Applied Biosafety, 7(2) pp. 52-63 © ABSA 2002

Biological Testing of a Laboratory Pathological Waste Incinerator

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Abstract

A pathological waste incinerator, consisting of a primary chamber operating at greater than 570°C and a secondary chamber operating at greater than 870°C with associated stack, was challenged with bacteriophage f2 of Escherichia coli K12 to demonstrate the decontamination efficiency.

A mean challenge of $10^{13.27}$ plaque forming units (pfu) of bacteriophage was sprayed into the primary chamber over a period of 30 seconds. A second, separate mean challenge of $10^{12.53}$ was sprayed into the incinerator stack. Matching challenges were done while the incinerator was at ambient temperature. Copper nitrate was used to compare the sampling efficiency when the incinerator was operating hot and cold.

A reduction of $10^{8.39}$ pfu of bacteriophage was demonstrated through the primary and secondary combustion chambers. A further reduction of $10^{7.65}$ pfu was achieved within the stack. The overall reduction through the chambers and the stack was $10^{16.04}$ pfu.

Introduction

Two identical carcass incinerators have been operating at this laboratory for 13 years. The decontamination efficacy of these carcass incinerators, with respect to decontamination of aerosols, has been assumed but not tested. Consequently, animal carcasses and laboratory waste have been steam-sterilised prior to incineration.

Proving the decontamination efficiency of the combustion process of one of the carcass incinerators may facilitate policy change with respect to direct incineration of infectious animal carcasses.

Material and Methods

Carcass Incinerators

The carcass incinerators were manufactured by Major Furnace Co., Moorabin, Victoria, Australia (Figures 1 and 4). The nominal maximum burning rate was 750 kilograms per hour. The incinerators consisted of a charge chute loaded from the autopsy room. The charge chute delivered waste into the primary chamber, which was cylindrically shaped with convex ends and 4.73 meters in length and approximately 22 cubic meters volume. The primary chamber was connected via a short mixing chamber to a secondary chamber, which was cylindrically shaped with convex ends and 4.65 meters in length and approximately 14.5 cubic meters volume.

Temperature and loading were controlled from start up by the microprocessor. Loading was inhibited, on start up, if the temperature in the primary chamber was below 670°C and on subsequent loads if the temperature was below 570°C. The temperature control point for the primary chamber was 770°C. Loading was also inhibited, from start up, if the secondary chamber temperature was below 840°C and below 780°C on subsequent loads. The control point for the secondary chamber was 900°C. Combustion temperature above 808°C was controlled by reducing the underfire air supply. Overfire air was controlled by an oxygen analyzer set point of 7%, operational at temperatures above 700°C.

During normal operation, a load was subject to drying, ignition and combustion in an air-lean atmosphere within the primary chamber. Partially oxidised gases rose through a mixing chamber, where additional air was introduced, into the secondary chamber. Additional gas burners ignited the partially oxidised gases in the presence of excess air. The combustion gases moved from the secondary chamber into a breeching piece across to the base of the 31.9 meter-high stack with a notional volume of 37 cubic meters.

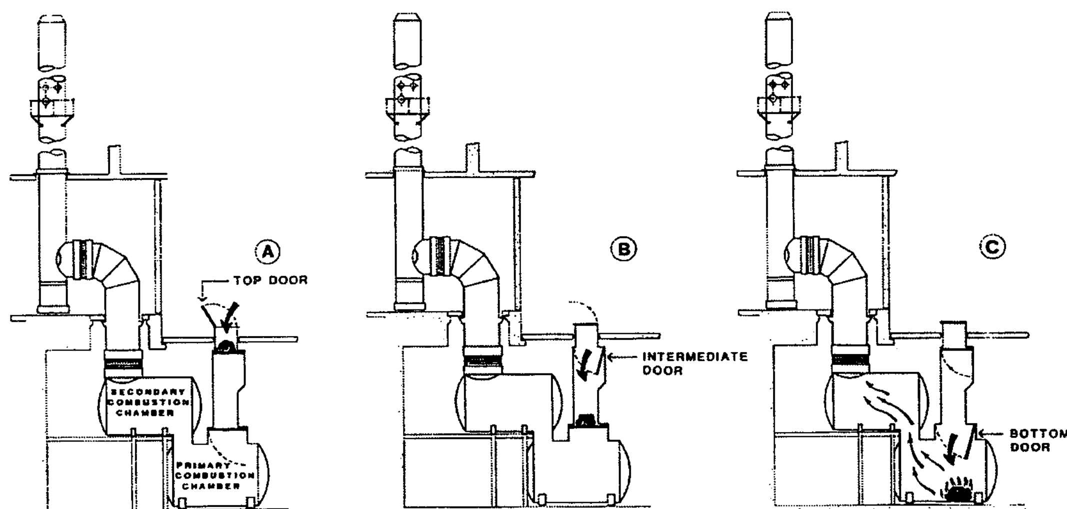
Purpose-designed ports were built into the breeching piece and stack wall to enable sampling devices to be fitted into the breeching piece and into the stack at a point 20.8 meters from the base. The cross-sectional area of the breeching piece was 900,000 mm^2. The cross-sectional area of the stack at its sampling port was 1,700,000 mm^2. During the testing, the primary chamber bed was free from the normally accumulated ash.

Test Equipment

The delivery and sampling methods used here for microorganism challenge were modified from unpublished work on air incineration plant carried out in the 1980s at this laboratory.

A delivery system for the challenge consisted of 10 litre Firestone pressure reservoirs manufactured by Mytton Rodd Limited. The reservoirs contained either the bacteriophage preparation or the copper nitrate solution described in Test and Collection Solutions below. Compressed air, regulated to 380 kPa-410 kPa, was provided from a reticulated system. The outlet from the reservoir was connected by flexible hose, with a valve, to a lance. The lance was supported in a housing that allowed it to be inserted into and retracted from the incinerator. The lance was tipped by a Unijet TX22, hollow-cone spray nozzle that was designed to produce volume mean diameter particles of 220 microns in a 78° angle spray.

Figure 1
Schematic of incinerator operation



Sampling Equipment

The sample-tube probe incorporated five separate tubes of internal diameter 12.7 millimeter and of varying lengths, designed to sample across the diameter of the breeching piece or the stack. The sample tube tip was bent at 90°, with as little radius as possible to face upstream. The resulting elliptical orifices had mean dimensions of 9.7 mm by 13.3 mm. The area of five orifices was approximately 500 square millimetres (Figure 2). Prior to starting the incinerator, the sample probe complete with venturi was placed into the sample port.

A stainless steel reservoir containing 20 litres of 1/10 strength nutrient broth was connected with hoses between the venturi and mechanical-gear pump in a returning loop. The venturi was actuated by liquid delivered at 500-600 kPa from an electrically driven, mechanical-gear pump. An industrial, HEPA-filtered vacuum cleaner was attached to an orifice on the reservoir to increase the sampling rate (Figure 3). The venturi, alone, produced a negative pressure of 1.8 kPa and with the vacuum cleaner operated at a negative pressure of 8.3 kPa.

Figure 2

Sample collection probe and position in the stack

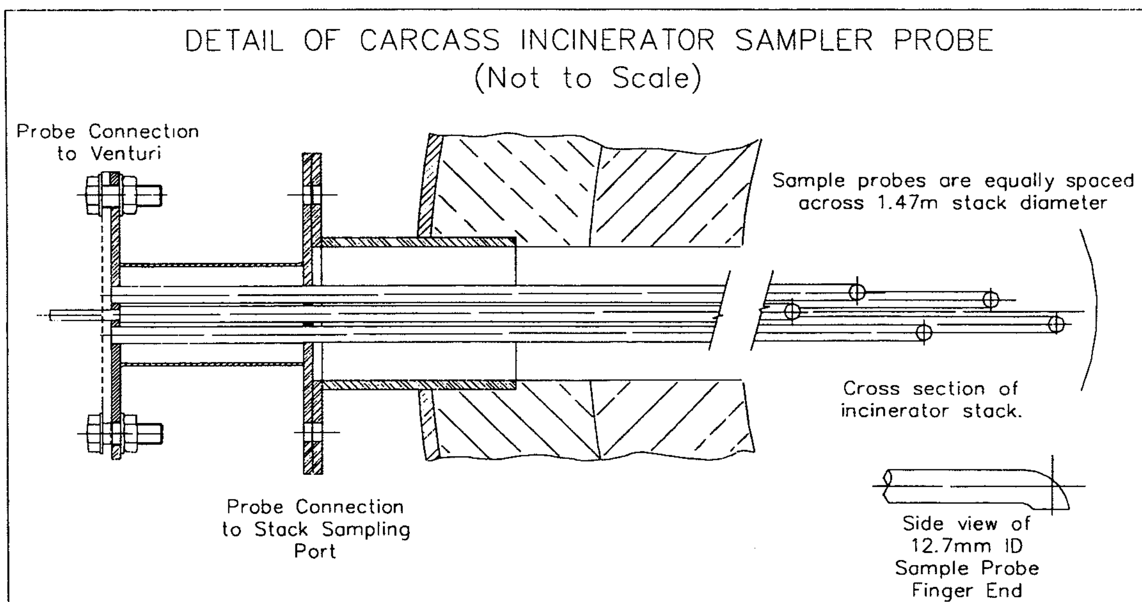
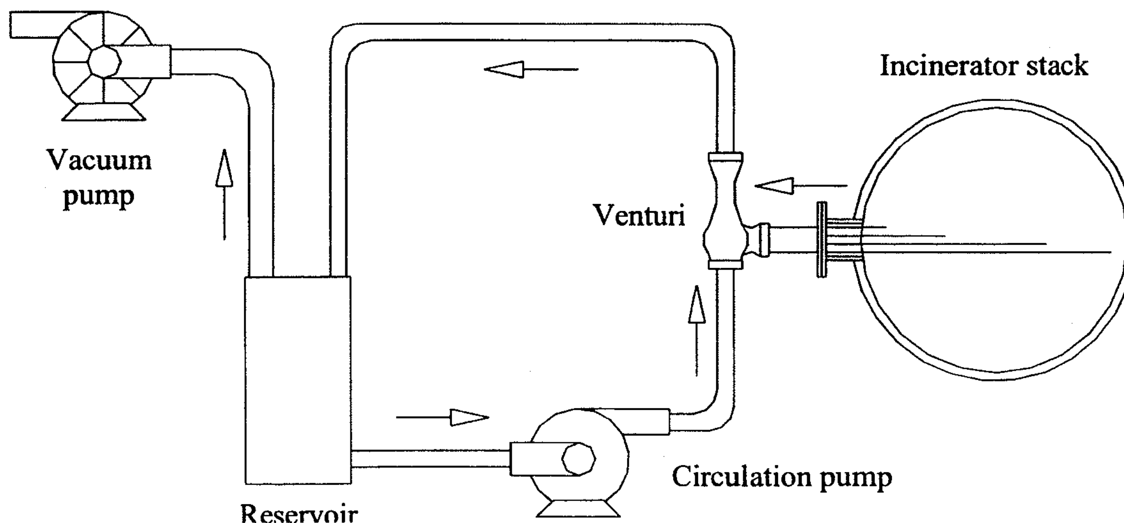


Figure 3

Schematic of sample collection apparatus



Production of Bacteriophage Challenge

The bacteriophage f2 (Loeb, 1960) with a host, wild type *Escherichia coli* strain p801 were obtained in 1982 from the Department of Microbiology and Immunology, University of Adelaide. The bacteriophage f2 had been selected for microbiological assessment of containment equipment in the Australian Animal Health Laboratory (AAHL) (Ludford & Denison, 1985). Criteria included ability to easily produce high titre in large quantities. Higher heat resistance than most viruses and, in particular, foot and mouth disease virus (FMDV). Heat resistance was examined at room temperature—65°C, 70°C, 75°C, and 80°C—which were temperatures used with FMDV by workers at the Animal Virus Research Institute, Pirbright, United Kingdom and the normal operating temperatures for the AAHL continuous flow sewage treatment temperature 97°C.

The bacteriophage f2 was generated from the 4th passage of the original material received from the University of Adelaide. The 4th passage stock was produced in February 1987 and refrigerated in vapour phase above liquid nitrogen until removed for this work. The *Escherichia coli* host bacterium was also the 4th passage from the original material received from the University of Adelaide and refrigerated in vapour phase above liquid nitrogen.

An inoculum of 10^9 pfu of bacteriophage f2 of *Escherichia coli* (*E. coli*) K12 and $10^{8.7}$ colony-forming units (cfu) of *E. coli* K12 bacteria (rough phase) were mixed into one litre of nutrient broth (Oxoid CM67). The culture was incubated at 37°C for 16 hours. Residual bacteria and debris were removed from the culture by centrifugation at 5,020 g for 20 minutes, in a JS 4.2 rotor within a Beckman J6 centrifuge. The supernatant was removed and stored at 4°C as the bacteriophage challenge preparation.

Titration of Bacteriophage Challenge

The bacteriophage culture was well mixed and titrated as 10-fold dilutions in 1/10 strength nutrient broth including negative and positive controls. *E. coli* K12, picked from a rough phase colony, was grown to exponential growth phase (4 hours) in nutrient broth. Two 0.1 ml volumes were mixed with two 1 ml replicates of each bacteriophage dilution and allowed to absorb at room temperature for 10 minutes. The mixture was transferred to 3 ml of soft (0.4%) Agar (Oxoid L13) at 52°C, mixed by swirling, and overlaid evenly on nutrient agar plates. Once solidified the plates were incubated at 37°C overnight. The plaques were counted and the mean titre calculated.

Test and Collection Solutions

A copper nitrate solution was made up as 68 g/l-74 g/l in reverse-osmosis permeate water (US Pharmacopoeia 23 standard). Sample collection fluid was 1/10 strength nutrient broth (Oxoid CM67).

Copper Assay

Copper assays were carried out using flame atomic absorption spectrophotometry by Faculty of Science and Technology, Deakin University, Geelong.

Test Method for Primary and Secondary Chambers

The delivery system for the challenge spray was assembled in the carcass-incinerator plant-room on building level 1. The lance with spray head was attached to the body of the incinerator in the dedicated flanged housing prior to starting the incinerator. The flange replaced a viewing port, that was located centrally on the convex end of the primary chamber, overlooking the area where the waste load was deposited (Figure 4).

The lance was extended into the incinerator immediately before the challenge liquid was discharged through the spray head. Discharge of bacteriophage f2 or copper nitrate solutions was initiated by opening a valve in the hose between the pressure vessel and the lance. An 850 ml volume of the test pool of bacteriophage f2 with a titre of $10^{13.33}$ pfu was sprayed into the combustion chamber of the carcass incinerator in 30 seconds.

Once delivery was complete, the spray head and lance were withdrawn from the incinerator into the flanged housing. As soon as practicable (7.5 minutes) after the bacteriophage challenge was completed, the second pressure reservoir of copper nitrate solution was attached to the spray head. The process was repeated in identical manner to deliver an 850 ml volume of copper nitrate solution.

Sample collection equipment was prepared for use in the after burner plant room on building level 3 (Figure 4).

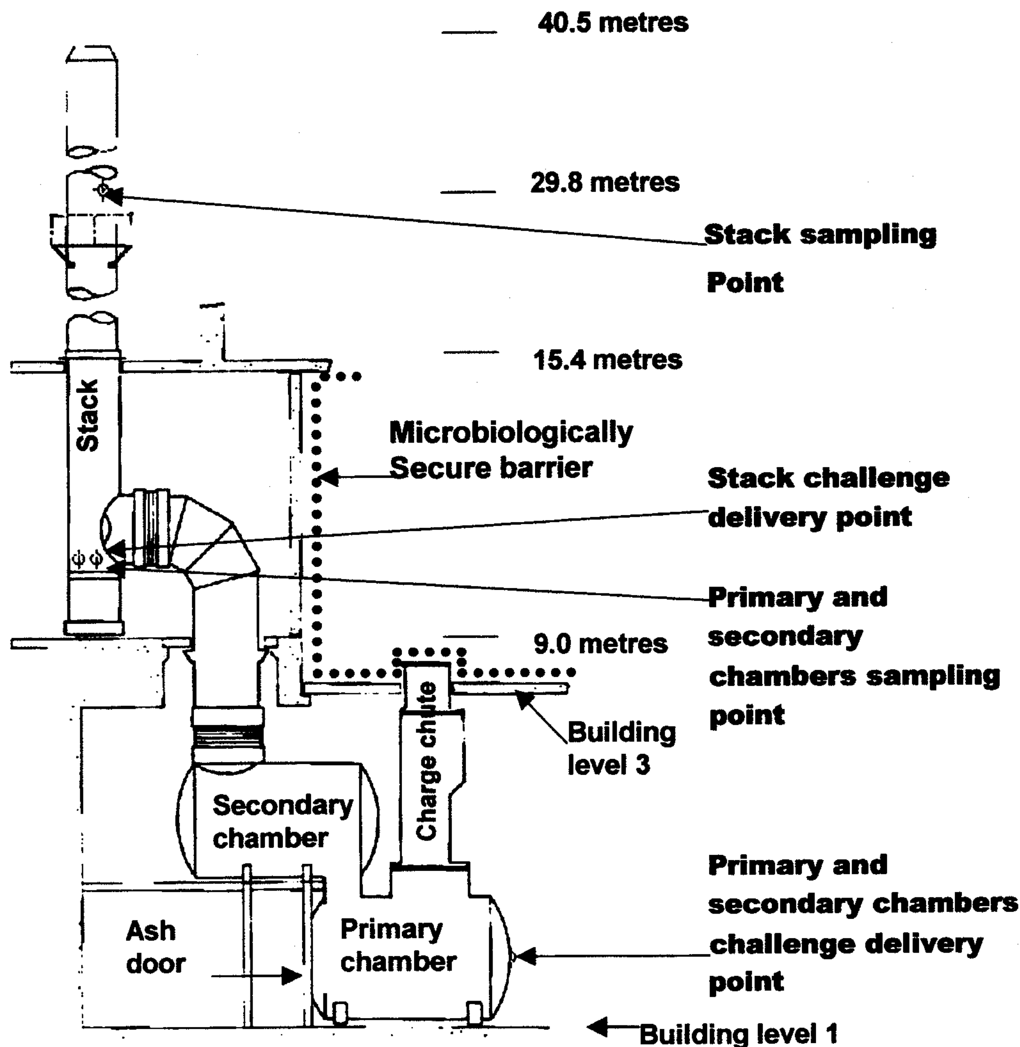
Operators in both plant rooms worked to a synchronised time schedule, referring to digital watches as the noisy environment precluded verbal communication by radio or telephone. The sampling duration was for the period from 1 minute before the challenge material was released until 2 minutes after the challenge had been delivered, a total of 3.5 minutes.

Samples of liquid were obtained by opening a valve positioned immediately after the mechanical-gear pump.

The incinerators operated "hot," that is, at the specified combustion temperatures under the normal operational controls. When the incinerators were challenged cold, the combustion-air fans were operated on manual override. The fans provided large airflows into ports on the primary incinerator chamber (underfire air) and into the duct between the primary and secondary chambers and the burner air ports (overfire air). The three fans had air delivery ratings of: primary burners 360 litre per second; secondary burners 360 litre per second; and underfire/overfire air 2,400 litre per second providing a theoretical 3.12 cubic meters per second through the incinerator.

Figure 4

Challenge delivery and collection points



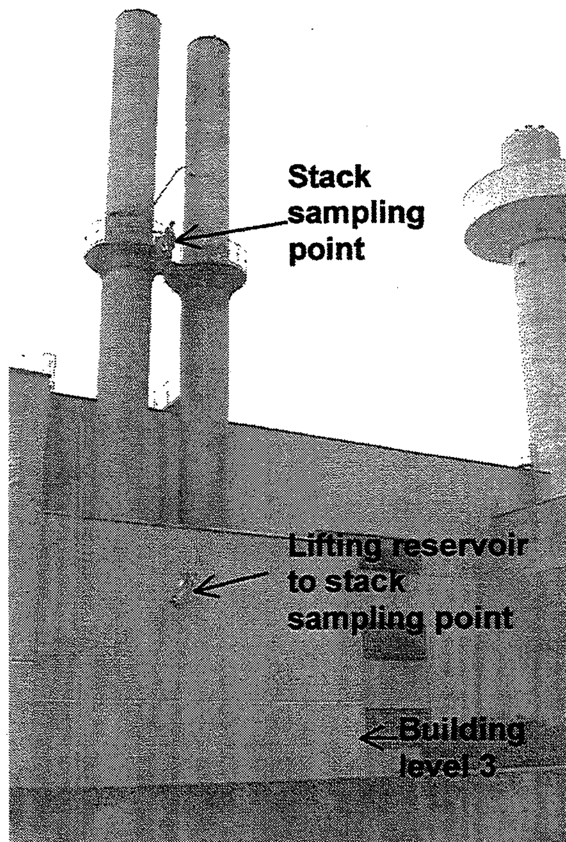
The tests were carried out with a hot test run first, followed by a cold test 4 days later when the incinerator was cold. The sampling equipment was decontaminated by running the collection system with 2% glutaraldehyde in the recirculating collection system. The equipment and sample probe were then removed, washed, and steam sterilized before the second hot test followed by a second cold test. The decontamination wash and sterilization were completed again before the third hot test and followed by a third cold test. The equipment was decontaminated, washed, and sterilized upon final removal.

Test Method on the Stack

Challenge delivery and sample retrieval equipment was similar to that described for the primary and secondary chambers. The spray head was inserted so that the tip extended 43 mm out from the refractory brick lining in the breeching piece.

Figure 5

Sample Collection from the Stack



The sample retrieval equipment was arranged on the service platform 25 meters up the stack. The sampling duration was for a period of 1 minute before the challenge material was released until 3.5 minutes after the challenge had been delivered, a total of 5 minutes.

Results

A pilot study showed that measurable quantities of copper ions were retrieved using the delivery and sampling apparatus. The mean of three samples taken from the 20-litre reservoir showed a copper concentration of 0.5 ppm. The collection efficiency was 1 in 2,540 or $10^{3.4}$.

Table 1 shows the results of samples of the collection liquid from the primary and secondary chamber tests assayed for copper ions. Randomization testing showed that the copper concentrations sampled from operations cold and hot did not differ significantly.

The results of the bacteriophage sampling are shown in Table 2. Samples were taken on three occasions when the incinerators were operated cold. On all three occasions, bacteriophage f2 was recovered. The mean titre was $10^{8.39}$ pfu. No bacteriophage was recovered, on three occasions, from samples of combustion gases from the incinerator at normal operating temperature. Temperature in the primary chamber was typically 633°C to 658°C and in the secondary chamber 871°C to 928°C. The temperatures in the stack were greater than 400°C. These temperatures were recorded at the laboratory's Central Monitoring Station.

Table 3 shows the copper concentration in samples taken during tests on the stack. Again, the concentration between the hot and cold tests did not differ significantly.

The results of bacteriophage tests on the stack are shown in Table 4. On three occasions when the incinerators operated cold, bacteriophages were recovered. The mean titre was $10^{8.33}$. No bacteriophages were recovered from the stack during sampling on three occasions when the incinerator was operating hot.

Discussion

Changes had been made to the microbiological barrier to delineate the carcass-incinerator plant-rooms

Table 1

Copper nitrate concentrations of test challenge and retrieved samples during tests of primary and secondary chambers.

Test conditions	Copper (Cu^{++}) liberated (g)	Copper concentration in sample retrieved (ppm)	Reduction
Trial cold run	57.80	4.73	$10^{-4.09}$
Cold run # 1	57.80	0.76	$10^{-4.88}$
Cold run # 2	57.80	1.43	$10^{-4.61}$
Cold run # 3	ND	ND	
Hot run # 1	57.80	1.70	$10^{-4.53}$
Hot run # 2	57.80	1.88	$10^{-4.49}$
Hot run # 3	57.80	1.82	$10^{-4.50}$

ND - Not done

Table 2

The titre of test challenge material and samples retrieved during tests of primary and secondary chambers.

Test conditions	Secondary chamber temperature °C	Bacteriophage concentration (pfu/ml)	Bacteriophage liberated in 850 ml	Bacteriophage retrieved	Temperature of collection reservoir
Trial cold run	RT	$10^{11.39}$	$10^{13.33}$	$10^{6.75}$	RT
Cold run # 1	RT	$10^{11.39}$	$10^{13.33}$	$10^{8.67}$	RT
Cold run # 2	RT	$10^{11.39}$	$10^{13.33}$	$10^{8.37}$	RT
Cold run # 3	RT	$10^{10.88}$	$10^{12.81}$	$10^{7.62}$	RT
Hot run # 1	928	$10^{11.39}$	$10^{13.33}$	No phage detected	NR
Hot run # 2	881 to 913	$10^{11.39}$	$10^{13.33}$	No phage detected	24.3°C
Hot run # 3	871 to 873	$10^{11.39}$	$10^{13.33}$	No phage detected	23.7°C

- NR-Not recorded.
- RT- Ambient air temperature

Table 3

Copper nitrate concentrations of test challenge and retrieved samples during tests of the stack.

Test conditions	Copper (Cu^{++}) liberated (g)	Copper concentration in sample retrieved (ppm)	Reduction
Cold run # 1	60.21	0.93	$10^{-4.81}$
Cold run # 2	60.21	2.06	$10^{-4.47}$
Cold run # 3	60.21	1.20	$10^{-4.70}$
Hot run # 1	60.21	1.20	$10^{-4.70}$
Hot run # 2	55.20	0.72	$10^{-4.88}$
Hot run # 3	55.20	0.58	$10^{-4.98}$

Table 4

The titre of test challenge material and samples retrieved during tests of stack.

Test conditions	Secondary chamber temperature °C	Bacteriophage concentration (pfu/ml)	Bacteriophage liberated in 850 ml	Bacteriophage retrieved	Temperature of collection reservoir
Cold run # 1	RT	$10^{9.64}$	$10^{12.57}$	$10^{8.44}$	RT
Cold run # 2	RT	$10^{9.65}$	$10^{12.58}$	$10^{8.35}$	RT
Cold run # 3	RT	$10^{9.62}$	$10^{12.55}$	$10^{8.13}$	RT
Hot run # 1	NR	$10^{9.62}$	$10^{12.55}$	No phage detected	26.1°C
Hot run # 2	908	$10^{9.62}$	$10^{12.55}$	No phage detected	24.8°C
Hot run # 3	923	$10^{9.44}$	$10^{12.37}$	No phage detected	NR

- NR-Not recorded.
- RT- Ambient air temperature

outside the high-containment area and to create a new airlock. The major benefit was to allow ash from the incinerators to be removed directly from the carcass-incinerator plant-rooms as safely treated waste. Previously, the ash had been subject to a further steam sterilization process across the microbiological barrier. Prior to the change, it was prohibited to release material from the high-containment area without treatment. Therefore, the changes made it permissible, from a biocontainment point of view, to generate a microbiological challenge from these plant rooms, now outside the high-containment area, and capture that untreated challenge further along the exhaust gas pathway. This provided a positive control for the decontamination by the combustion process.

Previous papers on incinerator testing (Barbeito & Gremillion, 1968; Barbeito & Shapiro, 1977) reported initial challenge and captured doses at various tempera-

tures in known portions of the airflow. The sampling efficiency of the process was not reported but relied upon bacterial spores released at lower operating temperature and perhaps flow rates.

The methodology used for the work reported here avoided the need for calculation of the initial dose, incinerator airflow rate, concentration of challenge microorganisms per volume of air passing through the incinerator, isokinetic sampling, and sampling rates. Simply, the sampling rates from incinerators operating cold and hot were equated by using copper ions as an indicator.

The error in synchronizing operations using digital watches may have been up to 5 seconds. This error was compensated by starting sampling 1 minute before the challenge was released. Continuing sampling for an extended period after the challenge was released reduced the chance of missing any challenge material as it moved through the incinerator.

The decontamination efficiency of the incinerators may be higher than the demonstrated reduction in infectious challenge. If a higher titre challenge in a short period could have been arranged or the sampling efficiency improved, then higher reductions might have been demonstrated.

A pilot study of collection efficiency was done to ensure that the sampling system operated in carcass incinerator number one before the tests proper began. This sighting, collection-efficiency test using copper nitrate showed that a 10^4 reduction occurred in copper concentration. This compares with the possible loss or reduction in copper concentration of $10^{3.25}$ expected from the relative geometry of the sampling apparatus relative to the breeching piece. The cross sectional area of all five sample tubes in the gas stream was 507 mm^2. The cross sectional area of the breeching piece was 900,000 mm^2. Therefore, the cross sectional area sampled was a ratio of 1:1,775 or $10^{3.25}$.

How a carcass burns and what particles are produced is not clear. A wet carcass may disintegrate, splattering boiling tissue fluids and fat. Alternatively, it may dry and ignite. Infectious aerosols, particles, or only combustion products may be generated from a carcass. It was not possible to dissipate an infectious carcass, in the cold test of the incinerators, to provide a genuine control for the aerosol generated as a carcass burns. Therefore, *in lieu* of a carcass, a spray was generated to approximate the splatter and aerosols that a carcass or other wet materials may exhibit when plunged into the hot primary chamber environment. The particle size of 200 microns was likely to be more robust and resistant to drying than a small particle size with a greater surface area to volume ratio. The spray angle was suitable to direct the spray into the air stream rather than against the wall of the stack.

The amount of virus in the spray challenge can be compared to virus concentration that might be present in carcass material. The test challenge spray produced bacteriophages at a rate of $10^{11.62}$ pfu/second. The incinerator was designed to burn 750 kilograms of carcass per hour or 208 grams per second. This is equivalent to a virus concentration of $10^{9.3}$ pfu (or infectious dose) per gram of carcass material that might completely become an aerosol.

The results show that the sampling and assay methods were able to detect $10^{8.3}$ bacteriophages when the incinerators were run without heat. No bacteriophages were detected when the primary and secondary chambers were operating hot. This is a 10^8 reduction. A similar 10^8 reduction was measured in the lower part of the stack. The reduction in bacteriophages in the two tested parts of the incinerator was 10^{16}.

The tests reported here assessed the ability of the incinerators to inactivate a high-titre microbiological droplet and aerosol cloud released in less than 1 minute. The challenge approximated the highest virus titres reported in infectious animal tissues. Virus titre in diseased animal tissue is not often reported. The data are usually qualitative; virus is present or not present in various tissues. Virus in some target tissues is found in quantities readily visualised by electron microscopy. Devine (1994) stated that electron microscopic visualisation of virus particles in clinical specimens requires an initial virus concentration of 10^6 to 10^8 particles per millilitre. Moreover, the concentration of viruses in most clinical specimens is lower than that. Table 5 shows the animal virus titres reported in various tissues and secretions.

The amount of the test bacteriophage released in these tests is considered to be more than the amount of virus that might be released by an incinerator burning a diseased animal carcass.

The stack tests were varied from the chamber tests in two ways. First, the sampling time after the chal-

Table 5

Animal virus titres reported in various tissues and secretions.

Virus	Tissue or secretion	Titre	Reference
Swine vesicular disease virus	skin	10^6 tissue culture infectious doses (TCID)/gram	Dawe 1974
	intercostal muscle	10^4 TCID/gram	
	rib bone and kidney cortex.	10^3 TCID/gram	
Foot and mouth disease virus	milk	$10^{5.9}$ pfu per millilitre	Walker et al. 1984
	blood	$10^{4.4}$ Mouse LD_{50}/ml	Cottral and Bachrach 1968
	skin	$10^{3.6}$ pfu per gram	Gailiunas and Cottral 1966
Newcastle disease virus	respiratory tract	10^4 EID_{50}*	Jungherr 1964
Duck hepatitis B virus	serum	10^{10} 50% Infectious dose (ID_{50})/ml	Jilbert et al.1996
Rabies	salivary glands (fox)	$10^{5.98}$ 50% mouse lethal dose per millilitre (MLD_{50}/ml)	Matouch 1978
	salivary glands (fox) salivary glands (dog)	$10^{6.9}$ MLD_{50}/g $10^{5.6}$ MLD_{50}/g	Wachendorfer and Frost 1978
West Nile virus	lung	$10^{4.60}LD_{50}$/ml	Nir et al. 1965
Rift Valley fever	blood	10^9 (pfu/ml)	Olaleye et al. 1996
Tick-borne encephalitis virus	blood	$10^{2.65}$to $10^{4.85}MLD_{50}$/ml	van Tongeren 1983
Bluetongue virus	blood	10^1 - $10^{2.8}$ ($TCID_{50}$).	Parsonson et al.1987
	blood	$10^{4.0}$ to $10^{7.0}$ ELD/ml	Goldsmit 1975
Border disease	blood mononuclear cells	$10^{3.26}$ $TCID_{50}$ per 10^6 cells	Woldehiwet and Hussin. 1994
Aujeszky's disease	nasal discharge	10^4 to 10^6 $TCID_{50}$/0.1 ml	Mocsári 1987

*Volume not stated.

lenge had been delivered was increased to 3.5 minutes. Similar amounts of copper were collected in these tests indicating that the small amount of copper ions recovered in earlier tests was not due to shorter sampling times. Second, the primary chamber temperature was ignored since the significant temperature is that of the secondary chamber, which exhausts into the breeching piece and stack that were under test.

The bacteriophages were not exposed to high temperatures after collection in the nutrient broth and further inactivation after collection is unlikely. The temperature of the reservoir of nutrient broth rose 4°C-8°C, as measured on the surface of the reservoir, and did not exceed 26.1°C.

Copper nitrate and bacteriophages were sprayed separately, to preclude a deleterious effect of copper

ions on the bacteriophages. The mean copper concentrations obtained after sampling from a cold and a hot stack gas stream are close. Therefore, the sampling efficiency was similar for all tests.

The results show that high titres of viable bacteriophages were collected when the incinerators were operated cold and were absent when the incinerators were at normal operating temperature. Animal carcasses were all steam sterilised at 121°C for 720 minutes and laboratory waste 121°C for 45 minutes prior to incineration. Testing of the incinerators provided information to support proposed policy changes whereby, in the future, infectious animal carcases may be disposed of by incineration alone, saving the additional energy costs and handling of steam-sterilised carcass material. The decontamination efficiency reported here compares with a particle reduction of $10^{7.25}$ for a single HEPA filter (Jamriska et al., 1997) and a 10^5 to 10^6 reduction validated by biological indicators in an autoclave (Joslyn, 1991). The stack retains the exhaust gasses for a further 10.6 meters from the stack sampling point to the top of the stack. It is reasonable to presume that further inactivation of infectious material would occur before gasses are diluted and cooled by mixing with the ambient air.

Conclusions

The carcass incinerators demonstrate a reduction in infectious microorganism titre of 10^{16} for a challenge delivered in 30 seconds. There is also additional retention time for the hot gas effluent in the stack past the stack sampling point in this study.

The reductions in virus titre, shown by the tests reported here, give a high level of confidence that infectious aerosols generated from an infected carcass disintegrating during incineration would be inactivated in the incinerator chambers and stack.

Acknowledgments

Thanks to Albert C. Trajstman and Vivienne Lewis of CSIRO Mathematical and Information Sciences for statistical assistance in preparation of this paper.

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