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SUBCELLULAR DISTRIBUTION OF CADMIUM WITHIN THE LUNG  
AND KIDNEY AFTER CADMIUM OXIDE INHALATION EXPOSURE<sup>1</sup>

Morris Blackstone, Phyllis Kaplan, Ph.D.,<sup>2</sup>

Natalie Richdale, B.S.

The University of Cincinnati

College of Medicine

Department of Environmental Health

Kettering Laboratory

Cincinnati, Ohio

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| 7. Author(s)<br>Blackstone, M., P. Kaplan, and N. Richdale                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |  |               |    | 6.                                                          |  |
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| 16. Abstract (Limit: 200 words)<br>Male Sprague-Dawley-rats were exposed for 5 or 7 weeks to cadmium-oxide (1306190) aerosols at relatively low levels of 35 to 275 micrograms/cubic meter (microg/m3) and sacrificed at 4 to 8 months of age. Cadmium was concentrated by the kidney and lung tissues in the cytoplasmic soluble fraction and in the connective tissue. Of the total cadmium present, 50 and 70 percent was associated with the kidney and lung supernatants, respectively. Even so, the total amount of the cadmium retained was far less than reported in the literature. Using an approximate lung volume of 2 cubic centimeters and a breathing rate of 80 times per minute, the percent retention in the lung was about 0.03 percent and in the kidney, about 0.003 percent. These low values may indicate an efficient clearance mechanism by the body which can be supported by the finding of virtually no cadmium present in the lung washings and very few particles present in the cell bodies or alveolar ducts on microscopic examination. However, after 6 weeks exposure, a slight amount of perivascular lymphoid hyperplasia was observed. |  |               |    |                                                             |  |
| 17. Document Analysis<br>a. Descriptors                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |  |               |    |                                                             |  |
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# ABSTRACT

Young male rats were exposed to cadmium oxide aerosols for varying periods of time and their lung and kidney tissues analysed for subcellular distribution patterns of cadmium, zinc and copper. Both organs concentrated cadmium in the cytoplasmic soluble fraction and in connective tissue. After inhalation exposure tissue subcellular fractionation revealed that 50% and 70% of the cadmium present was associated with the kidney and lung supernatants, respectively. These figures are in contrast to the cadmium subcellular distribution before inhalation where the majority was associated with the fibrous connective tissue and larger cell fragments. Distribution patterns for copper and zinc did not change as markedly as did cadmium. Retention values, based on the total quantity of cadmium recovered from each organ, indicate that very little of the inhaled cadmium is contained in either. The lung, being the major site of entry, retained ten times as much cadmium.



## INTRODUCTION

Although the absorption, distribution, metabolism, toxicity and excretion of cadmium has been the subject of extensive review,<sup>1,2</sup> fewer studies have been concerned with respiratory deposition and subsequent metabolism of cadmium containing dusts, aerosols or fumes.<sup>3-6</sup> The metabolic fate of cadmium containing compounds administered by other routes of entry, such as gastrointestinal,<sup>7-9</sup> intravenous,<sup>10,11</sup> subcutaneous,<sup>12,13</sup> or intraperitoneal,<sup>1,14</sup> has been investigated. The results indicate that cadmium is located initially in the plasma, followed by a later association with proteins in the blood cells, in turn followed by subcellular binding in the liver and kidneys. The cadmium-protein association, where examined, appears to be confined to low molecular weight components of serum,<sup>12,17</sup> liver and kidneys,<sup>6,13,15,16</sup> very often the protein, thionein. When found in the cell, cadmium appears to be associated mainly with the cytoplasmic soluble fraction. The present study was undertaken to locate cadmium absorbed into the lungs after short term exposures to CdO aerosol, and measure tissue levels of cadmium as a function of exposure times. These findings were compared with the measured concentrations of cadmium in the kidney, since cadmium appears to accumulate in that organ before its urinary excretion is markedly increased.<sup>1</sup>



### EXPERIMENTAL METHODS

All experiments were performed with male, albino, Sprague Dawley rats, (Laboratory Services), two months old at the start of each experiment. The animals used in this series were maintained on a standard diet (Purina Laboratory Chow) and housed in stainless steel cages. The rats were exposed for 5 to 7 week periods and therefore were sacrificed at ages ranging from approximately 4 to 8 months. After the appropriate inhalation exposure, the experimental as well as control animals were sacrificed after being anesthetized with sodium nembutal, the rib cage opened, and the lungs perfused with the buffer solution used for the succeeding cell homogenization. The perfusion fluid entered through the right ventricle of the heart and exited through the left auricle. The lungs were perfused until they were white in appearance, transferred from the body to ice-cold homogenizing medium and washed four successive times with 10 cc aliquots of homogenizing medium. Lung washing was a modification of standard procedures used in the collection of alveolar macrophages.<sup>19,20,21</sup> The kidneys were rinsed and transferred directly to the cold homogenization medium.

Preparation of the Homogenates: Both kidneys and lungs were homogenized at 4°C in a medium composed of: 0.17 M sucrose, 0.15 M mannitol and 0.005 M 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris), adjusted to a pH of 7.4 with HCl.<sup>19</sup> The procedure was an adaptation of published techniques.<sup>19,21-24</sup> The teflon pestle of the homogenization vessel (Arthur H. Thomas Co., Size C) was machined to provide

a 0.030 inch clearance. The tissue was homogenized for one minute at 800 rpm followed by one minute at 1000 rpm. The final volume of the homogenate was adjusted to give a 10% (w/v) solution. In the case of the lungs, this figure is approximate, as all lungs contained about 2 cc of the buffer used in the lung washings. The crude homogenates of three animals were pooled for each fractionation experiment. Subcellular fractionation was achieved through differential centrifugation<sup>25,26</sup> as modified by Reiss for rat lungs.<sup>24</sup> We have omitted filtration of the homogenate through bolting cloth, and washing of each individual fraction with excess buffer, in order to gain greater precision in the subsequent metal analyses. Examination of the individual fractions by electron microscopy indicates successful separation.

Animal Inhalation Exposures: The inhalation procedures used were those established by E. Bingham et al.<sup>27</sup> Animals were exposed to cadmium oxide aerosols for 8 hours per day, 5 days a week, in individual stainless steel cages. The concentration of the aerosol varied from 36 to 280  $\mu\text{g Cd/m}^3$  on a daily basis. Chemical analyses of the metal in the oxide were conducted daily on samples collected by filtration of the air through millipore filters. The CdO is generated by combustion of cadmium acetate aerosol, the desired concentration being maintained by controlling the volume of aerosol introduced into the combustion oven. The product is diluted with filtered air and passed into the inhalation chambers. The particulate matter in the chambers is sized using a 7-stage Anderson impactor and a 47 mm millipore back up filter. The mass median diameter is 0.15  $\mu$ , and 89% of all the particles are below 0.53  $\mu$ .<sup>28</sup>

## RESULTS AND DISCUSSION

Lung and kidney tissues, obtained from rats before and after a two hundred hour minimum exposure period, were homogenized in sucrose-mannitol buffer. Differential centrifugation of the homogenates separated the cell components into five fractions. Tables Ia and b list the distribution of metals throughout these fractions on a gram per gram dry weight basis and Table II presents the subcellular distribution in percentile terms. Nine to sixteen animals were used for each exposure period and the results are given as the mean plus or minus the standard deviation. Both the control and lower exposure groups contain animals ranging from four to eight months in age. Animals receiving 4 to 5 mgs of cadmium were all six months old. The animals are grouped according to the total amount of cadmium in the cadmium oxide because the variable daily exposure levels made some individual exposure periods shorter than others. All exposed animals were in the chambers 212 to 264 hours. Examination of both tables reveals that cadmium is concentrated in the fibrous connective tissue and the soluble cytoplasmic fraction of both lungs and kidneys. Due to procedural difficulties, the exact amount of buffer in the soluble supernatant is not measured and therefore not subtracted from the total weight of this fraction. Instead, the quantity of metal present is measured in percentile terms (Table II) to make the figure meaningful. Although there is some variation in the zinc and copper levels before and after CdO exposure, the relationship is largely obscured by the deviation from animal to animal. The majority of all three metals resides

in the supernatant and fibrous tissue. There is apparently a distinction between the lung and kidney with respect to cadmium binding in the microsomal fraction. The kidney microsomes selectively concentrate cadmium where their lung counterparts do not. This is best illustrated by Table IV where the results have been expressed in terms of a cadmium/zinc ratio (wt./wt.) in each fraction before and after exposure. Nine animals were used in each group; the exposure period lasted 232-242 hours. Cadmium accumulated in the lung at a faster rate than zinc in almost every fraction, but principally in the supernatant; in kidney, the same was true with respect to the microsomal fraction. Other workers have reported a similar preferential concentration of cadmium in the mitochondria of the liver after a single injection of a equimolar mixture of  $^{109}\text{Cd}$  and  $^{65}\text{Zn}$ .<sup>13</sup>

The exposed animals in these studies were all treated to relatively low concentrations of  $\text{CdO}$  aerosols ( $35\text{-}275\ \mu\text{g}/\text{m}^3$ ) at intervals of 8 hours per day, 5 days a week, over a minimum of 7 weeks. Although the aerosol particle size was small (median diameter  $0.15\ \mu$ ), and the particles were presumably soluble, the total quantities found in the lung and kidney were small (but well above that in the control animals) (Table III). A calculation of the percentage retention (based on the cadmium present in the chamber and the cadmium concentrations in the organs) indicates that far less is retained in the present work than reported in the literature.<sup>1</sup> Thus, using an approximate lung volume of 2 cc and a breathing rate of 80 times a minute, the percent retention in the lung is about 0.03%, and in the kidney, about 0.003%, a factor

of one hundred to a thousand fold less than those estimated for acute<sup>4</sup> as well as chronic<sup>6</sup> exposures. These low values, at this exposure level, may represent an efficient clearance mechanism by the body. In support of this possibility are the findings that: virtually no cadmium is present in the lung washings; and, microscopic examination of lung sections shows very few particles present in the cell bodies or alveolar ducts. A slight amount of perivascular lymphoid hyperplasia was noted after six weeks exposure, however.<sup>28</sup> It should be emphasized that the values used for the organ concentrations are a summation of concentrations measured in individual subcellular fractions. Since the precision with which the individual concentrations were measured was good (2-10% variation) and since all fractions are analysed, any cadmium lost in the fractionation procedure is considered small in proportion to that measured.

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Table Ia. Distribution of Metals in Rat Lung and Kidney Fractions  
(Gms./Gm. dry wt.) as a Function of Exposure to Cd<sup>a</sup>

| Total Cd Exposure               |                        |                                |                                |                                |
|---------------------------------|------------------------|--------------------------------|--------------------------------|--------------------------------|
|                                 | Gms x 10 <sup>-2</sup> | Cd                             | Zn                             | Cu                             |
| Lung:                           |                        |                                |                                |                                |
| Connective tissue <sup>b</sup>  | 0                      | 2.09 x 10 <sup>-6</sup> ± 1.23 | 8.55 x 10 <sup>-5</sup> ± 1.25 | 6.58 x 10 <sup>-6</sup> ± 1.76 |
|                                 | 1.89 - 2.48            | 2.96 x 10 <sup>-5</sup> ± 1.12 | 9.75 x 10 <sup>-5</sup> ± 3.28 | 9.47 x 10 <sup>-6</sup> ± 1.87 |
|                                 | 4.25 - 5.09            | 3.55 x 10 <sup>-5</sup> ± 0.81 | 5.46 x 10 <sup>-5</sup> ± 1.71 | 5.46 x 10 <sup>-6</sup> ± 1.72 |
| Cell Clumps and Large Fragments | 0                      | 1.05 x 10 <sup>-6</sup> ± 1.03 | 3.92 x 10 <sup>-5</sup> ± 1.13 | 2.15 x 10 <sup>-6</sup> ± 0.95 |
|                                 | 1.89 - 2.48            | 1.05 x 10 <sup>-5</sup> ± 0.32 | 4.84 x 10 <sup>-5</sup> ± 1.07 | 3.31 x 10 <sup>-6</sup> ± 1.00 |
|                                 | 4.25 - 5.09            | 1.49 x 10 <sup>-5</sup> ± 0.40 | 3.87 x 10 <sup>-5</sup> ± 0.96 | 2.49 x 10 <sup>-6</sup> ± 0.80 |
| Nuclei, Fragmented Cells        | 0                      | 1.88 x 10 <sup>-6</sup> ± 1.85 | 7.69 x 10 <sup>-5</sup> ± 9.14 | 4.91 x 10 <sup>-6</sup> ± 1.21 |
|                                 | 1.89 - 2.48            | 1.31 x 10 <sup>-5</sup> ± 0.48 | 4.03 x 10 <sup>-5</sup> ± 1.38 | 8.48 x 10 <sup>-6</sup> ± 3.55 |
|                                 | 4.25 - 5.09            | 1.82 x 10 <sup>-5</sup> ± 0.97 | 3.83 x 10 <sup>-5</sup> ± 2.10 | 6.92 x 10 <sup>-6</sup> ± 3.80 |
| Mitochondria                    | 0                      | 2.04 x 10 <sup>-6</sup> ± 1.50 | 3.16 x 10 <sup>-5</sup> ± 2.27 | 5.74 x 10 <sup>-6</sup> ± 2.65 |
|                                 | 1.89 - 2.48            | 1.41 x 10 <sup>-5</sup> ± 0.50 | 5.16 x 10 <sup>-5</sup> ± 2.74 | 1.84 x 10 <sup>-5</sup> ± 1.10 |
|                                 | 4.25 - 5.09            | 2.14 x 10 <sup>-5</sup> ± 0.38 | 4.01 x 10 <sup>-5</sup> ± 0.84 | 8.28 x 10 <sup>-6</sup> ± 1.97 |
| Microsomes                      | 0                      | 2.59 x 10 <sup>-6</sup> ± 3.23 | 6.17 x 10 <sup>-5</sup> ± 2.31 | 6.21 x 10 <sup>-6</sup> ± 3.41 |
|                                 | 1.89 - 2.48            | 1.44 x 10 <sup>-5</sup> ± 0.31 | 5.80 x 10 <sup>-5</sup> ± 2.62 | 1.27 x 10 <sup>-5</sup> ± 0.33 |
|                                 | 4.25 - 5.09            | 2.22 x 10 <sup>-5</sup> ± 0.34 | 7.84 x 10 <sup>-5</sup> ± 0.56 | 6.55 x 10 <sup>-6</sup> ± 3.04 |
| Macrophages                     | 0                      | 2.33 x 10 <sup>-6</sup> ± 1.62 | 1.50 x 10 <sup>-5</sup> ± 0.47 | 2.08 x 10 <sup>-6</sup> ± 1.02 |
|                                 | 1.89 - 2.48            | 7.09 x 10 <sup>-6</sup> ± 7.08 | 3.55 x 10 <sup>-5</sup> ± 2.89 | 8.67 x 10 <sup>-6</sup> ± 3.94 |
|                                 | 4.25 - 5.09            | 1.92 x 10 <sup>-5</sup> ± 0.87 | 5.85 x 10 <sup>-5</sup> ± 3.94 | 6.63 x 10 <sup>-6</sup> ± 2.91 |
| Lung Washings <sup>c</sup>      | 0                      | < limit                        | 1.15 x 10 <sup>-6</sup> ± 1.44 | 7.71 x 10 <sup>-7</sup> ± 1.38 |
|                                 | 1.89 - 2.48            | 3.9 x 10 <sup>-8</sup>         | no values                      | 2.94 x 10 <sup>-7</sup> ± 2.69 |
|                                 | 4.25 - 5.09            | 1.75 x 10 <sup>-7</sup> ± 0.79 | 2.09 x 10 <sup>-7</sup> ± 0.74 | 1.30 x 10 <sup>-7</sup> ± 1.04 |
| Supernatant <sup>c</sup>        | 0                      | 2.6 x 10 <sup>-7</sup>         | 1.22 x 10 <sup>-5</sup> ± 1.89 | 1.21 x 10 <sup>-6</sup> ± 1.06 |
|                                 | 1.89 - 2.48            | 1.41 x 10 <sup>-5</sup> ± 0.79 | 1.01 x 10 <sup>-5</sup> ± 0.51 | 2.08 x 10 <sup>-6</sup> ± 1.29 |
|                                 | 4.25 - 5.09            | 2.25 x 10 <sup>-5</sup> ± 0.81 | 7.09 x 10 <sup>-6</sup> ± 1.37 | 1.02 x 10 <sup>-6</sup> ± 0.40 |

a. The results are expressed as the mean ± the standard deviation from experimental groups consisting of fifteen, sixteen and nine rats for 0, 1.89 and 4.25 mg exposures, respectively.

b. Connective tissue is defined as that remaining in the tissue press after the rest of the organ has passed through.

c. Supernatant and lung washing values have not been corrected for buffer dilution. See text.

Table Ib. Distribution of Metals in Rat Lung and Kidney Fractions  
(Gms./Gm. dry wt.) as a Function of Exposure to CdO<sup>a</sup>

|         | Total Cd Exposure<br>Gms x 10 <sup>-2</sup> |  | Cd                             | Zn                             | Cu                             |
|---------|---------------------------------------------|--|--------------------------------|--------------------------------|--------------------------------|
| Kidney: | Connective Tissue <sup>b</sup>              |  |                                |                                |                                |
|         | 0                                           |  | 1.95 x 10 <sup>-6</sup> ± 1.46 | 1.02 x 10 <sup>-4</sup> ± 0.21 | 2.47 x 10 <sup>-5</sup> ± 0.54 |
|         | 1.89 - 2.48                                 |  | 3.81 x 10 <sup>-6</sup> ± 1.60 | 1.20 x 10 <sup>-4</sup> ± 0.21 | 3.17 x 10 <sup>-5</sup> ± 0.51 |
|         | 4.25 - 5.09                                 |  | 6.61 x 10 <sup>-6</sup> ± 2.76 | 1.10 x 10 <sup>-4</sup> ± 0.27 | 2.93 x 10 <sup>-5</sup> ± 1.61 |
|         | Cell Clumps and<br>Large Fragments          |  |                                |                                |                                |
|         | 0                                           |  | 1.46 x 10 <sup>-6</sup> ± 1.26 | 5.63 x 10 <sup>-5</sup> ± 1.36 | 1.56 x 10 <sup>-5</sup> ± 0.74 |
|         | 1.89 - 2.48                                 |  | 2.82 x 10 <sup>-6</sup> ± 1.13 | 5.73 x 10 <sup>-5</sup> ± 1.77 | 2.22 x 10 <sup>-5</sup> ± 0.88 |
|         | 4.25 - 5.09                                 |  | 3.26 x 10 <sup>-6</sup> ± 0.79 | 5.92 x 10 <sup>-5</sup> ± 0.97 | 1.44 x 10 <sup>-5</sup> ± 1.07 |
|         | Nuclei and Frag-<br>mented Cells            |  |                                |                                |                                |
|         | 0                                           |  | 1.13 x 10 <sup>-6</sup> ± 1.29 | 4.60 x 10 <sup>-5</sup> ± 3.16 | 2.40 x 10 <sup>-5</sup> ± 1.40 |
|         | 1.89 - 2.48                                 |  | 3.05 x 10 <sup>-6</sup> ± 1.75 | 4.88 x 10 <sup>-5</sup> ± 2.64 | 3.21 x 10 <sup>-5</sup> ± 1.00 |
|         | 4.25 - 5.09                                 |  | 2.10 x 10 <sup>-6</sup> ± 0.47 | 6.37 x 10 <sup>-5</sup> ± 2.80 | 1.80 x 10 <sup>-5</sup> ± 1.16 |
|         | Mitochondria                                |  |                                |                                |                                |
|         | 0                                           |  | 1.47 x 10 <sup>-6</sup> ± 1.64 | 4.80 x 10 <sup>-5</sup> ± 1.06 | 2.14 x 10 <sup>-5</sup> ± 1.06 |
|         | 1.89 - 2.48                                 |  | 3.64 x 10 <sup>-6</sup> ± 3.39 | 6.71 x 10 <sup>-5</sup> ± 4.90 | 2.76 x 10 <sup>-5</sup> ± 1.27 |
|         | 4.25 - 5.09                                 |  | 2.89 x 10 <sup>-6</sup> ± 0.54 | 4.74 x 10 <sup>-5</sup> ± 0.84 | 3.76 x 10 <sup>-5</sup> ± 2.33 |
|         | Microsomes                                  |  |                                |                                |                                |
|         | 0                                           |  | 1.34 x 10 <sup>-6</sup> ± 1.52 | 5.13 x 10 <sup>-5</sup> ± 2.42 | 1.04 x 10 <sup>-5</sup> ± 1.25 |
|         | 1.89 - 2.48                                 |  | 4.64 x 10 <sup>-6</sup> ± 5.43 | 6.24 x 10 <sup>-5</sup> ± 1.59 | 1.30 x 10 <sup>-5</sup> ± 0.26 |
|         | 4.25 - 5.09                                 |  | 3.19 x 10 <sup>-6</sup> ± 1.70 | 1.35 x 10 <sup>-4</sup> ± 0.73 | 6.92 x 10 <sup>-6</sup> ± 5.30 |
|         | Supernatant <sup>c</sup>                    |  |                                |                                |                                |
|         | 0                                           |  | 2 x 10 <sup>-7</sup>           | 3.53 x 10 <sup>-5</sup> ± 6.19 | 2.15 x 10 <sup>-5</sup> ± 3.14 |
|         | 1.89 - 2.48                                 |  | 5.23 x 10 <sup>-7</sup> ± 2.33 | 9.03 x 10 <sup>-6</sup> ± 3.88 | 1.14 x 10 <sup>-6</sup> ± 1.31 |
|         | 4.25 - 5.09                                 |  | 1.54 x 10 <sup>-6</sup> ± 0.97 | 9.31 x 10 <sup>-6</sup> ± 3.48 | 3.82 x 10 <sup>-6</sup> ± 1.69 |

a. The results are expressed as the mean ± the standard deviation from experimental groups consisting of fifteen, sixteen and nine rats for 0, 1.89 and 4.25 mg exposures, respectively.

b. Connective tissue is defined as that remaining in the tissue press after the rest of the organ has passed through.

c. Supernatant and lung washing values have not been corrected for buffer dilution. See text.

Table II. Distribution of Trace Elements in Normal and CdO Exposed Rat Lung and Kidney Fractions. Average results of 3 groups with standard errors of means.

| Results expressed as % of reconstituted total   |                         |                |            |                |            |                |                |
|-------------------------------------------------|-------------------------|----------------|------------|----------------|------------|----------------|----------------|
| Kidney                                          | Cd                      | Zn             | Cu         | Ni             |            |                |                |
| Exposure (mgs of Cd)                            | 0                       | 4.66<br>± 0.31 | 0          | 4.66<br>± 0.31 | 0          | 4.66<br>± 0.31 | 4.66<br>± 0.31 |
| Fibrous Tissue                                  | 10.3 ± 2.7 <sup>1</sup> | 8.8 ± 1.2      | 10.7 ± 2.7 | 11.9 ± 0.2     | 6.8 ± 1.54 | 12.0 ± 1.04    | 7.1 ± 2.2      |
| Cell Clumps,<br>Whole Cells,<br>Heavy Fragments | 69.9 ± 4.4              | 31.2 ± 5.4     | 43.1 ± 8.8 | 41.4 ± 0.6     | 37.1 ± 7.8 | 31.7 ± 5.2     | 71.4 ± 4.0     |
| Cell Fragments,<br>Nuclei                       | 7.0 ± 1.4               | 2.2 ± 0.3      | 4.9 ± 1.3  | 5.5 ± 1.6      | 6.2 ± 0.3  | 5.3 ± 0.1      | 5.9 ± 1.6      |
| Mitochondria                                    | 7.5 ± 2.3               | 2.2 ± 0.3      | 6.1 ± 1.5  | 6.8 ± 2.7      | 6.8 ± 0.9  | 4.2 ± 1.1      | 8.0 ± 1.7      |
| Microsomes                                      | 4.2 ± 1.4               | 2.1 ± 0.7      | 3.1 ± 1.3  | 7.6 ± 1.9      | 2.5 ± 0.3  | 1.5 ± 0.2      | 7.6 ± 0.7      |
| Supernatant                                     | 1.1 ± 1.1               | 53.7 ± 6.3     | 32.6 ± 9.6 | 26.8 ± 3.5     | 40.5 ± 9.1 | 45.1 ± 6.0     | < limit        |
|                                                 |                         |                |            |                |            |                |                |
| Lung                                            | Cd                      | Zn             | Cu         | Ni             |            |                |                |
| Fibrous Tissue                                  | 41.7 ± 8.4              | 13.0 ± 1.9     | 37.8 ± 3.0 | 21.6 ± 3.3     | 34.5 ± 3.6 | 22.9 ± 2.2     | 33.6 ± 5.3     |
| Whole Cells,<br>Cell Clumps,<br>Heavy Fragments | 41.1 ± 6.1              | 14.6 ± 2.1     | 34.2 ± 4.9 | 44.9 ± 2.2     | 25.1 ± 3.5 | 23.4 ± 4.0     | 54.4 ± 4.2     |
| Nuclei, Cell<br>Fragments                       | 4.0 ± 1.0               | 0.9 ± 0.1      | 2.0 ± 0.6  | 2.3 ± 0.8      | 3.1 ± 0.6  | 3.3 ± 0.4      | 5.1 ± 1.7      |
| Mitochondria,<br>Lysosomes                      | 4.5 ± 2.3               | 1.0 ± 0.2      | 0.6 ± 0.2  | 1.7 ± 0.2      | 2.5 ± 0.7  | 3.6 ± 0.9      | 3.4 ± 0.5      |
| Microsomes                                      | 2.4 ± 0.7               | 0.6 ± 0.2      | 1.0 ± 0.4  | 3.0 ± 0.8      | 1.7 ± 0.5  | 2.2 ± 0.9      | 3.8 ± 0.7      |
| Supernatant                                     | 2.1 ± 2.1               | 67.8 ± 3.4     | 20.5 ± 9.1 | 24.6 ± 2.7     | 20.7 ± 1.1 | 29.5 ± 2.8     | < limit        |
| Macrophages                                     | 4.1 ± 1.7               | 0.4 ± 0        | 0.6 ± 0.3  | 0.4 ± 0.1      | 1.3 ± 0.05 | 3.3 ± 1.6      | 3.4 ± 0.7      |
| Lung Washings                                   | < limit                 | 1.5 ± 0.5      | 3.2 ± 0.7  | 1.2 ± 0.6      | 11.0 ± 7.0 | 11.6 ± 5.4     | < limit        |

1. ± standard error

Table III. Retention of Cadmium in Lung and Kidney After Inhalation Exposure.  
Results are expressed as grams of cadmium in each cell fraction.

| Hours Exposure                        |                                 | 0                | 0                       | 232 <sup>a</sup>   | 248 <sup>a</sup>    | 264 <sup>a</sup>    |
|---------------------------------------|---------------------------------|------------------|-------------------------|--------------------|---------------------|---------------------|
| Grams Found                           |                                 | $\times 10^{-6}$ | $\times 10^{-6}$        | $\times 10^{-6}$   | $\times 10^{-6}$    | $\times 10^{-6}$    |
| <u>Lung:</u>                          |                                 |                  |                         |                    |                     |                     |
|                                       | Cell Component                  |                  |                         |                    |                     |                     |
|                                       | Connective tissue               | 0.540            | 0.811                   | 9.0                | 12.9                | 13.9                |
|                                       | Homogenate                      | 0.096            | 0.66                    | 2.45               | 2.93                | 2.29                |
|                                       | Cell clumps and large fragments | 0.398            | 0.47                    | 14.3               | 16.5                | 13.9                |
|                                       | Cell fragments and nuclei       | 0.033            | 0.054                   | 0.69               | 1.02                | 1.38                |
|                                       | Mitochondria and lysosomes      | 0.06             | 0.09                    | 0.56               | 1.06                | 1.69                |
|                                       | Microsomes                      | 0.018            | 0.024                   | 0.42               | 0.65                | 1.38                |
|                                       | Supernatant                     | <limit           | <limit                  | 46.9               | 82.0                | 88.3                |
|                                       | Macrophages                     | 0.100            | 0.017                   | 0.345              | 0.65                | 1.38                |
|                                       | Lung Washings                   | <limit           | <limit                  | 0.905              | 1.25                | 3.02                |
|                                       | TOTAL                           | 1.211            | 2.127<br>(Avg. = 1.665) | 73.90 <sup>b</sup> | 117.29 <sup>b</sup> | 125.57 <sup>b</sup> |
| <u>Kidney:</u>                        |                                 |                  |                         |                    |                     |                     |
|                                       | Connective tissue               | 0.180            | 0.402                   | 0.76               | 1.68                | 2.21                |
|                                       | Homogenate                      | 0.122            | <limit                  | 0.42               | 0.75                | 0.66                |
|                                       | Cell clumps, large fragments    | 1.450            | 1.79                    | 3.15               | 5.40                | 5.99                |
|                                       | Nuclei and cell fragments       | 0.180            | 0.114                   | 0.25               | 0.41                | 0.46                |
|                                       | Mitochondria                    | 0.068            | 0.188                   | 0.20               | 0.45                | 0.48                |
|                                       | Microsomes                      | 0.044            | 0.084                   | 0.26               | 0.23                | 0.71                |
|                                       | Supernatant                     | <limit           | <limit                  | 3.79               | 7.73                | 18.5                |
|                                       | TOTAL                           | 2.044            | 2.578<br>(Avg. = 2.311) | 6.52 <sup>b</sup>  | 14.34 <sup>b</sup>  | 26.70 <sup>b</sup>  |
| Grams of cadmium inhaled <sup>c</sup> |                                 | -                |                         | 0.2844             | 0.3342              | 0.3824              |
| Percent cadmium retained:             |                                 |                  |                         |                    |                     |                     |
|                                       | Lung                            |                  |                         | 0.026              | 0.035               | 0.033               |
|                                       | Kidney                          |                  |                         | 0.002              | 0.004               | 0.007               |

a. 232, 248, 264 hour exposures deliver  $4.25$ ,  $4.68$  and  $5.05 \times 10^{-2}$  gms Cd respectively, to the chamber. Weight figures have been adjusted to represent only the cadmium present in the oxide.

b. The figures representing the grams recovered have been corrected for the cadmium present before the experiment began.

c. The calculations are based on an average lung volume of 2 cc and an inhalation rate of 80/minute. There are 3 rats in each exposure grouping.

Table IV. The Cadmium-Zinc Ratio for Lung and Kidney Fraction  
Before and After Exposure to Cadmium Oxide Aerosol<sup>1,2</sup>

| Subcellular Fraction            | Lung    |         | Kidney  |         |
|---------------------------------|---------|---------|---------|---------|
|                                 | Control | Exposed | Control | Exposed |
| Connective Tissue               | 0.0244  | 0.507   | 0.0191  | 0.031   |
| Cell Clumps, Large<br>Fragments | 0.0268  | 0.257   | 0.0259  | 0.056   |
| Nuclei, Cell<br>Fragments       | 0.0244  | 0.515   | 0.0246  | 0.076   |
| Mitochondria                    | 0.0646  | 0.226   | 0.0306  | 0.060   |
| Microsomes                      | 0.0420  | 0.289   | 0.0261  | 0.139   |
| Supernatant                     | 0.0213  | 1.308   | 0.0057  | 0.0615  |
| Macrophages                     | 0.155   | 0.141   |         |         |

1. The exposure period lasted  $235 \pm 5$  hours.
2. The median figure for the analysis of 9 animals was used for each calculation.

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