AMRL-TR-72-72

RESEARCH PROGRAM ON BERYLLIUM OXIDE ANALYSIS AND TOXICITY

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AEROSPACE MEDICAL RESEARCH LABORATORY
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13. ABSTRACT

The principal objective of this program was to devise reaction conditions for the conversion of low-fired BeO and high-fired BeO such that the reaction mixture was in a form suitable for the gas chromatographic measurement of beryllium, and to study the applicability of the technique for the conversion of the oxides in blood and tissue matrices. The conversion was accomplished by dissolution of the oxides in hot 75% sodium hydroxide, neutralizing the reaction mixture, buffering, and extracting the solution with benzene containing 1,1,1-trifluoro-2,4-pentanedione [trifluoroacetylacetone, H(tfa)]. Method adaptation studies were greatly facilitated by the use of radioactivity measurements employing high-fired and low-fired BeO containing ^{7}BeO to monitor the efficiency of all stages of the dissolution and extraction scheme. Method verification studies were conducted on dog blood and rat liver homogenate spiked to contain both high-fired and low-fired BeO containing ^{7}BeO. The final extracts were analyzed by gas chromatography and by radioactivity measurements. The overall recovery by gas chromatography was 104%; the standard deviation calculated from 60 measurements was ±5.0%. A limited number of measurements were also made on liver homogenates of rats which had received intratracheal injections of beryllium oxides containing ^{7}BeO, and which were sacrificed 21 days after injections. Examination by the base solution technique and by direct reaction of the homogenate with H(tfa) revealed that translocated beryllium was, at least partially, in a readily chelatable form. Spleen, kidney and blood samples were also removed, analyzed for beryllium content by radioactivity measurements, and stored for a future investigation.

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FOREWORD

This research was initiated by the Chemical Hazards Branch, Toxic Hazards Division, of the Aerospace Medical Research Laboratory. The work was performed by the Dayton Laboratory of Monsanto Research Corporation between June 1971 and June 1972. William G. Scribner served as project leader for Monsanto Research Corporation; he was assisted by Thomas Ctvrtnicek. Captain George M. Frame served as project engineer for the Aerospace Medical Research Laboratory.

This technical report has been reviewed and is approved.

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TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

LIST OF TABLES

SECTION I

INTRODUCTION

In a recently completed program, the beryllium levels in blood, liver, spleen, and other organs of rats were determined as a function of time for rats which had been injected intravenously with beryllium(II) containing $^{7}Be(II)$. These samples of known beryllium levels permitted the evaluation of the accuracy of an AMRL method for the gas chromatographic determination of beryllium in biological samples (Taylor and Arnold, 1971). The method is based on the formation and subsequent gas chromatographic measurement of the volatile chelate of beryllium trifluoroacetylacetonate.

In another phase of that program, low-fired (500°C) beryllium oxide containing ^{7}BeO was intratracheally administered to rats. Beryllium was found in the liver, spleen, and kidney at 10 days and thereafter. Gas chromatographic analysis of liver and spleen yielded lower recoveries when the chelation scheme was identical to that employed for blood. More severe chelation conditions yielded improved (but not quantitative) recoveries.

In the final phase of the program, preliminary experiments revealed that high-fired beryllium oxide could be quantitatively dissolved by neat trifluoroacetylacetone [H(tfa)] in 1 hour at 175°C.

The carcinogenic activity of BeO has been shown to be a function of the temperature to which the beryllium has been exposed (Spencer et al., 1968). Low-fired BeO (500°C) is highly carcinogenic while high-fired BeO (1600°C) is essentially inert. Beryllium is being considered for use by the Air Force as a propellant additive in solid-fuel rocket motors. Examination of the toxicological properties of various motor exhaust products indicated that some products resemble high-fired BeO in their lack of carcinogenic activity, while others contain considerable quantities of water soluble beryllium and vary in toxicity.

The principal objective of this program was to devise reaction conditions for the conversion of low-fired BeO and high-fired BeO such that the reaction mixture was in a form suitable for the gas chromatographic measurement of beryllium, and to study the applicability of the technique for the conversion of the oxides in blood and tissue matrices.

It was essential that the conversion reaction not be accompanied by the formation of side reaction products which would interfere with the gas chromatographic measurement of the volatile beryllium chelate.

Two conversion techniques were studied in detail: (1) direct reaction of the oxides with H(tfa) in sealed ampoules followed by dissolving the reaction products in benzene and (2) the "base solution technique"; i.e., dissolution of the oxides in hot 75% sodium hydroxide followed by neutralization and buffering of the reaction solution and formation of $Be(tfa)_2$ via solvent extraction of the buffered solution with benzene containing H(tfa). Optimization of dissolution conditions and evaluation of the quantitative aspects of the gas chromatographic method were accomplished by the use of low-fired and high-fired BeO containing ^{7}BeO.

For brevity in this report, the formula ^{7}BeO is used to designate beryllium oxide containing ^{7}BeO in lieu of the more correct $BeO \cdot {}^{7}BeO$.

SECTION II

PREPARATION AND CHARACTERIZATION OF BERYLLIUM OXIDES AND BERYLLIUM TRIFLUOROACETYLACETONATE

BERYLLIUM OXIDES

To provide samples of beryllium oxide containing ^{7}BeO for gas chromatographic and dissolution studies, low-fired and high-fired oxide was prepared by igniting beryllium hydroxide under prescribed time-temperature conditions (Spencer et al., 1967 and 1968).

Lot 1

A weighed amount (15.37 g) of $Be(NO_3)_2 \cdot 3H_2O$ (Fisher Purified Lot 773535) was dissolved in distilled water and the contents of two vials of $^{7}BeCl_2$ (in 0.5N HCl, New England Nuclear, Lot 4711) were added. Each vial initially contained 2 mCi of ^{7}Be, but the combined amount decayed to a total of 3 mCi on the synthesis date. The vials were washed with distilled water and the washings were added to the beryllium nitrate solution. Total volume was 75 ml. The pH was adjusted to 11.5 by the dropwise addition of 10% sodium hydroxide; total final volume was 140 ml. The beryllium hydroxide was filtered in two portions using S&S 589 black ribbon filter paper. The precipitates were washed repeatedly with distilled water and then with hot distilled water until the washings were neutral. Total volume of filtrate plus washings was about 250 ml in each case.

The filtrates had an activity of ~400 cpm/g; thus the precipitation of beryllium-7 was essentially quantitative. The washed precipitates were transferred to 100-ml platinum dishes and partially dried over a weekend at 42°C on a hot plate.

Low-fired ^{7}BeO was prepared by placing the platinum dish containing the dried hydroxide in a Lindberg furnace preheated to 300°C. The furnace was immediately reset to 500°C; this temperature was reached in 1 hour. Exactly 10 hours later, the furnace was shut off. The beryllium oxide was allowed to cool in the furnace for 13 hours. The weight of calcined, low-fired oxide was 0.8747 g.

Low-fired beryllium oxide was ground and sieved in the following manner. The oxide, a mortar and pestle, several beakers, ethanol, medicine droppers, tissues, a 20-micron sieve and pan, and two lead bricks were placed inside a glove bag and the bag was closed. The oxide from a vial was put into the mortar in three batches. Each batch was wetted with ethanol to form a paste. The paste was ground for about 30 minutes. Each batch was then transferred to the 20-micron sieve and brushed through.

Most of the ground oxide passed through the 20-micron sieve. Material which did not pass through the sieve was discarded into a radioactive waste vessel. Material in the receiving pan was transferred to a beaker with ethanol. Light microscopic examination of the ground and sieved material revealed that the material was mostly in the 1-5 micron range, although some ∿10 micron particles were present. After evaporation of the ethanol on a steam bath, and drying the product at 105°C overnight, it was ground again and reserved for chelation studies.

High-fired beryllium oxide was prepared by placing the dried hydroxide contained in a platinum dish into a Lindberg furnace preheated to 300°C. The furnace was reset to 500°C and, when attained, the oxide was calcined for 1 hour at this temperature. The material was removed from the furnace, cooled, transferred to an alumina crucible, and placed in a Lucifer furnace which had been preheated to 482°C. The oxide then experienced the time-temperature environment shown in table I, which included a 10-hour residence at 1582 to 1591°C.

The high-fired oxide was sieved and ground in a manner identical to that described for the low-fired oxide. More of this material failed to pass the 20-micron sieve. Light microscopic examination of the material which passed the 20-micron sieve revealed that most of the material was in the 1- to 10-micron range, with a considerable number of 10-micron particles, some greater than 10 microns, and none 20 microns or greater.

The material was ground again for 1 hour as an ethanol slurry. The additional grinding resulted in a definite revised distribution of the particles toward the sought-for 1-5 micron range. However, the material was considered to be mostly in the 1-10 micron range with the mean at about 5 microns.

A gravity separation of the larger particles was performed. An ethanol slurry of the material was placed onto an 18 cm x 2.5 cm column of ethanol to give a total column length of 25 cm. Turbulence during the addition caused the added slurry to penetrate to a depth of about 10 cm into the column. [The time required for a 10-micron particle of density 2.89 g/cm^3 to fall 20 cm through a fluid of viscosity 0.011 poise and a density of 0.789 g/ml was calculated from Stoke's Law to be 0.53 hour. The time for a 5-micron particle to fall 10 cm was calculated to be 1.07 hours.] After 32 minutes, the turbid supernatant liquid above the settled particles was removed by decantation. Light microscopic examination of the material revealed that although a few 10-micron particles were still present, the product was satisfactory, i.e., the distribution was principally in the 1-5 micron range.

Table I

TIME-TEMPERATURE ENVIRONMENT
FOR PREPARATION OF HIGH-FIRED ^{7}BeO, LOT 1

Date	Time (Hr)	Temperature (°C)	Tap	Amps	Remarks
8/31	1330	482	2	8.4	
	1400	593	3	14.5	
9/1	0800	1204	3	11.5	Tap changed
	2245	1493	4	13.8	
9/2	1330	1493	4	13.8	Terminals tightened
	1430	1507	4	14.4	
	1530	1527	4	14.3	
	1630	1543	4	14.3	
	2230	1582	4	14.1	Start 10-hr residence
	2330	1588	4	14.1	
9/3	0030	1593	4	14.0	
	0130	1591	4	13.9	
	0230	1588	4	13.9	
	0330	1588	4	13.9	
	0430	1588	4	14.0	
	0530	1588	4	14.0	
	0630	1588	4	14.0	
	0730	1591	4	14.1	
	0830	1591	4	14.0	10-hr residence completed; furnace turned off.
9/4	0900				Oxide removed.

The yield of high-fired beryllium oxide after the 500°C ignition was 0.983 g. After ignition at 1590°C for 10 hours, the material weighed 0.8816 g, which represents a weight loss of about 10%. Considerable losses of material occurred during the grinding, sieving, regrinding, gravity separation, etc., because of the numerous transfers which were required. It is estimated that the final yield of 1-5 micron material was only about 100 mg. The combined yield of low- and high-fired oxide (before grinding, etc.) was 1.76 g (85%). The maximum theoretical yield was 2.06 g.

It is of interest to compare the calculated and found values for the activity of the oxides. The specific activity of the oxide on the preparation date was calculated to be about 0.71 mCi/g which is equivalent to 1576 dpm/μg. The activity of carefully weighed portions of high-fired and low-fired beryllium oxides was determined to be 59.1 cpm/μg and 60.4 cpm/μg, respectively. The efficiency of the well-type scintillation counter, therefore, is about 3.8% for ^{7}Be. The efficiency of this counter for ^{60}Co (1.17 and 1.33 MeV γ) has been previously determined to be 35%. These results are consistent with information (Lambie, 1964) reporting that the response of a gamma scintillation counter to ^{60}Co is approximately 11 times greater than it is for ^{7}Be.

Lots 2 and 3

Additional lots of both low-fired and high-fired ^{7}BeO were prepared as required for the program as described above. In these cases, it was possible to avoid the Stoke's Law separation of the larger particles of high-fired ^{7}BeO by more thorough grinding of the sample prior to sieving.

Each additional lot of high-fired ^{7}BeO experienced essentially the same time-temperature environment as Lot 1.

BERYLLIUM TRIFLUOROACETYLACETONATE

Purified beryllium trifluoroacetylacetonate, needed for a gas chromatographic standard, was prepared in the following manner: Trifluoroacetylacetone (Pierce Chemical Co.) was distilled in a 45-cm, heated column packed with glass helices. The fraction boiling from 105.5 to 106.0°C (750 mm) was collected. The distilled product was stored at -18°C in a Teflon bottle.

Beryllium metal (0.4778 g, Alfa Inorganics, Inc., -200 mesh, $m_3n_5t_2n$) was placed in a 200-ml round-bottomed flask, and 24 ml (30 g) of distilled trifluoroacetylacetone was added. The mixture was refluxed for 15 hours, after which the reaction mixture was filtered to remove unreacted Be. It was necessary to dilute the mixture with hot solvent (benzene and/or isooctane) to complete the transfer and to unplug the filter paper. The

clean filtrate was heated to remove solvents. A dark orange oil remained which solidified on cooling. The crude product was recrystallized once from benzene, filtered, and allowed to air dry over a weekend.

The recrystallized material was purified further by sublimation at 75 to 85°C (0.08 mm) and resublimed at 68-81°C (0.10 mm). The twice-sublimed material had a melting point of 109 to 113.5°C. Consequently, it was recrystallized one more time from benzene, yielding material with a melting point of 114-114.5°C. The yield of twice sublimed-recrystallized material was 8.47 g (50%).

APPARATUS AND EQUIPMENT

High-Temperature Furnace

Lucifer Furnaces, Inc., Model 6030-4P, modified to contain five super Kanthal heating elements; 2.24 KW; chamber size 3" x 3" x 6"; overtemperature controller, Type T platinum thermocouple assembly and separate contactor.

Metabolic Cages

Hoeltge Inc. HB11M, with HB-17 Urine-Feces separator and HB-66 tunnel feeder.

Gamma Counting Equipment

Nuclear Measurements DS 1B Scaler with US-1B Well.

SECTION III

PREPARATION OF REACTION AMPOULES FOR GAS CHROMATOGRAPHIC STUDIES: ^{7}BeO AND TRIFLUOROACETYLACETONE

Numerous ampoules were prepared consisting of microgram portions of each type of BeO reacted with H(tfa) or benzene H(tfa) in the presence and absence of water at various temperatures for varying times. These reaction mixtures, with known beryllium content as determined by radioactivity counting, were intended to serve as standards for the optimization of conditions for the conversion of the oxide to $Be(tfa)_2$ and for the optimization of the gas chromatographic procedure.

EXPERIMENTAL

Portions of low-fired and high-fired ^{7}BeO were weighed on a microbalance and transferred with washing to glass-stoppered 100 ml graduated cylinders. The contents of the cylinders were diluted with distilled water to such a volume that a 0.2 ml aliquot would contain ∿10 µg ^{7}BeO. Several glass beads were added and the cylinders were shaken vigorously to suspend the ^{7}BeO prior to removal of aliquots by a "calibrated" Pasteur pipet.

The aliquots were transferred to "diSPo" pipets which had been previously sealed with an oxygen-gas torch at a distance of 75 to 80 mm below the constriction. Following the addition of H(tfa), the ampoules were sealed at the constriction, counted twice, mixed, coded, and reacted for prescribed times and temperatures.

Standards consisting of weighed quantities of each type of oxide were counted daily, and a factor, cpm/µg ^{7}BeO, was calculated. This factor was used to calculate the amount of ^{7}BeO present in each ampoule. The standards were also contained in glass ampoules of the same size and type as the reaction ampoules, thereby minimizing geometry effects on the accuracy of radioactivity assays.

In many instances, water was evaporated prior to adding H(tfa). This was accomplished by overnight drying of the suspension in the ampoules at 95°C in a forced circulation oven.

RESULTS

Series I: ^{7}BeO Suspensions; ∿150°C; neat H(tfa)

Thirty ampoules were prepared consisting of 0.2 ml of suspension of both low-fired and high-fired beryllium oxide. These were

treated with 0.1 ml of H(tfa) and reacted for 0.5 and 3 hours at ∿150°C. No gas chromatographic peak attributable to $Be(tfa)_2$ was found in any of the reaction mixtures.

Series II: ^7BeO Suspension; 150°C; Benzene-H(tfa)

To determine whether or not the presence of benzene would promote the formation of $Be(tfa)_2$ a series of ampoules containing the suspension of low-fired ^7BeO was prepared. The ^7BeO was reacted with 0.1 ml of 25% H(tfa) in benzene or 50% H(tfa) in benzene for three hours. No gas chromatographic peak of $Be(tfa)_2$ was observed in any of these mixtures.

Series III: Dried ^7BeO Suspensions; 150°C; H(tfa) and Benzene-H(tfa)

Because no chelation of ^7BeO was detected when suspensions of the oxide were reacted with either neat H(tfa) or benzene-H(tfa) it was decided to remove the water by evaporation prior to the addition of the chelating reagent. This was easily accomplished by overnight residence in a forced circulation oven set at 95°C. Tissue wipes of the inside of the oven revealed no contamination of the oven by ^7Be. Hence, the evaporation proceeded smoothly without bumping even though the suspensions were confined in ampoules.

A series of 18 ampoules was prepared containing low-fired ^7BeO. The contents of the ampoules were dried overnight at 95°C, treated with 0.1 ml of neat H(tfa) or 25% H(tfa) in benzene or 50% H(tfa) in benzene and heated at 150°C for 0.5 or 3 hours.

Radioactivity and gas chromatographic data presented in table II reveal that both neat H(tfa) and 25% H(tfa) in benzene had dissolved 80 to 90% of the oxide and that $Be(tfa)_2$ had formed. Chelate formation with 50% H(tfa) in benzene had also occurred but with significantly less efficiency. This, however, may have been a consequence of a change in response of the chromatographic detector.

An identical series of experiments with high-fired ^7BeO did not produce any detectable $Be(tfa)_2$.

In an effort to obtain dissolution and chelation of the high-fired oxide, a series of reaction ampoules was prepared, dried, treated with 0.20 ml of neat H(tfa), sealed, and allowed to react overnight at 150 or 175°C. Gas chromatographic examination of the reaction mixture indicated that there was some evidence for the presence of $Be(tfa)_2$. However, the amount could not be quantitated because the peak was masked by a large interference peak which eluted only 2 mm before the $Be(tfa)_2$ peak. The height

Table II

DISSOLUTION OF LOW-FIRED ^{7}BeO (DRIED SUSPENSION) BY H(tfa) AND BENZENE-H(tfa)

Ampoule	Time, Hrs	µg ^{7}BeO - Low-Fired			µg ^{7}BeO Found, GC	% Recovery
		Neat	25% H(tfa)	50% H(tfa)		
1	3	8.44			6.8; 8.1	81; 96
2	3	4.46			3.1	70
3	3	9.54			8.9; 9.5	93; 100
4	0.5	7.58			6.4	84
5	0.5	7.90			6.5	82
6	0.5	7.72			6.8; 7.2	88; 93
7	3		9.52		8.35	88
8	3		5.50		5.3	96
9	3		7.07		5.5	78
10	0.5		4.54		-	-
11	0.5		4.75		-	-
12	0.5		7.02		-	-
13	3			10.2	7.5	74
14	3			13.6	8.6	63
15	3			4.97	3.3	66
16	0.5			10.8	4.2	39
17	0.5			5.61	2.8	50
18	0.5			8.72	5.0	57

of the interference peak was proportional to the amount of H(tfa) and the temperature of heating. This peak was subsequently found to be present when H(tfa) only was heated in two ampoules (from different manufacturers) and was not present when standards were injected.

At this time, an additional series of reaction ampoules were prepared [dried suspension, 0.20 ml neat H(tfa)] and reacted for 3 and 17 hours. The interference peak prevented gas chromatographic quantitation of $Be(tfa)_2$ in all samples. This was especially perplexing since good quantitation of similar (3-hr) samples had been obtained.

In an effort to reduce the interference peak height, additional reaction ampoules were prepared consisting of dried suspensions of low-fired and high-fired ^{7}BeO reacted with only 0.025 ml of neat H(tfa) at 135° and 150°C. The reaction time in each case was 17 hours (table III). The interference peaks were indeed reduced but still interfered; quantitations were erratic and low (14 to 78%).

Further study of the gas chromatographic method by AMRL personnel indicated that the interference peak and the $Be(tfa)_2$ peak could be separated by the use of a lower column temperature (95°C rather than 115°C). To provide assayed samples for the evaluation of quantitation at the revised GC conditions, ampoules were prepared consisting of both the high-fired and low-fired oxide (dried suspension) reacted with 0.025 and 0.100 ml of H(tfa) for 16 hours at 150°C. The results (table IV) revealed that the gas chromatographic quantitation was still erratic; no significant difference was found between the two H(tfa) concentrations.

Following additional studies of the gas chromatographic method by AMRL personnel, further ampoules were prepared consisting of freshly prepared, higher specific activity low-fired and high-fired ^{7}BeO (dried suspension). The oxide was reacted with 0.10 ml of H(tfa) at 150°C for 16 hours. Gas chromatographic analysis of the reaction mixture gave excellent recoveries with low-fired oxide on the first day (average 95%), but poorer recoveries when examined on the second day (average 52%). For the high-fired oxide, recoveries averaged 71% the first day and 57% the second day.

Table III

REACTION OF LOW-FIRED AND HIGH-FIRED ^{7}BeO WITH 0.025 ml OF H(tfa) FOR 17 HOURS

µg ^{7}BeO Low-Fired		µg ^{7}BeO Found, GC		% Recovery	
135°C	150°C	135°C	150°C	135°C	150°C
9.14	9.65	6.10	6.69	67	69
13.6	10.3	8.3	6.26	61	61
10.6	10.7	8.05	8.35	76	78
15.8	11.3	2.22	4.73	14	42
µg ^{7}BeO High-Fired					
135°C	150°C				
10.5	12.2	4.44	6.12	42	50
10.9	10.7	3.88	2.78	36	26
9.64	9.42	2.78	3.36	29	36
12.0	11.2	3.33	2.22	28	20
12.3	11.3	9.40	4.73	76	42

Table IV

REACTION OF LOW-FIRED AND HIGH-FIRED ^{7}BeO WITH H(tfa) FOR 16 HOURS; REVISED GC CONDITIONS

		µg ^{7}BeO		
Oxide Type	[H(tfa)]	Taken	Found	% Recovery
Low-Fired	0.025	10.8	5.6	52
	0.025	9.51	7.25	76
	0.100	8.70	4.74	54
	0.100	8.23	5.60	68
High-Fired	0.025	7.96	2.1	26
	0.025	10.7	2.1	20
	0.025	12.0	5.6	47
	0.100	11.1	7.25	65

SECTION IV

OPTIMIZATION OF CONDITIONS FOR THE DISSOLUTION OF HIGH-FIRED ^{7}BeO BY H(tfa)

Earlier work indicated that high-fired ^{7}BeO could be quantitatively solubilized by 0.20 ml of neat H(tfa) in 30 minutes at 175°C*. Experiments were conducted to optimize the conditions; i.e., to find the minimum H(tfa) concentration, time, and temperature for quantitative dissolution of milligram quantities of ^{7}BeO.

EXPERIMENTAL

Approximately 1 mg portions of high-fired ^{7}BeO powder were put into disposable pipets which had been sealed at the tapered end. Various amounts (in the range 0.05-0.2 ml) of neat H(tfa) were added, and pipets were sealed at the other end. The next day they were counted twice for 5 minutes and thus the amount of ^{7}BeO in each ampoule was determined. Each ampoule was wrapped in heavy duty aluminum foil and placed vertically into an oven. After a selected length of time the ampoules were taken out of the oven, unwrapped, and opened in a hood. Each ampoule was centrifuged for 5 minutes. The H(tfa) was then pipetted out with a disposable pipet. The slurry on the bottom of each ampoule was washed with benzene, the ampoule was centrifuged again and the benzene phase pipetted out. This was repeated 3 times. H(tfa) and benzene washes were placed into vials which were counted; dissolved BeO was thus determined. Also the open ampoules were counted and undissolved BeO was determined.

Evaporation of benzene under a gentle stream of nitrogen prior to counting the solubilized fractions yielded higher activities than when the benzene solutions were counted. This geometry more closely matches that of the ampoule containing unreacted BeO at the bottom of the ampoule. This mode of operation led to improved total recoveries of activity; i.e., "soluble" plus "undissolved" more nearly equalled the amount taken initially.

RESULTS

Present studies did not confirm earlier data, and more severe conditions were employed (table V). Milligram amounts of high-fired ^{7}BeO were found to be quantitatively solubilized in 16 hours in a forced circulation oven (Blue M) at 175 or 150°C by 0.05, 0.10, or 0.20 ml of H(tfa).

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*W. G. Scribner, Final Report, Contract F33615-71-C-1008, February 1971.

Table V

EFFECT OF TIME AND TEMPERATURE ON THE DISSOLUTION OF HIGH-FIRED ^{7}BeO BY NEAT H(tfa)

Temp °C	Time, Hrs	H(tfa) ml	% Solubilized Blue M Oven	Cenco Oven
100	16	0.05	7.9	
		0.10	9.5	
		0.20	16.1	
135	16	0.05	76.8	
		0.10	81.2	
		0.20	80.0	
150	16	0.05	99.7	
		0.10	99.8	
		0.20	99.8	
	3	0.20	27.2	
	1	0.05		39.8
		0.10		54.3
		0.20		58.2
	0.67	0.05		14.7
		0.10		27.0
		0.20		39.1
	0.33	0.05		9.5
		0.10		16.5
		0.20		15.7
175	16	0.05	99.8	
		0.10	99.8	
		0.20	99.6	
	3	0.05	34.3	
		0.10	68.1	
		0.20	58.1	
	2	0.05	31.1	69.8
		0.10	36.1	57.9
		0.20	46.4	69.3
	1	0.05	11.8	25.3
		0.10	12.9	27.8
		0.20	18.0	28.9
	0.5	0.05	5.0	
		0.10	7.1	
		0.20	9.1	13.6

A curious difference was noted in the results obtained from ampoules heated in the forced circulation oven as compared to ampoules heated in a smaller convection oven (Cenco). More effective solubilization at otherwise constant conditions was found for the samples heated in the convection oven. Apparently, more effective heat transfer is occurring in the convection oven. It appears that quantitative dissolution should occur at a lower temperature than 150°C and at a residence time of less than 16 hours in the convection oven.

GAS CHROMATOGRAPHIC EXAMINATION OF REACTION PRODUCTS

In order to confirm that dissolution of beryllium oxide was accompanied by (or a consequence of) the formation of $Be(tfa)_2$ certain reaction mixtures (175°C) were diluted to 100 ml with benzene and 5 ml aliquots were given to AMRL personnel for gas chromatographic examination (activity less than 0.02 μCi). The diluted reaction mixtures contained 5.06 and 0.33 μg of beryllium as the oxide.

The samples were further diluted 1 ml to 25 ml with benzene and 0.3 ml of this solution was passed through a Sephadex column. The column was washed with 0.9 ml of benzene. Gas chromatographic analysis of the column eluate yielded 88 and 81% recovery of the stronger solution and no detectable $Be(tfa)_2$ in the less concentrated solutions. The less concentrated solution was then passed through the column without dilution; recovery was 62 and 82%. A repeat run on these solutions on the following day gave recoveries of 83, 100, and 73% on the stronger solution (Sephadex column) and 100, 104, and 93% recovery of the more dilute solution after removal of excess H(tfa) by extraction with 1:5 NH_4OH. These results indicate that dissolution is indeed accompanied by formation of $Be(tfa)_2$.

Additional solutions consisting of 4.785 and 4.402 μg BeO/ml were prepared and examined gas chromatographically:

	% Recovery	
[BeO]	Sephadex Column	1:5 NH_4OH Extraction
4.785 μg/ml	82	99
	82	92
	98	100
4.402 μg/ml	81	99.5
	57	98.5
	81	96.5

These data give added proof for the formation of $Be(tfa)_2$ on reaction of high-fired BeO with H(tfa) in a sealed ampoule.

SECTION V

EVALUATION OF THE BASE SOLUTION TECHNIQUE: DISSOLUTION OF BERYLLIUM OXIDES WITH SODIUM HYDROXIDE

As discussed in section III numerous ampoules were prepared consisting of evaporated suspensions of low-fired and high-fired ^{7}BeO reacted with trifluoroacetylacetone for varying times at varying temperatures. The reaction mixtures were dissolved in benzene, treated to remove excess H(tfa), and examined gas chromatographically for the presence of $Be(tfa)_2$. An interference peak was observed, the adverse effect of which could be minimized by the use of a lower GC column temperature. Nevertheless, recoveries by GC were still erratic.

Radiotracer studies of the efficiency of H(tfa) for the dissolution of high-fired ^{7}BeO revealed that a reaction time of 16 hours at 150°C would yield quantitative dissolution of ^{7}BeO. However, it was obvious that considerable work would be required to refine the GC conditions for the quantitative measurement of beryllium in such reaction mixtures. Further, whether or not the dissolution reaction would occur when the oxide was present in a blood or tissue matrix would need to be verified.

Because of the difficulties and uncertainties outlined above, a radical departure from the original program was explored. At this time, there appeared in the literature (Eisentraut et al., 1971), a novel method for the dissolution of rocket exhaust (high-fired BeO). This method involves a two-minute reaction of the oxide with 75% sodium hydroxide at its boiling point. Neutralization of the reaction mixture yields an aqueous solution of Be(II) which is readily amenable to chelation-extraction of $Be(tfa)_2$.

In the published procedure for the dissolution of rocket exhaust product the statement is made that the oxide "can be quantitatively dissolved by simply boiling with a 75% NaOH solution containing the suspended material for 2 minutes." It seemed worthwhile to confirm the efficiency of this step and the subsequent chelation-extraction steps by radiotracer techniques. We are indebted to Dr. Kent J. Eisentraut for the exact experimental details of the dissolution step.*

*K. J. Eisentraut, Aerospace Research Laboratories, Wright-Patterson Air Force Base, Ohio, private communication, January 1972.

HIGH-FIRED ^{7}BeO

A mixture of 0.29 ml of water and 0.86 g of sodium hydroxide was heated to the boiling point in a 10 ml Erlenmeyer flask covered with a microscope slide cover. A portion of high-fired ^{7}BeO (3.32 mg) was added to the mixture. After heating for 2 minutes at the boiling point with swirling, the flask and contents were allowed to cool and the contents were diluted with distilled water and transferred to a 150 ml beaker. The Erlenmeyer flask was washed with conc. nitric acid and the washes were added to the beaker. After three washes the solution in the beaker was acidic. The acid solution was filtered through S&S 589 blue ribbon filter paper. The filtrate was collected in a 100 ml volumetric flask.

The beaker was thoroughly washed and the washes were also passed through the filter paper. The filter paper was also thoroughly washed. The filtrate and washings were diluted to 100 ml and a 5.00 ml aliquot was removed and counted; the filter paper was also counted:

	net cpm
Filter Paper	12,016
Aliquot	24,556

The activity data indicate that 97.6% of the oxide had been dissolved.

Three 5.00 ml aliquots of the filtrate were placed in polyethylene bottles for chelation and extraction. The solutions were neutralized with 0.5 N sodium hydroxide and 2.0 ml of 0.05 M EDTA, and 2.0 ml of acetate buffer (pH 5) was added. The contents were shaken for five minutes and then heated in a water bath at 95°C for another five minutes. After cooling to room temperature 10.00 ml of 1% H(tfa) in benzene (0.082 M) was added and the mixtures were shaken on a high-speed shaker for 15, 30, and 60 minutes. Equal-volume aliquots of each phase were removed and counted. The pH of one aqueous phase was checked and found to be 5.0.

Data below confirm that analytically useful extractions occur in 15 minutes.

Equilibration Time, min	net cpm Aqueous Phase	net cpm Organic Phase	% Extracted
15	180	11,588	98.4
30	11	11,773	99.9
60	15	11,905	99.9

HIGH-FIRED ^{7}BeO - DOG BLOOD MIXTURES

This section presents the chronological study of the dissolution of ^{7}BeO in an oxide-dog blood matrix. Slight procedural variations were employed in each series with the dual objective of simplifying the procedure and minimizing the sample volume to increase sensitivity. Of necessity then, experimental details and observations are discussed in each subsection.

Preliminary Experiment

Approximately 1 mg of high-fired ^{7}BeO was mixed with 1.0 ml of dog blood. One aliquot (0.2 ml) of the mixture was removed, placed in a 10 ml Erlenmeyer flask and treated with ∿0.65 g of sodium hydroxide. The mixture was heated, diluted, transferred, neutralized and filtered as described above except that the filtrate volume was 50 ml. The filter paper and a 5.00 ml aliquot of the filtrate were counted:

	net cpm
Filter Paper	977
Aliquot	2,613

From these data one can calculate that 96.4% of the high-fired ^{7}BeO had dissolved.

Five ml aliquots were removed for chelation-extraction as described above. A 15 minute equilibration time was used for the extraction step. Activity measurement indicated that 97.2% of the beryllium had been transferred to the organic phase.

The preliminary examination indicated that dog blood does not prevent the dissolution of BeO by sodium hydroxide. Further, any dog blood-base reaction products do not interfere with the subsequent chelation-extraction step.

Replicate Series

A more thorough radiotracer study of the dissolution of ^{7}BeO in a dog blood matrix by sodium hydroxide was performed. All benzene

phases from the final extraction step were counted and then analyzed gas chromatographically.

In this series, 1.8 cm O.D. pyrex test tubes were used for the dissolution step. These were cut to a length of 9 cm to fit into the well of the gamma counter.

A suspension of high-fired ^{7}BeO and dog blood was prepared in a small vial. Aliquots of the suspension (0.2 ml) were placed into the test tubes and were counted. Following the addition of 0.6 g of sodium hydroxide, the mixtures were heated with intermittent agitation in a sand bath at the boiling temperature for 2 minutes. After cooling somewhat, the contents were diluted with distilled water and transferred to 25 ml volumetric flasks. The test tubes were washed with conc. nitric acid and the washes were also transferred to the corresponding volumetric flask. The flasks were diluted to the mark and three 5 ml aliquots were removed for chelation-extraction as previously described. The acid-washed test tubes and aliquots of the diluted sample and the extracts were counted.

Activity measurements of the initial oxide suspension and an aliquot of the diluted acidified reaction mixture were used to calculate the percent dissolution of the oxide (table VI). These measurements confirm the initial study wherein dissolution efficiency was based on the activity of filtered (undissolved) material and the activity of the filtrate.

In addition, table VI presents the results of the chelation-extraction study. Activity measurements confirmed that analytically useful extraction of the beryllium(II) had occurred in 15 minutes. Benzene solutions from the extraction study were examined for beryllium trifluoroacetylacetonate by gas chromatography. The results, expressed as μg BeO/ml, are also presented in table VI.

The mean of the percent recovery values of the gas chromatographic (GC) vs radioactivity (R) values in table VI is 99.4% and the standard deviation is ±4.1%.

We have observed in every case a precipitate in acidified solutions of the beryllium oxide-sodium hydroxide reaction mixtures. It was suspected that this was merely silicic acid resulting from attack of glass by the hot strong caustic. To confirm this, the acidified solution from experiment 5 was filtered and washed thoroughly. Examination of the precipitate by emission spectrographic analysis indicated that the following inorganic species were present: silicon, major constituent; iron and sodium, high trace; magnesium, aluminum, trace; nickel and copper, low trace.

Table VI

HIGH-FIRED ^{7}BeO - DOG BLOOD SUSPENSION

No.	Taken μg ^{7}BeO	Reaction Vial μg ^{7}BeO	Aqueous Solution μg ^{7}BeO	% Dissolved	Extraction: Aqueous μg ^{7}BeO	Extraction: Benzene μg ^{7}BeO	% Extraction	% Recovery	μg ^{7}BeO/ml Benzene: R	μg ^{7}BeO/ml Benzene: GC	% GC vs R	% GC vs Taken
1	85.7	ND	85.4	99.6								
A					0.21	8.95	97.7	104	1.79	1.92	107	112
B					0.11	8.67	98.7	101	1.73	1.94	112	113
C					0.09	8.97	99.0	105	1.79	1.73	97	101
2	107.3	ND	105.3	98.1								
A					0.07	11.1	99.4	103	2.22	2.21	99	103
B					(0.01)	10.8	100	101	2.15	2.11	98	98
C					0.10	11.3	99.1	105	2.27	2.02	89	94
3	92.2	ND	80.1[a]	-								
A					0.38	7.72	95.3	-	1.54	1.64	106	
B					0.27	7.87	96.7	-	1.57	1.68	107	
C					0.22	7.84	97.3	-	1.57	1.41	90	
4	78.9	ND	77.8	98.6								
A					0.26	8.17	97.0	104	1.63	1.59	98	101
B					0.22	7.95	97.3	101	1.59	1.41	89	89
C					0.09	7.90	98.9	100	1.58	1.59	101	101
5	106.0	ND	105.3	99.3								
A					0.03	10.4	99.7	98	2.08	2.06	99	97
B					-	10.4	100	98	2.08	2.08	100	98
C					0.22	10.3	97.8	97	2.06	2.03	99	96

[a]Spilled before diluting to volume

HIGH-FIRED BeO - RAT LIVER HOMOGENATE

The sodium hydroxide method for the dissolution of high-fired BeO was proved by radioactivity measurements to be a rapid, effective method for the solubilization of high-fired BeO in a blood matrix. Gas chromatographic measurements of benzene-H(tfa) extracts of reaction solutions have verified that low concentrations of the oxide can be quantitated.

To extend the method, a detailed study of the dissolution efficiency, extraction efficiency, and gas chromatographic quantitation was performed when the oxide was present initially in a rat liver homogenate. The experimental details were identical to those described for the replicate dog blood experiments. Results are presented in table VII.

The data reveal excellent dissolution efficiency (expressed as percent recovery), extraction efficiency, and good gas chromatographic quantitation. The mean of the gas chromatographic values compared with the radioactivity values is 108.1%; the standard deviation is ±3.9%.

LOW-FIRED ^{7}BeO - DOG BLOOD SUSPENSION AND LOW-FIRED ^{7}BeO - RAT LIVER HOMOGENATE

Because the base solution technique was found to be applicable to the highly refractory high-fired ^{7}BeO, it was presumed to be applicable also to low-fired ^{7}BeO. Blood and tissue mixtures were prepared, processed, and analyzed by radioactivity counting and by gas chromoatography.

Results presented in table VIII confirm that the low-fired oxide will be dissolved by reaction with sodium hydroxide when it is present in a dog blood matrix, that the neutralized reaction mixture can be extracted with benzene-H(tfa) to form the chelate, and that the beryllium content of the extract can be quantitated by the gas chromatography method. The mean recovery of the GC data vs the radioactivity data is 105.8%; the standard deviation is ±4.2%

Similar conclusions can be drawn from the examination of the data for the low-fired ^{7}BeO - rat liver homogenate study (table IX). The mean of the gas chromatographic recovery data is 103.1%; standard deviation is ±2.6%

ANALYSIS OF VARIANCE

Data in tables VI, VII, VIII, and IX on the percent recovery of the gas chromatographic data vs the values determined by radioactivity counting were subjected to an analysis of variance (table X).

Table VII

HIGH-FIRED ^{7}BeO - RAT LIVER HOMOGENATE

No.	Taken µg ^{7}BeO	Reaction Vial µg ^{7}BeO	Aqueous Solution µg ^{7}BeO	% Dissolved	Extraction: Aqueous µg ^{7}BeO	Extraction: Benzene µg ^{7}BeO	% Extraction	% Recovery	µg ^{7}BeO/ml Benzene: R	µg ^{7}BeO/ml Benzene: GC	% GC vs R	% GC vs Taken
1	222.6	0.2	216.0	97.0								
A					-	21.97	100	99	4.39	4.42	101	99
B					-	22.09	100	99	4.42	4.40	100	99
C					-	21.51	100	97	4.30	4.54	106	102
2	205.8	0.2	198.3	96.4								
A					0.06	20.41	99.7	99	4.08	4.50	110	109
B					0.70	20.43	96.7	99	4.09	4.56	112	111
C					-	20.61	100	100	4.12	4.84	118	118
3	208.4	0.1	204.5	98.1								
A					0.13	20.57	99.4	99	4.11	4.59	112	110
B					0.36	20.89	98.3	100	4.18	4.50	108	108
C					0.18	20.31	99.1	97	4.06	4.23	104	101
4	125.2	0.9	114.2	91.2								
A					0.72	10.62	93.7	85	2.12	2.24	106	90
B					2.54	10.52	81.1	84	2.10	2.25	107	90
C					1.01	10.53	91.2	84	2.11	2.38	113	95
5	136.4	ND	134.4	98.5								
A					0.37	15.65	97.7	115	3.13	3.44	110	126
B					0.31	15.61	98.1	114	3.12	3.30	106	121
C					0.18	15.71	98.9	115	3.14	3.42	109	125

Table VIII

LOW-FIRED ^{7}BeO - DOG BLOOD SUSPENSION

No.	Taken µg ^{7}BeO	Reaction Vial µg ^{7}BeO	Aqueous Solution µg ^{7}BeO	% Dissolved	Extraction: Aqueous µg ^{7}BeO	Extraction: Benzene µg ^{7}BeO	% Extraction	% Recovery	µg ^{7}BeO/ml Benzene: R	µg ^{7}BeO/ml Benzene: GC	% GC vs R	% GC vs Taken
1	181.5	0.1	178.0	98.1								
A					0.21	18.45	98.9	102	3.69	4.11	111	113
B					0.22	18.04	98.8	99	3.61	4.26	118	117
C					-	18.46	100	102	3.69	3.92	106	108
2	302.6	0.3	297.4	98.3								
A					0.57	30.90	98.2	102	6.18	0.56	106	108
B					0.18	32.03	99.4	106	6.41	6.45	101	107
C					0.42	31.01	98.7	102	6.20	6.50	105	107
3	105.5	ND	102.6	97.3								
A					0.18	11.21	98.4	106	2.24	2.31	103	110
B					0.32	10.54	97.1	100	2.11	2.32	110	110
C					0.42	10.77	96.2	102	2.15	2.41	112	114
4	489.0	0.2	483.0	98.8								
A					1.3	49.85	97.5	102	9.97	10.05	101	103
B					3.3	49.06	93.8	100	9.81	10.52	107	108
C					1.3	49.55	97.4	101	9.91	10.17	103	104
5	418.7	0.2	412.3	98.5								
A					5.3	42.59	89.0	102	8.52	9.05	106	108
B					4.5	42.84	90.5	102	8.57	8.87	104	106
C					0.26	42.11	99.4	101	8.42	7.95	94	95

Table IX

LOW-FIRED ^{7}BeO - RAT LIVER HOMOGENATE

No.	Taken µg ^{7}BeO	Reaction Vial µg ^{7}BeO	Aqueous Solution µg ^{7}BeO	% Dissolved	Extraction: Aqueous µg ^{7}BeO	Extraction: Benzene µg ^{7}BeO	% Extraction	% Recovery	µg ^{7}BeO/ml Benzene: R	µg ^{7}BeO/ml Benzene: GC	% GC vs R	% GC vs Taken
1	340.9	ND	339.3	99.5								
A					0.11	34.20	99.7	100	6.84	7.43	109	109
B					0.33	33.65	99.0	99	6.73	6.91	103	101
C					0.39	33.97	98.9	100	6.79	7.03	104	103
2	369.4	ND	364.5	98.7								
A					-	36.22	100	98	7.24	7.67	106	104
B					0.42	36.69	98.9	99	7.34	7.67	105	104
C					0.12	36.71	99.7	99	7.34	7.65	104	104
3	748.3	ND	729.2	97.4								
A					0.17	75.05	99.8	100	15.0	15.1	101	101
B					0.33	74.68	99.6	100	14.9	14.8	99	99
C					0.04	74.32	99.9	99	14.9	14.7	99	98
4	232.8	ND	231.9	99.6								
A					-	25.12	100	108	5.02	5.30	106	114
B					0.16	26.08	99.4	112	5.22	5.41	104	116
C					0.30	24.13	98.8	104	4.83	5.00	104	107
5	433.1	ND	431.3	99.6								
A					-	44.55	100	103	8.91	9.26	104	107
B					0.19	43.92	99.6	101	8.78	9.06	103	105
C					0.20	44.24	99.5	102	8.85	8.51	96	98

Table X

ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-ratio
Mean	1	216777.8	216777.8	
Treatments:				
A (sample type)	1	46.2	46.2	1.15
B (firing mode)	1	2.6	2.6	0.06
AB	1	167.6	167.6	4.20
Error	16	645.3	40.3	
Total	20	217211.9		

The analysis of variance indicates that sample type and firing mode have no significant effect (95% confidence level) on the relative error of the method within the range of concentrations studied. This conclusion is based on the fact that for every treatment the computed F-ratio is less than the corresponding tabled value of $F_{(1,16;\ 0.05)} = 4.49$.

SECTION VI

INTRATRACHEAL INJECTION OF RATS WITH ^{7}BeO: RADIOTRACER MEASUREMENT OF BERYLLIUM TRANSLOCATION AND ELIMINATION

The applicability of the sodium hydroxide dissolution method, or base solution technique, for the conversion of beryllium oxides to an ionic, chelatable form has been demonstrated for both forms of the oxide when present in laboratory-prepared mixtures of blood or liver homogenate. The resulting solutions were readily chelated, extracted, and analyzed by gas chromatography for beryllium content. Final verification of the technique for biological samples, however, required that in vivo specimens be obtained and analyzed. Consequently two series of experiments were performed wherein 16 rats received intratracheal injections of low-fired ^{7}BeO or high-fired ^{7}BeO.

INJECTION PROCEDURE

Weighed, anesthesized, white female rats were injected intratracheally (Gross, 1958 as cited in Spencer et al., 1967) with a 10% saline suspension of high-fired or low-fired ^{7}BeO. The suspension was prepared by adding a weighed portion of the oxide and several glass beads to sodium chloride solution (Baxter, Travenol, sterile, non-pyrogenic) and shaking for 1/2 to 2 1/2 hours.

The approximate dosage was 50 mg/kg and the suspension was shaken each time prior to filling the syringe. The syringe was pumped 2 or 3 times and rotated and shaken immediately prior to the injection through a large bore needle.

Following the injections the rats were placed in individual metabolic cages (figure 1, Hoeltge Inc., HB 11 M Cage with HB 17 Urine-Feces Separator and HB-66 Food Tunnel). Four control rats were also placed in metabolic cages at the same time.

EXCRETION OF BERYLLIUM

Urine and feces samples were collected after 1, 2, 7, and 21 days from each rat in each series that had received an intratracheal injection of ^{7}BeO. The beryllium content of the specimens was measured by counting the samples and calculated as ^{7}BeO by comparison with the activity of weighed standards each day (tables XI and XII). As indicated, for convenience, the data are calculated as ^{7}BeO although there is no evidence to prove that the beryllium was eliminated in this form.

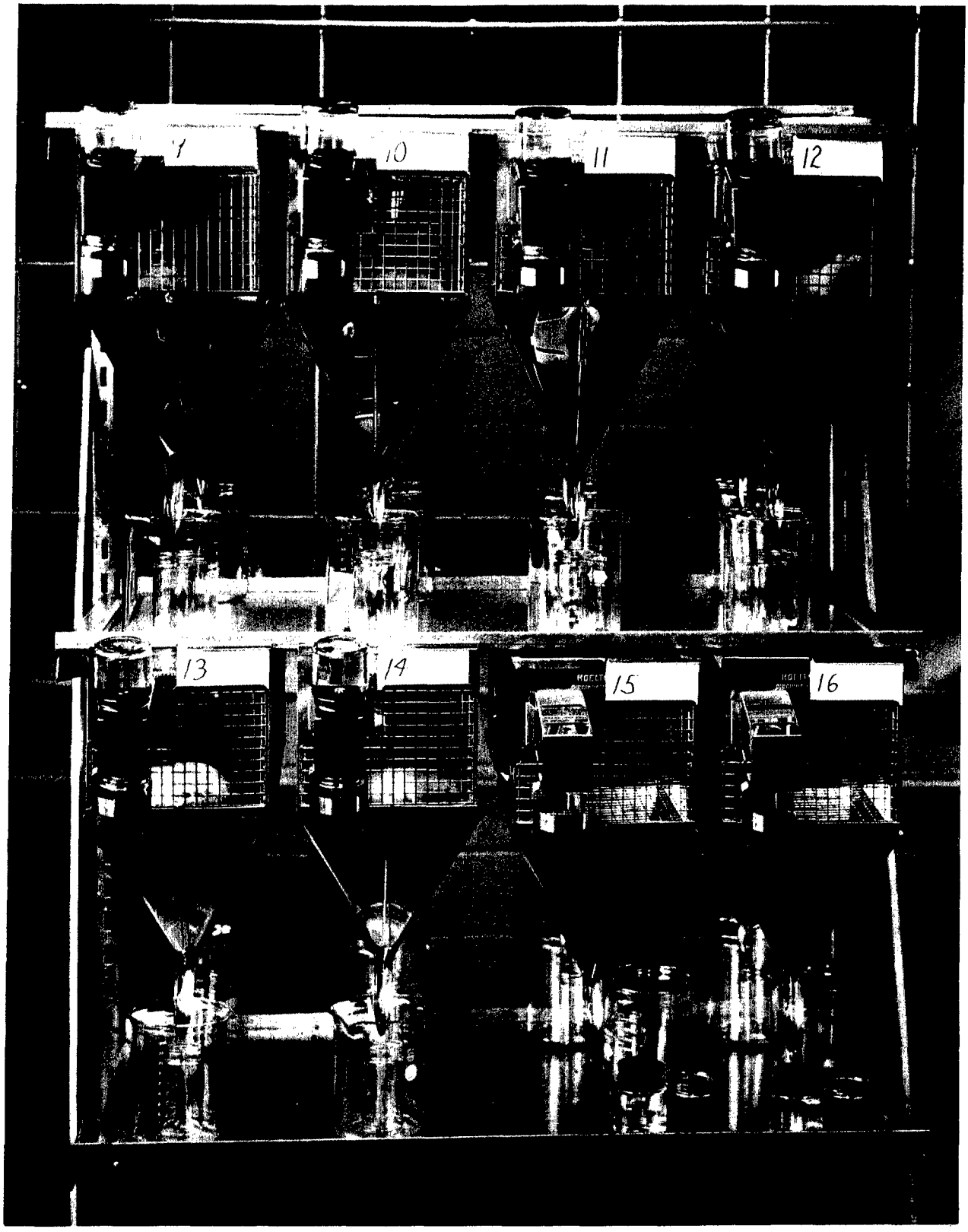

Figure 1. Metabolic Cages.

Table XI

EXCRETION OF ^{7}BeO AS A FUNCTION OF TIME

Series I

High-Fired ^{7}BeO

Rat	Weight, g	mg ^{7}BeO Injected	mg/kg	Urine Day 1 cm[a]	Urine Day 1 µg BeO	Urine Day 2 cm	Urine Day 2 µg BeO	Urine Day 7 cm	Urine Day 7 µg BeO	Urine Day 21 cm	Urine Day 21 µg BeO	Feces Day 1 cm	Feces Day 1 µg BeO	Feces Day 2 cm	Feces Day 2 µg BeO	Feces Day 7 cm	Feces Day 7 µg BeO	Feces Day 21 cm	Feces Day 21 µg BeO
1	375	19	51	2.8	0.4	3.3	19.6[b]	7.5	0.5	7.5	0	3.0	620	6.3	1089	2.9	20	4.5	3.7
2	348	17	49	7.2	1.6	7.5	0.7	6.2	<0.1	7.5	0	5.4	2323	7.0	1014	3.5	16	6.5	3.6
3	357	18	50	5.1	0.4	6.2	0.7	7.5	<0.1	7.5	0	3.0	4145	4.7	3198	2.0	11	6.0	2.6
4	332	16	48	4.8	41	6.5	0.9	7.5	0.4	7.5	0	2.0	250	5.4	1952	2.0	7	7.5	1.7
5	363	18	50	5.5	0.5	7.5	0.8	7.5	0.3	7.5	0	4.0	607	7.5	569	5.0	2	4.5	0.8
6	330	16	49	2.5	1.3	3.0	0.5	7.5	0.4	7.5	0	4.4	2585	0	0	3.5	6	3.0	1.6
7	291	14	48	3.9	3.3	7.5	0.6	7.5	0.1	7.5	0.8	4.1	2168	7.5	1517	3.0	10	5.5	2.5
8	315	16	51	3.7	3.2	7.0	0.5	7.5	0.2	7.5	0	3.8	386	6.4	122	3.0	0.8	3.5	0.6
									Low-Fired ^{7}BeO										
9	323	16	50	3.0	2.1	3.0	1.6	6.9	0.1	7.5	0.8	P	0.4	0.8	0.4	6.5	32	6.0	2.4
10	395	20	51	2.1	4.9	2.6	5.3	3.2	1.3	7.5	0.4	0	0	1.8	0.4	5.0	39	5.0	3.7
11	316	16	51	3.4	3.6	7.5	1.3	5.5	<0.1	7.5	0	2.5	1193	4.9	263	6.5	2	6.0	1.0
12	275	14	51	4.0	3.2	7.5	0.8	7.5	<0.1	7.5	0	4.6	5457	4.4	1111	5.4	3	6.5	0
13	325	16	49	1.7	2.7	3.8	2.4	7.5	1.0	7.5	0.9	P	0.1	0	0	2.9	9	7.0	1.2
14	410	20	49	1.9	3.7[b]	4.3	5.8	7.5	0.7	7.5	1.3	2.3	17	1.5	1681	6.0	32	6.5	2.5
15	282	14	50	3.5	1.9	2.8	1.8	7.5	2.2	7.5	1.9	0	0	2.4	882	7.5	31	5.5	4.4
16	325	16	49	3.0	1.7	4.9	3.4	7.5	1.1	7.5	1.2	0	0	0	0	4.5	37	5.5	1.8

[a]Depth of urine sample in a 4 dram vial

[b]Urine sample contaminated with feces

Table XII

EXCRETION OF ^{7}BeO AS A FUNCTION OF TIME

Series II

Rat	Weight, g	mg ^{7}BeO Injected	mg/kg	Urine Day 1 cm	Urine Day 1 µg BeO	Urine Day 2 cm	Urine Day 2 µg BeO	Urine Day 7 cm	Urine Day 7 µg BeO	Urine Day 21 cm	Urine Day 21 µg BeO	Feces Day 1 cm	Feces Day 1 µg BeO	Feces Day 2 cm	Feces Day 2 µg BeO	Feces Day 7 cm	Feces Day 7 µg BeO	Feces Day 21 cm	Feces Day 21 µg BeO
High-Fired ^{7}BeO																			
1	290	14	48	4.3	13.0	5.3	5.6	7.5	0.1	7.5	0.7	2.3	1470	3.5	2499	2.2	12	4.0	4.5
2	323	16	50	4.0	4.9	7.5	3.2	7.0	0.3	7.5	0.3	2.0	1469	5.0	2188	5.6	8.4	7.0	2.1
3	300	15	50	3.8	2.2	5.2	0.7	7.5	0	7.5	0	1.2	1920	3.4	4865	6.2	9.4	4.0	7.5
4	276	14	51	5.1	2.2	4.4	0.7	7.5	0.2	7.0	1.6	3.0	1775	3.5	4827	4.7	28	4.0	11.8
5	273	14	51	3.8	3.8	5.5	1.2	7.5	0	4.5	0.5	1.5	1442	2.8	1477	4.6	6.9	3.0	5.7
6	314[c]	16	51	4.6	12.0	3.2	2.3	7.5	0.1	7.0	1.9	3.8	3992	1.9	1187	4.3	14	5.5	
7	280	14	50	4.6	5.7	6.8	2.0	7.5	0	7.5	1.8	3.0	1668	3.1	1473	6.0	9.3	4.0	3.3
8	312	16	51	4.3	2.5	7.5	0.8	7.5	0	7.5	1.9	2.3	308	5.4	1396	4.4	8.5	5.5	1.2
Low-Fired ^{7}BeO																			
9	311	16	51	2.0	2.9	3.6	2.9	7.5	0.7	7.5	1.3	0	0	1.2	1447	5.6	12	3.0	3.7
10	306	15	49	2.8	3.2	2.7	2.0	7.5	0.1	6.0	0.1	1.4	0	2.0	6020	5.6	10	2.0	8.4
11	295	15	51	2.7	3.8	2.3	1.6	7.5	0.3	6.5	2.0	0	0	1.2	356	5.6	31	2.5	14.1
12	327	16	49	3.1	5.3	3.8	1.8	7.5	1.3	7.0	4.2	1.6	0.5	3.7	3044	5.6	34	3.5	13.6
13	272	14	52	2.8	3.1	5.8	2.5	7.5	0.6	6.5	3.4	2.5	1054	5.8	871	5.6	14	4.0	8.5
14	267	13	49	2.8	2.8	5.0	3.5	7.5	0.1	7.0	2.0	0	0	4.7	1695	5.6	21	5.0	11.2
15	290	15	52	4.0	2.9	6.4	2.8	7.5		7.5	1.3	1.3	652	4.7	3120	5.6	7.9	6.0	8.8
16	304[c]	15	49	4.1	31.1	3.7	10.8	7.5	0.2	7.0	0.6	2.8	1608	2.9	729	5.6	7.8	5.0	5.4

[c]Rejected

The data indicate, in general, a relatively high amount of BeO in the feces one and two days after injection and a marked decrease in amount thereafter. The presence in the feces is probably a consequence of coughing up and swallowing the oxide by the rats.

No further examination of the urine and feces samples was made.

TRANSLOCATION OF BERYLLIUM

Twenty one days after the start of each series the rats were given anesthesia and blood samples were obtained by cutting off a portion of the tail. Each animal was then killed and the liver, spleen, kidneys, and lungs were collected into separate vials and weighed (series II, only). All animal remains were collected and disposed of as radioactive waste. The tissue and blood samples were counted, stored in a refrigerator, and subsequently transferred to AMRL (lungs excepted).

The beryllium content, expressed as the oxide, of tissue and blood samples of series I and series II experiments are presented in tables XIII and XIV, respectively. Tissue and blood weights of series II samples are given in table XV.

The large number of samples to be counted prohibited the use of counting times of sufficient length for accurate quantitation. The short counting times coupled with the decay of ^{7}Be during the experiments and the resultant decrease in the specific activity of the ^{7}BeO seriously affected the sensitivity of detection. Values less than 1 μg ^{7}BeO in tables XIII and XIV are reported for information only and should be considered highly suspect. (For example, Rat 3 liver gave 4798 counts in 15 minutes; the background was 4655 counts in 15 minutes for a net cpm of 9.5. The error in cpm for a 4800 total count in 15 minutes is 13 cpm. Therefore, the quantitation of 9.5 cpm as 0.26 μg ^{7}BeO is highly suspect.)

Beryllium was detected in the liver, spleen and kidneys of rats which had been injected with either high-fired or low-fired ^{7}BeO. In general, the amount of oxide present after 21 days was found to be higher for the low-fired series as compared with the high-fired series.

Table XIII

TRANSLOCATION OF BERYLLIUM OXIDE, SERIES I

Rat	Lungs	Liver	Kidney	Spleen	Blood
			µg High-Fired BeO		
1	10952	-	-	-	-
2	6883	5.1	-	2.3	-
3	6060	0.26	-	0.10	0.25
4	3717	0.49	0.11	0.15	0.24
5	181	1.00	0.81	0.59	0.82
6	2736	0.43	0.31	0.21	0.31
7	5445	0.10	-	-	-
8	72.2	-	-	-	-
			µg Low-Fired BeO		
9	3821	43.3	5.4	4.8	0.1
10	4408	23.2	1.07	2.34	0.0
11	101	1.87	0.11	0.14	0.09
12	80	-	-	-	-
13	1145	27.4	0.32	2.05	0.19
14	3770	24.2	1.14	2.07	0.18
15	3357	28.3	0.49	3.29	0.25
16	3419	57.2	2.06	5.88	0.28

Table XIV

TRANSLOCATION OF BERYLLIUM OXIDE, SERIES II

Rat	Lungs	Liver	Kidney	Spleen	Blood
			µg High-Fired BeO		
1	7794	12.68	0.32	1.08	-
2	5368	46.48	0.54	6.42	-
3	5252	-	0.40	-	0.11
4	5915	10.07	0.07	1.27	-
5	4771	0.22	0.11	0.27	-
6	-	-	0.66	0.05	-
7	2592	0.24	0.54	0.05	-
8	1899	0.38	0.12	-	-
			µg Low-Fired BeO		
9	3972	22.0	1.19	0.61	-
10	923	1.81	0.42	0.12	-
11	2912	17.6	1.41	1.81	0.01
12	3414	14.4	1.83	1.01	0.27
13	2808	58.7	0.88	9.72	0.28
14	2729	17.4	1.06	2.22	0.26
15	1582	3.8	0.85	0.56	-
16	-	-	-	-	-

Table XV

SERIES II: TISSUE AND BLOOD WEIGHT

Rat	Weight, Grams				
	Lungs	Liver	Kidney	Spleen	Blood
1	2.014	10.589	1.911	0.609	5.294
2	2.188	13.192	2.362	0.785	8.593
3	1.957	11.132	2.318	0.623	7.240
4	2.195	8.858	2.045	0.523	5.466
5	1.910	9.267	1.723	0.584	6.731
6	2.011	10.446	2.138	0.687	6.913
7	2.040	11.987	2.148	0.696	8.048
8	2.200	11.390	2.598	0.699	8.789
9	2.737	11.139	2.328	0.613	7.218
10	2.681	10.453	2.225	0.653	5.850
11	2.428	9.082	1.959	0.563	6.679
12	2.639	10.535	2.080	0.644	5.005
13	2.652	8.825	1.647	0.727	4.363
14	2.963	10.857	1.954	0.688	7.909
15	2.780	11.791	2.062	0.661	6.911
16	2.193	10.988	2.272	0.731	6.135
17	2.180	11.364	2.844	0.675	7.957
18	2.043	12.286	2.685	0.617	7.364
19	2.233	12.835	2.775	0.726	8.755
20	2.169	15.022	2.961	0.680	5.842

SECTION VII

GAS CHROMATOGRAPHIC DETERMINATION OF BERYLLIUM IN RAT LIVER FROM ^{7}BeO-INJECTED RATS

BASE SOLUTION - EXTRACTION

To provide samples for the gas chromatographic examination of beryllium in tissue, the livers of rats 9 and 16 (series I) and rat 13 (series II) were homogenized and processed. Exact details of the dissolution-extraction procedure are documented below.

Homogenates were transferred to weighed vials which had been counted for one hour, weighed to determine the homogenate weight, and counted for one hour. The net cpm were converted to µg ^{7}BeO from the activity of weighed standards counted on the same day.

Rat	µg ^{7}BeO/g homogenate
9	0.909
16	1.186
13	1.904

Weighed portions (∿0.2 g) of each homogenate were placed in 10 ml Erlenmeyer flasks and 6 pellets (∿0.75 g) of sodium hydroxide were added. The top edge of the neck of the flask was treated with a small amount of silicone lubricant and closed almost completely with a microscope cover glass. The mixtures were heated on a hot plate and allowed to boil for 2 minutes with intermittent shaking. The flasks were then placed on a wooden board and allowed to cool.

After cooling, about 1 ml of water was added from a Pasteur pipet to dissolve the reaction mixture. The contents of the flask were transferred to a 25 ml volumetric flask through a small funnel and the flask was washed twice with 2 ml portions of water. The flask was then washed twice with 2 ml portions of conc. nitric acid and these washings were added to the volumetric flask through the funnel. The cover glass was also washed with nitric acid. Finally, the flask and funnel were washed several times with distilled water.

After cooling to room temperature the contents of the flask were diluted to 25 ml and mixed well.

Five ml aliquots were placed in small polyethylene bottles and 5 drops of phenolphthalein were added. The solution was partially neutralized with 50% NaOH, 10% NaOH, and finally taken to a red

color with 0.5 N sodium hydroxide. Then 2 ml of acetate buffer, pH 5 and 2 ml of 0.05 M EDTA were added. This solution was heated in a boiling water bath for 5 minutes and cooled. Ten ml of 1% H(tfa) in benzene was added and the mixture was shaken for 15 minutes on a platform shaker.

The benzene extract, after treatment to remove free H(tfa), was injected into a gas chromatograph for measurement of beryllium content.

In addition, several weighed portions of homogenate were placed directly into polyethylene bottles, treated with buffer and EDTA, heated, cooled, and extracted with 10 ml of benzene-H(tfa), and examined gas chromatographically to determine whether or not ionic beryllium was present in the homogenate.

GAS CHROMATOGRAPHIC ANALYSIS

Gas chromatographic analysis of direct-reaction and base solubilized-extracted portions of rat 13 liver homogenate are presented in table XVI. Of special interest is the fact that ∿42-45% of the beryllium was found in the benzene extracts performed directly on the homogenate at room temperature. This observation suggests that translocation occurred at least partially by a dissolution process since the beryllium is chelatable. The 45% recovery may merely be a consequence of incomplete reaction with H(tfa) caused by competition of protein fractions for the Be(II). Also of importance is the fact that all of the beryllium was recovered from the aliquots of homogenate that were treated with base and then extracted.

These observations on the translocation of beryllium and the form (i.e., ionic or oxide) should be confirmed in a definitive study. This work could be performed in conjunction with <u>in-vitro</u> experiments to determine the mode of participation of phagocytic cells in the translocation; i.e., does the translocation occur initially as a soluble or unsoluble substance?

CONTAMINATION DIFFICULTIES

Data cited in the section immediately above were the end result of an extensive, systematic investigation into the source of contamination observed when numerous aliquots of the liver homogenates of rats 9 and 16 were processed. The beryllium concentration in the initial studies of rats 9 and 16 were on the following order of magnitude

Table XVI

GAS CHROMATOGRAPHIC DETERMINATION OF BERYLLIUM IN RAT 13 HOMOGENATE: DIRECT REACTION AND BASE-SOLUTION TECHNIQUE

Aliquot Wt, g*	µg ^{7}BeO in Aliquot	µg ^{7}BeO/ml Extract	µg ^{7}BeO/ml Direct Reaction	µg ^{7}BeO/ml Base Solution	% Recovery
0.2138	0.407	0.0407	0.0185		45
0.2068	0.394	0.0394	0.0168		43
0.2456	0.468	0.0468	0.0196		42
Blank	-		nil		
0.2002	0.381	0.00762		0.00895	117
0.2049	0.390	0.00780		0.00922	118
0.2453	0.467	0.00934		0.0118	126
Blank				nil	

*Homogenate contains 1.904 µg ^{7}BeO/g by radioactivity counting.

Rat	Extraction	GC µg BeO/ml	Radiotracer µg/ml
9	Direct	3-5	0.01-0.02
9	Base Solution	1-3	0.003-0.005
16	Direct	1-2.5	0.020-0.025
16	Base Solution	0.2-2	0.004-0.005

Additional extracts were examined by gas chromatography with no dilution of the sample prior to NH_4OH treatment to remove excess H(tfa).

Rat	Extraction	GC µg BeO/ml	Radiotracer µg BeO/ml
9	Direct	0.03-0.06	0.01-0.02
9	Base Solution	0.01-0.03	0.003
16	Direct	0.04-0.06	0.020-0.025
16	Base Solution	0.01-0.03	0.004-0.005
13	Direct	0.018-0.019	0.039-0.044
13	Base Solution	0.019-0.028	0.007-0.009

High results by a factor of 3 to 10 were still observed.

At this stage, fresh buffer and EDTA were prepared and blanks were processed consisting of various combinations of the old and new reagents. Sizable contamination was noted in all cases and the source, therefore, could not be ascribed to any single reagent.

The blanks, however, were no longer obtained when the buffer- EDTA- and benzene-H(tfa) reagents were processed in new polyethylene bottles. The source of the contamination (for determinations at the nanogram/ml level) was subsequently traced to the reuse of those polyethylene bottles which had been previously used for spiked blood and tissue homogenate work at the µg/ml level. These bottles had been cleaned but the procedure obviously was not sufficient to remove all of the adsorbed beryllium.

APPENDIX

EVALUATION OF EPA METHOD FOR DISSOLUTION OF ROCKET EXHAUST

In a recent issue of the Federal Register (1971) a notification of "proposed rule making" for national emission standards for the hazardous air pollutants asbestos, beryllium, and mercury is presented. Included in this proposal is an analytical procedure for the isolation, dissolution, and quantitation of beryllium. This procedure, which involves treatment with nitric acid followed by addition of sulfuric-perchloric acid mixture and heating to dryness, is specifically stated to be applicable to samples taken at rocket motor firing sites in addition to other beryllium sources.

According to Spencer (1968), high-fired BeO is much less soluble in 6 N hydrochloric acid than is low fired oxide by about a factor of 38 [i.e., high fired oxide released 0.12 g Be(II) per 100 g of "sample in 30 minutes at room temperature; low-fired oxide released 4.6 g Be/100 g sample]. These data, although interesting, do not reveal the relative behavior of these oxides under acid conditions that would be employed by the chemist attempting to dissolve oxide samples. Cotton and Wilkinson (1962) state that a high temperature form of BeO (calcined above 800°C) is "virtually insoluble in aqueous acids or bases and is only with difficulty soluble in fused salts."

In view of the statements of Cotton and Wilkinson, there was reasonable justification to question the effectiveness of the proposed EPA method for the dissolution of rocket exhaust BeO. Low-fired and high-fired ^{7}BeO, prepared under this contract, permitted facile measurements of the extent of dissolution.

Milligram portions of low-fired ^{7}BeO and high-fired ^{7}BeO were placed in separate 150 ml beakers and treated with 35 ml of concentrated nitric acid. After boiling on a hot plate for 10 minutes the solutions were allowed to cool to room temperature and 5 ml of concentrated sulfuric acid and 5 ml of concentrated (70%) perchloric acid were added. The solutions were replaced on the hot plate and evaporated to dryness with occasional swirling. The cooled residues were dissolved in 10 ml of 25% hydrochloric acid (3 N) by heating to near boiling. (Note: Directions in the Federal Register state "dissolve the residue in 10 ml of 25% hydrochloric acid", we would suggest that the phrase - by heating to boiling, if necessary - be added.)

The hydrochloric acid solution was diluted to about 40 ml and filtered through a 0.45 μ Millipore filter paper. The beaker was thoroughly policed and washed and the washings were also

filtered. Finally, the filter paper was washed thoroughly. The filtrate was diluted to 100 ml and 5.00 ml aliquots were counted. The filter paper, which should contain all of the undissolved ^{7}BeO, was also counted.

Results presented below indicate that both low-fired and high-fired beryllium oxide are dissolved essentially quantitatively for analytical purposes.

	net cpm		
Sample	Filtrate	Filter Paper	% BeO Dissolved
Low-Fired	54,500	36	99.9
	77,174	114	99.9
High-Fired	58,460	79	99.9
	167,914	2456	98.6
	133,168	311	99.8
	158,664	442	99.7

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