

A Study of Air-tightness in Australian High-level Bio-containment Facilities

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Abstract

To assess the air-tightness of physical containment level 3 (PC3) bio-containment facilities in Australia, the seal integrity of 18 PC3 facilities was quantified by means of an equilibrium pressure air-tightness test conducted at positive pressure. The results of the test, which measured the leakage of air from the facility, indicate a variation in the air-tightness of the tested facilities which correlated with the facilities' age and method of construction. The results of the test also provided information on the contribution of different types of penetrations of the facility barrier to the overall air-tightness of a PC3 facility. These results demonstrate that while newer facilities constructed using modern technology had the greatest air-tightness, older facilities constructed using cheaper construction materials and methods also achieved a high level of air-tightness. Possible risks to health and human safety from facilities with decreased air-tightness are discussed.

Introduction

Within Australia, high-level bio-containment facilities (laboratories, animal and plant facilities of physical containment (PC) levels 3 and 4) are used to work with organisms and micro-organisms that pose a risk to human health and/or environmental safety. While the maintenance of these facilities is the responsibility of the organization that operates the facility, the use of organisms within the facility is regulated at the federal level by legislation that includes the *Gene Technology Act (2000)* for genetically modified organisms (GMOs) and the *Quarantine Act (1908)* for organisms that are considered a quarantine hazard to Australia.

The level of risk associated with the use of hazardous organisms in a containment facility can largely be mitigated by the physical containment level provided by the facility and the training and procedures adopted by those conducting work within the facility. According to the *Australian and New Zealand Standard for Safety in Laboratories: Microbiological Aspects (AS/NZS 2243.3 2002)*, work with organisms that belong to Risk Group (RG) 2, which are defined as posing a low individual and com-

munity risk, must be conducted in a facility that meets the requirements for a PC2 facility. By contrast, work with organisms that have been assessed on the basis of factors such as host range, pathogenicity, and transmissibility as posing a high individual but low community risk, and which therefore belong to RG3, must be conducted in PC3 facilities.

The requirements for the construction of bio-containment facilities specified in *AS/NZS 2243.3 (2002)* and by containment facility guidelines published by the Office of the Gene Technology Regulator (OGTR) are very similar to those specified by the United States, Canada, and the World Health Organization, though the Australian requirements utilize the nomenclature of PC instead of BSL (thus, the designation PC3 instead of BSL-3) (CDC-NIH, 1999; Health Canada, 2004; WHO, 2004).

In Australia, more than 40 PC3 facilities are certified by OGTR. The process of certification establishes a facility's compliance with guidelines issued by the Gene Technology Regulator (OGTR, 2006). As in Canadian and U.S. bio-containment guidelines, the major structural difference between PC2 and PC3 facilities is that PC3 facilities are constructed so that potentially contaminated air is actively removed from the facility by extraction through a high efficiency particulate air (HEPA) filter before being discharged into the atmosphere. To achieve this, facilities are maintained under negative pressure whereby the pressure within the work area of the facility, in which work with the organisms is being conducted, is at least 50 Pascals (Pa) below that of adjacent areas outside the facility, such as access corridors which are not subject to any negative pressure requirements.

One requirement stipulated both by *AS/NZS 2243.3 (2002)* and by OGTR is that PC3 facilities must be able to be decontaminated by gaseous fumigation. A variety of gaseous decontaminants are available. These include formaldehyde, chlorine dioxide and more recently, vapourised hydrogen peroxide (VHP). In Australia, formaldehyde remains the gaseous decontaminant of choice for microbiological containment facilities. This is due primarily to its efficacy against the majority of pathogenic micro-organisms and the low cost associated with its use, especially compared to other decontaminating agents such as VHP or chlorine dioxide.

Gaseous decontamination agents are efficacious against a wide variety of micro-organisms, and have been used as space decontaminants for almost 40 years (Hoffman & Spiner, 1971). However, there are hazards associated with the use of these chemicals. In 2005 the International Agency for Research on Cancer (IARC) declared formaldehyde to be carcinogenic to humans on the basis of induction of nasopharyngeal cancer (IARC, 2006). Both chlorine dioxide and VHP have also been shown to be hazardous to health. Chlorine dioxide has been shown to be fatal at a concentration of 19 ppm (approximately $60\mu\text{g}/\text{m}^3$) and dangerous to life or health at a concentration of 10 ppm (Elkins, 1959; United States Environmental Protection Agency, 2000). At lower concentrations, it is irritating to the respiratory system (Henneberger et al., 2005). While VHP is also toxic and an irritant to mucous membranes, skin, and the respiratory system, it is less hazardous due to its spontaneous breakdown into H_2O and O_2 .

Theoretical studies of the gaseous diffusion of formaldehyde across barriers concluded that gaseous decontamination of facilities which comprised a single barrier (i.e., that were not contained within another laboratory) could result in the diffusion of hazardous levels of formaldehyde into adjacent areas, even in facilities with a leakage rate coefficient of $10^{-5} \text{ m}^3/\text{Pa}\cdot\text{s}$ (Pickering, 1982; see also *Veterinary Containment Facilities: Design and Construction Handbook*, 2006). It should be noted, however, that these results were calculated under a scenario of positive pressure, whereas high-level containment facilities are maintained at a negative pressure of -50Pa relative to adjacent areas. Generally, high-level containment facilities would be unlikely to experience conditions such as that under which the results proposed by Pickering were calculated.

Therefore, the use of gaseous decontaminants such as formaldehyde poses a hazard to personnel who may be exposed during the decontamination process. This may be either to those personnel who are conducting the decontamination or to personnel who are inadvertently exposed to the gaseous decontaminants and who do not wear any personal protective equipment. The risk of the exposure occurring is largely dependent on the ability of the fumigant to leak across the barrier of the facility being decontaminated and could occur either because of a failure to seal the facility during decontamination or from defects or holes in the containment barrier that have arisen over time.

AS/NZS 2243.3 (2002) recommends a maximum air-leakage rate of $10^{-5} \text{ m}^3/\text{Pa}\cdot\text{s}$ for PC3 and PC4 laboratories. International guidelines also provide an indication of the acceptable leakage rates for PC3- and PC4-equivalent facilities. Section 7.1 of the Canadian Food Inspection Agency/Agriculture and Agri-food Canada *Containment Standards for Veterinary Facilities* (1996) quotes a leakage rate of $12.5\text{Pa}/\text{min}$. at a pressure of 500Pa for PC4 laboratories and PC3 and PC4 animal containment

facilities. This is equivalent to approximately $10^{-6} \text{ m}^3/\text{Pa}\cdot\text{s}$. No recommended leakage rate is provided for PC3 laboratories or animal facilities in which animals are housed in containment cages.

The air leakage from a facility can be used as a measure of the facility's air-tightness and structural integrity. This may vary over time and could represent a measure of the effects that maintaining the facility under a constant negative pressure have on the facility.

Very little information is available on the changes in air-tightness of facilities over time. This presents a unique problem for regulatory agencies such as OGTR when developing facility containment guidelines. This is because while approvals to use GMOs in research are based on an assessment of the hazards that the GMOs pose to the safety of human health and/or the environment, the facility's ability to contain the GMOs must also be taken into account. If a facility does not meet accepted or proposed air-tightness criteria, this may have implications for the ability of the facility to contain micro-organisms or GMOs in the event of an accidental spill outside primary containment. In addition, if containment guidelines mandate the use of toxic fumigants, then a facility's decreased air-tightness may pose additional risks to personnel during gaseous decontamination of that facility.

To address the information gap regarding the air-tightness of PC3 containment facilities and to assess other factors that may affect structural integrity, the level of air-tightness of 20 PC3 laboratory and small-animal containment facilities of differing ages and construction was assessed. The results of the testing indicated that, in general, air-tightness decreased with the increasing age of the facility, but also that the construction method used appeared to be a major contributing factor to the air-tightness of the facility. The results of the test also provided an indication of the number of facilities that may pose a health risk to workers in adjacent areas during gaseous decontamination.

Materials and Methods

Facility Pressurization and Measurements

Air leakage from facilities can be measured by pressurization of a facility under negative or positive pressure. For this test, facilities were positively pressurized to 200Pa ($\pm 5\text{Pa}$) by means of a single centrifugal fan pressurization system attached to a door template (either plywood or metal). The template was fitted into the internal jamb of each door. This door template was fixed in place by fitting cross braces (either wood or metal) to the external door jamb and by tightening these with bolts fitted to the door templates. A strip of foam rubber was fitted to all areas of the door template that made contact with the external door jamb. The door template was taped in place with PVC duct tape (48mm) and adhesive aluminium-backed tape (80mm). The differential pres-

sure across an orifice plate (30mm-70mm diameter) and within the facility was measured using a TSI micro-manometer at 60-second intervals. Isolation dampers on supply and exhaust ducts were closed when possible, and laboratory duct outlets were sealed. Leaks in the taping of door templates and supply and exhaust outlets during pressurization were detected by the use of a Dräger Flow Check™ apparatus and sealed. Door templates and supply and exhaust ducts were monitored for leaks throughout the period of the test. The air leakage of the work area (facility without the airlock) and of the whole facility (work area plus airlock) were tested twice for 20 minutes per test at the test pressure of +200Pa, the standard pressure at which the equilibrium pressure test is conducted.

The average of the two equilibrium pressure tests was used to calculate the final air-tightness measurement (L/min.). The leakage rate coefficient (β) for flow through a leak ($\text{m}^3/\text{Pa}\cdot\text{s}$) for each facility was also calculated using the formula $\beta=q/(\Delta p)$ where q = volume of air lost (m^3/sec) and Δp = pressure difference (Pa).

Results

Air-tightness Testing of Laboratory and Small Animal Containment Facilities

The data gathered from the air-tightness testing of 18 PC3 laboratories and small-animal facilities with an age range of <1 year to 40 years are shown in Table 1 and Figure 1. A comparison of the leakage rate from the facility as a function of age and construction type is shown in Figure 2. The walls and ceilings of the tested facilities were constructed from a range of building materials including a single layer of gyprock (plasterboard), a double overlapping layer of gyprock, brick and plaster, or sandwich panel. Wall surface area (Column C), floor area (Column D), and volume of the area tested (Column E) were calculated for each facility tested. Information on the number of penetrations of the barrier was also recorded (data not shown). Data from an additional two laboratories were not able to be collected because of minor structural failure during pressurization which resulted in the separation of the facility's gyprock ceiling from the walls.

Table 1
Results of air-tightness testing.

A Facility	B β -Value ($\text{m}^3/\text{Pa}\cdot\text{s}$)	C Wall Surface Area (m^2)	D Facility Volume (m^3)	E Average Leakage Rate @ 200Pa (L/s)	F Average Leakage Rate @ 200Pa (L/min)	G Facility Age (yrs)	H Age of Building (yrs)	I Construction Type
1	8.05×10^{-6}	59.29	79.69	1.61	96.6	0.25	0.35	SP
2	8.30×10^{-6}	49.01	42.2	1.66	99.6	5	20	G*
3	1.14×10^{-5}	76.61	48.11	2.27	136.2	5	20	G*
4	1.29×10^{-5}	70.15	57.82	2.65	159	0.16	0.16	SP
5	2.02×10^{-5}	76.61	48.11	4.03	241.8	5	20	G*
6	3.74×10^{-5}	46.46	50.83	7.48	448.8	5	5	G
7	7.21×10^{-5}	62.78	143.07	14.42	865.2	3	3	G*
8	7.37×10^{-5}	68.55	61.97	14.73	883.8	3	3	SP
9	7.92×10^{-5}	60.56	67.35	15.84	950.4	3	3	SP
10	9.47×10^{-5}	52.3	48.91	18.94	1136.4	17	17	G
11	1.18×10^{-4}	59.94	82.8	23.68	1420.8	7	7	G
12	1.24×10^{-4}	97.75	112.83	24.82	1489.2	8	8	G
13	1.26×10^{-4}	48.12	42.04	25.2	1512	7	30	G
14	1.34×10^{-4}	52.76	49.15	26.8	1608	17	17	G
15	1.57×10^{-4}	54.22	66.86	31.33	1879.8	15	15	G
16	2.83×10^{-4}	143.93	156.68	56.6	3396	7	40	G
17 ¹	4.20×10^{-4}	66.47	97.44	NC	NC	40	40	G
18 ²	1.07×10^{-3}	53.43	26.71	NC	NC	7	7	G

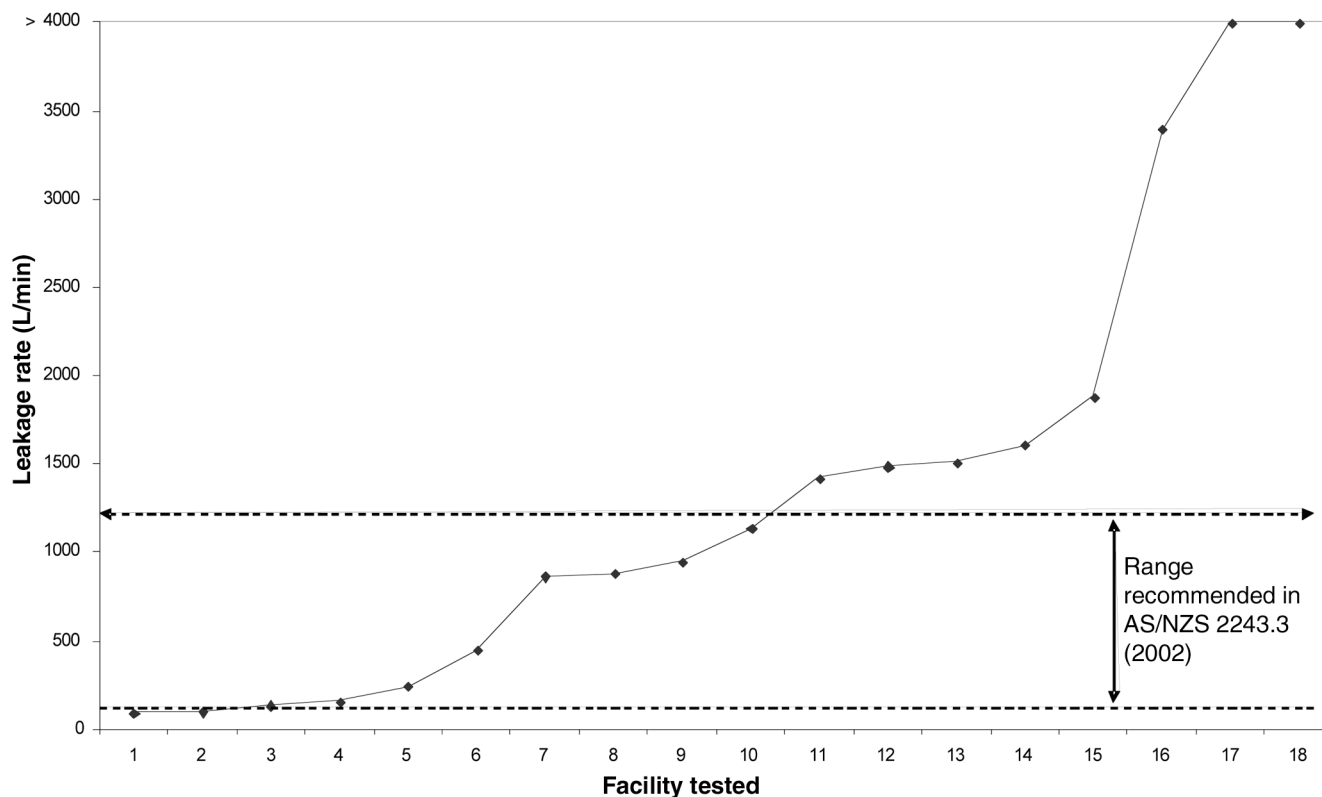
G Gyprock (Plasterboard)
 G* Overlapping double layer gyprock
 L/min Litres of air per minute
 L/s Litres of air per second
 NC Not calculated due to leaks within the facility

SP Metal faced sandwich panel
 yrs Years
¹ Facility only pressurised to 87Pa
² Facility only pressurised to 36.7Pa

Figure 1

Air leakage from PC3 facilities tested. The facilities were sealed with tape and polythene and the pressure increased to 200Pa until stable. The pressure was maintained at 200Pa for a period of 20 minutes and the amount of air used to maintain this pressure was calculated by measuring the differential pressure across an orifice plate (30mm-70mm diameter). Results are shown for 18 facilities.

The remaining facilities were not able to be tested due to structural defects. The recommended air-leakage rates from PC3 and PC4 facilities (120-1200 L/min.) in AS/NZS 2243.3 (2002) is shown.



Contribution of Different Types of Penetration to Air-leakage Rate

During testing, one facility, constructed of single gyprock wallboards between a concrete floor and ceiling, was used to test the contribution of different types and sizes of penetrations of the gyprock barrier to the air-leakage from the facility. The results are shown in Table 2. The difference in leakage rates when the leaks were sealed compared to when unsealed were calculated using data collected over a 5-minute period. With the exception of the autoclave control plate, all of the tested sources of leakage were due to penetrations in the gyprock wall. The contribution of pipe penetrations to overall leakage rate was not measured.

Validation of Testing Methodology

The methodology used during facility testing was validated because the air-leakage from one of the facilities (No. 1) had previously been independently tested prior to commissioning by the same positive pressurization method. The average leakage rate calculated during the previous test was 99 L/min. The results obtained during this test produced an average leakage rate of 96 L/min.

The variation of 3% between independent tests suggested that the results presented here would likely be consistent with those obtained by other testers.

Discussion

The equilibrium pressure test was used in 20 PC3 laboratories or small-animal containment facilities to calculate their air-tightness. Air-tightness was measured by calculating the amount of air leaking from the facility when pressurized to a positive pressure of 200Pa over a 20-minute period. Figure 1 shows that of those facilities tested, 10 (60%) had an air-leakage rate within or better than the range of 120 L/min. -1200 L/min. (10^{-5} - 10^{-4} m³/Pa.s) suggested for PC3-level containment facilities in AS/NZS 2243.3 (2002), and 8 facilities (40%) had an air-leakage rate of greater than 1400 L/min. (1.16×10^{-4} m³/Pa.s). Results for the remaining 2 facilities were unavailable due to structural failure. These results demonstrate a correlation between the age of the facility and air-leakage from the facility, and also provide an indication of the type of construction which may be suitable for high-level containment facilities.

Table 2
Contribution of penetrations to leakage rate.

Source of Leakage	Initial Leakage Rate (L/s)	Leakage Rate Following Removal of Tape (L/s)	Contribution of Penetration to Leakage Rate (L/s)
Starting leakage rate	26.1		
Leakage rate with all leaks sealed	14.1		
Single Powerpoint	14.1	14.55	0.45
Thermostat/static pressure alarm	14.63	19.76	5.13
Unsealed 50mm gap around surveillance camera	13.28	13.93	0.65
950mm crack between vertical partitions	13.93	19.97	6.04
Integral barrier seal autoclave control panel	12.85	13.9	1.05
All tape removed	13.9	26	12.1

Air-tightness Decreases with Facility Age

The facilities tested varied in age. The most recently constructed facility (No. 1) had been in use for less than 4 months, while the oldest facility (No. 17) was approximately 40 years old. When tested, facility No. 1 had an average air-leakage rate of 96 L/min. ($8.05 \times 10^{-6} \text{ m}^3/\text{Pa.s}$) with a lowest recorded leakage rate of 46.2 L/min. ($3.85 \times 10^{-6} \text{ m}^3/\text{Pa.s}$). By contrast, the rate of air-leakage from facility No. 17 was calculated as $4.2 \times 10^{-4} \text{ m}^3/\text{Pa.s}$, though this was calculated with the pressure of the facility at 87Pa due to leaks in the facility structure. Facility No. 1 was tested independently during commissioning. The result obtained during the current test (96 L/min., $8.05 \times 10^{-6} \text{ m}^3/\text{Pa.s}$) correlated well with that achieved during the commissioning test 4 months previously (99 L/min., $8.25 \times 10^{-6} \text{ m}^3/\text{Pa.s}$).

Figure 2 shows the relationship between the air-tightness of the facilities, the age of the facilities, and the material used to construct the walls of the facility. Facilities Nos. 16 and 17 are examples of the way in which age affects a facility's air-tightness. These facilities are located in buildings which are approximately 40 years old, although facility No. 16 was constructed only 7 years ago and retrofitted into the 40-year-old building. It is possible that the retrofitting of containment facilities into older pre-existing buildings is complicated by advancing structural deterioration. This theory is supported by observations of air-leakage within facilities Nos. 16 and 17 which were located on the building envelope. Air-leakage in these facilities was pronounced around surfaces adjacent to the building envelope such as windows. Leakage from similar surfaces was also observed in other facilities located on the perimeter of older buildings (e.g., facilities Nos. 10 and 14).

Although facility or building age appeared to be a major contributing factor to the decreased ability to maintain air-tightness in the facilities tested, sealing around penetrations was also a major contributory factor to decreased air-tightness. Facility No. 18 could be pressurized only to 36.7Pa with a calculated leakage rate coefficient (β -value) of $1.07 \times 10^{-3} \text{ m}^3/\text{Pa.s}$. Unlike other

facilities with a high air-leakage rate, this 7-year-old facility was not located on the building perimeter and had not been refurbished or retrofitted. The leaks that were apparent in this facility occurred around penetrations such as power points, pipes, viewing panels, and exhaust vents. Because no major unsealed penetration was visible in the facility, the combination of leaks around all penetrations was considered responsible for the inability to test the facility at the required test pressure.

Construction of Facilities

While age appeared to contribute to a decrease in the ability of the tested facilities to maintain air-tightness, another factor considered was the material used to construct the facilities. Three common construction types were used: single gyprock (plasterboard) sheeting fastened to wood or metal supporting structures (studs); double overlapping gyprock sheets; and insulated metal-faced sandwich panel. The floors of the facilities were invariably formed by the concrete slab, which separated the levels of the building, covered in welded vinyl sheets. Facility ceilings were formed either by the concrete slab of the level above or by a gyprock sheet.

Table 1 and Figure 2 show that in the majority of facilities constructed more than 5 years previously, the walls were constructed mainly of single gyprock sheets. The joints between wall and ceiling sheets were sealed either by some form of epoxy paint or through the use of plaster cornices. Of the nine facilities whose walls were constructed using single gyprock sheets, only one (No. 10) had an air-leakage rate of less than 1200 L/min. (1136.4 L/min. [$9.75 \times 10^{-5} \text{ m}^3/\text{Pa.s}$]). The remainder had leakage rates $>1369 \text{ L/min.}$ ($1.18 \times 10^{-4} \text{ m}^3/\text{Pa.s}$), with the highest leakage rate exceeding 4000 L/min. ($1.07 \times 10^{-3} \text{ m}^3/\text{Pa.s}$). If these facilities were originally constructed to comply with leakage rate requirements, this would indicate that this type of construction deteriorated more significantly, leading to a relatively higher increase in air-leakage rate. However, it is unknown whether they were constructed to meet any air-tightness requirements.

One source of leakage observed in facilities whose

walls were constructed of a single gyprock sheet was due to penetrations through the barrier into the cavity formed between the gyprock sheets. Noticeably, penetrations created subsequent to the commissioning of the facility in order to accommodate communication devices, thermostat housings, or additional power points were frequently unsealed. This is shown in Table 2, which provides details of the contributions to air-leakage rate in this type of facility.

Leakage also occurred around wall-wall joints and wall-ceiling joints, even in those facilities in which all services such as pipes and cables were mounted on the surface of the wall. One possible explanation for this is that the single gyprock sheet does not provide sufficient strength or rigidity to withstand the stress generated by the negative pressure that could destabilize or weaken the contact between the gyprock sheeting and underlying supporting structures. Furthermore, any building movements would likely cause cracking at joints. This was observed in most of the facilities of this age and construction type.

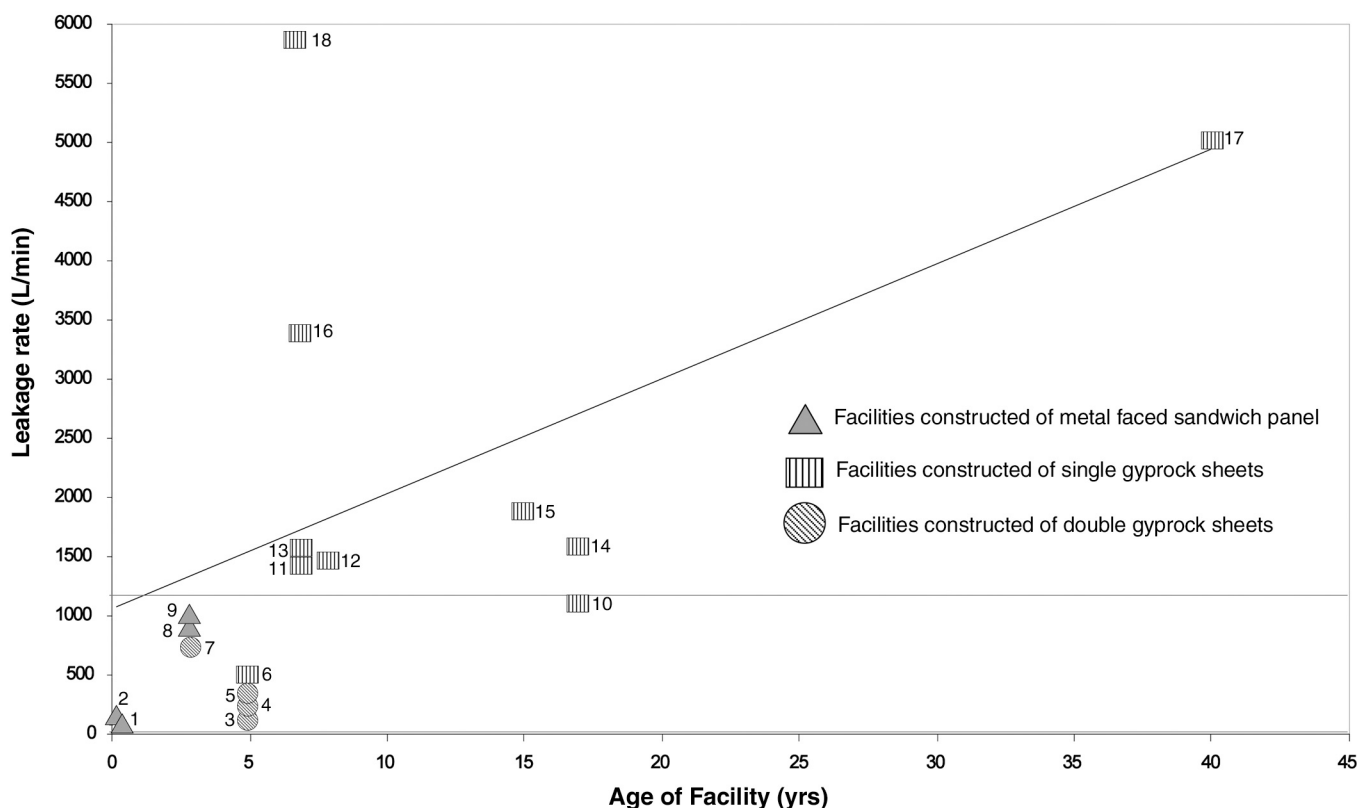
The remaining facility that was constructed in this manner (No. 6) had a leakage rate of 448.8 L/min. ($3.74 \times 10^{-5} \text{ m}^3/\text{Pa.s}$). This facility was approximately 5 years old, with all power points and cables surface mounted, ceiling-wall joints sealed by a plaster cornice, and the joint between the floor and the wall completely sealed.

Minor leakages in this facility were noted around the integral barrier-seal autoclave and through ceiling penetrations such as lights and smoke/fire-sensing equipment. Because the air-leakage from this facility had not previously been tested, it is not possible to make any assumptions about the air-tightness of the facility at commissioning. However, it was apparent that the quality of the finishes in floor, wall, and ceiling joints were important factors in the low level of air-leakage observed in this facility.

In contrast to the facilities constructed with single gyprock sheets, three of the facilities (Nos. 2, 4, and 5) utilized double gyprock sheets to form the walls of the facility. These three facilities provided some of the lowest air-leakage results, ranging from 99.6 L/min. ($8.3 \times 10^{-6} \text{ m}^3/\text{Pa.s}$) to 241.8 L/min. ($2.02 \times 10^{-5} \text{ m}^3/\text{Pa.s}$). The characteristics of these facilities were the sealing of wall and ceiling surfaces with vinyl paint and the mounting of services on the surface of the walls into conduits which did not penetrate the gyprock barrier. All of these facilities were approximately 5 years old and had been retrofitted into a 20-year-old building. None of the facilities contained an integral barrier-seal autoclave. While all facilities were located on the building perimeter, none of the facilities had external windows. Furthermore, none of the wall-ceiling joints was corniced, indicating that the joints were adequately sealed without resorting to cornices.

Figure 2

Leakage rate versus age of facility. The air leakage rate (L/min.) is plotted as a function of the age (years) of the facility and the material used for construction of the walls and ceiling of the facility. The horizontal line indicates the upper leakage rate of 1200L/min. recommended in AS/NZS 2243.3 (2002).



One other tested facility (No. 7) was also constructed of overlapping double gyprock sheets. Although only 3 years old, the facility had an air-leakage rate of 865.2 L/min. ($7.21 \times 10^{-5} \text{ m}^3/\text{Pa}\cdot\text{s}$). While the wall-ceiling joint did not employ a cornice, the major source of air-leakage in this facility appeared to be through the barrier-seal autoclave to the cavity above which housed the steam generator. Sealing of the area around the autoclave greatly reduced the air-leakage (data not shown).

The remaining facility construction method observed during this test involved the use of interlocking sandwich panels that comprise two metal faces and a fully insulating core, sealed together by a flexible setting compound. Originally used in the construction of cool rooms because of the quality of insulation provided by the panels, the technology has been more recently applied to the construction of clean rooms and PC3 and PC4 containment facilities. Of the facilities tested, four (Nos. 1, 4, 8, and 9) were constructed using this material for walls and ceilings.

The results obtained during testing of facilities Nos. 1 and 4, both of which were less than 3 months old at the time of testing, indicated that both facilities achieved an air-tightness that exceeded the performance criteria for PC3 containment facilities in AS/NZS 2243.3 (2002). Facility No. 1 achieved an average air-leakage rate of 96 L/min. ($8.05 \times 10^{-6} \text{ m}^3/\text{Pa}\cdot\text{s}$) with a lowest recorded leakage rate of 46.2 L/min. ($3.85 \times 10^{-6} \text{ m}^3/\text{Pa}\cdot\text{s}$), while No. 4 achieved a leakage rate of 159 L/min. ($1.29 \times 10^{-5} \text{ m}^3/\text{Pa}\cdot\text{s}$). In both of these facilities, sandwich panels were sealed to each other at wall-ceiling joints and cornice was then sealed to these panels. Neither facility contained a barrier seal autoclave. By contrast to the above, facilities Nos. 8 and 9, which are adjacent within the same building and which were constructed at the same time, produced results that were higher than expected. These facilities had an air-leakage rate of 883 L/min. ($7.37 \times 10^{-5} \text{ m}^3/\text{Pa}\cdot\text{s}$) and 950 L/min. ($7.92 \times 10^{-5} \text{ m}^3/\text{Pa}\cdot\text{s}$), respectively.

The major difference between facilities Nos. 1 and 4 and between facilities Nos. 8 and 9 was facilities Nos. 8 and 9 being located on the building envelope. Although exterior windows were present, careful testing did not reveal any leaks around the windows. Another apparent difference was the way in which the cornice was sealed to the wall and ceiling panels. Testing with smoke apparatus revealed that leaks had occurred behind the cornice in facilities Nos. 8 and 9. This may represent a construction method in which the wall and ceiling panels were sealed only to the cornice and not to each other. It is also possible that in the 3-year period since the facility was constructed, the negative pressure may have broken the seal between wall and ceiling panels within the facility. One other area of leakage, apparent in facility No. 9, was around the door frame. This was able to be visualized due to the aggregation of dust and other dirt around the leakage site. This was unexpected as it was

assumed that the door frame would have been sealed to the wall panel or that the insulation afforded by the sandwich material would have prevented any leakage from this area. Taping up the door frame resulted in a drop in air-leakage (data not shown). Because no data on air-tightness were gathered at commissioning, it is unknown to what extent the leakage in facilities Nos. 8 and 9 deteriorated over the 3-year period.

Structural Failure of Facilities During Positive Pressurization

Two facilities which were to have been tested could not be pressurized to enable any measurements to take place. This was due to a structural failure in which the gyprock ceiling boards separated from structural supports. Under normal circumstances, ceiling boards would have been fixed onto a roof stud from inside the facility and then sealed. However, in this construction method, it appeared that there was no support around the edge of the ceiling boards.

This structural failure raises the question of whether the test should be conducted at a positive pressure of 200Pa and how relevant this test is to facilities that are normally under negative pressure. To calculate the leakage rate from a facility, the tests should ideally be conducted at a number of different pressures to ascertain whether there is a linear relationship among measurements at different test pressures. Even though one other facility tested could be pressurized only to approximately 40Pa, it was still possible to calculate the leakage rate from the facility. Therefore, tests could be conducted at lower pressure eventually reaching the test pressure of +200Pa.

Since high-level containment facilities are maintained under negative pressure, how relevant is a test under positive pressure? There are two justifiable reasons for conducting the test in this way. First, a net flow of microorganisms out of the facility would be likely to occur only under conditions of positive pressurization, such as when the exhaust fan failed and the inlet fan continued to operate. Second, the action of positive pressurization is to force joints apart rather than "pull" them together, as would likely occur under negative pressurization. If the equilibrium pressure test was used as a measure of structural integrity, an air-tightness test using positive pressure is arguably more appropriate. However, it is important to consider that facilities that were constructed to withstand negative pressures may not simultaneously have been constructed to withstand positive pressures and that different results may be obtained if the pressure test was conducted under negative pressure.

Air-leakage Through Barrier-seal Autoclaves

Of the facilities tested, four contained barrier-seal autoclaves. In all cases, some degree of air-leakage occurred around the autoclave. It is unknown whether the leak extended through the barrier into adjacent clean areas or occurred as a result of a failure of seals around

cables or pipework. In either case, leakages should be sealed as they may pose a risk to personnel operating on the clean side of the barrier during decontamination of the facility. Furthermore, the observations highlight the need to ensure that any penetrations of the barrier wall are effectively sealed and that the seals are checked at regular intervals to ensure seal integrity. Since autoclaves generate heat and pressure, this will have a deleterious effect on seals around the autoclave. It is unknown with what frequency seals around autoclaves are maintained and checked for integrity within high-level containment facilities. However, one way to avoid this problem would be to use a standalone autoclave within a facility. This would obviate any problem associated with loss of seal integrity.

Air-leakage Through Facility Penetrations

The contribution of a number of different types of penetrations through the facility barrier to air-leakage from the facility was measured. The results are shown in Table 2. These included power points, cracks between wallboards, and the seal around the autoclave. By sealing individual penetrations, it was possible to measure the relative contribution to overall air-leakage from each of these penetrations. Air-leakage due to any pipe penetrations was not quantified. However, even with all the visible penetrations sealed, air still leaked from the facility at an average rate of 12.1 L/s ($6.05 \times 10^{-5} \text{ m}^3/\text{Pa}\cdot\text{s}$). Some of this leakage may have been due to gaps around pipe work that penetrated the facility barrier.

Maintenance of Facilities

The continued certification of high-level containment facilities that use GMOs, in Australia at least, requires that they meet maintenance standards at all times. This includes an annual inspection of the facilities and annual or biannual maintenance of containment equipment such as biological safety cabinets (BSCs) or autoclaves. At present conducting air-tightness testing of facilities is not required, though regulatory agencies may be considering the need to impose this restriction. However, the imposition of an air-tightness test may have to be justified by an assessment of the risk posed by the organisms used in the facility.

The Role of Air-tightness in Protecting Human Health and Safety

From the data presented, the air-tightness of facilities varies due to the combination of a number of factors such as age of the facility, construction method, number of penetrations through the barrier, and attention to detail regarding the finishes on surfaces during construction. However, does a high level of air-leakage mean that a facility should not be used as a bio-containment facility? There is not a simple answer to this question because it depends on a number of variables including the nature of the organism used, the ease with which that organism

can be rendered non-viable, the purpose for which the facility was constructed, whether or not the room forms the primary containment barrier, and whether the heating, ventilation, and air-conditioning control system will always prevent positive pressurization of the facility.

Exposure to RG3 organisms poses a moderate to high individual risk. The risk from exposure can be minimized by appropriate training and the use of a Class II biological safety cabinet (BSC). This is a mandatory requirement in some guidelines (OGTR, 2006). The use of a BSC to manipulate RG3 organisms results in a situation in which even in the event of the facility's positive pressurization, the net negative pressure generated by the BSC ensures that any contaminated aerosols are filtered by a HEPA filter before exiting the BSC, thereby minimizing exposure to the operator or to any individuals outside the facility.

While the use of a BSC for handling RG3 organisms substantially mitigates risk to operators from exposure to these hazardous organisms, at least two other scenarios may expose operators: when there is a spill of the hazardous organisms outside the BSC; and when use of a BSC is not possible such as in large-animal facilities where the animals cannot be kept in BSCs or in HEPA-ventilated cages. In these situations, the air-tightness of a facility is an important consideration both to prevent escape of the micro-organisms and, if the facility is to be decontaminated with toxic gaseous decontaminants, to prevent escape of the decontaminant.

Because PC3 and PC4 facilities are under negative pressure, aerosols containing micro-organisms under normal operating conditions have little chance of escape. However, in a situation where the pressure within the facility became positive due to failure of the exhaust fan, the air-tightness of the facility would be a consideration in preventing the possible escape of micro-organisms.

There is also a possibility that micro-organisms or the decontaminant itself could escape during the decontamination process. Any pressure variations induced during the decontamination process, either resulting from a fan or from the heating of the decontaminant, could accelerate leakage through the containment barrier prior to complete inactivation of the micro-organism. The likelihood that any micro-organism or decontaminant will escape from a facility increases as the air-leakage rate from the facility increases.

While the risk associated with the escape of micro-organisms in the above scenario can largely be mitigated by decontaminating a spill with a liquid decontaminant as soon as it occurs and by maintaining the facility under negative pressure during this process, more risk may be associated with the gaseous decontamination process. Diffusion of formaldehyde across a single barrier to adjacent areas has been calculated to occur to levels of 3500 ppm/hour even in facilities with an air-leakage rate of 120 L/min. ($10^{-5} \text{ m}^3/\text{Pa}\cdot\text{s}$) (Pickering, 1982). Even though successful decontamination can be achieved

using much lower concentrations of formaldehyde (600–800 ppm, per AS/NZS 2243.3 (2002), decontamination of facilities that have a leakage rate of greater than 120 L/min. (10^{-5} m³/Pa.s) may pose a risk to the health and safety of unprotected personnel from exposure to toxic levels of the gaseous decontaminant. The results of this study indicate that of the 18 facilities tested, 16 (89%) have an air-leakage rate greater than 120 L/min. (10^{-5} m³/Pa.s) and 8 have a leakage rate greater than 1200 L/min. (10^{-4} m³/Pa.s). Based on these results, considering whether gaseous decontamination of these 16 facilities is necessary is advisable, and if so, what additional precautions should be taken.

Air-tightness as a Measure of Structural Integrity

The results presented here demonstrate that PC3 facilities need not be constructed from expensive materials to withstand negative pressure or to maintain a high degree of air-tightness. Instead, a construction technique that used less expensive building materials, combined with surface-mounted services, produced a high degree of air-tightness even after 5 years of constant negative pressure applied to the containment facility.

The results also indicate a correlation among the air-tightness of facilities, the age of the facility, and the method of facility construction. Air-tightness testing could therefore be used as a longitudinal measure of the structural integrity of a facility. This would enable more targeted maintenance of a facility so that it could continue to meet any regulatory requirements. Data gathered in this way would also be informative for facilities constructed using modern construction methods. Regardless of whether this kind of data is seen as useful, periodic air-tightness testing may be more suitable for, and therefore targeted to, those facilities that require decontamination due to the nature of the micro-organism under study or to those facilities that form the primary containment measure.

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Author's Note

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