LA-UR-79-2737

MASTER

CONF-791002--1

TITLE:

FLOW CYTOMETRIC ANALYSIS OF RESPIRATORY TRACT CELLS EXPOSED TO OIL SHALE AND SILICA PARTICULATES

AUTHOR(S):

John A. Steinkamp and Julie S. Wilson

SUBMITTED TO:

To be published in the Proceedings of the 19th Annual Hanford Life Sciences Symposium on Pulmonary Toxicology of Respirable Particles, held in Richland, Washington (October 22-24, 1979) (to be published in the U. S. Department of Energy Symposium Series)

University of California

LOS ALAMOS SCIENTIFIC LABORATORY
Post Office Box 1663 Los Alamos, New Mexico 87545
An Affirmative Action/Equal Opportunity Employer

Form No. 836 R3
St. No. 2629
12/78

UNITED STATES
DEPARTMENT OF ENERGY
CONTRACT W-7405-ENG. 36

FLOW CYTOMETRIC ANALYSIS OF RESPIRATORY TRACT CELLS EXPOSED TO OIL SHALE AND SILICA PARTICULATES

John A. Steinkamp and Julie S. Wilson

Life Sciences Division, Los Alamos Scientific Laboratory

University of California, Los Alamos, New Mexico 87545

ABSTRACT

Flow cytometric techniques were used to measure the cytological and biochemical damage to respiratory tract cells in animals exposed to particulates. Hamsters were exposed to raw and spent oil shale particulates and silica by intratracheal instillation. Exfoliated lung cells were obtained by sacrificing the animals and lavaging the respiratory tract posterior to the trachea with saline. Cell samples were fixed in ethanol and stained with mithramycin for fluorescence analysis of DNA content. DNA content distributions from hamsters exposed to spent oil shale and silica particulates showed atypical changes 28 to 35 days later. Cell counts and total numbers of macrophages, leukocytes, and epithelial cells in the lavage fluid also showed marked changes related to time after exposure.

INTRODUCTION

The application of advanced flow cytometric instrumentation (Steinkamp et al., 1973; Mullaney et al., 1974) to measure cytological and biochemical properties of respiratory tract cells provides a new approach for assessing damage to lung epithelium exposed by inhalation to toxic environmental pollutants associated with the production of synthetic fuels (Steinkamp et al., 1979). This includes the development of automated cytological methods for determining the presence of atypical cells exfoliated from the respiratory tract of experimental animals, with the end objective being examination of sputum samples from exposed humans. To develop analytical flow-analysis methods for quantitative assessment of cellular damage, automated cell-analysis and sorting instrumentation is being applied to study respiratory tract cells from hamsters exposed to particulates of oil shale and silica. This includes (1) acquisition of exfoliated lung cells by lavaging the respiratory tract with normal saline; (2) utilization of fluorescence staining methods for measurement of cellular parameters; (3) initial characterization of respiratory tract cells using flow instrumentation methods; and (4) exposure of experimental animals to physical and chemical toxicants. Examples of the results from initial studies involving measurement of DNA content in normal and exposed respiratory tract cells are presented, along with cell counts and total numbers of macrophages, leukocytes, and epithelial cells as a function of time after exposure. This technology provides a new approach for studying the mechanisms of damage to respiratory lung cells, with future anticipated results serving to assist in estimating the risks, in evaluating the dose-damage relationships, and in establishing guidelines for determining exposure levels to humans.

MATERIALS AND METHODS

Thirty Syrian hamsters were injected intratracheally with 10 mg of ball-milled (2- to 7-μm diameter range) raw oil shale suspended in 0.2 ml of normal saline and 30 with the same amount and size of spent oil shale. Thirty hamsters were similarly instilled with 10 mg of silica (4-μm mean diameter) suspended in 0.2 ml of normal saline. Control hamsters (10) received 0.2 ml of normal saline alone.

The hamsters were anesthetized intramuscularly with methohexital sodium (20 mg) prior to intratracheal instillation of particulates and saline via the oral cavity and then were returned to the colony. Animals exposed to particulates of oil shale and silica were then sacrificed by pentobarbital injection in groups of three for each type of exposure at 4, 7, 14, 21, 28, 35, 42, 49, 60, and 90 days later. The lungs were lavaged four times with saline to obtain exfoliated macrophages, leukocytes, and epithelial cells, which were fixed in 35% ethanol prior to staining for DNA content with mithramycin (Crissman and Tobey, 1974; Crissman et al., 1976). Cell samples were then analyzed using flow cytometric methods, where cellular fluorescence (DNA content) was measured on a cell-by-cell basis as frequency distribution histograms. Cell counts (cells/ml) also were made on lavage samples using a hemocytometer. Cytology was performed to determine the number of different cell types present.

RESULTS AND DISCUSSION

Figure 1 shows a typical DNA content distribution of cells taken by lavaging the respiratory tract of a normal (control) hamster during the 4- to 90-day postexposure period. Peak 1 represents cells having 2C DNA content and peak 2 binucleated cells and doublets having 4C DNA content. Examples of DNA content distributions of lung cells from hamsters exposed to raw and spent oil shale and silica particulates for up to 90 days are shown in Fig. 2. The DNA

content distributions 4 and 7 days postexposure (Figs. 2A and 2B) appear normal with the exception that a region of cells to the left side of peak 1 is present. These are most likely dead cells. Peak 1 also is broadened compared to controls. By 14 to 21 days after exposure, the DNA content distributions of respiratory tract cells exposed to silica (Figs. 2C and 2D) began to show marked changes, with a definite shoulder appearing on peak 1. DNA content distributions recorded on hamsters exposed to raw and spent oil shale particulates during this period were similar to those recorded for the 4- to 7-day postexposure period (Figs. 2A and 2B). Respiratory tract cells from silica-exposed hamsters (Fig. 2E) showed marked changes at 28 days, with peak 1 split into two peaks. Experiments are under way to determine which cells are present in each peak. The 28-day DNA content distribution of respiratory tract cells exposed to raw shale again was similar to those previously recorded for the 4- to 21-day exposure period. The DNA content distribution for hamster lung cells exposed to spent shale for 28 days began to show a definite shoulder on peak 1 that was similar to the data recorded from lung cells exposed to silica for 14 to 21 days (Figs. 2C and 2D). By 35 to 49 days postexposure to spent shale (Figs. 2F through 2H), peak 1 had split into two definite regions similar to lung cells exposed to silica for 28 days (Fig. 2E). The DNA content distributions recorded from lung cells exposed to raw shale for 35 to 49 days showed no change. The DNA content distributions of respiratory tract cells exposed to silica during this period appear to have reverted to normal. However, by 49 days and continuing through 90 days post-exposure to silica, peak 1 of the DNA content distributions was again divided into two regions (Figs. 2H through 2J). The DNA content distributions of cells exposed to spent shale similarly were bimodal but appeared to be reverting to normal as the time after exposure increased. At 49 days

postexposure to raw shale, the DNA content distribution was similar to those previously recorded but, at 60 to 90 days, bimodal distributions began to appear (Figs. 2I and 2J).

Cell counts in the lavage fluid from normal and exposed animals are shown in Fig. 3. The mean cell count for controls (Fig. 3A) was 1.56 x 10^6 cells/ml. The total numbers of macrophages, leukocytes, and epithelial cells also were determined from differential cell counts and were plotted as a function of time after exposure for control and exposed hamsters. These data are shown in Fig. 4. Cell counts from hamsters exposed to raw shale (Fig. 3B) showed an increase (3.21 x 10^6 cells/ml mean) compared to controls. Plots of the total numbers of cells (Fig. 4B) reflect a slight increase in the numbers of macrophages, with a more definite increase in leukocytes and epithelial cells as a function of time. For hamsters exposed to spent shale, the cell count in the lavage fluid (Fig. 3C) initially was elevated when compared to controls but then decreased slightly with time. The mean value was 2.0 x 10^6 cells/ml. The total numbers of macrophages initially were elevated, whereas leukocytes and epithelial cells remained nearly constant throughout the exposure period (Fig. 4C). Cell counts performed on hamster respiratory tract cells exposed to silica (Fig. 3D) were elevated by an order of magnitude (mean 21.8 x 10^6 cells/ml) compared to controls. The results of total cell number counts (Fig. 4D) show that macrophages and leukocytes increased substantially during the 90-day exposure period, indicating an inflammatory response. Epithelial cells also increased slightly in number during the first 30 days and then decreased to near zero at 40 to 60 days.

The above results demonstrate the potential of using flow cytometric analysis methods to study the response of lung cells from exposure to particulates. Future experiments will be designed to correlate cytology with

DNA content measurements through sorting and identifying cells within peak 1 (bimodal distribution). From differential cell counts and plots of total numbers of cells (Fig. 4), we have been unable to identify positively the cells within the two separate regions of peak 1. DNA content measurements also may be coupled with other cellular parameters (e.g., protein, enzymes, etc.) using multiparameter methods to study further cellular damage related to toxic agents.

ACKNOWLEDGMENT

This work was performed under the auspices of the U. S. Department of Energy, with joint support from the U. S. Environmental Protection Agency (interagency agreement EPA-IAG-D5-E681).

REFERENCES

Crissman, H. A., and R. A. Tobey, 1974, Cell Cycle Analysis in Twenty Minutes. Science 184: 1297-1298.

———, M. Oka, and J. A. Steinkamp, 1976, Rapid Staining Methods for Analysis of DNA and Protein in Mammalian Cells. J. Histochem. Cytochem. 24: 64-71.

Mullaney, P. F., J. A. Steinkamp, H. A. Crissman, L. S. Cram, and D. Holm, 1974, Laser Flow Microphotometers for Rapid Analysis and Sorting of Individual Mammalian Cells, In Laser Applications in Medicine and Biology (M. L. Wolbarsht, Ed.), Vol. 2, pp. 151-204, Plenum Press, New York-London.

Steinkamp, J. A., M. J. Fulwyler, J. R. Coulter, R. D. Hiebert, J. L. Horney, and P. F. Mullaney, 1973, A New Multiparameter Separator for Microscopic Particles and Biological Cells. Rev. Sci. Instrum. 44: 1301-1310.

———, J. S. Wilson, and Z. V. Svitra, 1979, Detection of Early Changes in Lung Cell Cytology by Flow-Systems Analysis Techniques. Los Alamos Scientific Laboratory report LA-7983-PR. [Available from National Technical Information Service, U. S. Department of Commerce, Springfield, VA 22161.]

Fig. 1. Frequency distribution histograms (DNA content per cell) of normal hamster respiratory tract cells fixed in 35% ethanol, stained with mithramycin, and analyzed for fluorescence.

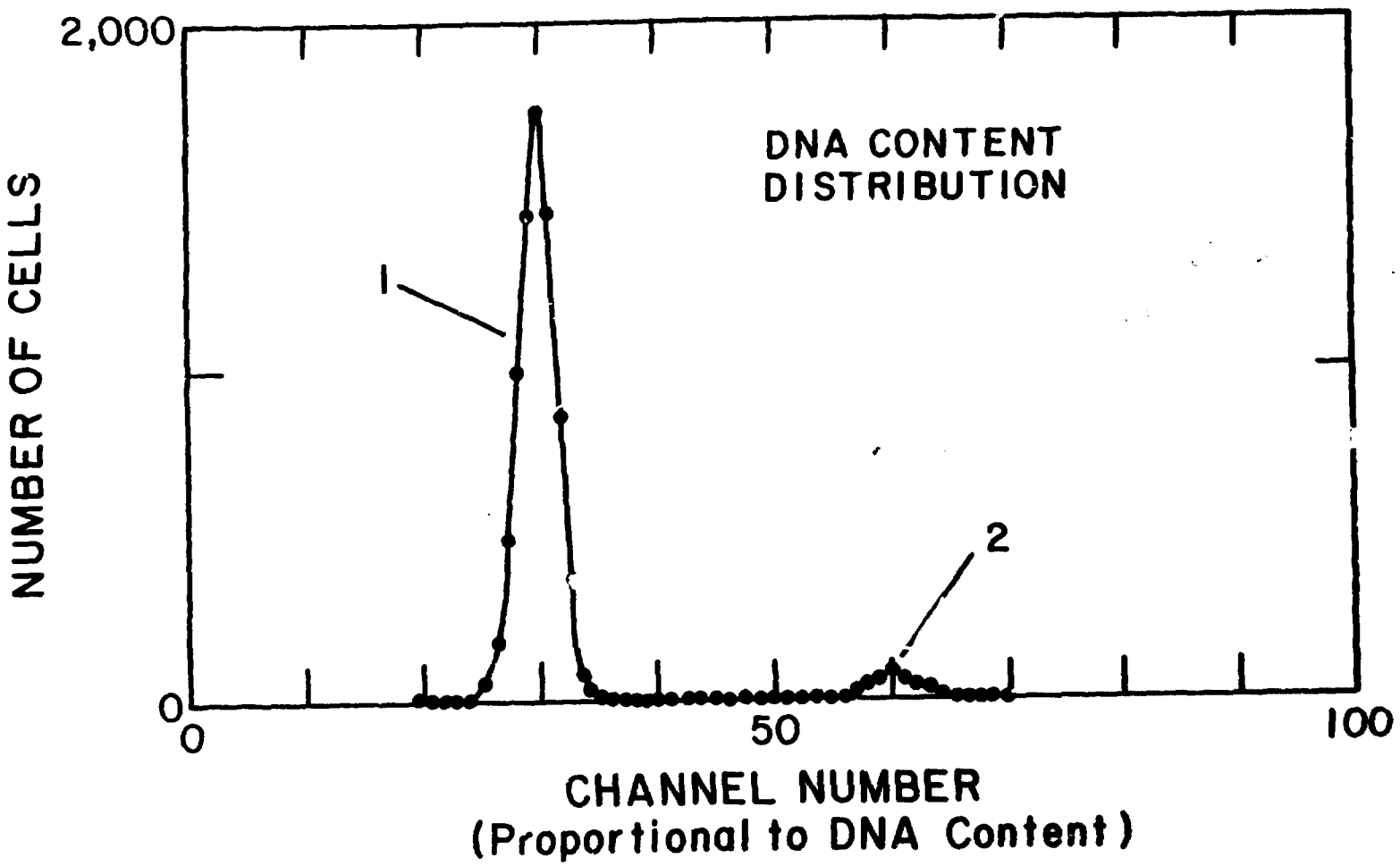
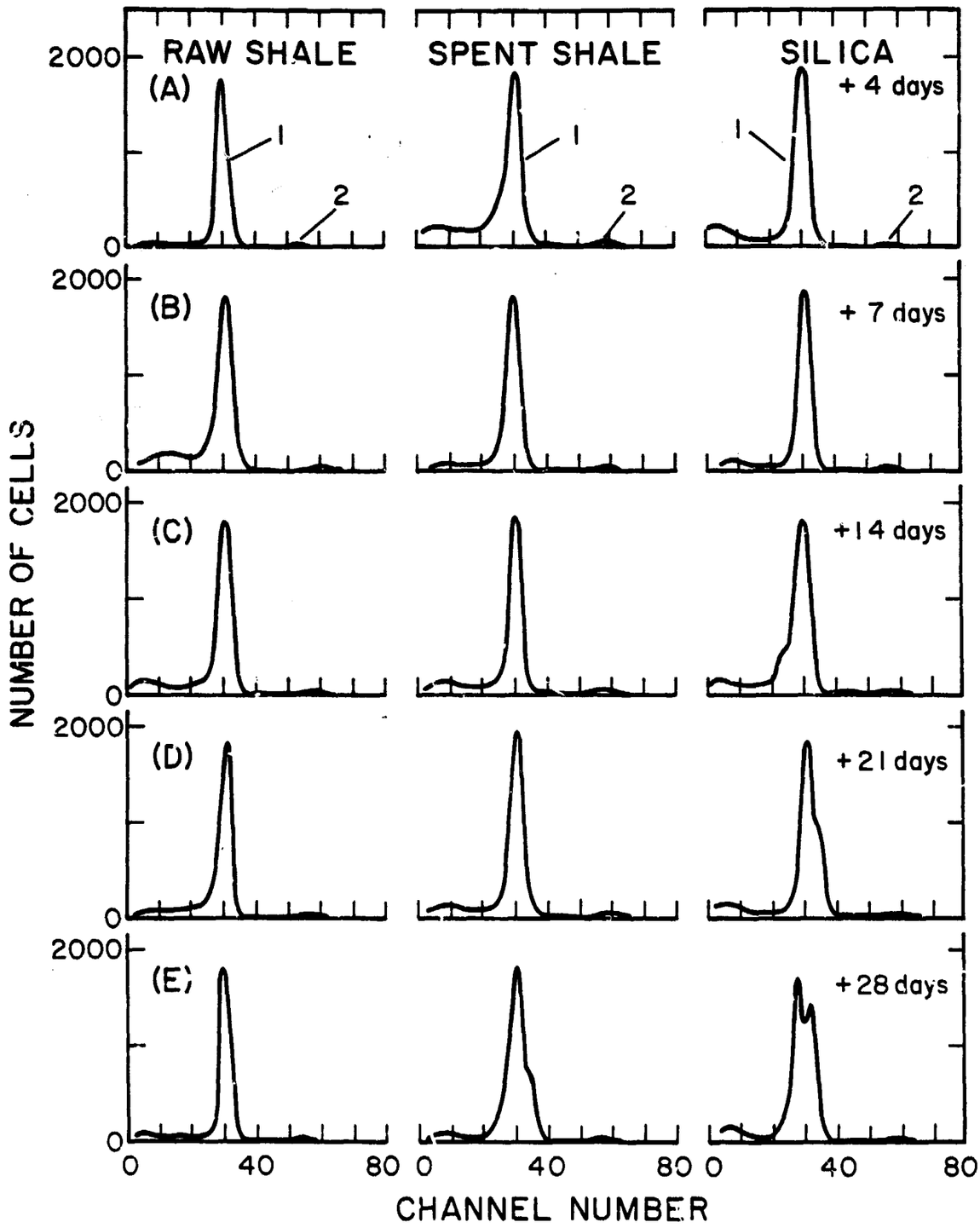

2,000
DNA CONTENT
DISTRIBUTION
1
2
NUMBER OF CELLS
0
0
50
100
CHANNEL NUMBER
(Proportional to DNA Content)

Fig. 2. Typical DNA content frequency distribution histograms of hamster respiratory tract cells exposed to raw and spent oil shale and silica particulates via intratracheal injection at day 0. The hamsters were sacrificed in groups at 4, 7, 14, 21, 28, 35, 42, 49, 60, and 90 days later. Cell samples were obtained by lung lavage, fixed in 35% ethanol, stained with mithramycin, and analyzed for fluorescence. Each DNA content distribution represents data from a single hamster exposed and sacrificed x days later.


RAW SHALE
SPENT SHALE
SILICA
(A)
+ 4 days
(B)
+ 7 days
(C)
+14 days
(D)
+21 days
(E)
+28 days
1
2
2000
0
0
40
80
NUMBER OF CELLS
CHANNEL NUMBER

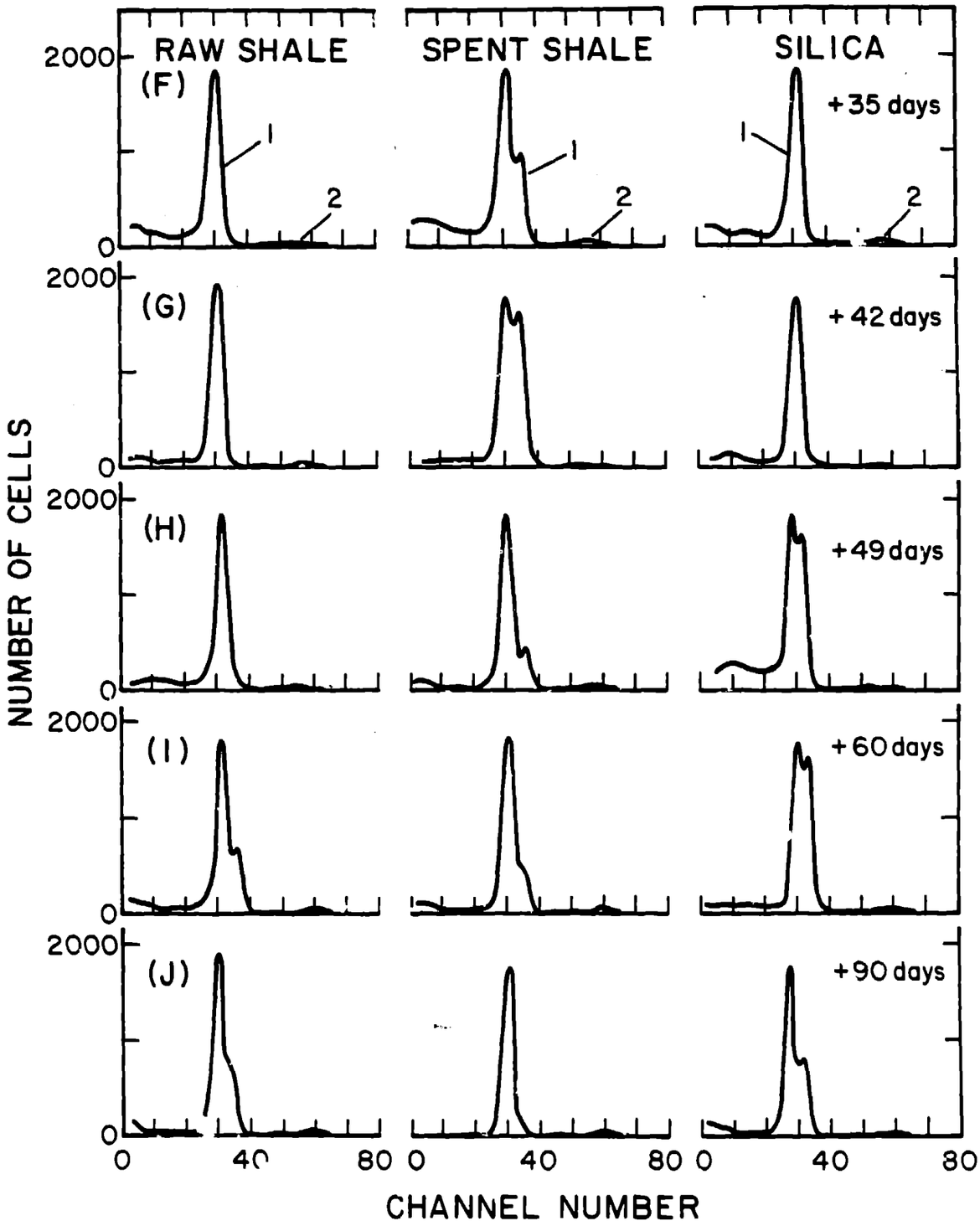

RAW SHALE
SPENT SHALE
SILICA
(F)
+35 days
(G)
+42 days
(H)
+49 days
(I)
+60 days
(J)
+90 days
1
2
2000
0
0
40
80
NUMBER OF CELLS
CHANNEL NUMBER

Fig. 3. Plots of cell counts vs time after exposure of hamster respiratory tract cells (and controls) to raw shale, spent shale, and silica. Each data point for the control represents one animal, whereas each point on the remaining plots represents the average cell count from three hamsters.

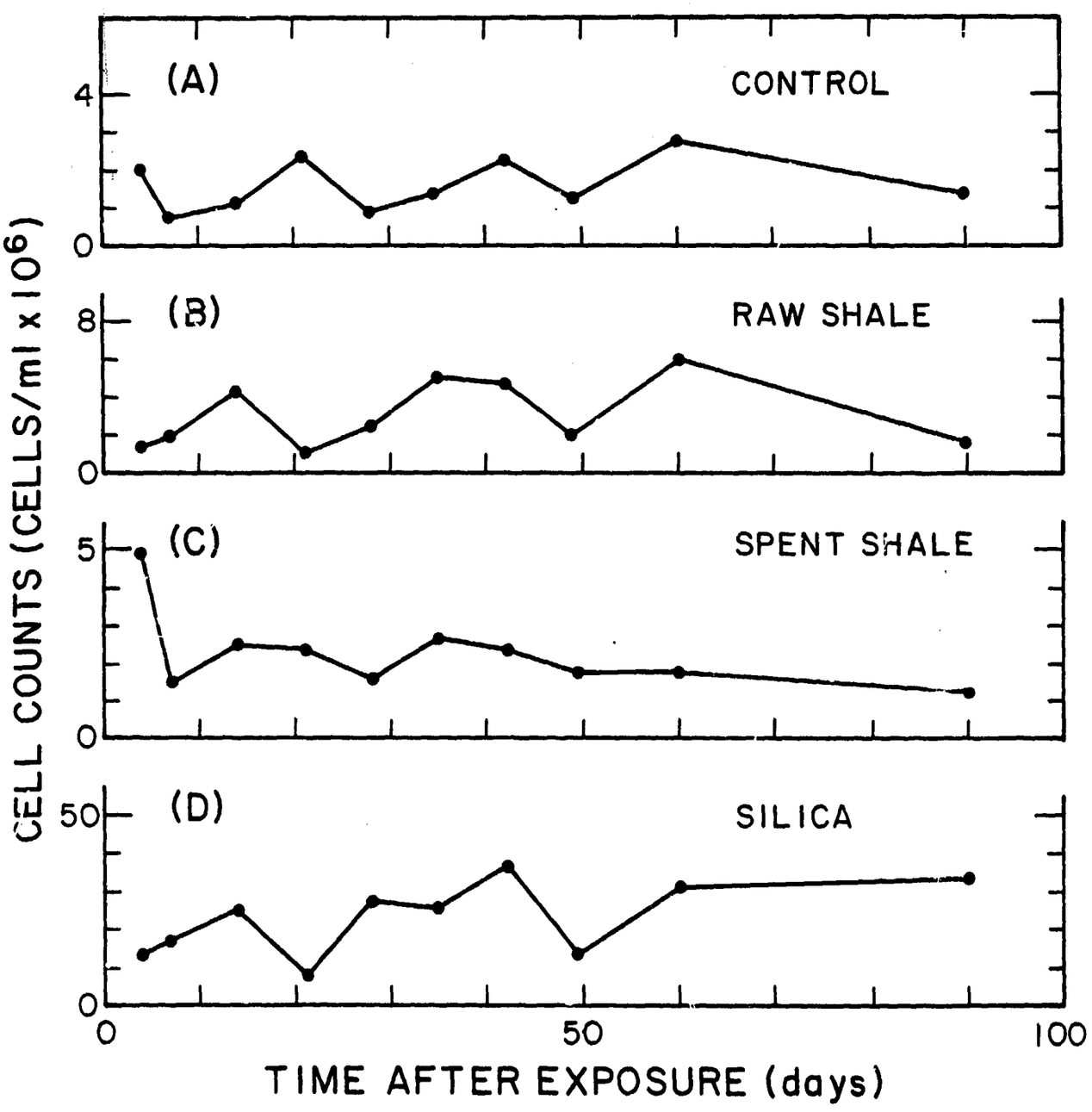
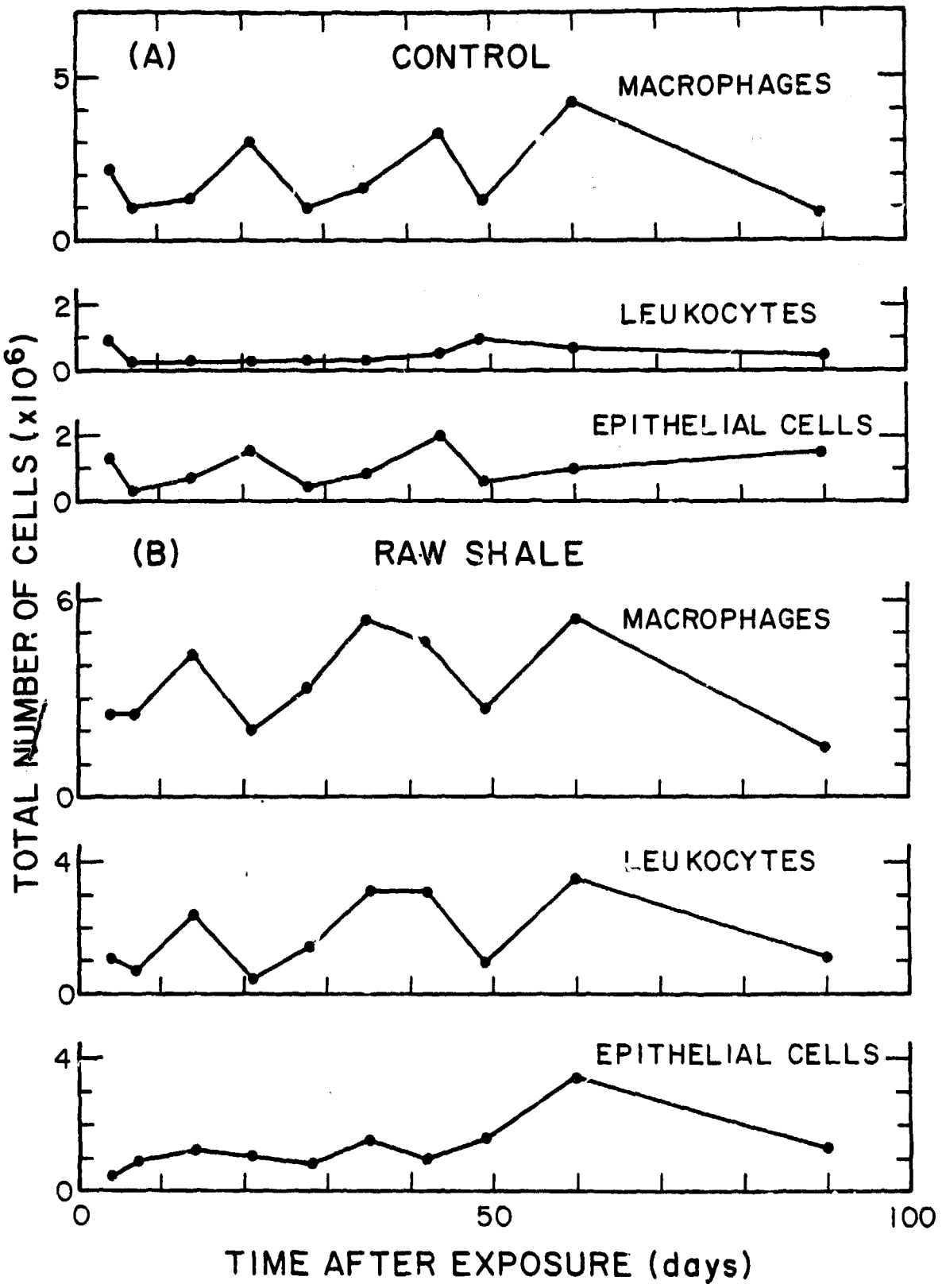
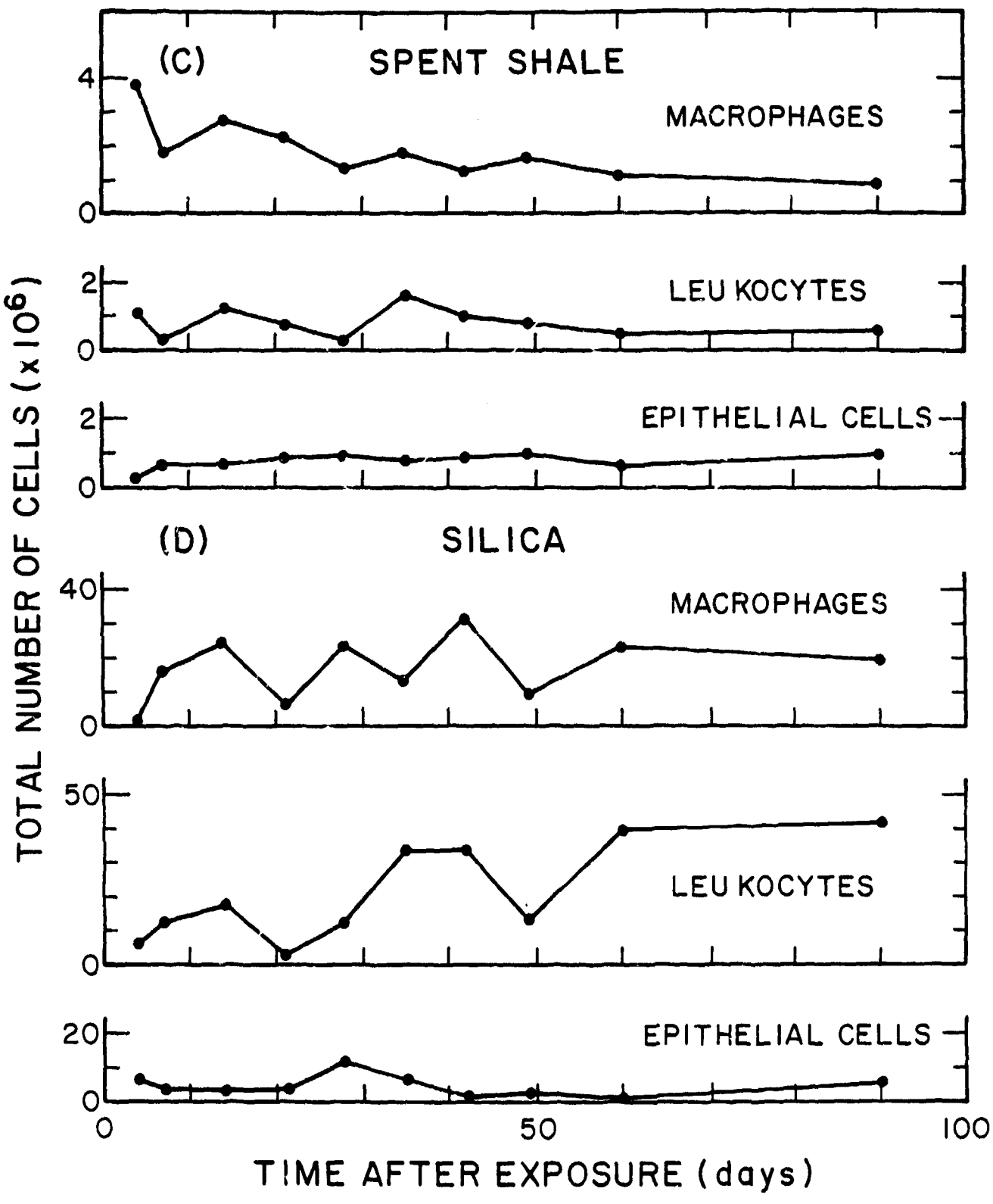

(A)
CONTROL
4
0
(B)
RAW SHALE
8
0
(C)
SPENT SHALE
5
0
(D)
SILICA
50
0
0
50
100
CELL COUNTS (CELLS/ml x 10⁶)
TIME AFTER EXPOSURE (days)

Fig. 4. Plots of total numbers of macrophages, leukocytes, and epithelial cells vs time after exposure of hamster respiratory tract cells (and controls) exposed to raw shale, spent shale, and silica. Each data point for the control represents one animal, whereas each point on the remaining plots represents the average number of cells from three hamsters, obtained by multiplying the percentages from differential cell counts times the individual animal cell counts.


(A)
CONTROL
MACROPHAGES
LEUKOCYTES
EPITHELIAL CELLS
(B)
RAW SHALE
MACROPHAGES
LEUKOCYTES
EPITHELIAL CELLS
CELLS (x10⁶)
TOTAL NUMBER OF
TIME AFTER EXPOSURE (days)
0
50
100


(C)
SPENT SHALE
MACROPHAGES
LEUKOCYTES
EPITHELIAL CELLS
CELLS (x10⁶)
(D)
SILICA
TOTAL NUMBER OF
MACROPHAGES
LEUKOCYTES
EPITHELIAL CELLS
TIME AFTER EXPOSURE (days)