indicated in parentheses

TABLE 2 LAVAGED FREE CELL NUMBER AND ESTIMATED NUMBER OF ALVEOLAR MACROPHAGES (AM), POLYMORPHONUCLEAR LEUKOCYTES (PMN), LYMPHOCYTES (LY), MONOCYTES (MONO), AND "OTHER" CELLS (OTH) * FOLLOWING INSTALLATION OF MICROSPHERES (MI) IN EXPERIMENTAL GROUP 2

Sacri- fice times** (days)	Total no. of free cells harvested (x10/4)	No. of AM-s (x10/4)	No. of PMN-s (x10/4)	No. of Ly-s (x10/4)	No. of Mono-s (x10/4)	No. of Oth-s (x10/4)	Viabil- ity of cells (x10/4)
1(6)	med. 465.0	239.4	1.95.1	29.8	2.0	0.0	318.0
	min. 375.0	137.3	0.60.4	21.5	0.93	0.0	262.0
	max. 520.0	380.1	2.12.1	34.3	5.6	0.6	437.0
7(6)	med. 1092.5	840.0	0.46.7	172.7	1.3	0.0	887.5
	min. 860.0	593.7	0.06.6	106.4	0.0	0.0	698.0
	max. 1450.0	1203.6	0.78.9	291.2	3.6	0.0	1358.0
30(6)	med. 1237.5	1079.3	15.8	105.5	1.8	0.0	1050.5
	min. 860.0	709.3	3.2	80.0	0.0	0.0	688.0
	max. 1440.0	1324.8	40.0	165.8	3.6	0.0	1166.0
80(5)	med. 925.0	761.9	31.3	81.7	1.9	0.0	825.0
	min. 640.0	580.0	6.4	52.0	0.0	0.0	530.0
	max. 1572.0	1408.9	37.3	126.0	3.1	0.0	1225.0
150(6)	med. 572.5	510.8	37.2	35.5	1.4	0.0	501.0
	min. 410.0	356.1	4.2	14.8	1.0	0.0	295.0
	max. 900.0	777.3	68.2	81.0	4.5	0.7	810.0

* Values represent median, maximum and minimum

** Number of animals studied is in parentheses

TABLE 3 LAVAGED FREE CELL NUMBER AND ESTIMATED NUMBER OF ALVEOLAR MACROPHAGES (AM), POLYMORPHONUCLEAR LEUKOCYTES (PMN), LYMPHOCYTES (LY), MONOCYTES (MONO) AND "OTHER" CELLS (OTH)* FOLLOWING INSTILLATION OF CO-MIXTURE OF PARTICLES AND ASBESTOS FIBER (MIX) IN EXPERIMENTAL GROUP 3

Sacri- fice times** (days)	Total no. of free cells harvested (x 10/4)	No. of AM-s (x 10/4)	No. of PMN-s (x 10/4)	No. of Ly-s (x 10/4)	No. of Mono-s (x 10/4)	No. of Oth-s (x 10/4)	Viabil- ity of cells (x 10/4)
1(6)	med.	427.5	209.8	168.2	22.7	1.2	0.0
	min.	330.0	192.2	4.9	12.7	0.8	0.0
	max.	590.0	305.2	38.1	38.1	3.6	0.5
7(6)	med.	782.5	641.7	27.7	90.6	0.0	0.0
	min.	585.0	519.1	21.2	43.8	0.0	0.0
	max.	900.0	715.0	64.3	150.7	3.3	0.0
30(6)	med.	752.5	664.1	21.8	79.1	3.3	0.0
	min.	680.0	529.0	8.7	33.1	0.0	0.0
	max.	905.0	789.6	54.7	106.8	4.3	0.0
80(6)	med.	1625.0	1319.12	61.1	168.7	5.9	0.0
	min.	1085.0	915.4	24.4	44.5	4.0	0.0
	max.	2110.0	1724.9	170.1	316.5	25.4	0.0
150(6)	med.	522.5	442.6	17.9	50.2	2.3	0.0
	min.	480.0	389.4	8.6	35.4	0.6	0.0
	max.	585.0	523.5	42.6	62.1	4.8	0.0

* Values represent median, maximum and minimum

** Number of animals studied is indicated in parentheses

TABLE 4 LAVAGED FREE CELL NUMBER AND ESTIMATED NUMBER OF ALVEOLAR MACROPHAGES (AM), POLYMORPHONUCLEAR LEUKOCYTES (PMN), LYMPHOCYTES (LY), MONOCYTES (MONO), AND "OTHER" CELLS (OTH)* FOLLOWING INSTILLATION OF ASBESTOS FIBERS (ASB) IN EXPERIMENTAL GROUP 4

Sacri- fice times** (days)	Total no. of cells harvested (x 10/4)	No. of AM-s (x 10/4)	No. of PMS-s (x 10/4)	No. of Ly-s (x 10/4)	No. of Mono-s (x 10/4)	No. of Oth-s (x 10/4)	Viabil- of cells (x 10/4)
1(6)	med.	370.0	304.2	28.7	19.7	1.0	247.5
	min.	310.0	126.8	7.3	5.2	0.3	195.0
	max.	455.0	346.1	303.1	26.4	2.4	337.0
7(6)	med.	627.5	496.5	31.9	74.1	0.2	505.5
	min.	435.0	337.1	9.3	58.2	0.0	365.0
	max.	675.0	541.8	191.5	138.5	1.6	540.0
30(6)	med.	1107.5	881.5	30.4	114.9	2.0	911.5
	min.	890.0	692.8	23.4	78.1	0.0	712.0
	max.	1250.0	1143.7	68.5	240.8	5.8	1088.0
80(6)	med.	687.5	559.5	23.4	66.4	1.4	580.0
	min.	590.0	505.5	9.0	30.9	0.0	430.0
	max.	800.0	734.0	64.2	134.1	9.0	648.0
150(6)	med.	580.0	496.3	16.2	68.4	2.3	427.0
	min.	455.0	397.5	10.5	25.5	0.7	341.0
	max.	735.0	633.9	35.3	104.8	3.5	592.0

* Values represent median, maximum and minimum

** Number of animals studied is indicated in parentheses

TABLE 5 NUMBER OF ALVEOLAR MACROPHAGES (AM), POLYMORPHONUCLEAR LEUKOCYTES (PMN), CONTAINING MICROSPHERES (MI) AND THE NUMBER OF PARTICLES PER LUNG AND LYMPH NODE TISSUE FOLLOWING INSTILLATION OF MICROSPHERES IN EXPERIMENTAL GROUP 2

Sacrifice times (days)		No. of AM-s containing microspheres x10/4	No. of PMN-s containing microspheres x10/4	No. of particles (lung) x10/4	No. of particles (lymph node) x10/4
1	med.*	141.695 (6)**	10.18 (6)	473.5 (6)	5.8 (6)
	min.	22.030	3.92	176.0	4.3
	max.	249.620	11.52	549.0	7.7
7	med.	828.791 (6)	0.69 (6)	363.00 (5)	39.8 (5)
	min.	451.03	0.36	308.0	34.1
	max.	1297.93	6.59	712.0	50.2
30	med.	541.59 (6)	0.45 (6)	816.0 (5)	20.9 (5)
	min.	275.03	0.00	560.0	18.2
	max.	1415.32	0.35	1342.0	24.6
80	med.	387.50 (5)	0.00 (5)	343.0 (5)	15.10 (5)
	min.	116.0	0.00	236.0	13.4
	max.	1066.07	0.17	447.0	17.0
150	med.	139.38 (6)	2.42 (6)	183.0 (5)	14.30 (5)
	min.	95.97	0.25	95.0	12.5
	max.	363.80	9.1	277.0	20.3

* Values represent median, maximum and minimum

** Number of animals studied is indicated in parentheses

TABLE 6 NUMBER OF ALVEOLAR MACROPHAGES (AM), POLYMORPHONUCLEAR LEUKOCYTES (PMN) CONTAINING MICROSPHERES (MI) AND THE NUMBER OF PARTICLES PER LUNG AND LYMPH NODE TISSUE FOLLOWING INSTILLATION OF CO-MIXTURE OF PARTICLES AND ASBESTOS FIBERS IN EXPERIMENTAL GROUP 3

Sacrifice times (days)		No. of particles in lavaged AM x10/4	No. of particles in lavaged PMN x10/4	No. of particles in lung tissue x10/4	No. of particles in lymph nodes x10/4
1	med.*	44.16 (6)**	8.98 (6)	1975.0 (5)	6.0 (5)
	min.	14.24	0.17	1115.0	2.3
	max.	60.17	31.87	2793.0	10.5
7	med.	46.45 (6)	0.34 (6)	162.0 (5)	4.0 (5)
	min.	15.25	0.12	126.0	3.4
	max.	129.22	2.57	200.0	5.3
30	med.	56.04 (6)	0.99 (6)	488.0 (5)	9.1 (5)
	min.	14.99	0.20	102.0	5.9
	max.	74.63	4.27	883.0	26.1
80	med.	86.39 (6)	0.54 (6)	624.0 (5)	9.9 (5)
	min.	34.74	0.12	427.0	8.0
	max.	192.99	13.61	669.0	26.0
150	med.	38.46 (6)	0.51 (6)	634.0 (5)	41.0 (5)
	min.	27.45	0.09	394.0	5.6
	max.	57.97	2.13	712.0	49.6

* Values represent median, maximum and minimum

** Number of animals studied is indicated in parentheses

TABLE 7. DISTRIBUTION OF MICROSPHERES IN ALVEOLAR MACROPHAGES (AM) IN VARIOUS CATEGORIES BURDEN FOLLOWING INSTILLATION OF MICROSPHERES IN EXPERIMENTAL GROUP 2

		PARTICLE BURDEN PER AM IN VARIOUS CATEGORIES																					
PRICE TOTAL No. OF AM/BAL	s) $\bar{X}$ ($\times 10^4$)	CATEG. 0 0 PART./AM		CATEG. I 1-2 PART./AM		CATEG. II 3-4 PART./AM		CATEG. III 5-6 PART./AM		CATEG. IV 7-8 PART./AM		CATEG. V 9-10 PART./AM		CATEG. VI 11-12 PART./AM		CATEG. VII 13-14 PART./AM		CATEG. VIII 15-16 PART./AM		CATEG. IX >16 PART./AM			
		$\bar{X}(\times 10^4)$	%	$\bar{X}(\times 10^4)$	%	$\bar{X}(\times 10^4)$	%	$\bar{X}(\times 10^4)$	%	$\bar{X}(\times 10^4)$	%	$\bar{X}(\times 10^4)$	%	$\bar{X}(\times 10^4)$	%	$\bar{X}(\times 10^4)$	%	$\bar{X}(\times 10^4)$	%	$\bar{X}(\times 10^4)$	%		
5)*	251,69	225,75	89,69	10,43	4,1	4,85	1,92	3,15	1,25	2,45	0,96	1,59	0,62	0,67	0,26	0,93	0,36	0,57	0,22	1,32	0,52		
6)	883,69	681,66	77,13	97,56	11,04	38,94	4,40	25,74	2,91	14,57	1,64	11,40	1,3	5,53	0,63	3,03	0,34	2,81	0,31	2,45	0,28		
6)	1019,53	808,19	79,27	133,19	13,06	35,43	3,48	16,75	1,64	10,90	1,07	8,01	0,78	3,12	0,31	2,76	0,27	0,20	0,02	0,98	0,10		
(5)	891,93	703,26	78,84	129,90	14,56	31,27	3,5	16,25	1,82	3,88	0,43	4,22	0,47	0,82	0,1	1,41	0,16	0,0	0	0,94	0,10		
(6)	530,76	478,82	90,21	34,89	6,6	5,69	1,1	3,45	0,65	1,98	0,37	1,86	0,35	1,42	0,27	0,68	0,13	0,80	0,15	1,17	0,22		

* NUMBER OF ANIMALS STUDIED IS INDICATED IN PARENTHESES

TABLE 8. DISTRIBUTION OF MICROSPHERES IN ALVEOLAR MACROPHAGES (AM) IN VARIOUS CATEGORIES BURDEN FOLLOWING INSTILLATION
CO-MIXTURE OF MICROSPHERES AND ASBESTOS FIBERS IN EXPERIMENTAL GROUP 3

CRIFICE MES AYS)	TOTAL No. OF AM/BAL $\bar{X}(x10^4)$	PARTICLE BURDEN PER AM IN VARIOUS CATEGORIES																			
		CATEG. 0 0 PART./AM $\bar{X}(x10^4)$ %		CATEG. I 1-2 PART./AM $\bar{X}(x10^4)$ %		CATEG. II 3-4 PART./AM $\bar{X}(x10^4)$ %		CATEG. III 5-6 PART./AM $\bar{X}(x10^4)$ %		CATEG. IV 7-8 PART./AM $\bar{X}(x10^4)$ %		CATEG. V 9-10 PART./AM $\bar{X}(x10^4)$ %		CATEG. VI 11-12 PART./AM $\bar{X}(x10^4)$ %		CATEG. VII 13-14 PART./AM $\bar{X}(x10^4)$ %		CATEG. VIII 15-16 PART./AM $\bar{X}(x10^4)$ %		CATEG. IX 17-16 PART./AM $\bar{X}(x10^4)$ %	
1(6)*	232,83	213,98	91,90	13,70	5,88	2,61	0,53	1,16	0,49	0,51	0,21	0,22	0,09	0,0	0	0,0	0	0,0	0	0,15	0,06
7(6)	636,15	608,62	95,67	21,16	3,32	3,97	0,62	0,90	0,14	0,94	0,14	0,14	0,02	0,29	0,04	0,14	0,02	0,0	0	0,0	0
10(6)	673,08	647,94	96,26	19,43	2,88	3,38	0,50	0,97	0,14	0,98	0,14	0,22	0,03	0,0	0	0,17	0,02	0,0	0	0,0	0
10(6)	1306,01	1255,61	96,14	39,25	3,00	4,96	0,37	1,62	0,12	0,99	0,07	0,42	0,03	0,96	0,07	2,21	0,16	0,12	0,02	0,0	0
10(6)	446,35	427,81	95,84	14,57	3,26	2,41	0,53	0,47	0,10	0,50	0,11	0,12	0,02	0,25	0,04	0,12	0,02	0,12	0,02	0,0	0

* NUMBER OF ANIMALS STUDIED IS INDICATED IN PARENTHESES

TABLE 9. DISTRIBUTION OF MICROSPHERES IN POLYMORPHOCLEAR LEUKOCYTES (PMN) IN VARIOUS CATEGORIES BURDEN FOLLOWING
INSTILLATION OF MICROSPHERES IN EXPERIMENTAL GROUP 2

SACRIFICE TIMES (DAYS)	TOTAL No. OF PMN/BAL $\bar{X}(x10^4)$	PARTICLE BURDEN PER PMN IN VARIOUS CATEGORIES											
		CATEG. 0		CATEG. 1		CATEG 2		CATEG. 3		CATEG. 4		CATEG. 5	
		0 PART./PMN		1 PART./PMN		2 PART./PMN		3 PART./PMN		4 PART./PMN		5 PART./PMN	
		$\bar{X}(x10^4)$	%	$\bar{X}(x10^4)$	%	$\bar{X}(x10^4)$	%	$\bar{X}(x10^4)$	%	$\bar{X}(x10^4)$	%	$\bar{X}(x10^4)$	%
1 ₍₆₎ *	166,42	160,75	96,6	3,93	2,4	0,99	0,6	0,49	0,3	0,26	0,2	0,0	0,0
7 ₍₆₎	45,83	44,58	97,27	0,87	1,9	0,09	0,2	0,27	0,6	0,0	0	0,0	0
30 ₍₆₎	18,80	18,73	99,63	0,06	0,32	0,02	0,1	0,0	0	0,0	0	0,0	0
80 ₍₅₎	25,66	25,63	99,88	0,04	0,2	0,0	0	0,0	0	0,0	0	0,0	0
150 ₍₆₎	39,62	37,74	95,25	1,01	2,55	0,37	0,93	0,11	0,3	0,15	0,4	0,25	0,6

* NUMBER OF ANIMALS STUDIED IS INDICATED IN PARENTHESES

TABLE 10. DISTRIBUTION OF MICROCSDHERES IN POLYMORPHONUCLEAR LEUKOCYTES (PMN) IN VARIOUS CATEGORIES BURDEN FOLLOWING INSTILLATION OF CO-MIXTURE CF MICROSPHERES AND ASBESTOS FIBRES IN EXPERIMENTAL GROUP 3

SACRIFICE TIMES (DAYS)	TOTAL No. OF PMN/BAL $\bar{X} (x10^4)$	PARTICLE BURDEN PER PMN IN VARIOUS CATEGORIES											
		CATEG. 0		CATEG. 1		CATEG. 2		CATEG. 3		CATEG. 4		CATEG. 5	
		0 PART./PMN		1 PART./PMN		2 PART./PMN		3 PART./PMN		4 PART./PMN		5 PART./PMN	
		$\bar{X}(x10^4)$	%	$\bar{X}(x10^4)$	%	$\bar{X}(x10^4)$	%	$\bar{X}(x10^4)$	%	$\bar{X}(x10^4)$	%	$\bar{X}(x10^4)$	%
1 ₍₆₎ *	179,41	170,93	95,27	6,83	3,81	0,83	0,46	0,28	0,15	0,54	0,30	0,0	0
7 ₍₆₎	34,04	33,53	98,50	0,37	1,10	0,07	0,21	0,07	0,21	0,0	0	0,0	0
30 ₍₆₎	26,38	26,03	98,67	0,49	1,86	0,16	0,61	0,17	0,64	0,0	0	0,01	0,038
80 ₍₆₎	72,38	71,05	98,16	0,66	0,91	0,25	0,35	0,0	0	0,43	0,59	0,0	0
150 ₍₆₎	24,76	22,24	89,82	0,37	1,50	0,15	0,61	0,01	0,04	0,0	0	0,0	0

* NUMBER OF ANIMALS STUDIED IS INDICATED IN PARENTHESES

FIG. 1. TOTAL NUMBER OF FREE CELL POPULATION LAVAGED FOLLOWING INSTILLATION OF PBS, PARTICLES, CO-MIXTURE AND ASBESTOS FIBERS

FIG. 2. NUMBER OF ALVEOLAR MACROPHAGES (AM) LAVAGED FOLLOWING INSTILLATION OF PBS, PARTICLES, CO-MIXTURE AND ASBESTOS FIBERS

FIG. 3. NUMBER OF POLYMORPHONUCLEAR LEUKOCYTES (PMN) LAVAGED FOLLOWING
INSTILLATION OF PBS, PARTICLES, CO-MIXTURE AND ASBESTOS FIBERS

FIG. 4. NUMBER OF LYMPHOCYTES (LY) LAVAGED FOLLOWING INSTILLATION OF PBS, PARTICLES, CO-MIXTURE AND ASBESTOS FIBERS

FIG.5. NUMBER OF ALVEOLAR MACROPHAGES (AM) CONTAINING PARTICLES LAVAGED FOLLOWING
INSTILLATION OF PARTICLES AND CO-MIXTURE OF PARTICLES AND ASBESTOS FIBERS

FIG. 6. NUMBER OF POLYMORPHONUCLEAR LEUKOCYTES (PMN) CONTAINING PARTICLES LAVAGED FOLLOWING INSTILLATION OF PARTICLES AND CO-MIXTURE OF PARTICLES AND ASBESTOS FIBERS

FIG. 7. ESTIMATED PERCENTAGES OF ALVEOLAR MACROPHAGES (AM) CONTAINING PARTICLES IN VARIOUS BURDEN CATEGORIES FOLLOWING INSTILLATION OF MICROSPHERES IN EXPERIMENTAL GROUP 2.

FIG. 8. ESTIMATED PERCENTAGES OF ALVEOLAR MACROPHAGES (AM) CONTAINING PARTICLES IN VARIOUS BURDEN CATEGORIES FOLLOWING INSTILLATION OF CO-MIXTURE IN EXPERIMENTAL GROUP 3.

FIG. 9. ESTIMATED PERCENTAGES OF POLYMORPHONUCLEAR LEUKOCYTES (PMN) CONTAINING PARTICLES IN VARIOUS PARTICLE CATEGORIES FOLLOWING INSTILLATION OF MICROSPHERES IN EXPERIMENTAL GROUP 2.

FIG.10. ESTIMATED PERCENTAGES OF POLYMORPHONUCLEAR LEUKOCYTES (PMN) CONTAINING PARTICLES IN VARIOUS BURDEN CATEGORIES FOLLOWING INSTILATION OF CO-MIXTURE IN EXPERIMENTAL GROUP 3.

FIG .11. NUMBERS OF PARTICLES IN THE LUNG TISSUE FOLLOWING INSTILLATION OF PARTICLES
AND CO-MIXTURE OF PARTICLES AND ASBESTOS FIBERS

FIG.12. NUMBER OF PARTICLES IN THE LYMPH NODE TISSUE FOLLOWING INSTILLATION OF PARTICLES AND CO-MIXTURE OF PARTICLES AND ASBESTOS FIBERS

AIM

Association for Information and Image Management

1100 Wayne Avenue, Suite 1100
Silver Spring, Maryland 20910
301/587-8202

Centimeter

Inches

MANUFACTURED TO AIM STANDARDS
BY APPLIED IMAGE, INC.

DATE

FILMED

12/16/94

END