“Beryllium Oxide Digestion Optimization at the Savannah River Site”

by

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November, 2002

A paper submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Master of Public Health in the School of Public Health

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Abstract

Chronic beryllium disease (CBD) is a debilitating lung disorder affecting an estimated 4.6% of past nuclear weapons and beryllium manufacturing workers. The Chronic Beryllium Disease Prevention Program, as set up by the U.S. Department of Energy (DOE), establishes guidelines for maintaining acceptable exposure levels in all beryllium manufacturing facilities. These exposure levels are based on the measuring of total beryllium which includes beryllium metal, beryllium salts, beryllium alloys and beryllium oxide. The most sensitive detection instruments used in industrial hygiene sampling measure beryllium in a solubilized form. Beryllium oxide, in particular, has been shown to resist solubilization unless it is performed in the presence of strong acid(s). This poses a problem for industrial hygienists because beryllium oxide has been shown to have a high level of toxicity due to its small size and moderate solubility. The current digestion method (EPA 3050B) for all metals at the Savannah River Site involves the use of nitric and hydrochloric acids. It was hypothesized that this method was inadequate for the complete digestion of beryllium oxide.

This study examines this issue by comparing method 3050B with two other acid digestion methods. Once the most effective of the three methods was determined, optimization factors such as settling time, heating/reflux time, and hydrogen peroxide addition were examined. It was found that treatment with a combination of nitric, hydrochloric and hydrofluoric acids with overnight settling, a one hour heating/reflux time, and hydrogen peroxide addition was the most effective means of digesting beryllium oxide for measurement by inductively-coupled plasma emission spectroscopy.
Beryllium Oxide Digestion Optimization

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November, 2002

Background

Beryllium (Be) is listed as the fourth element on the periodic table, with an atomic mass of 9.01218 g/M. Trace amounts can be found in virtually everything, including soil, plants, water, and air. At two-thirds the bulk density of aluminum, with six times the stiffness of steel\(^1\), beryllium has become popular in many manufacturing arenas. In its natural form, it is found as beryl and bertrandite ores (silicates), which are mined in several locations around the United States. After extraction, it is chemically purified to form beryllium hydroxide and soluble beryllium salt byproducts, such as beryllium fluoride, beryllium chloride, beryllium sulfate, and beryllium nitrate. The beryllium hydroxide then undergoes additional treatment to become beryllium oxide, pure beryllium metal, and beryllium alloys (beryllium-aluminum, copper-beryllium [\(<2\%\)Be], etc.), which are the more commercially important forms\(^1\). This enhancement allows a wider range of uses for beryllium than other common metals. For example, beryllium oxide is used in thermal ceramics because of its high heat capacity and conductivity, while copper-beryllium is used in golfing equipment because it adds stiffness to the usually soft copper—without the weight or corrodability of steel. Likewise, pure beryllium metal often is used in high-flux reactors and fiber optics.
Given all these factors, it is easy to see why beryllium use has grown in recent years and likely will continue to do so. Unfortunately, this popularity has brought with it some cause for concern among industrial health professionals. The major issue centers on the potential for beryllium workers to develop Chronic Beryllium Disease (CBD)—a debilitating lung disorder affecting as many as 4.6% of past workers at nuclear weapons and beryllium mining facilities.

The majority of beryllium inhaled by an individual is cleared from the lungs by the mucociliary escalator and airway macrophages. The remainder is usually deposited in nearby lymph nodes and the pulmonary interstitium. The signs of CBD often mimic those of sarcoidosis in which the lungs develop non-caseating granulomas. This is brought on by the attack of insoluble beryllium particulates by host lymphocytes. Symptoms may include shortness of breath, chronic coughing, night sweats, fatigue, weight loss and, in extreme cases, digital clubbing. One of the difficulties with diagnosing and treating CBD lies in the fact that there are no easily identifiable patterns of susceptibility. Epidemiological study has shown no dose-response relationship between beryllium exposure and CBD. Clinical symptoms may not appear for an average of ten years after exposure. In fact, many individuals die from other illnesses long before symptomatic CBD appears. Also, it is not necessary for exposure to be continuous. One case study tells of a 29 year old male with CBD who was exposed for just two weeks before presenting with the illness thirteen years later.

Recently, researchers have found certain factors that might contribute to beryllium disease. Size and solubility of particles are two factors that may have a
large impact on disease development. Larger particles found in beryl ore dust and beryllium metal are unable to penetrate as deeply into the lungs as the sm. This makes them much less likely to cause lasting illness. Also, the oxide and salts are more easily solubilized in pulmonary fluid relative to larger particles. This facilitates crossing of the pulmonary epithelium into the blood. Lastly, temperature has been shown to affect toxicity. Oxide formed at 500° C has been found to be much more soluble and, therefore, toxic in canine lungs than oxide formed at 1000° C. Unfortunately, understanding these contributing factors has not led to improvements in diagnostics and treatment of this disease.

Around the 1950's CBD was measured using a skin patch test involving a beryllium sulfide paste. This was proven ineffective, however, when subjects with no known history of beryllium sensitivity suddenly showed a positive test result after the paste was applied. This left researchers and industrial hygienists with the dilemma of finding a reliable, non-invasive test that could distinguish CBD from other lung ailments. This would not happen until the late 1970's. It was around this time that doctors developed a technique for extracting viable lung cells from living patients using a bronchoalveolar lavage technique. This allowed scientists to culture lung cells in the presence of various toxins, including beryllium, to determine their effect. It was found that lymphocytes of subjects with CBD showed a dramatic increase in proliferation when exposed to a beryllium-containing solution as compared to unsensitized cells. This was the basis of what has become known as the Beryllium Lymphocyte Proliferation Test (BeLPT)—the current standard in diagnosing CBD. A confirmed diagnosis of
beryllium sensitization involves two independently confirmed positive BeLPT's. Critics of this test challenge its usefulness by pointing to inconsistencies in results from several independent laboratories\(^9\). Currently, the only available alternative is to use a less invasive blood test. Unfortunately, this presents the same potential for discrepancies. Scientists have also discovered a possible genetic marker (Glu69) for beryllium sensitivity but, as yet, no test has been developed\(^\text{10}\). With such uncertainty surrounding CBD testing, focus has shifted to ensuring that proper prevention standards be in place to decrease exposure to beryllium in all forms.

In 1999, the U.S. Department of Energy issued a final ruling that established the Chronic Beryllium Disease Prevention Program, which defined the responsibilities of beryllium manufacturers to their employees and set exposure limits for total beryllium (metal, salts, silicates, alloys, and oxide) in workplaces. Adherence to these standards hinge on the ability of testing methods to accurately measure total beryllium concentrations. Because the most sensitive measuring devices measure only soluble beryllium, all solid forms must be digested and put into solution. As a result, considerable effort has been devoted to finding the most effective means of accomplishing this. Of particular concern here at Savannah River Site (SRS) and other facilities is the ability to successfully digest and analyze samples that may contain beryllium oxide (BeO). This form of beryllium has gained significant attention within the industrial hygiene community in recent years because of its high reported toxicity, small size, and only moderate solubility.
The issues addressed by this beryllium oxide digestion study involve the current U.S. Environmental Protection Agency (EPA) digestion method. The working hypothesis was that this method, which utilizes HNO₃ and HCl, was not sufficient to fully digest BeO in samples. This hypothesis was tested by comparing three different digestion techniques being used for beryllium—the EPA 3050B, ORNL, and HF methods. EPA method 3050B utilizes HNO₃, HCl, and H₂O₂, and is the current standard for all solid samples digested in a hot-block at SRS. The ORNL method is used at Oak Ridge National Laboratories and calls for a mixture of H₂O₂ and H₂SO₄. The HF method was designed to use a combination of HNO₃, HCl, and HF. Once the most effective method was determined, the effects of settling time (post-digestion period during which the sample sits at room temperature), heating/reflux time, and the addition of hydrogen peroxide were examined. The results reported are based on hot-block digestion followed by analysis with inductively coupled-plasma atomic emission spectroscopy (ICP-AES) at a wavelength of 313.107.

Materials and Methods

Materials:

- OPTIMA 3000XL ICP spectrometer using 313.107 wavelength (Perkin Elmer)
- 100-mL cups w/ attached lids (Environmental Express)
- Ghost Wipes™ moistened swipes (Environmental Express)
- Beryllium oxide (99% pure) (Alfa Aesar #10001)
- Concentrated HCl, HNO₃, and H₂SO₄
- Hydrogen peroxide, 30%
- Hydrofluoric acid, 50%
- Hot block heated to 95° C
- Metals standard—EM-Std 1-Solution A (High Purity Standards)
Methods:

1. **3050B** – EPA Method 3050B

2. **ORNL (Oak Ridge National Laboratories Adaptation)** – BeO samples with and without swipes were treated with 4 mL of 30% H₂O₂ and 4 mL of concentrated H₂SO₄ and heated at 95°C for 20 minutes. An additional 4 mL of 30% H₂O₂ was added, and the samples were allowed to dry down to approximately 5 mL. Digested samples were diluted and analyzed using ICP atomic emission spectroscopy.

3. **HF** – BeO samples with and without swipes were treated with 6 mL of a 1:4:1 mixture of concentrated HCl, concentrated HNO₃, and 50%HF, respectively. The samples then were heated at 95°C to near dryness, followed by the addition of 3 mL concentrated HCl. After an additional 10 to 15-minute heating, the samples were diluted in ddH₂O to 50 mL and analyzed by ICP atomic emission spectroscopy.

All experiments involved digesting pure beryllium oxide in the presence or absence of Ghost Wipes®. To examine settling time, two sets of five samples (BeO + swipes) were allowed to sit in 50–100 mL of deionized water at room temperature for periods of one and two days, respectively, while a third set was run immediately after digestion. For determination of heating/reflux time, three sets of three samples (BeO + swipes) were allowed to reflux for periods of 1, 30, and 60 minutes, respectively. This was repeated three times, with the third time involving an overnight settling period. Finally, for peroxide addition,
comparisons were made between two sets of five samples (BeO + swipes) treated or not treated with peroxide after 20 minutes of initial refluxing.

Results

The results below are to be used as preliminary data for the optimal digestion of beryllium oxide. Results for the experiments on settling time, reflux time, and peroxide use all were obtained using the HF method of digestion, which was shown to have the greatest effect.

Table 1 and figure 1 show the percent recovery for beryllium oxide digested without swipes. The results would seem to indicate that use of the ORNL method produced an approximate 27% increase in recovery over the 3050B method for the three treatment conditions. Likewise, the HF method showed an increase of about 32%. Each percent recovery given is an adjusted average of three similarly treated samples that differed only in the amount of beryllium oxide present. Adjustments were made by extrapolating all mean intensity values for each treatment back to the lowest mean ICP standard intensity among the three groups. For example, if the method 3050B mean standard value was 5,000,000 counts per second (cps) while the ORNL and HF standard values were 5,500,000 and 6,000,000-cps, respectively, all mean values making up the averages would be recalculated using the 5,000,000-cps value.
Table 1: Percent Recovery\(^1\) for Beryllium Oxide Digestion Without Swipes\(^2,3\)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>3050B</th>
<th>ORNL</th>
<th>HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. All run according to protocol with three-day settling period</td>
<td>48%</td>
<td>86%</td>
<td>86%</td>
</tr>
<tr>
<td>2. Digested for extended times (~45 minutes with reagents doubled)</td>
<td>69%</td>
<td>91%</td>
<td>97%</td>
</tr>
<tr>
<td>3. Run immediately without overnight settling</td>
<td>58%</td>
<td>80%</td>
<td>89%</td>
</tr>
</tbody>
</table>

1. Adjustments were made by extrapolating all mean intensity values for each treatment back to the lowest standard mean intensity among the three groups.
2. All values given are an average of three independent samples.
3. Samples diluted 1:25,000

Figure 1:

When swipes were added to the HF digestion tubes and the digestates were allowed to settle for three days, as shown in table 2 and figure 2, there appeared to be a dramatic increase in beryllium recovery. Compared to the HF digested samples that were run immediately, the settled samples showed an approximate 12–23% increase in recovery. Surprisingly, no significant increase was shown in samples digested with the ORNL method.
Table 2: Percent Recovery for Beryllium Oxide Digestion with Swipes

<table>
<thead>
<tr>
<th>Sample Treatment</th>
<th>HF (run immediately)</th>
<th>ORNL (three-day settling)</th>
<th>HF (three-day settling, dilute)</th>
<th>HF (three-day settling, concentrated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Be standard (liquid standard added to swipes)</td>
<td>Not tested</td>
<td>105%</td>
<td>110%</td>
<td>110%</td>
</tr>
<tr>
<td>2. BeO</td>
<td>83%</td>
<td>83%</td>
<td>100%</td>
<td>108%</td>
</tr>
<tr>
<td>3. Be standard + BeO</td>
<td>85%</td>
<td>76%</td>
<td>97%</td>
<td>103%</td>
</tr>
</tbody>
</table>

1. "HF-run immediately" results are an average of two identical runs.  
2. "HF dilute" samples were settled in 100 mL deionized water and "concentrated" samples were brought up to 15–20 mL deionized water.  
3. All three-day incubation results are representative of one sample each.

Figure 2:

The additional experiments involving settling time, heating/reflux time, and peroxide are summarized in tables 3, 4, and 5. Unlike the previous results, these are meant to show comparisons between treatment conditions rather than treatment methods.
Table 3: Effects of Post-Digestion Settling on Percent Recovery

<table>
<thead>
<tr>
<th>Settling Period (days)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>90%</td>
</tr>
<tr>
<td>1</td>
<td>91%</td>
</tr>
<tr>
<td>2</td>
<td>92%</td>
</tr>
</tbody>
</table>

1. Adjustments were made by extrapolating all mean intensity values for each treatment back to the lowest standard mean intensity among the three groups.
2. Results are based on three independent sets of five samples each, including blanks.

Table 4: Effects of Heating/Reflux Time on Percent Recovery

<table>
<thead>
<tr>
<th>Time</th>
<th>First Run</th>
<th>Second Run</th>
<th>Second Run Repeat w/ overnight settling</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 min.</td>
<td>79%</td>
<td>80%</td>
<td>88%</td>
</tr>
<tr>
<td>30 min.</td>
<td>87%</td>
<td>91%</td>
<td>101%</td>
</tr>
<tr>
<td>60 min.</td>
<td>85%</td>
<td>91%</td>
<td>100%</td>
</tr>
</tbody>
</table>

1. Adjustments were made by extrapolating all mean intensity values for each treatment back to the mean standard intensity of the first run.

Table 5: Effects of Peroxide Addition on Percent Recovery

<table>
<thead>
<tr>
<th>Sample</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control¹</td>
<td>95%</td>
</tr>
<tr>
<td>No Treatment</td>
<td>95%</td>
</tr>
<tr>
<td>Peroxide</td>
<td>90%</td>
</tr>
</tbody>
</table>

1. Control results based on one set of two samples run without peroxide.

Discussion

3050B

The results from table 1 seem to indicate that this method is the least effective of the three tested. At no point in any of the digestions did the beryllium oxide totally dissolve; as a result, subsequent filtration was necessary. This was time consuming and somewhat cumbersome when working within the limited confines of the safety hood. Extending digestion times did not seem to help.
Because of the poor results obtained from the first set of tests (table 1), it was deemed unnecessary to include this method in the digestions with swipes.

**ORNL**

This method was quite comparable in effectiveness to the HF method. In most cases, no visible BeO remained undisolved, and no additional filtration was needed. Also, this method worked very well with the liquid beryllium standard, as shown by the >100% recovery. There were some drawbacks, however. The first was that the reactions tended to be somewhat violent. The effervescence often would be visible above the rim of the digestion cups. Additionally, when the swipes were added, the reactions tended to become hot to the point of charring the samples. This generated sticky tar-like deposits on the inside of the cups. In some cases, it also resulted in an incomplete digestion of the swipes, which created the need for filtration. Although the samples could be somewhat cleared up with the addition of peroxide, to the point of taking on an “iced tea” appearance, the overall process was inconvenient.

**HF**

This method seemed to produce the best overall results of the three. The small working volume and lack of effervescence made it very easy to use. In all cases, the BeO was fully dissolved and required no filtration. The additional experiments concerning settling showed that the amount of recovery was enhanced with longer settling periods. While this does not seem significant in the results of table 3, it is dramatic in the “overnight” result found in table 4. As for the varying heating/reflux times, it appears that a near-optimal amount of
digestion is achieved after only 30 minutes. In fact, there seems to be a slight decrease in yield for the more refluxed samples. This may be misleading, however, because incomplete dissolution of the swipe may have left minute organic particles in the samples. These particulates may have interfered with the vaporization process, which affected the reading. This also would help explain the relatively high yields found in the one-minute digestion. Results from the peroxide tests seem to fall along these same lines. Because the peroxide more fully dissolves the organic material, a lesser but perhaps more true value is given. Additional optimization based on these results may include an incubation period of at least 12 hours to enhance yields, a 60-minute refluxing period, and the addition of peroxide.

Limitations

It is important to point out several limitations to this preliminary study. The first was that the working amounts of BeO were far in excess of what would be found in the vast majority of working environments. Amounts ranging from 12–366 mg were tested, with most samples falling in the range of 20–40 mg. All samples had to be diluted to, at least, 1:5000 so they would fall under the maximum detection limit of the ICP. Although results are not shown here, cursory experiments were conducted to test effectiveness at a dilution of 1:100 (standard working dilution). The recovery from swipes spiked with liquid Be was high (>95%), but beryllium oxide could not be tested (in very small quan because necessary amounts would have fallen below the 10 mg minimum-weight accuracy of the scale. It is quite possible that any of the given methods would be able to
handle the minute amounts usually associated with industrial hygiene sampling. However, without the proper materials to measure out the <7 mg necessary for a 1:100 dilution, it is difficult to make any concrete conclusions.

It also should be noted that the ORNL digestion method was developed specifically for use at Oak Ridge National Laboratories. Modifications were necessary to adapt this method to the SRS testing lab. For example, Oak Ridge uses a microwave oven for its digestion procedures, with as little as 1.2 mL of acid solution. This may optimize the procedure and alleviate charring while producing higher recoveries, but it would be difficult to assess without further testing.

Finally, obvious errors were shown in recoveries of more than 100% of the added BeO (tables 2 and 4). This likely was caused by fluctuations in scale readings during measurement, by low reading of the ICP standard concentrations, or by both. These should not alter the integrity of the results, however. All samples from a given test were weighed and run under identical conditions, and any error would have been seen in all results equally–as evidenced by the >100% recovery of Be standard using the ORNL method.

**Conclusion**

Based on these preliminary results, it would appear that the HF method of digestion is the most appropriate for use at the Savannah River Site. The small working volumes—together with the mild reactions and one-step process—make this a simple and effective technique. A proposed protocol for use with this new method is attached (see attachment 1). Based on results from the additional
optimization experimentation, changes were made to include peroxide and a 60-minute reflux period, as well as a 12 to 48-hour settling time. Preliminary results using this new method have shown an average of 97% recovery in six independent samples (results not shown).

**Implications for Public Health**

It has been well established that the most effective means of curing environmental diseases involves effective prevention. Adding the issue of diagnostic uncertainty, in the case of chronic beryllium disease, only stresses this fact. This project was designed to test current policy surrounding beryllium detection procedures. The results have shown them inadequate for the detection of beryllium oxide in environmental samples. Because the entire Chronic Beryllium Disease Prevention Program hinges on the ability of industrial hygienists to detect total beryllium in the working environment, a shift in policy would seem to be the most likely course of action. Although the findings of this study are not sufficient for the basis of such a shift, it is hoped that it will provide the impetus for further study into this issue. Any future policy should be based on strong scientific evidence that is in step with the latest technologies.
Reference:


Attachment 1:

PROCEDURE FOR THE TOTAL DIGESTION OF BERYLLIUM IN SOIL AND SWIPE SAMPLES TO BE ANALYZED BY ICP EMISSION SPECTROSCOPY

*Materials:
- 100ml plastic cups with attached lid (Environmental Express)
- 49% concentrated hydrofluoric acid (HF)
- Concentrated hydrochloric acid (HCl)
- Concentrated nitric acid (HNO3)
- Hot block with 95°C heat capability
- 30% Hydrogen peroxide (H2O2)

Method:

1. **MIX** soil samples thoroughly to obtain homogeneity.
2. **WEIGH** to the nearest 0.01 gram and **TRANSFER** 1 gram of sample to digestion tube.
3. For **SWIPE SAMPLES**, transfer aseptically to a 100 mL plastic cup if not already in one.
4. **ADD** 4 mL of conc. HNO3 and 1 mL of conc. HCl.
5. Allow **brown fumes** to dissipate before adding 1 mL of 49% HF.
   *CAUTION: Swipe samples may strongly effervesce during desolvation. Wait for completion before adding HF.
6. **HEAT/REFLUX** samples for 20 minutes at 95°C using a watch glass or similar device to prevent fast evaporation.
7. **COOL** and **ADD** 2 ml of 30% H2O2.
8. Continue heating (using watch glass) at 95°C until samples are nearly dry (1-2 mL). Using similar equipment, this should take approximately 50-60 minutes.
*NOTE: Avoid complete drying as this may dramatically affect readings.

9. **COOL** samples and bring volumes up to 2 mL with ddH2O if not already so.

10. **ADD** 2mL conc. HCl.

11. **HEAT/REFLUX** samples at 95°C for an additional 10-15 minutes using watch glass. **COOL** samples.

12. **WASH** the watch glass into the digestion tube and bring volume up to 50 mL with ddH2O.

13. **MIX** and **SETTLE** samples for a period of 12-48 hrs.

   NOTE: Longer incubation periods (up to 48 hrs.) may enhance analyte recovery.

14. Alternatively, if particulates are still present **TRANSFER** samples to centrifuge tubes.

15. **WASH** the watch glasses and digestion tubes into the corresponding centrifuge tubes and fill to 50ml with ddH2O.

16. **CAP**, **MIX** and **CENTRIFUGE** samples at 2000-3000 rpm for 10 minutes.

17. **TRANSFER** 15-25 mL of the clear solution into a clean sample tube for analysis.

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Approved by: __________________________

Title: __________________________

Date: __________________________