TOXICOLOGY OF INHALED LOW-FIRED AND
HIGH-FIRED BERYLLIUM OXIDES
IN THE RAT AND HAMSTER

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SUMMARY

Seventy rats and 70 hamsters were exposed to aerosols of high-fired BeO for 3 hours and examined at intervals for 63 days after exposure. The BeO exhibited an MMAD of about 1 μ m. From 77 to 85% of the BeO was deposited in the tracheobronchial tree and 15 to 23% in the alveolar region, amounting to 150 μ g of Be in rats and 20 μ g Be in the alveoli of hamsters. Pathological alterations were limited primarily to the alveolar macrophage which rapidly concentrated BeO particles following exposure. The BeO was very insoluble as seen by lack of translocation of Be to liver and bone and the small amount excreted in urine. The BeO particles were tenaciously retained in alveolar macrophages with only 12 to 21% of alveolar deposited BeO being cleared from the alveoli in 63 days. No significant differences were noted between sexes or between rat and hamster.

Toxicology of Inhaled Low-Fired and High-Fired
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The development of pulmonary disease in humans has been associated with exposures during the mining, processing and manufacturing of beryllium-containing compounds. Beryllium oxide (BeO) is the air contaminant common to most situations in which berylliosis has been seen in humans (Breslin, 1966). Low-fired BeO is more toxic than is high-fired BeO (Hall et al., 1950; Spencer et al., 1967). Studies with inhaled BeO in quinea pigs and rats, demonstrated the production of granulomata, interstitial fibrosis, metaplasia and epithelial neoplasia (Policard, 1950; Vorwald, 1953 and 1958; Schepers, 1961 and 1957). Inhaled BeSO_4 in rats is the most potent beryllium compound carcinogen tested and also the most soluble (Reeves, Deitch and Vorwald, 1967). A greater in vivo solubility of a beryllium compound is associated with a greater toxicity in the lung.

Differences among rodent species in carcinogenic action of inhaled beryllium dust have been reported, with the rat exhibiting a high incidence of lung tumors as compared to none seen in the hamster (Wagner et al., 1969). Differences have also been observed between the sexes with regards to lung clearance rates and translocation of inhaled beryllium to pulmonary lymph nodes (Reeves and Vorwald, 1967).

METHODS

Male and female Wistar SPF albino rats and Syrian golden hamsters were obtained from suppliers and placed in isolation quarters for 3 weeks prior

to their exposure to BeO. All animals were about 70 days of age at time of exposure. Beryllium oxide was obtained from ORNL as a high-fired (about 1000°C) compound.

Exposure and Aerosol Characterization - The animal exposure system is designed to provide nose-only exposure of rodents to BeO. The rodents are contained in Coca Cola bottles which have the bottoms and part of the neck removed and ground. Large rubber stoppers seal off the bottoms of the bottles after the animals are inserted. Other rubber stoppers with holes cut in the center are placed over the bottle necks and then are inserted into circular ports in the aerosol chamber. The aerosol chamber is constructed of clear Lucite plastic in cylindrical form, with the exposure ports arranged in 7 tiers of 11 ports each. Circular shelves separate each tier of ports. Of the 77 ports in the chamber, 7 are used for locating aerosol sampling equipment, while 70 are used for animal exposures.

The aerosol enters the chamber at the top end through a diffuser which breaks up streamlines, enhancing the uniform distribution of aerosol throughout the chamber. The aerosol is exhausted from the bottom of the chamber into an absolute filter. The aerosol chamber along with the associated aerosol generating equipment and sampling equipment are all contained within a Lucite glove box (Figure 1). A continuous flow of room air enters this box and is exhausted through an absolute filter into a double filtered exhaust system. Four sets of rubber gloves, sealed to ports in the containment box, allow access to all interior points within the box. All items, including animals, entering or leaving the containment box do so via a pass-through chamber. The two doors on the chamber leading into the containment box and into the room are never open at the same time, reducing the possible release of

aerosols into the room.

The aerosol is generated by a Wright Dust Feed Mechanism, employing a metal cup into which BeO is packed with a Carver Press. The mounted cup turns against a stationary blade which scrapes a thin layer of BeO from the pack. Air under pressure, blows the loosened dust through a jet, creating the aerosol. BeO is stored in a vacuum desicator prior to packing for aerosol generation. The aerosol generation rate is determined by a system of gears which alters the rate that the blade cuts into the pack (Figure 2).

The aerosol is then passed through a cyclone elutriator (Figure 2) before its introduction into the chamber, in order to remove large particles from the aerosol, which are not respirable by rodents.

During exposures 3 types of aerosol samples are taken: 1) Filter samples to determine total aerosol concentration; 2) Electrostatic precipitator samples to obtain representative samples for viewing with the electron microscope and sizing with the TGZ 3 Zeiss particle size analyzer and 3) Mercer cascade impactor samples, which classify particles in the aerosol according to their aerodynamic diameters into eight fractions. The particle size distribution is then determined by computer analysis in which the distributions are compared to log-normal distributions and median diameters and geometric standard deviation values calculated.

Table I summarizes the exposure conditions and aerosol characterization. The mass median aerodynamic diameter (MMAD) was about 1.0. Electron micrographs of BeO particles collected by electrostatic precipitation show considerable coagulation of particles (Figure 3). Individual particles were mostly in the size range of 0.05-0.10 μm diameter. The calculated initial alveolar deposition of BeO in hamsters compares well with what was actually

observed (Tables I and V).

Table I. Aerosol Characterization for BeO

<u>Parameter</u>	<u>Rat</u>	<u>Hamster</u>
Mean Chamber Concentration ($\mu\text{g Be/liter air}$)	44	12
Exposure Time (minutes)	180	150
MMAD (μm)	1.2	0.89
Estimated Total Deposition ($\mu\text{g Be}$)	770	116
Estimated Initial Alveolar Deposition ($\mu\text{g Be}$)	120	17

Biological Sampling - Five rats of each sex were killed at 1, 7, 14, 21, 35, 49 and 63 days after exposure; five hamsters of each sex were killed at 1, 7, 21, 35, 49 and 63 days after exposure. Death was by overdose of chloroform. Cardiac blood was obtained and mixed with EDTA; red and white blood cell counts were performed using a Model F_n Coulter Counter.. White cell differentials were performed from Wright stained blood smears. The trachea was cannulated with P.E. 60 polyethylene tubing and the lung was washed with 2 x 10 ml lavages of Isoton (Coulter Electronics). The mixed 2 lavages from each animal were diluted in Isoton, and the cell count determined with the Coulter Counter.

Four animals per species per sex per time period were lavaged; one animal underwent tracheal cannulation with the lung being instilled with Karnovsky's fixative for electron microscopic examination. Small pieces of lung were post-fixed in 1% osmium tetroxide, dehydrated in ethanol and embedded in plastic. Silver sections were stained with uranyl acetate and lead

citrate and examined with a RCA-EMU electron microscope. The cardiac lobe of all others animals was fixed in 10% neutral buffered formalin, embedded in paraffin and sections stained with hematoxylin and eosin for examination under the light microscope. Weights of lung, liver, spleen, kidney, adrenal and total body were determined.

Five female and 5 male rats and hamsters were placed in individual metabolism cages immediately following exposure. Feces and urine were collected from 0-3 days and at weekly intervals up to 63 days after exposure. The Be contents of lung, lung lavage, pulmonary lymph nodes (associated with thymus, trachea and main stem bronchi), liver, skeleton and remaining tissues was determined along with Be contents of excreta samples.

Analytical Determination of Beryllium - BeO-containing samples were muffled at 400°C, mixed with HNO_3 and brought to dryness. The ash was digested in several steps with 8 M HNO_3 , 12 M HF and 6 M HCL. The converted salts were dissolved in acid and diluted to 25 ml with distilled water, such that the final dilution contained about 2 M HNO_3 and 0.2 M HCL. The solution was aspirated into a Perkin-Elmer 303 atomic absorption spectrophotometer and absorbancy was read at a wavelength of 2349Å. A Be hollow cathode lamp was used in conjunction with a nitrous oxide-acetylene flame. A deuterium background corrector was used to correct for interference from high salt levels in some samples. Analytical sensitivity was 0.01 ppm or 0.25 µg Be per 25 ml sample. Working standard curves were determined using a HARLECO Be standard containing 1000 ppm aqueous $\text{Be}(\text{NO}_3)_2$. From 103 to 106% of Be spiked into tissue samples was recovered by this procedure. Lung samples, repeatedly rerun over several months, showed good reproducibility: the mean Be contents $\pm$ standard deviation for lung samples run 19 times and 16 times

were $0.98 \pm 0.02 \mu\text{g}$ and $4.43 \pm 0.08 \mu\text{g}$, respectively. No background interference was seen in Be determinations on tissues or urine. Significant interference was seen with fecal samples, requiring appropriate corrections in Be determinations.

RESULTS

Biological Effects: General - The body weights of male rats ranged from 406 g at 1 day after exposure to 532 g at 63 days; female rats ranged from 301 g to 370 g over this period, while male and female hamsters exhibited body weights that ranged from 108 g to 130 g. Body weights, and weights of spleen, liver, kidney and lung were not significantly different from those of unexposed control animals. There was an approximate doubling in adrenal weights of both male and female rats and hamsters at 21-49 days after exposure; adrenal weights returned to near normal by 63 days. Red and white blood cell counts were not significantly different from unexposed control animals.

Biological Effects: Lung - There was little BeO-related pulmonary pathology in rats or hamsters up to 63 days after exposure. The only alterations were confined to the alveolar macrophage and limited areas in peribronchiolar regions of the lung, which exhibited a mild granulomatous reaction characterized by an accumulation of BeO-laden foamy macrophages, inflammatory mononuclear cells and limited alveolar interstitial fibrosis. It is emphasized that these lesions were small and few in number. Under the electron microscope, there was observed in alveoli an apparent accumulation of eosinophils in capillary spaces and a small increase in membranous debris in alveolar air spaces.

The only other alterations were confined to alveolar macrophages, residing in alveolar air spaces. BeO particles were observed with the light and electron microscope to be nearly always in alveolar macrophages during the 63 day observation period. BeO particles were seen within phagosomes of macrophages within the first day after exposure. Macrophages, within a few weeks, developed numerous, large, clear vacuoles in their cytoplasm. After several more weeks, these cells had become enlarged, exhibiting a highly vacuolated appearance, the cytoplasmic vacuoles containing lysosomal-like material, BeO particles, membranous debris and other cellular debris (Figure 4). BeO particles were not observed in type I or II alveolar epithelium or in other cells of the septal walls, although other metal oxides have been observed in type I epithelial cells (Sanders, 1969; Sanders and Adey, 1970; Sanders et al., 1971). The fine structural alterations in lung due to deposited Be compounds has been the subject of several publications, one of which describes the presence of Be in cells of the alveolar walls (Robins, Schaffner and Trachtenberg, 1968; Conradi et al., 1971; Potapova and Seleznev, 1966 and 1967).

Surprisingly, there was not observed a significant reduction in cells found in lung lavages following inhalation of BeO (Table II). This would indicate that either the structural alterations seen in BeO-laden macrophages did not result in significantly increased cell death, or that macrophage replacement kept up with any increased cell death.

Table II. Recovery of alveolar cells and BeO in lung lavages of rats. Values are $\bar{x}$ (n,4-5) $\pm$ S.D.

Time After Exposure (Days)	Alveolar Cells/ml $\times 10^3$		Be Content of Lavage (μg)	
	Female	Male	Female	Male
Unexposed Control	133 $\pm$ 36	156 $\pm$ 46	---	---
1	156 $\pm$ 69	172 $\pm$ 56	---	2.99 $\pm$ 1.64
7	223 $\pm$ 65	304 $\pm$ 141	4.88 $\pm$ 2.75	6.05 $\pm$ 3.85
14	160 $\pm$ 55	220 $\pm$ 63	1.82 $\pm$ 0.99	1.70 $\pm$ 0.57
21	196 $\pm$ 75	274 $\pm$ 49	1.56 $\pm$ 0.57	2.78 $\pm$ 1.28
35	215 $\pm$ 120	174 $\pm$ 47	1.95 $\pm$ 0.86	2.49 $\pm$ 1.48
49	176 $\pm$ 91	---	1.71 $\pm$ 1.17	---
63	177 $\pm$ 115	113 $\pm$ 49	2.35 $\pm$ 1.74	0.89 $\pm$ 0.60

The removal of inhaled BeO in lung lavages tended to follow the number of cells found in lavages. The greatest amount of Be was found in lavages at 7 days after exposure, corresponding to the time of highest cell count, while the least amount of Be was found in males at 63 days, corresponding to the lowest recorded cell count (Table II). Damage to macrophages from phagocytized BeO, resulting in a greatly increased cell size, may have decreased the ability of lavage to mechanically remove them from alveoli. Studies with inhaled PuO_2 in rats have demonstrated a 10% removal of Pu lung burden during the first month after exposure by 2 x 10 ml lavages; the macrophages found in these lavages were not significantly larger than those found in lavages of unexposed control rats (Sanders, 1969; Sanders, unpublished data).

Fate of Inhaled BeO - A total of 483 μg Be and 918 μg Be were excreted by female and male rats, respectively, during the 63 day period following exposure; of these amounts, 92% and 95% were excreted in the feces, in females and males respectively (Table III). Of the total amount of Be excreted in the feces, 97% was excreted the first 3 days after exposure, and

only 3% from 4-21 days after exposure. Be was below detectable limits in urine samples taken after 7 days and in fecal samples taken after 15-21 days.

Table III. Excretion of Inhaled BeO in Rats

Time After Exposure (Days)	<u>Amount of Be (μg)</u>			
	<u>Urine</u>		<u>Feces</u>	
	<u>Female</u>	<u>Male</u>	<u>Female</u>	<u>Male</u>
0- 3	38.3	43.2	428	842
4- 7	1.6	2.6	11.2	20.9
8-14	N.D.	N.D.	4.2	8.0
15-21	N.D.	N.D.	N.D.	0.6
22-63	N.D.	N.D.	N.D.	N.D.

The average total amount of Be inhaled by rats was 605 μ g in females and 1041 μ g in males. Of these amounts, 22.9% and 15.0%, or 138 μ g Be and 156 μ g Be, were initially deposited in the alveoli of female and male rats, respectively (Table IV). Limited amounts of Be were cleared from the alveoli during the 63-day observation period, amounting to only 12 to 20% of initial alveolar Be burdens. About 2% of initial alveolar Be lung burden was translocated to pulmonary lymph nodes by 63 days after exposure; no differences were seen between male and female rats (Table IV). The behavior of BeO in hamster lung was similar to that seen in rats. Detectable amounts of Be were not found in any liver or skeleton samples of hamster or rat.

Table IV. Deposition, pulmonary clearance and translocation of inhaled BeO in rats.

<u>Parameter</u>	<u>Female</u>	<u>Male</u>
<u>% Total Lung Deposition¹</u>		
Tracheobronchial Deposition ²	77.1	85.0
Alveolar Deposition	22.9	15.0
<u>% Initial Alveolar Deposition³</u>		
Alveolar Clearance ⁴	12.3	20.6
Lung ⁵	85.2	76.6
Pulmonary Lymph Nodes ⁵	1.7	1.6
Other Tissues ⁵	0.8	1.2

¹All excreta and tissues

²All excreta from 0-3 days

³All excreta from 4-63 days + tissues at 63 days

⁴All excreta from 4-63 days

⁵At 63 days

No statistically significant clearance of inhaled BeO from the pulmonary alveoli was evident when examining groups of serially sacrificed male or female rats or hamsters at from 1 day to 63 days after exposure (Table V). There was, however, an indication of greater alveolar clearance of BeO in hamsters than in rats, possibly due to the lesser amount of BeO deposited in the hamster lung, than in the rat lung. Data from excretion studies did not show a great difference in lung retention of BeO between the two species. The retention half-life for BeO in either rodent species may approach several hundred days. This is a rather unexpected finding, considering the general lack of pathological reaction to the BeO. Damage to BeO-laden macrophages may be sufficient to inhibit their movement to the ciliated portions of the upper respiratory tract.

Table V. Retention of inhaled BeO in rat and hamster lung. Values are $\bar{x}$ (n,4-5) $\pm$ S.D.

Time After Exposure (Days)	Amount of Be In Lung (μ g)			
	Rat		Hamster	
	Female	Male	Female	Male
1	150.8 $\pm$ 25.7	138.3 $\pm$ 54.8	20.1 $\pm$ 7.8	15.3 $\pm$ 4.2
7	91.0 $\pm$ 18.9	107.7 $\pm$ 36.7	11.2 $\pm$ 4.7	14.1 $\pm$ 10.1
14	113.0 $\pm$ 20.8	88.1 $\pm$ 20.8	---	---
21	87.9 $\pm$ 26.3	105.7 $\pm$ 32.9	5.0 $\pm$ 1.6	8.1 $\pm$ 3.1
35	87.7 $\pm$ 30.2	113.6 $\pm$ 35.7	8.5 $\pm$ 2.1	7.2 $\pm$ 3.3
49	91.5 $\pm$ 40.6	---	7.0 $\pm$ 2.7	9.2 $\pm$ 2.9
63	117.6 $\pm$ 62.2	119.5 $\pm$ 44.1	10.2 $\pm$ 5.0	8.5 $\pm$ 4.1

There was a slow accumulation of Be in pulmonary lymph nodes (Table VI), although the amount translocated from lung into lymph nodes was small. No differences were seen in translocation between male and female rats. No detectable amounts of Be were found in hamster pulmonary lymph nodes.

Table VI. Accumulation of inhaled BeO in pulmonary lymph nodes of rats. Values are $\bar{x}$ (n,4-5) $\pm$ S.D.

Time After Exposure (Days)	Amount Be in Pulmonary Lymph Nodes (μ g)	
	Female	Male
1	0.69 $\pm$ 0.40	1.03 $\pm$ 0.55
7	1.08 $\pm$ 1.38	1.73 $\pm$ 0.88
14	2.14 $\pm$ 1.32	1.35 $\pm$ 1.22
21	1.64 $\pm$ 1.25	2.28 $\pm$ 1.10
35	1.68 $\pm$ 0.83	0.97 $\pm$ 0.74
49	2.39 $\pm$ 1.97	---
63	2.33 $\pm$ 1.01	2.56 $\pm$ 1.35

COMMENTS

As has been previously reported, high-fired BeO did not illicit a general pathological-granulomatous response in the lung of either rats or hamsters, such as has been seen with more soluble beryllium compounds. There was no evidence of in vivo solubilization of high-fired BeO in the lung, since detectable amounts of Be were not found in liver or bone. The tenacious retention of inhaled high-fired BeO in the lung of rats and hamsters was a surprise and has not been previously described in the literature. The prolonged retention of high-fired BeO was probably due to the rather specific damage produced in BeO-laden alveolar macrophages, which rapidly phagocytized and concentrated these particles. Why BeO should have such a profound effect on macrophages and resultant lung clearance and not on other pulmonary cells is perplexing. Lifespan studies will perhaps answer this question, and will provide needed data on long-term retention and fate of high-fired BeO in the lung.

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FIGURE LEGEND

- Figure 1. Exposure system for BeO inhalation studies in rodents.
- Figure 2. Close-up view of Wright Dust Feed Mechanism and cyclone elutriator.
- Figure 3. Electron micrograph of BeO obtained from aerosol chamber by electrostatic precipitation. Grid shadowed with chromium at 22.5 degree angle. X20,000
- Figure 4. Electron micrographs of alveolar macrophages following inhalation of BeO particles. Arrows point to subcellular location of BeO particles within phagolysosomal structures.
- A. 21 days after exposure. X10,600
- B. 49 days after exposure. X10,600

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Figure 4. Electron micrographs of alveolar macrophages following inhalation of BeO particles. Arrows point to subcellular location of BeO particles within phagolysosomal structures.

A. 21 days after exposure. X10,600

B. 49 days after exposure. X10,600

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