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TOXICOLOGICAL STUDIES ON A BERYLLIUM CONTAINING EXHAUST PRODUCT

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INTRODUCTION

The major toxicological portion of the beryllium propellant development program of the U. S. Air Force Rocket Propulsion Laboratories was performed by The Dow Chemical Company during the period from 1963 to 1968 (Spencer et al., 1968). Results of long-term studies on rats, injected intratracheally with well-characterized key samples of beryllium oxide prepared by calcining beryllium hydroxide for 10 hours at 500, 1100, and 1600 C, respectively, showed clearly that there is a definite gradation in biological response depending upon the oxide administered. Thus, the oxide calcined at 500 C was highly active as Judged by histopathological examination of the lungs, incidence of tumors, and translocation of beryllium from the lungs to other tissues. In contrast, the oxide calcined at 1600 C showed much less severe effects. Dose-response studies were carried out, using carefully prepared subsamples of "respirable particle size" of the three key oxides; results showed a definite gradation in response which diminished with decreasing dosages of the administered oxide. Investigations on motor exhaust products have shown that most samples have chemical, physical, and toxicological properties similar to the beryllium oxide calcined at 1600 C. On the other hand, other samples were heterogeneous, contained considerable quantities of water-soluble beryllium, and varied in toxicity.

The present research study* was designed to define the physical and chemical characteristics and toxicological properties of an exhaust product collected from a beryllium-fueled NASA-JPL High Energy Upper Stage (HEUS) motor. Comparisons have been made with selected exhaust products previously studied as well as with key beryllium oxide samples of varying physical properties and biological activity. The sample studied will hereafter be identified as "BeO exhaust product".

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SAMPLE STUDIES

The physical and chemical properties determined on the "BeO exhaust product" are given below, along with the analytical methods employed:

Specific surface area - nitrogen adsorption.
Average crystallite size - X-ray diffraction.
Crystallinity - polarized light microscopy.
Refractive index - dispersion staining.
Density - sink-float method.
Major components of sample - X-ray powder diffraction.
Trace elements in sample - emission spectroscopy and X-ray fluorescence.
Sample solubility - gravimetric method.
Determination of major and trace components in soluble and insoluble portions - X-ray powder diffraction and emission spectroscopy.
Determination of amount of beryllium soluble in water, 0.1 N HCl and 6 N HCl - emission spectroscopic analysis of filtrate.
Elemental analysis -
beryllium by gravimetric method
carbon by microcombustion
hydrogen by microcombustion
oxygen by neutron activation
chloride by microvolumetric method
Particle characterization - light microscopy, transmission electron microscopy, and scanning electron microscopy.
Particle size - photomicrographs and Coulter Counter technique.

The physical properties of the "BeO exhaust product" are presented in table I. Data on the key samples of BeO calcined at 500, 1100, and 1.600 C are included for comparative purposes. Surface area of the "BeO exhaust product" is slightly greater than that of the key BeO calcined at 1100 C, and density is midway between that of the key BeO calcined at 500 C and the density of the 1100 C material. The "BeO exhaust product" most closely resembles the key BeO calcined at 1600 C in average crystallite size and refractive index. It is slightly less crystalline than the key BeO samples calcined at 1100 and 1600 C.

The physical properties of the "BeO exhaust product" are compared with those of a group of selected exhaust products previously studied in table II. The "BeO exhaust product" is higher in surface area, smaller in average crystallite size, and slightly less crystalline than the exhaust products previously studied. Other exhaust products previously studied, with properties similar to those of ARC Nos. 1, 22, and 24, were not measured for surface area due to limited

TABLE I

PHYSICAL PROPERTIES OF "BeO EXHAUST PRODUCT" COMPARED WITH KEY SAMPLES OF BeO

PROPERTY	BeO CALCINED FOR 10 HOURS AT: 500 C	1100 C	1600 C	"BeO EXHAUST PRODUCT
Specific Surface Area M^2/G	58.8	2.2	1.3	2.51
Average Crystallite Size Å	150	1500	1600	2000 ± 300
Crystallinity %	<10	100	100	95
Refractive Index	1.683 ± 0.003	2.704 ± 0.002	1.711 ± 0.007	1.712 ± 0.002
Density G/ML	2.87 ± 0.07	2.98 ± 0.02	3.00 ± 0.03	2.92 ± 0.03

TABLE II

PHYSICAL PROPERTIES OF "BeO EXNAUST PRODUCT" COMPARED WITH EXHAUST PRODUCTS PREVIOUSLY STUDIED

PROPERTY	ARC NO. 1	ARC NO. 22	ARC NO. 24	"BeO EXHAUST PRODUCT"
Surface Area M^2/G	<2.5	0.8	0.7	2.51
Average Crystallite Size Å	~5000	~5000	~5000	2000 ± 300
Crystallinity %	100	100	100	95
Refractive Index	1.704 ± 0.004	1.709 ± 0.003	1.709 ± 0.003	1.712 ± 0.003
Density G/ML	2.80 ± 0.05	2.99 ± 0.01	2.93 ± 0.07	2.92 ± 0.03

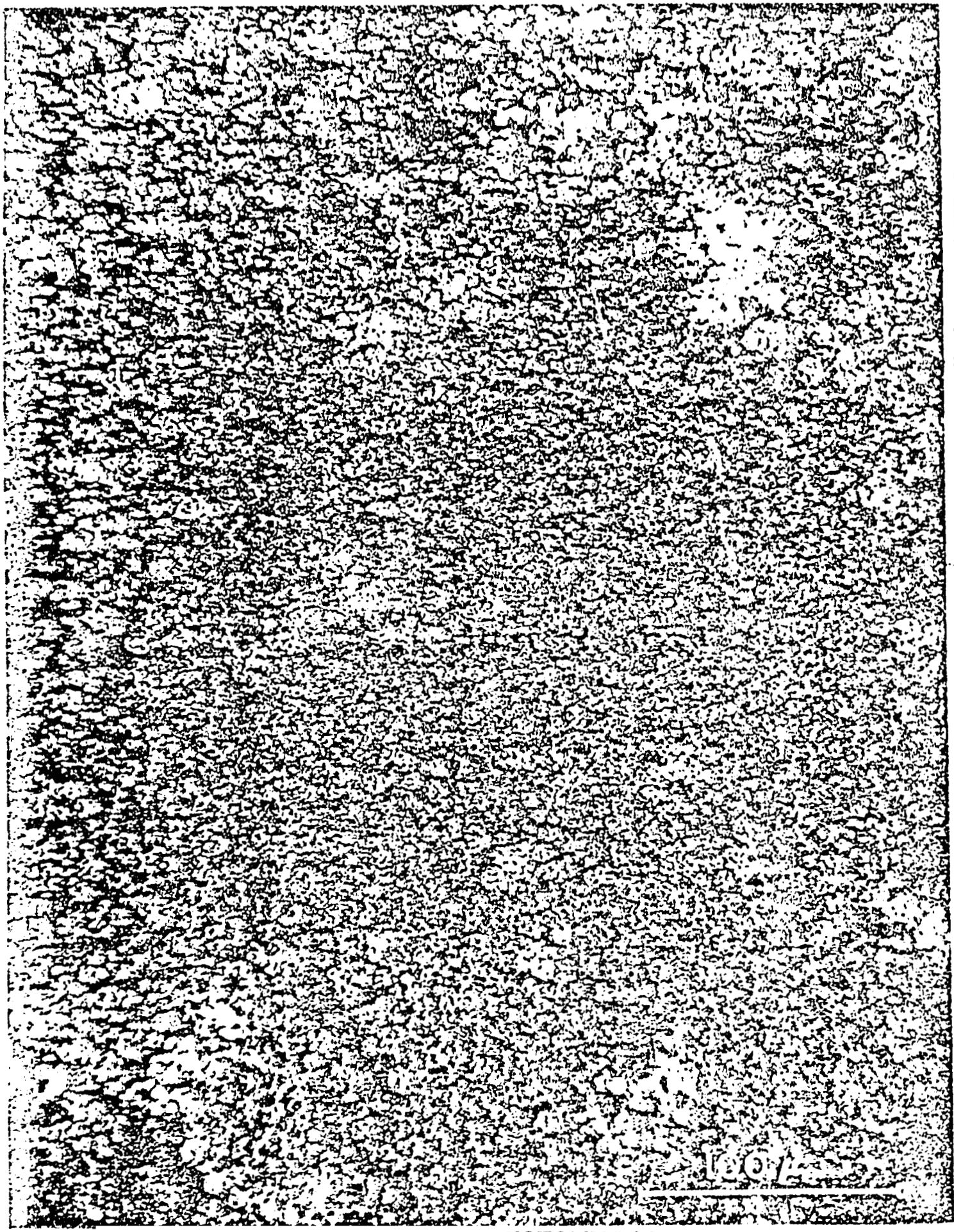

Figure 1. SCANNING ELECTRON PHOTOMICROGRAPH OF "BeO EXHAUST PRODUCT". x 500

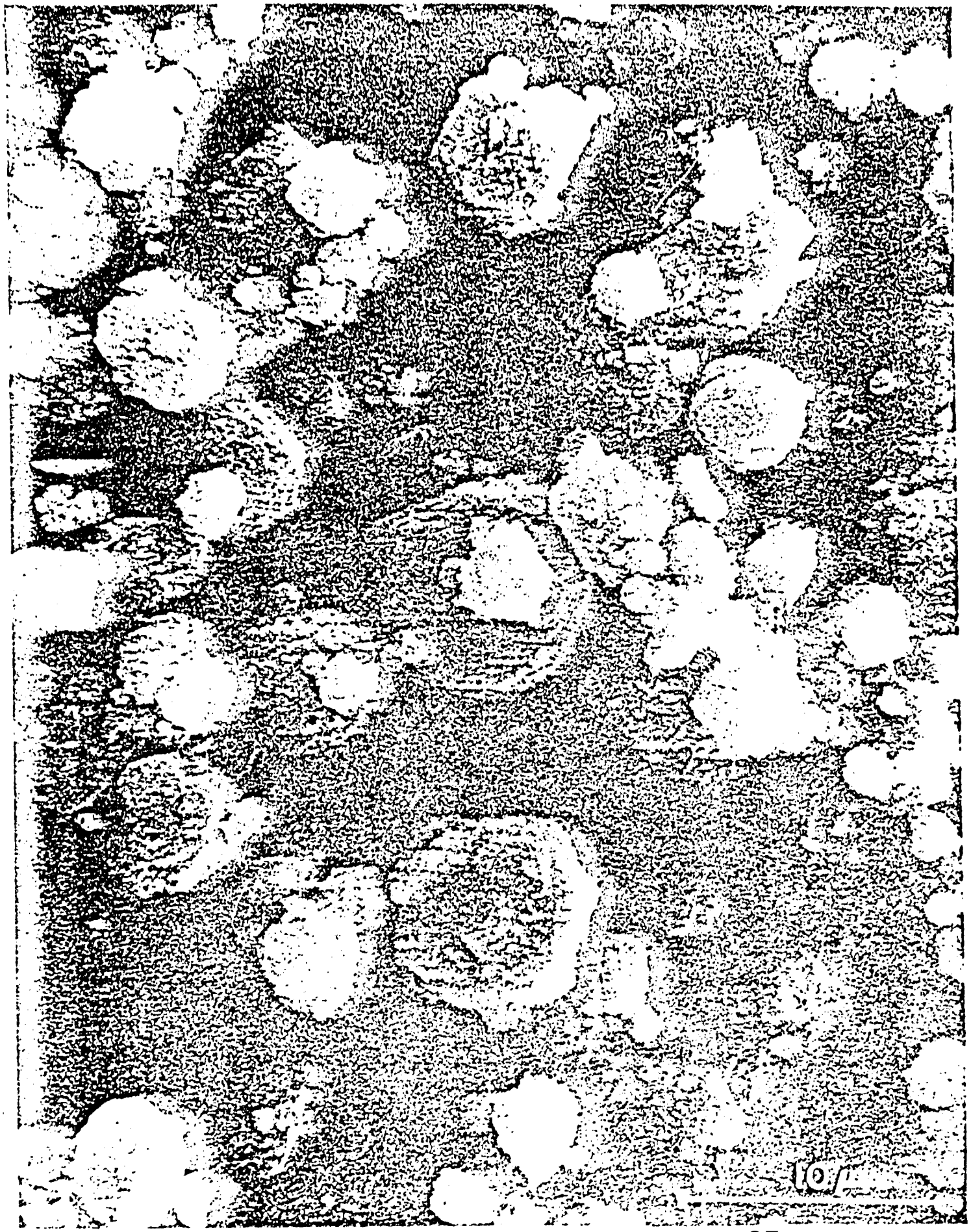

Figure 2. SCANNING ELECTRON PHOTOMICROGRAPH OF "BeO EXHAUST PRODUCT". x *5000*

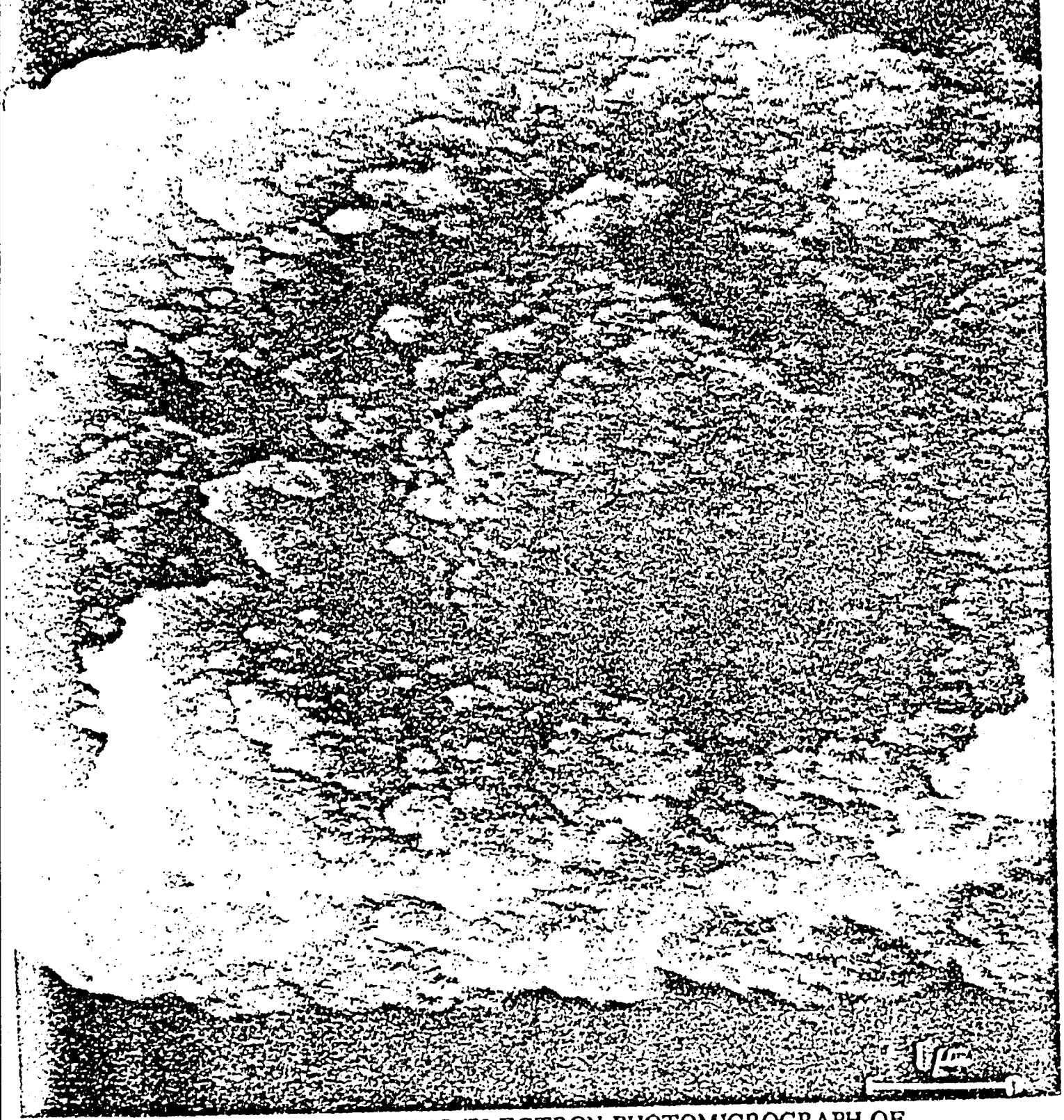

Figure 3. SCANNING ELECTRON PHOTOMICROGRAPH OF "BeO EXHAUST PRODUCT". x 25, 000

TABLE III

EXPERIMENTAL DESIGN FOR FEMALE RATS RECEIVING "BeO EXHAUST PRODUCT" BY INTRATRACHEAL ADMINISTRATION

SAMPLE	DOSE (Mg/Kg)	NUMBER OF RATS TREATED	NUMBER OF RATS SCHEDULED FOR NECROPSY			
			25 Weeks	50 Weeks	75 Weeks	100 Weeks
"BeO Exhaust Product"	50	60	10	10	10	All Survivors
	10	60	10	10	10	All Survivors
	2	60	10	10	10	All Survivors
BeO Calcined at 500 C	50	60	10	10	10	All Survivors
Saline Control	0	60	10	10	10	All Survivors
TOTAL		300	50	50	50	

TABLE IV

BERYLLIUM CONCENTRATION IN TISSUES OF RATS KILLED 25 WEEKS FOLLOWING INTRATRACHEAL ADMINISTRATION OF "BeO EXHAUST PROWCT"

SAMPLE	DOSE Mg/Kg	BERYLLIUM CONCENTRATION (µg/g TISSUE)			
		LIVER	KIDNEY	SPLEEN	DONE
"BeO	50	1.7	0.01	1.7	0.52
		0.07	0.01	0.09	0.45
		0.16	0.01	0.26	0.47
		0.07	0.01	0.10	0.31
	10	<0.01	<0.01	0.03	<0.1
		0.02	<0.01	0.04	<0.1
		1.3	<0.01	0.84	<0.1
		0.32	<0.01	0.40	<0.1
	2	<0.01	<0.01	<0.01	<0.1
		<0.01	<0.01	<0.01	<0.1
		<0.01	<0.01	<0.01	<0.1
		<0.01	<0.01	<0.01	<0.1
Saline Control	0	<0.01	<0.01	<0.01	<0.1
		c0.01	<0.01	<0.01	<0.1
		<0.01	<0.01	<0.01	<0.1
		<0.01	<0.01	<0.01	<0.1

samplc quantitics and are, therefore, not included in the comparative tabulation. Thcsc samples werc **ARC** No. **3,** AC No. **2** (Aerospace Corporntlon), "Aerospace Corporation BeO Snmplc from Large Motor Firings" rcceived from Wright-Pnttcrson Air Force Base in October, 1966, and a snmplc of bcrylllum exhaust products from Acrospncc Corporation - "BeO, June 29, 1967".

Dctcrmination **of** the otlicr properties studicd on the "BeO cxhaust product" gave unremarkable findings. The sample is primarily BeO, with low lcvels **of** impurities. Water solubility of the sample **is low,** with 0.71% of the total **sample** soluble in water (chiefly $CaSO_4 \cdot \frac{1}{2} H_2O$) and only **4** ppm soluble beryllium. Determination of particle **size** distribution by Coulter Counter showed **87% of** the sample mass to be less than 11μ in diameter, whilc examination **by** microscopy revealed that most of the particles were less than 5μ in diameter.

Scanning electron photomicrographs of the "BeO **exhaust** product" **are** prcscntcd in figures **1, 2,** and **3.** Photomicrographs of key samples of BeO and exhaust products previously studicd may be found in the report of the earlier work (Spenccr et al., 1968). Figure **1** (x500) can be compared with figures 21, **24,** 91, 93, and 96 **of** the rcport **by** Spencer et al. (1968); figurc 2 (x5000) **can** be compared with figures **22,** 25, **92,** 94, and 97; and figure 3 (x25, 000) with figures **23, 26, 27, 95,** and 98 of the **1968** rcport.

ANIMAL STUDIES

The biological activity of the "BeO cxhaust product" was evaluated by intratracheal administration of single closes of 50, 10, and 2 mg/kg **of** the sample to groups of female rats, followed by a 100-week test period. Another group of rats received 50 mg/kg of the key samplc of BeO calcined **3t** 500 C as a positive control, and a final group received saline as a negative control. **The** experimental design is summarized in table III. Pathological examinations were conducted on groups **of** rats necropsied 25, 50, 75, and **100** weeks following treatment. Studies on the translocation of beryllium to extrapulmonary tissues werc carried out at 25 and 100 **wecks.**

Results of translocation studies on the "Be0 cxhaust product" **25** weeks following treatment are prcsentcd in table IV. Beryllium was dctcctable in all **4** tissues analyzed at the 50 mg/kg dose, while only liver and spleen showed mcasurablc amounts at the 10 mg/kg dose. Tissues frorn rats treatcd with **2** mg/kg showed no detectable quantities of beryllium. Translocation studies 100 **wecks** following treatment **with** "BeO cxhnust product" showed a similar decrease in beryllium concentration with decreasing dose (table V). The analyses conducted after 100 weeks also showed slight increases in beryllium concentration over tne levels at 25 wccks.

TABLE V

BERYLLIUM CONCENTRATION IN TISSUES OF RATS KILLED 100 WEEKS FOLLOWING INTRATRACHEAL ADMINISTRATION OF "BeO EXHAUST PRODUCT"

SAMPLE	DOSE Mg/Kg	BERYLLIUM CONCENTRATION (μg/g TISSUE) LIVER	KIDNEY	SPLEEN	BONE
"BeO Exhaust Product"	50	0.26	0.02	1.0	0.51
		11.0	0.04	34.0	1.3
		1.2	0.03	0.89	0.49
		0.22	0.02	0.41	0.51
	10	1.5	<0.01	3.0	<0.1
		0.07	<0.01	0.12	<0.1
		0.02	<0.01	0.05	<0.1
		0.15	<0.01	0.25	<0.1
	2	0.01	<0.01	0.02	<0.1
		0.01	<0.01	8.03	<0.1
		1.4	<0.01	1.6	<0.1
		0.02	<0.01	0.04	<0.1
Saline Control	0	<0.01	<0.01	<0.01	<0.1
		<0.01	<0.01	<0.01	<0.1
		<0.01	<0.01	<0.01	<0.1
		<0.01	<0.01	<0.01	<0.1

Translocation of beryllium from the "BeO exhaust product" 25 weeks following treatment is compared with data obtained after similar time periods on key BeO samples and exhaust products previously studied in table VI. Levels of beryllium in tissues from rats treated with the key BeO calcined at 500 C are greater than those found in tissues from rats treated with "BeO exhaust product", while the latter sample in turn shows slightly greater translocation than found from the key BeO calcined at 1600 C or ARC Nos. 22 and 24. (Note that the doses of the key BeO calcined at 1600 C, ARC No. 22 and ARC No, 24, were approximately twice that of the "BeO exhaust product".) A comparison of the 100-week translocation data on the "BeO exhaust product" with long-term data on key BeO samples and exhaust products previously studied is presented in table VII. Although the data are more variable, the same general comparative relationship that was observed in the 25-week data is evident. (Note again the differences in dose.)

TABLE VI

TRANSLOCATION TO EXTRAPULMONARY TISSUES OF BERYLLIUM FROM "BeO EXHAUST PRODUCT" AT 25 WEEKS COMPARED WITH KEY BeO SAMPLES AND EXHAUST PRODUCTS PREVIOUSLY STUDIED

SAMPLE	DOSE Mg/Kg	WEEKS POST TREATMENT	BERYLLIUM CONCENTRATION (μg/g TISSUE) LIVER	KIDNEY	SPLEEN	BONE
Key BeO Calcined at 500 C	50	25	3.2	0.11	3.6	6.0
			1.3	0.12	1.1	5.0
			0.15	0.11	0.18	5.0
			0.21	0.11	0.36	6.5
"BeO Exhaust Product"	50	25	1.7	0.01	1.7	0.52
			0.07	0.01	0.0)	0.45
			0.16	0.01	0.26	0.47
			0.07	0.01	0.10	0.31
Key BeO Calcined at 1600 C	~100	a4	0.021	<0.003	0.10	0.10
			0.033	<0.003	0.11	0.06
			0.070	0.003	0.26	0.11
			0.028	0.004	0.16	0.11
			0.010	0.004	0.010	0.14
			0.007	0.004	0.038	0.09
ARC No. 22	~100	21	0.010	<0.003	0.019	0.28
			0.005	0.004	0.037	0.60
			0.004	<0.003	0.015	0.23
			0.010	<0.003	0.063	0.48
ARC No. 24	~100	21	0.006	0.004	0.014	0.43
			0.004	0.004	0.029	0.16

TABLE VII

TRANSLOCATION TO EXTRAPULMONARY TISSUES OF BERYLLIUM FROM "BeO EXHAUST PRODUCT" AT 100 WEEKS COMPARED WITH KEY BeO SAMPLES AND EXHAUST PRODUCTS PREVIOUSLY STUDIED

SAMPLE	DOSE Mg/Kg	WEEKS POST TREATMENT	BERYLLIUM CONCENTRATION (μg/g TISSUE) LIVER	KIDNEY	SPLEEN	BONE
Key BeO Calcined at 500 C	50	100	0.11	0.IS	0.39	3.2
		100	0.46	0 27	1.3	5.7
		100	0.24	0.20	0.97	5.2
		100	1.4	0.30	79	8.5
"BeO Exhaust Product"	50	100	0.26	0.02	1.0	0.51
	50	100	11.0	0.04	34.0	1.3
	50	100	1.2	0.03	0.89	0.49
	50	100	0.22	0.02	0.44	0.51
Key BeO Calcined at 1600 C	50	80	1.1	0.009	0.92	0.4
	50	80	0.029	<0.003	0.12	0.5
	~100	98	0.60	0.008	10	0.75
	~100	98	0.60	0.009	0.90	0.30
	~100	90	0.34	0.006	0.64	0.20
	~100	98	0.43	0.007	0.70	0.70
ARC 22	~100	77	0.012	0.004	0.022	0.2
	~100	77	0.006	0.003	0.037	0.3
	~100	77	0.005	0.004	0.042	0.4
ARC 24	50	55	0.008	<0.003	0.009	0.4
	50	55	0.007	<0.003	0.035	0.5
	50	65	0.013	<0.003	0.036	0.4

Histopathological examination revealed a typically severe pulmonary response in rats treated with 50 mg/kg key BeO calcined at 500 C. This reaction can be described as a progressive proliferative response charactcrized in the earlier phases by fibrotic and epithelial metaplastic changes, advancing into neoplastic changes as indicated by the development of pulmonary carcinomas. The associated lymph nodes from these rats showed evidence of fibrotic proliferation. The pulmonary lesions observed in the lungs of rats treated with 50 mg/kg "BeO exhaust product" were less severe than those induced by the key BeO calcined at 500 C, but were similar in nature. The severity of the pulmonary rcsponsc diminished with a decrease in dose of the "BeO exhaust product", with only focal, minimal changes observed at the 2 mg/kg dose. No evidence of a proliferative response was observed in the associated lymph nodes from rats treatcd with even the highest dose of "BeO exhaust product".

The occurrence of primary pulmonary tumors in rats treated with 50 mg/kg key BeO calcincd at 500 C or with 50 mg/kg "BeO cxhaust product" is prcscntcd in tnblc VIII. The vast majority of all these tumors were carcinomas, with occasional metastases to the associated lymph nodes. A icw tumors werc morc appropriatcly classified as adenomas. Since the earliest primary pulmonary tumor was noted 58 weeks following trcatmcnt in the group of rats that had received 50 mg/kg of the key BeO calcined at 500 C, the tabulation was started at that point. For comparative purposes, 58 weeks was used as the starting time in tabulation of tumors in the group treated with 50 mg/kg "BeO cxhaust product", although the first tumor was not noted in this group until the 75-week necropsy. The tabulation shows a higher occurrence of tumors in the group of rats treated with 50 mg/kg key BeO calcined at 500 C than was found at the same dose of the "BeO exhaust product". Only one pulmonary tumor was observed in the group of rats treated with 10 mg/kg "BeO exhaust product". None of the rats treated with 2 mg/kg "BeO exhaust product" had pulmonary tumors.

TABLE VIII

PRIMARY PULMONARY TUMORS IN RATS FOLLOWING SINGLE INTRATRACHEAL ADMINISTRATION OF "BeO EXHAUST PRODUCT" AND KEY BeO CALCINED AT 500 C

WEEKS POST TREATMENT	CUMMULATIVE TOTALS					
	KEY BeO 500 C 50 Mg/Kg			"BeO EXHAUST PRODUCT" 50 Mg/Kg		
	NUMBER RATS EXAMINED	NUMBER RATS W/TUMORS	% RATS WITH TUMORS	NUMBER RATS EXAMINED	NUMBER RATS W/TUMORS	% RATS WITH TUMORS
58	1	1	100	1	0	0
67	5	2	40	1	0	0
73	7	3	43	3	0	0
75	17	9	53	13	5	38
80	21	11	52	16	6	38
86	23	11	48	19	7	37
87	27	15	56	23	9	39
a9	28	16	57	28	10	36
93	30	18	60	29	10	35
95	31	19	61	29	10	35
98	32	20	62	29	10	35
100	37	25	68	39	19	49

In comparison with the three key BeO samples (calcincd at 500, 1100, and 1600 C), the "BeO exhaust product" is less active, as far as tumorigenicity is concerned, than the key BeO calcined at 500 C, but is morc active than the key BeO samples calcined at 1100 and 1600 C. Datn regarding the tumorigenicity of the three key samples of BeO are prescntcd in the rcport of the earlier work (Spcnccr et al., 1968).

SUMMARY

Physical and chemical characterization studies on an exhaust product collected from a beryllium-fueled NASA-JPL High Energy Upper Stage motor ("BeO exhaust product") showed the material to be similar to the key samples of BeO calcined at 1100 and 1600 C, and to a group of exhaust products previously studied (ARC Nos. 1, 22, and 24). A slightly higher surface area, smaller crystallite size, and lower crystallinity of the "BeO exhaust product" were the notable differenccs from the exhaust products previously studied.

The biological activity was evaluated during a 100-week period following intratracheal administration of the "BeO exhaust product" to rats in single doses of 50, 10, and 2 mg/kg. Criteria used for judgment were analysis of beryllium in extrapulmonary tissues and histopathological examination of the lung.

Studies of the translocation of beryliium to extrapulmonary tissues (liver, kidney, spleen, and bone), conducted 25 and 100 weeks following treatment, showed the translocation of beryllium from the "BeO exhaust product" to be considerably lower than that of the key BeO calcined at 500 C, but slightly greater than that of the key BeO calcined at 1600 C and ARC Nos. 22 and 24.

Histopathological examination 25, 50, 75, and 100 weeks following treatment rcvealcd a pulmonary response less scverc than that induced by the key BeO calcined at 500 C, but with lesions similar in nature. Evaluation of tumorigenicity of the "BeO exhaust product" showed fewer tumors than were found in rats treatcd with the key BeO calcincd at 500 C. The tumors found in rats treated with the "BeO exhaust product" also occurred later than those induccd by the key BeO calcined at 500 C.

ACKNOWLEDGMENTS

The authors wish to acknowledge the valuable contributions of Dr. G. L. Sparschu and Dr. Paul Gross in the pathological evaluations, and to thank the personnel in various analytical laboratories of The Dow Chemical Company for their work on the physical and chemical characterization of the sample.

REFERENCE

1. Spencer, H. C., R. H. Hook, J. A. Blumenshine, S. B. McCollister, S. E. Sadek, and J. C. Jones; "Toxicological Studies on Beryllium Oxides and Beryllium Containing Exhaust Products"; AMRL-TR-68-148, Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio, December, 1968.

DISCUSSION

DR. **THOMAS** (Aerospace Medical Research Laboratory): Was that a perfect static firing, or was that the one which blew up half way?

DR. BACK (Aerospace Medical Research Laboratory): No, this was a perfect firing. For those of you who don't know, the ARC (Atlantic Research) material was fired in a tank at altitude and that's why it's so clean. It was fired in a tank at altitude through a water scrubber. It was I suppose almost a hard vacuum. If they fire in a closed system, they have to fire into a vacuum. That's why it's so clean. Now, this other thing was fired outside and maybe the reason it looks so amorphous is because of the way it finally cooled. Cooling one way through a water scrubber at altitude as opposed to cooling out in the tropics, outside in the ambient air, probably gave us the differences. That's what we're looking for, obviously. We just made three new samples with the good help of Dow Chemical. But we don't know whether somebody is going to let us work on them or not since beryllium is a bad name. But if we ever get any money to do it and if somebody will let us, we are certainly going to put two years effort into this, and I hope we can continue this work. Since we are this far along, it would be a shame not to get the full answer.