

A METHOD FOR RADIOACTIVE CONTAINMENT DURING BIOLOGICAL INACTIVATION OF RADIOACTIVE-BIOMEDICAL MIXED WASTE

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ABSTRACT

A waste container that can contain the radioactive component of a radioactive-biomedical mixed waste while the biological component of the waste is inactivated was designed, fabricated, and validated. *Bacillus stearothermophilus* spore indicator strips were used to validate the biological inactivation through autoclaving of a given biomedical waste mixture. Repeated experiments indicated that a ninety minute autoclave sterilization cycle was sufficient to effect biological inactivation of the biomedical waste.

The experiments also validated that any radioactivity placed within the container was contained during the autoclaving process. Any volatilization of radionuclides was contained within the container or adsorbed onto a charcoal filter column attached to the container. Experiments were performed using varying activities of three common radionuclides used in research at the National Cancer Institute-Frederick Cancer Research and Development Center (NCI-FCRDC). The majority of this research was performed using ^{125}I ; subsequent validation studies were also conducted using ^3H and ^{35}S .

INTRODUCTION

Traditionally, steam has been used to inactivate infectious or potentially infectious biomedical waste. Steam inactivation of radiolabeled biomedical waste can be problematic. The release of volatile radioactive substances can lead to radioactive contamination of the sterilizer and the environment when the steam is exhausted. When the sterilizer exhaust is released to the environment, there is potential for radioactive air emissions. Furthermore, the Nuclear Regulatory Commission (NRC) regulates radioactive air emissions, through a facility-specific licensing procedure. Also, additional risk is placed on personnel handling the mixed radioactive-biomedical waste. These personnel may lack the

training or the equipment to safely handle infectious or potentially infectious biomedical waste. To reduce these occupational risks, many institutions decontaminate the biological component of the waste within the laboratory before it is released for collection and disposal.

The goal of this project was threefold: (1) design and fabricate an enclosure in which mixed radioactive-biomedical waste can be steam sterilized; (2) validate that biomedical waste placed in this container can be completely biologically inactivated using steam sterilization; and (3) demonstrate that radioactive compounds that potentially could be volatilized during routine autoclave procedures, can be completely contained within this secondary containment enclosure.

The Volatility of Isotopes Used in This Project

The majority of the radiological work for this project was done with proteins labeled with ^{125}I ; however, validation studies were also conducted using the radionuclides ^3H and ^{35}S . The volatility of compounds labeled with these radionuclides is widely known (Bolton, 1980), (Hamilton and Button, 1980), (Meisenhelder and Hunter, 1988), (Klein, et al., 1990), and (Clinton and Scougall, 1995).

Volatile substances labeled with ^{35}S or ^3H will adsorb reasonably well to activated carbon and will remain bound even at high temperature and humidity. In contrast, bound iodine does not adsorb well to pure activated carbon at high temperature and high humidity, such as is found in the autoclave. Therefore, carbon must be impregnated with an adjuvant with which the iodine will combine even in the presence of water. One such compound is triethylenediamine (TEDA). TEDA-impregnated carbon works by converting the radioactive gas into an ionic form that is readily adsorbed onto the carbon:



Sorption of iodine on TEDA-impregnated carbon is effective with airstreams of high moisture content as well as high temperature, such as those found in the autoclaving process (Shapiro, 1981).

The State of the Art of Radioactive-Biomedical Mixed Waste Treatment

Stinson, et al., (1990) examined the problem of autoclaving biomedical waste that contained radionuclide contamination. In the initial phase of this work, non-infectious biomedical waste material consisting of bench paper, gloves, pipette tips, and Eppendorf™ microfuge tubes were inoculated with various activities of several radionuclides, and autoclaved for 30 minutes at 121°C. As expected, autoclave contamination occurred to varying degrees from the more volatile radionuclides (Stinson, et al., 1990).

Next, isotopes of known activity were placed in a double bag system. One polypropylene autoclave bag was placed inside a second. Each bag was filled with biomedical waste. An activated carbon filter, to capture volatile radioactive vapors released as pressure in the system equilibrated to the surrounding environment, was securely tied into the opening of each double bag system. All trials validated that this filtered double bag system was able to contain all of the radioisotopes during the sterilization process. No contamination was found outside the bags nor on the surface of the autoclave interior (Stinson, et al., 1990).

MATERIALS AND METHODS

Container Fabrication

Our goal was to use a modification of the Stinson technology to design and fabricate a durable, reusable container that would contain volatile radionuclides while the waste was biologically inactivated. The radioactive waste container was fabricated out of 16 gauge 304 stainless steel. Figure 1 is a scale drawing of the container. The container bottom is an open top box 18" long x 18" wide x 18" tall. Each of the four sides has a clamping mechanism (Master Carr, Cat. No. 102) that connects with the lid so that the lid is held tightly on the box bottom. The box bottom has a one-quarter inch stainless steel lip around the opening perimeter. The lid has the dimensions: 18" wide x 18" long x 3" tall. The lip of the lid is gasketed with a silicon rubber gasket (Phelps Rubber and Gasket, Cat No. 53300210) with a 70

durometer, which is the same material used to gasket autoclave doors. In the center of the box lid is a 3" x one-quarter inch outer diameter nipple that has a 90° bend at 1.0 inch. This is the attachment point for a carbon filter. This filter and its connecting tube are fabricated out of an autoclavable material. Nalgene 689™ one-quarter inch inner diameter polypropylene tubing (Nalge Corporation) is used as the connecting tube for the carbon filter. Nalgene™ 8" length x 5/8" ID polypropylene drying tubes with a three-eighths inch nipple (Nalge Corporation) are filled with a carbon substrate suitable to collect any volatile radionuclides. Triethylenediamine impregnated carbon (Flanders Filter Corporation, Cat. No. KA3510, Lot 97-003) is employed to capture ¹²⁵I, while non-impregnated activated carbon (Fisher Scientific Corporation, Cat. No. 05-685A, Lot 3444) is used to capture all other radionuclides.

Biological Validation

In all the trials of the waste container, all solid waste was autoclaved at 123°C and 17 psig for at least 60 minutes in a Kuhlman Pharmed (Model PH263660/D) microcomputer controlled steam sterilizer. This is a large autoclave, with a working volume of approximately 100 ft³. Autoclave operating parameters were confirmed during every operation with a Diack™ thermal indicator, and an instrument readout of drain temperature and chamber pressure was given for each minute of the cycle.

The biomedical-radiological mixed solid waste sterilization cycle, which was used in this project, was as follows:

- Vacuum air removal - 3 minutes
- Chamber heating to 123°C and 17 psig - 5 minutes
- Sterilizing time - 60, 75, 90, or 120 minutes as the given experiment dictated
- Slow exhaust - (0.2 psi/min) 85 minutes
- Cool down - 5 minutes

Once the sterilization cycle was complete, the autoclave was exhausted over an 85 minute period to reduce the risk of off-gassing of volatile compounds labeled with radionuclides, and to increase the contact time between the steam exiting the container and the carbon in the filter.

The parameters investigated during the biological validation process were: autoclave run time, mass of water added to the waste, and mass of absorbent needed to absorb the water. These

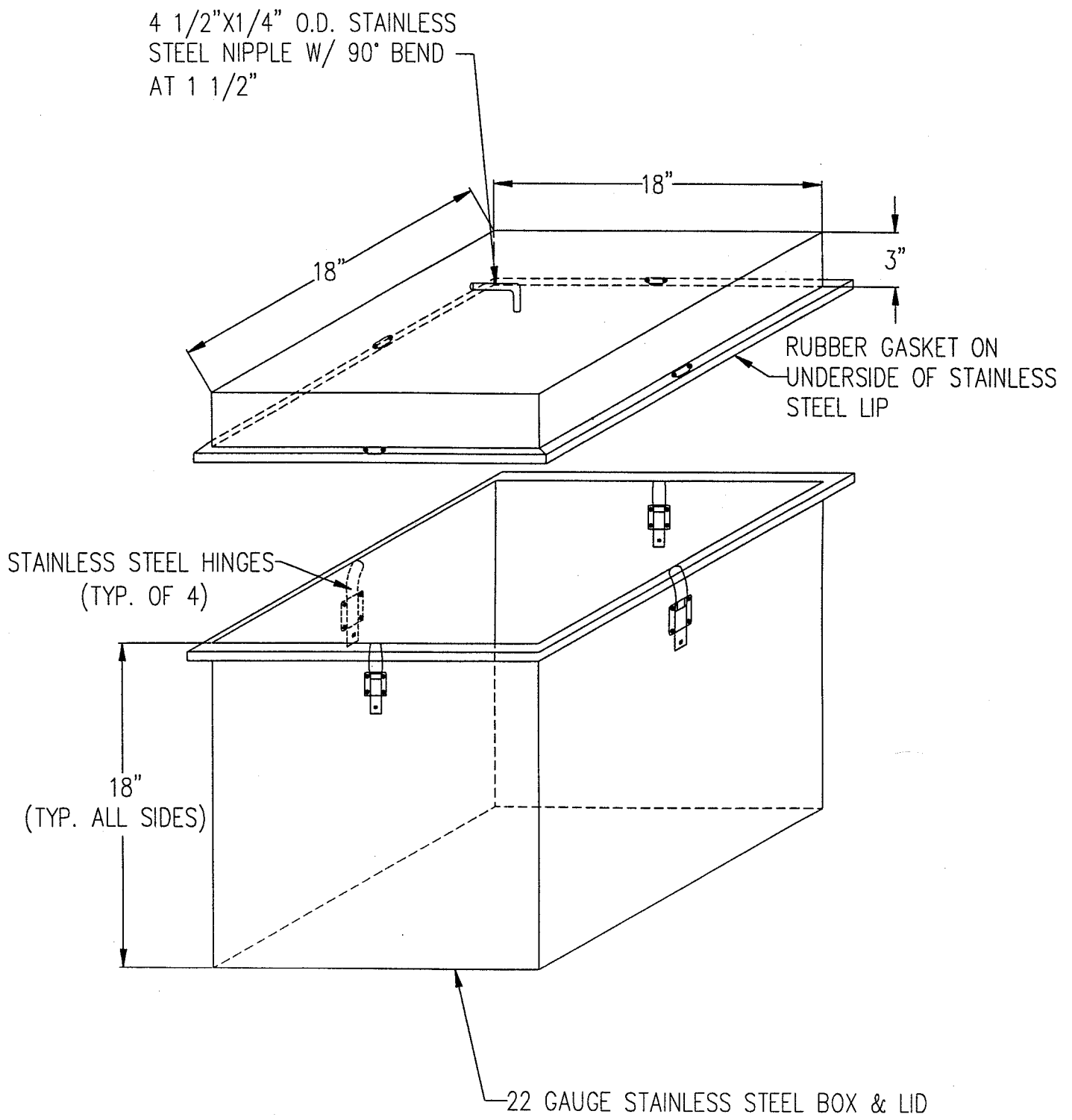


FIGURE 1

RADIOACTIVE-BIOMEDICAL
 AUTOCLAVABLE WASTE
 CONTAINER
 SCALE 1/8"=1"

experiments evaluated three criteria: (1) amount of absorbent needed to absorb any water added; (2) amount of water needed, and (3) length of run time needed to insure complete inactivation of the biological indicators that were placed in each load of waste. The biological validation was performed by loading a 2 mil 30" x 36" autoclave bag (Leonard Paper, Balt. MD, Cat. No. 81051031) with a standardized amount of biomedical waste, and autoclaving it within the container. The waste was obtained from a Biosafety Level-1 (BSL-1) laboratory at the NCI-FCRDC. The standardized waste bag contained the following:

- X *Bacillus stearothermophilus* Spordex™ biological indicators (Steris Corporation, Lot No. HP065A)
- Y ml water
- Z g clay absorbent (Floridin Co., Tallahassee, Florida)
- 9 x 1 L media bottles with caps
- 5 x 100 ml bottles or conical tubes with caps
- 5 x pipetting buffer trays
- 20 x gloves
- 2 x quart size plastic bags

This biomedical waste material represents a typical waste stream from the laboratories at the NCI-FCRDC. The autoclave bag was approximately one-quarter full and weighed approximately 1.5 kilograms.

Water and Time Optimization

Varying quantities of water and absorbent were placed in the autoclave bag for various sterilization cycle times as shown in Table 2. *B. stearothermophilus* biological indicator spore strips were introduced into the autoclave bag to determine the efficacy of the sterilization experiment. The indicator strips were taped on various waste items or were placed loose in the autoclave bag. After completion of the experiment, the *B. stearothermophilus* spore indicators were aseptically collected and incubated in 4.5 ml of tryptic soy broth (Remel, Lot No. 5516) for seven days at 56°C to validate the sterilization process. Any growth of the biological indicator in broth was interpreted as inadequate biological inactivation. Trials using different parameters continued until the sterilization process was optimized while maintaining biological inactivation.

Radiological Validation

The procedure for the radiological validation of

the container paralleled the biological procedure. The only additional parameter added to the waste stream was the radiolabeled compound. Three radioisotopes were used during this research: ¹²⁵I, ³⁵S, and ³H. An iodinated HIV-1 P24 protein was introduced into the autoclave bag during the ¹²⁵I validation experiments. An ³⁵S labeled methionine stock vial (New England Nuclear, Dupont Company, Boston, MA, Cat. No. NEG 072, Lot. No.100796EX) that had been discarded as radioactive waste was introduced into the autoclave bag during the ³⁵S validation experiments. A ³H labeled leucine stock vial (New England Nuclear, Dupont Company, Boston, MA, Cat. No. NET 460, Lot No. 3248860) was introduced into the autoclave bag during the ³H validation experiments.

A given activity of each of the radionuclide labeled compounds was diluted in a buffer. Ten microliters of this solution were placed in each uncapped 1 ml polystyrene tube (Skatron Corp), and allowed to dry. The concentration of the activity of each radioactive compound in the buffer and the number of Skatron™ tubes placed in the autoclave bag determined the total radioactivity placed in the bag. This varied from experiment to experiment. This resulted in varying the activities, and activity concentrations within the waste bag throughout the series of these experiments (Table 1).

For each given experiment mentioned in Table 1, the given number of radioactive Skatron™ tubes was placed inside the standardized biomedical waste bag. The bag was then autoclaved using the sterilization cycle used in the biological validation. So as to reduce the investigator risk to radiation exposure while opening the autoclave bag, the biological validations were performed separately from the radiological validations. Therefore, no biological indicators were placed in the waste bags used for the radiological validations.

In the case of iodine use, a Geiger counter (Ludlum model 14 C, Ludlum Instruments Inc., Sweetwater, TX) with a pancake probe survey was performed on the exterior and interior of the container as well as the interior of the autoclave to verify that all radiation levels were at background before the experiment was started. In all radiation validations, swipes using parafilm™ or cotton tip swabs were taken on 100 cm² areas on the exterior and interior of the container as well as the interior of the autoclave to verify that all radiation

TABLE 1
Radioisotopes and Activities Used in This Research

EXPERIMENT	ISOTOPE	TOTAL ACTIVITY (microcuries)	NUMBER OF TUBES PER BAG	ACTIVITY PER TUBE (microcuries)
1	¹²⁵ I	14.4	12	1.2
2	¹²⁵ I	28.8	24	1.2
3	¹²⁵ I	57.6	48	1.2
4	¹²⁵ I	115.2	60	1.92
5	¹²⁵ I	30	96	0.3
6	¹²⁵ I	30	384	0.08
7	¹²⁵ I	19	100	0.19
8	³⁵ S	35	96	0.36
9	³ H	15	96	0.14

levels were initially at the background level.

The beta radiation activity on the swipes was determined by placing each parafilm™ wipe in a 20 ml scintillation vial, adding 12 ml of scintillation fluid (Ecoscint O™, National Diagnostics, Lot No. 02-97-35), and measuring the activity for two minutes with a Beckman LS6000LL (Beckman Corp.) liquid scintillation detector. The gamma radiation activity on the swipes was determined by measuring each parafilm™ wipe in a Searle Model 1185 (Searle Analytical Inc.) gamma counter for 10 minutes.

Carbon in 2 g increments, separated by a swatch of cotton, was placed into each filter. TEDA treated carbon was used for iodine experiments, and activated carbon was used for all other experiments.

Upon completion of the autoclave cycle, the exterior of the container, the sides and ceiling of the autoclave interior were swiped to determine any radioactive contamination present. In the case of iodine use, the autoclave interior was also monitored with a Geiger counter. In the experiments using ¹²⁵I the carbon filter was removed and the activity from each 2 g section of carbon from the filter was determined. The efficiency of the instruments used was calculated using the equation:

$$EFF = \frac{\text{Standard(CPM)} - \text{Background(CPM)}}{(\text{Standard Activity (microcuries)}) \times 2.22 \text{ E6 DPM per microcurie}} \quad (2)$$

The activity on the swipes or carbon was determined using the equation:

$$ACT = \frac{\text{Sample(CPM)} - \text{Background(CPM)}}{(\text{Efficiency}) \times (2.22 \text{ E6 DPM per microcurie})} \quad (3)$$

RESULTS AND DISCUSSION

Time and Water Optimization for Biological Inactivation

Table 2 shows the data obtained from biological inactivation experiments by varying autoclaving time and the amount of water and absorbent introduced into the container. For the biological inactivation of each spore coupon to be considered adequate, no growth after 72 hours can be seen. It was not until a sterilization cycle of ninety minutes was utilized that consistent 100 percent inactivation of the biological indicators was observed (Table 2). It was therefore concluded that ninety minutes was the optimal time needed to sterilize the contents of the container.

Water volumes from zero ml to 300 ml were used in the ninety minute sterilization cycle. Surprisingly, successful sterilizations were afforded

TABLE 2
Experimental Data Evaluating the Relationship of Time and Water to the Sterilization Process

Time (min)	Water (ml)	Absorbent (g)	Bio Indicators	% Inactivation
60	1000	900	6	0
60	100	300	5	40
75	100	300	4	100
75	30	150	4	75
90	300	300	4	100
90	200	300	4	100
90	50	150	4	100
90	50	150	4	100
90	50	150	4	100
90	30	150	4	100
90	30	150	4	100
90	0	0	4	100
90	0	0	4	100
90	0	0	4	100
90	0	0	4	100
90	0	0	4	100
90	0	0	4	100
90	0	0	4	100
120	300	300	4	100
120	300	300	4	100
120	300	300	4	100
120	200	300	4	100
120	135	300	4	100

without water. This raises the question whether it was truly necessary to add water to attain sterilization.

This question can be answered mathematically. The volume of the container is 118 L. The total volume of the bag was about 50% of the total volume of the container. If the packing density of the biomedical waste was approximated at 30% of the total volume occupied inside the bag, the remaining container volume was approximately

100 L. Data from an engineering steam table show that to produce 100 L of saturated steam at 17 psig and 123°C, the volume of water that is needed is roughly 125 ml. It was assumed that since sterilization was afforded in this process, this volume of water entered the container during the autoclaving process. It was hypothesized that the port of entry was the carbon filter, since the gasket seal between the lid and bottom of the container was measured to hold pressure up to 20 psig. At

the conclusion of each experiment 20 - 50 ml of condensate was found inside the container regardless of the initial amount of added water. Using the ideal gas equation, it can be shown that this condensate found in the container at the end of the operation is in the order of magnitude that would be theoretically expected.

Since the biological indicators were inactivated in repeated experiments without water, it can be concluded that no added water is needed to effect the inactivation of the contents in the autoclave bag. This finding may have important cost saving ramifications. It is expensive to compact and store radioactive waste. The more the radioactive waste can be minimized, the more the cost saving to the institution. Since absorbent takes up valuable space, by eliminating the water, and therefore the need for absorbent, the volume of radioactive waste placed into storage is minimized.

The Containment of Volatile Radionuclides Within the Waste Container During the Autoclaving Process

Using a Geiger counter survey for ¹²⁵I, and 100 cm² swipe tests with parafilm wax paper or cotton tipped swabs for all radioisotopes used, no radioactive contamination was found outside the container after any of the 13 experiments, thus effectively demonstrating the ability of the container to contain volatile radionuclides during the autoclaving process.

Table 3 displays the data determining the ¹²⁵I activity captured on the first 2 gram section of the TEDA impregnated carbon filters used during the radiological validation phase of this project. The TEDA impregnated carbon filters performed efficiently; effectively capturing 95 -98 per-cent of the volatilized bound ¹²⁵I moieties in the first 2 gram section of the filter.

TABLE 3

Experimental Data Determining the Activity of ¹²⁵I Captured on the First Two Gram Section of a TEDA Impregnated Carbon Column After Autoclaving an ¹²⁵I Labeled HIV P24 Protein for Ninety Minutes.

Total Activity (microCi)	Number of Tubes	Activity per Tube (microCi)	Captured Activity (microCi)
14.4	12	1.2	1.1 E (-3)
28.8	24	1.2	2.6 E (-3)
57.6	48	1.2	8.3 E (-3)
115.2	60	1.9	1.7 E (-2)
30	96	0.3	7.3 E (-3)
30	384	0.08	5.1 E (-3)
19	96	0.18	6.1 E (-3)

Problems Experienced while Utilizing the Containment Container

Though the overall results of this project were positive: there were two problems experienced during this research. First, it was discovered during the initial testing that four clamps were not sufficient to maintain an effective seal. The container began leaking as soon as any air pressure was introduced. This was relieved by placing C-type clamps at each of the four corners of the container. Once these clamps were in place, the container could take up to 20 psig of air pressure

before the gasket began leaking. An air pressure of this magnitude was much greater than any back pressure that would be exhibited by the carbon filter during the autoclave process. If an investigator is contemplating building his own radiation container, an effective clamping mechanism must be considered so that an effective seal between the lid and the bottom is implemented.

Another related problem was that the container would bow inward with repeated usage due to the pressure changes throughout the

operation. As this bowing proceeded, the container seal would begin to leak. The size of this container was chosen to hold a large waste load. It is hypothesized that the above mentioned clamping problem as well as the sidewall bowing inward problem could both be solved by building a smaller container with less surface area. A second container fabricated at the NCI-FCRDC was designed as a cylinder, 16 inches long and 8 inches in diameter. Three clamps hold the circular top on the container, and none of the above mentioned problems has been experienced.

In our case this bowing was corrected by blowing the bowing outward with air pressure, or pounding it out with a mallet. If a large size container is necessary to autoclave the waste, and this additional step is not acceptable, it would be suggested to fabricate a container constructed of heavier gauge thicker steel than that of the above describe container.

CONCLUSIONS

The use of a durable, reusable container was found to be an effective method of inactivating the biological component of radioactive-biomedical mixed waste. The containment of radionuclides up to the activity of 115 microcuries was achieved. A ninety minute autoclave sterilization cycle was found to be optimal. Some minor problems were experienced, but minor modifications to the container resolved these problems.

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