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# USE OF LOW-OXYGEN ATMOSPHERES IN QUARANTINE APPLICATIONS TO ERADICATE *MUS MUSCULUS*, THE HOUSE MOUSE

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## ABSTRACT

The feasibility of using low-oxygen ($O_2$) atmospheres to eradicate infestations of mice (*Mus musculus*) in order to meet quarantine standards was investigated. Drilling equipment and transportable buildings were enclosed within standard plastic fumigation sheets both with and without floor sheets. The enclosures, 45–189 m$^3$, were dosed with a low-$O_2$ atmosphere ($\approx$1.5% $O_2$) generated by a membrane nitrogen separation system. Purging was observed to follow a simple free mixing model in form. The atmospheres were created using about 20% less gas than the amount predicted on the basis of free mixing alone. Applying this model allows selection of generators of suitable size. Leakage from sheeted containers was very slow after purging (0.04% d$^{-1}$ or less), showing that a high degree of gas retention was easily achieved.

Atmospheres containing less than 2.5% $O_2$ were obtained and maintained within the enclosures for a minimum of 6 h. Published literature suggests this would have caused 100% mortality of exposed mice. Work is continuing to establish the most cost-effective exposure regime.

Application of this treatment as a quarantine measure against rodents is discussed and its costs compared with conventional methods of disinfestation using methyl bromide.

## INTRODUCTION

In 1994, the Stored Grain Research Laboratory was commissioned by West Australian Petroleum Pty Limited (WAPET) to find an effective replacement for methyl bromide (MB) for control of rodents in its quarantine operations. This chemical has been used for rodent control world-wide since the 1940's (Borg, 1944; Lembrez, 1966) and is still internationally recommended for this purpose (Bond, 1984).

WAPET is an oil and gas company for exploration and production, operating on two islands on the northwest shelf of Australia. Both islands, Barrow and Thevenard, are nature reserves serving as refuges for rare or endangered wildlife. Consequently, the

Company's operations are constrained by both voluntary controls and those mandated by the Environmental Protection Authority on imported material, including plants and animals.

The company, which has won industry recognition for its environmentally friendly operations, has gained two awards for its environmental policies. Even after 30 years of operation, Barrow Island is still a refuge for a number of animal species, such as the Burrowing Bettong, that have disappeared from their original habitats on the mainland. If rabbits, cats, foxes, rats, mice, snails or weeds were allowed to enter these islands, they could seriously, perhaps permanently, disturb the ecology of the native wildlife, possibly causing extinctions through predation and deprivation.

WAPET enforces a quarantine program to ensure that exotic animals, specifically mice, are not carried from the mainland to the islands. Equipment that can be infested by rodents and other vermin is subjected to MB fumigation. Items such as transportable offices, accommodation units, tool sheds, equipment used for food storage, mobile messes, etc. are commonly treated before they are shipped from the mainland to the islands. Also, because non-native mice have become established on Thevenard Island, similar restrictions apply when equipment is transported from there to Barrow Island.

In keeping with WAPET's environmental concerns, and with occupational health and safety concerns, the company desired to replace MB for this particular application. However, the company wanted any potential replacement for MB to be environmentally safe; safer for workers than the existing system; compatible with exploration and production operations for oil and gas; compatible, in terms of speed of operation, with the existing method of disinfestation; and suitable for use at remote locations.

After visiting and inspecting WAPET's on- and off-shore operations it was concluded that three potential alternatives could be considered. These were use of heat/high temperatures, high carbon dioxide ($CO_2$) atmospheres and low-oxygen ($O_2$) atmospheres.

### Heat as an alternative

This was ruled out because of potential damage to the heat sensitive equipment used during the company's operations. More importantly, it posed a potential, and serious, fire hazard when used in the vicinity of gas and oil, whether wells or storage vessels.

### Carbon dioxide as an alternative

Early reports of the potential use of $CO_2$ in the storage environment to control mice and other rodents were published by Pieniazek and Christopher (1947) and Southern (1954). It has been used to control mice both in seed and feed stores and in cold storages, for which an atmospheric concentration of 25% $CO_2$ was applied for periods extending up to 2 h (Hamel, 1986). It is widely used in euthanasia for killing a wide range of animals, including rats and mice (Andrews *et al.*, 1993; Hansen *et al.*, 1991; Hornett and Haynes, 1984). However, its use in this particular application was constrained by the logistics and costs involved in transporting the large quantities of $CO_2$ that would be required.

**Low-$O_2$ atmospheres as an alternative**

Use of nitrogen ($N_2$) to generate low-$O_2$ atmospheres was considered most appropriate because the proposed system met most of WAPET's replacement criteria. $N_2$ is already used in oil and gas exploration and production to remove air in tanks and pipes in order to prevent fires and explosions. A membrane generation system was chosen because such systems are reliable, small and portable, and they are already used by the industry elsewhere. Also, in our experience, low-$O_2$ atmospheres are more easily contained in plastic enclosures sealed with sand or sand snakes than are high $CO_2$ atmospheres.

$N_2$ is widely used for euthanasia of a wide range of animals, including rats and mice (Andrews *et al.*, 1993). Rats collapse in approximately 3 min, and stop breathing in 5–6 min, in low-$O_2$ atmospheres produced with $N_2$ flowing at a rate of 39% of chamber volume per minute, thus falling to about 3% $O_2$ after 5 min (Hornett and Haynes, 1984). For mink, in 100% $N_2$ the time to death is 134 sec, but Hansen *et al.* (1991) recommend an extended exposure period of 5 min. Dogs are killed in less than 5 min by $N_2$ atmospheres containing 1.5% $O_2$ (Herin, 1978; Quine, 1980). When a group of 72 animals consisting of cats, kittens, rabbits and dogs was placed in a $N_2$ atmosphere (final $O_2$ concentration declining to ≤4.0%), 70 collapsed within 1 min of the $O_2$ concentration falling to 10%; 66 suffered respiratory arrest within 2 min of collapsing; and 66 had no detectable heart beat at 6 min, or less, after the $O_2$ concentration fell to <10% (Quine *et al.*, 1988).

However tolerance to hypoxia, in the short exposure periods used for euthanasia, has been observed in newborn mice, rats, rabbits, kittens and puppies (Blackmore, 1993; Herin, 1978; Quine, 1980; Quine *et al.*, 1988).

Barrere (1980) reported that atmospheres containing 99% $N_2$ killed rodents in less than half an hour in grain storages, but he gave no indication of a specific dosage regime for these pests. Pryor *et al.* (1974) in working with mice found that deaths occurred at 7.5% $O_2$ in 4 h experiments. Levin *et al.* (1987) calculated $LC_{50}$ values for rats exposed to low-$O_2$ atmospheres for 30 min in an atmosphere of 7.5% $O_2$.

This paper reports on a trial undertaken to assess the feasibility and effectiveness of using low-$O_2$ atmospheres to eradicate infestations of mice in equipment used by the oil and gas exploration and production industry.

## MATERIALS AND METHODS

The equipment disinfected during this trial consisted of drilling pipes (in racks), a toolshed container, an equipment container and transportable offices both with and without wheels. Dimensions and volumes of the seven lots of equipment after enclosure under fumigation sheets are given in Table 1. The sheets used were made from either yellow nylon reinforced laminated PVC or black Valeron (cross laminated HDPE). All equipment was positioned on cement stabilised sand prior to being enclosed and disinfested.

To obtain an indication of gas loss downward through the ground, three of the seven treatments were carried out using a gastight floor sheet. This was laid on the ground and the equipment placed on it before being enclosed and sealed for treatment.

TABLE 1
Dimensions and volumes of equipment treated, and type of enclosure sheet used

| Treatment no. | Equipment | Dimensions (m) length | width | height | Volume ($m^3$) | Fumigation sheet | Ground sheet |
|---|---|---|---|---|---|---|---|
| 1 | Pipe racks 1 | 10.2 | 5 | 1.1 | 56 | Valeron | – |
| 2 | Pipe racks 2 | 10.2 | 5 | 1.1 | 56 | Reinforced laminated PVC | – |
| 3 | Container 1; Toolshed | 6.0 | 3 | 2.5 | 45 | Valeron | – |
| 4 | Container 2; Kumo shack | 12.2 | 3 | 2.5 | 92 | Valeron | – |
| 5 | Container 3; Transportable office on wheels | 13.5 | 4 | 3.5 | 189 | Valeron | Valeron |
| 6 | Container 4; Jan's office rooms | 13.5 | 3 | 3.1 | 126 | Reinforced laminated PVC | Valeron |
| 7 | Container 5; Transportable office on wheels | 13.5 | 4 | 3.5 | 189 | Valeron | Valeron |

Four pipe racks were treated. These were enclosed as pairs under a fumigation sheet, and each pair was disinfested as a single unit. All other items were enclosed and treated as single units. Sharp edges and protrusions from the equipment were covered to prevent their penetrating and tearing the fumigation sheets. The fumigation sheets were sealed and anchored to the ground, or to the floor sheet, using sand heaped approximately 0.5 m high continuously along the perimeter of the sheeted enclosures at the base of the structures. All visible holes and tears in the fumigation sheets were patched or sealed prior to treatment. Ropes were tied around the middle of each enclosure to reduce billowing and consequent gas "pumping" in the wind.

$N_2$ for the treatment was supplied from a Prism (Permea Inc., St Louis, Missouri, USA) $N_2$ membrane system supplied by Oxair Australia, Perth, WA. It had a rated output of 18 $m^3$ $h^{-1}$ at 98% $N_2$ and consisted of an electrically powered compressor, an air storage tank, the membrane system and a $N_2$ receiving tank.

In this trial it was decided to obtain and hold the $O_2$ levels below 5.0% and maintain these levels long enough to initially ensure complete dispersal of the atmosphere throughout the equipment and thereafter to disinfest the equipment.

$N_2$ for the treatments was piped from the generator via a 5-cm i.d. nylon-reinforced plastic pipe to a manifold fitted with three ball valves from which it was distributed to the enclosed equipment. These valves provided full (100%) flow with a 90° turn. For the purposes of this trial it was assumed that a 30° turn of the valve delivered 33.3% of the full flow and a 45° turn delivered 50% of the full flow. Subsequent calculations confirmed this assumption.

To prevent excess ballooning of the enclosures during the purge in treatments 1–3 (Table 1), gas was allowed to vent via a small (≈5 cm i.d.) hole in the sheeting approximately 1 m from the ground at the point furthest from the gas input line. In treatments 4 and 5 (Table 1), no vent was provided.

At the end of the treatment time, if the equipment was immediately required for shipment, the fumigation sheets were removed and the containers allowed to air until $O_2$ levels had been restored to 20–21%. Otherwise the containers remained enclosed until required. In such cases, the opportunity was used to monitor $O_2$ levels to determine the rate of leakage and permeation of air through the fumigation sheets after the $N_2$ purge was terminated.

$O_2$ concentrations achieved during the treatments were measured by sampling the atmosphere inside the enclosures. The samples were drawn out through 3-mm i.d. nylon tubing from various points in the enclosures (Figs. 1–4). The sampling lines were cleared

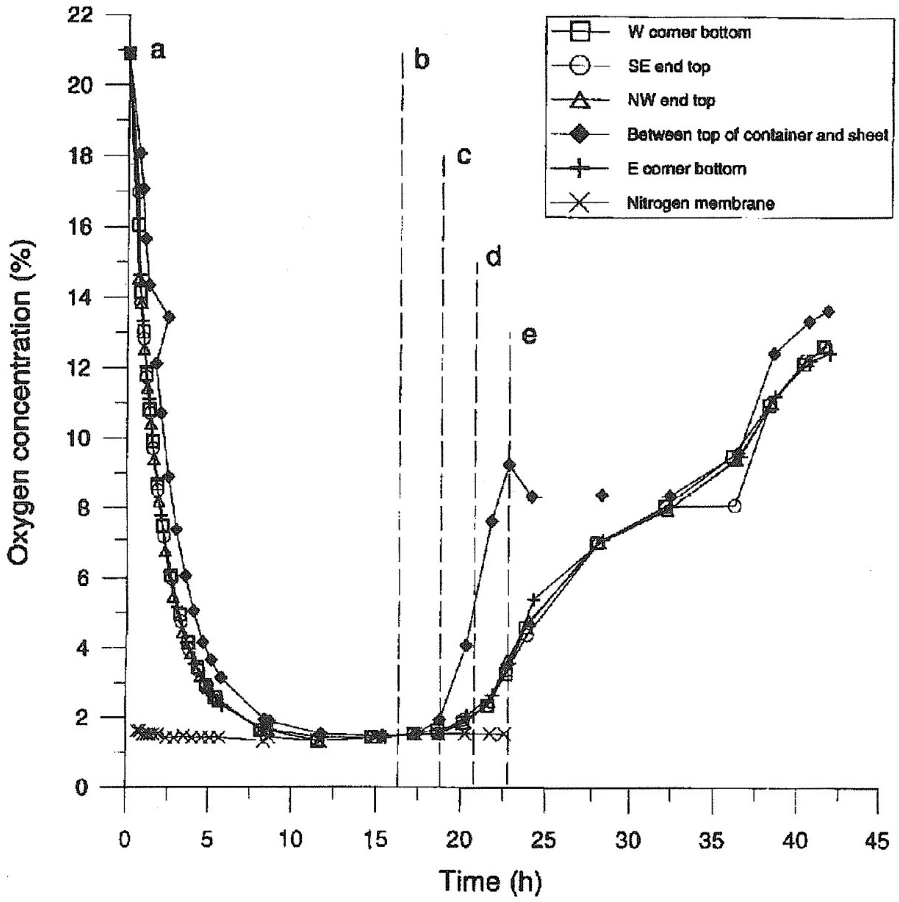


Fig. 1. $O_2$ concentrations during and after purge of container 1, the toolshed without floorsheet. Assumed flow rates during purge: a = 100%, b = 33%, c = flow rate reduced, d = flow rate reduced then increased and e = end of purge.

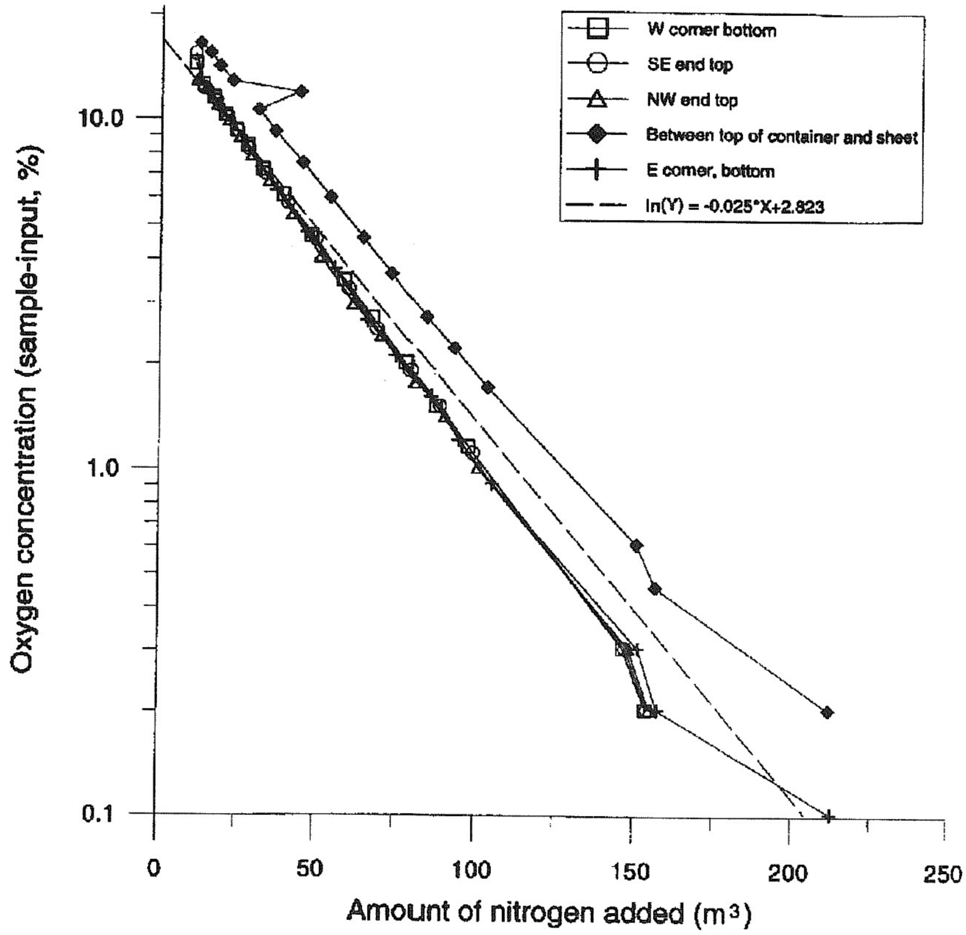


Fig. 2. Plot of $O_2$ concentration to show rate of $O_2$ decrease during purge of container 1, the toolshed without floorsheet.

by pumping for a period of 3–5 min at a rate of 150 ml $min^{-1}$ using a Masterflex (Barnant Co, Illinois, USA) peristaltic pump until a steady $O_2$ concentration reading was obtained. $O_2$ concentrations were measured using either an Otox 90 (Neotronics, Bishops Stortford, UK) or an Oxywarn 100 (Draeger) $O_2$ meter. During the treatments, the calibration of these meters was checked regularly against ambient air and industrial grade $N_2$ (BOC, $N_2 = 99.9\%$, $O_2 < 10$ ppm).

Ambient temperature as well as temperatures attained within the enclosures were measured using T-type copper/constantan thermocouples and recorded on a data logger (Data Electronics, Datataker DT100). Temperatures within the enclosures were measured at the same points from which gas samples were withdrawn (Figs. 1–4).

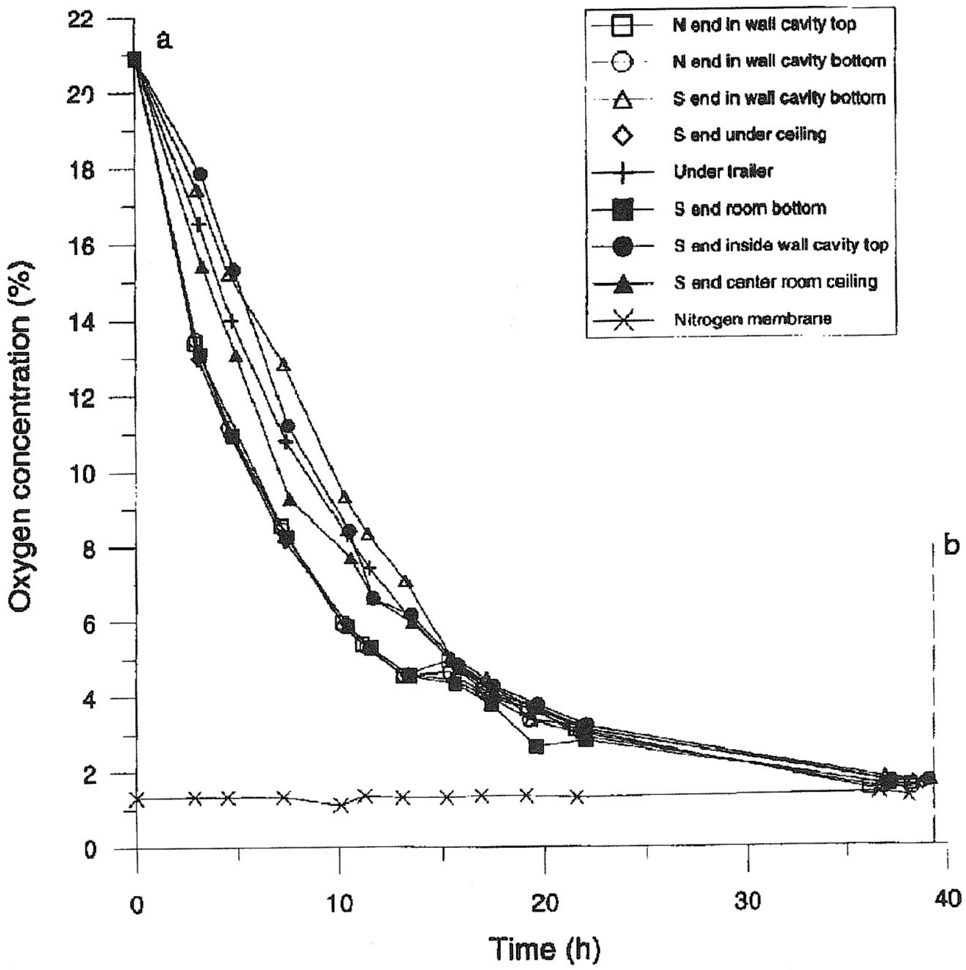


Fig. 3. $O_2$ concentrations during and after purge of container 5, transportable office on wheels with floorsheet. Flow rates during purge: a = 100% and b = end of purge.

## RESULTS

The $N_2$ output from the membrane system provided an atmosphere with an $O_2$ concentration varying from 1.3–2.0% and averaging 1.5% $O_2$ (Figs. 1 and 3).

The $O_2$ concentrations achieved during the purges and the rate of $O_2$ decrease obtained are shown for container 1 (the toolshed without floor sheet) in Figs. 1–2 and for container 5 (the transportable office on wheels with floor sheet) in Figs. 3–4. The required $O_2$ concentrations ($\leq$5.0%) were achieved within a 5-h treatment period for container 1 and a 16-h treatment period for container 5, the volume of the latter being approximately 4 times that of the former (Figs. 1 and 3).

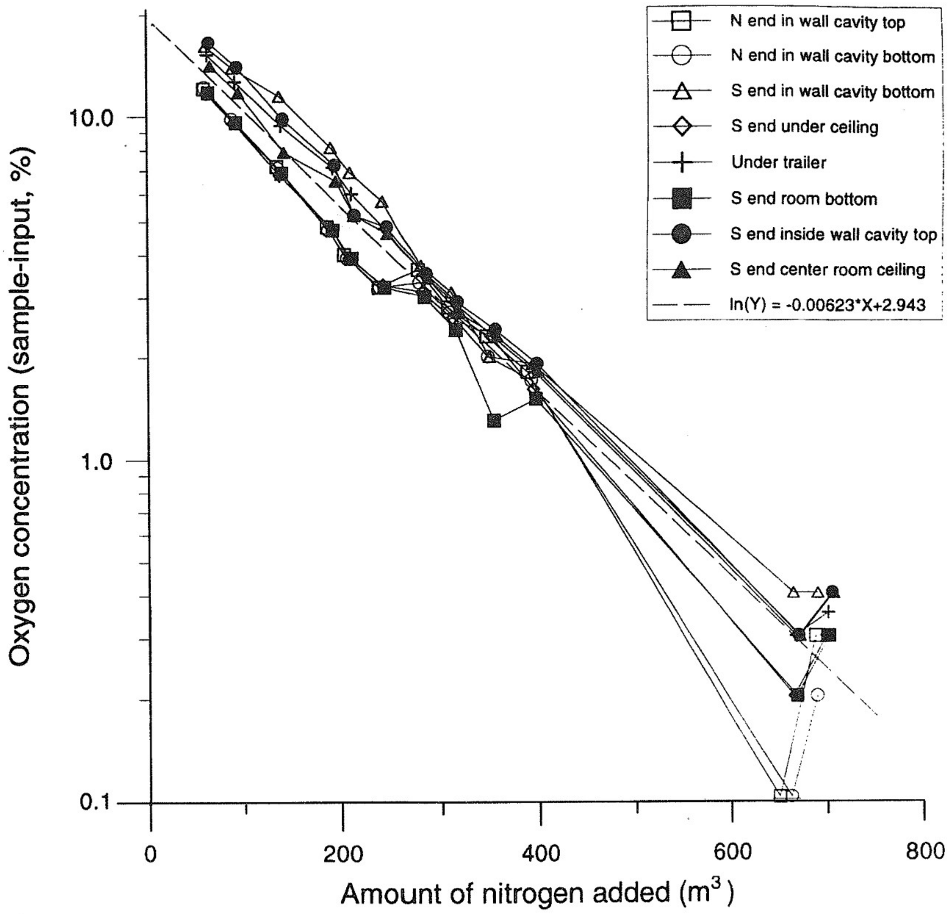


Fig. 4. Plot of $O_2$ concentration to show rate of $O_2$ decrease during purge of container 5, transportable office on wheels with floorsheet.

The amount of $N_2$ used during the purges was calculated from the rated output of the membrane system (18 $m^3$ $h^{-1}$ at 98% $N_2$) and the degree of opening of the valves on the manifold regulating $N_2$ input into the enclosures. Table 2 compares the amount of $N_2$ required to reduce the $O_2$ level to 5% for each treatment as well as the rate and efficiency of the purge. Purging efficiency was calculated using Banks' (1979) formula, modified to take into account the fact that the purge gas, $c_p$, contained about 1.5% $O_2$. This formula gives 100% efficiency if free mixing occurs throughout the space being purged:

$$Efficiency\ (\%) = 100 \times \frac{volume\ of enclosure}{volumeof gas\ added} \times \ln \frac{21 - c_p}{5 - c_p}$$

The ventilation rate, $k$, a measure of the rate of gas loss from a system, was calculated

TABLE 2
Purge data for the seven treatments

| Treatment no. | Equipment | Total $N_2$ used ($m^3$) | Amount of $N_2$ required to reduce $O_2$ to 5% ($m^3$) | Treatment time at $O_2$ ≤5% (h) | Slope of pull-down line | Efficiency of purge[1] (%) |
|---|---|---|---|---|---|---|
| 1 | Pipe racks 1 | 235 | 67 | 13 | –0.0190 | 129 |
| 2 | Pipe racks 2 | 165 | 55 | 6 | –0.0187 | 157 |
| 3 | Container 1; Toolshed | 329 | 38 | 20 | –0.0250 | 183 |
| 4 | Container 2; Kumo shack | 394 | 127 | 11 | –0.0080 | 112 |
| 5 | Container 3; Transportable office on wheels | 416 | 211 | 12 | –0.0048 | 138 |
| 6 | Container 4; Jan's office rooms | 406 | 116 | 27 | –0.0086 | 168 |
| 7 | Container 5; Transportable office on wheels | 707 | 172 | 30 | –0.0062 | 170 |

[1]Calculated assuming 10% of the volume within enclosure filled with impervious objects.

using the formula given by Banks and Annis (1984),

$$\ln \frac{21 - c}{21 - c_0} = -kt$$

where $c$ and $c_0$ are the $O_2$ concentrations at time $t$ and at start of ventilation. Figure 5 shows the return of $O_2$ by natural leakage into the purge enclosure after the purge gas addition ceased.

The averages of the ambient temperatures and those attained within the enclosures during the treatments are given in Table 3.

## DISCUSSION

The purge data for the seven treatments carried out during this trial are given in Table 2. In all cases, purging efficiencies in excess of 100%, as calculated on the basis of free mixing, were observed, suggesting there was some $O_2$ removal by direct displacement. Up to 50% less $O_2$ was needed than was expected.

Decrease in $O_2$ concentrations (Figs. 1, 3) during the purges was uniform at all points within the treated equipment. This included the wall and ceiling cavities of the transportable office. Even at roof height, areas which can provide nesting and breeding sites for rodents were subjected to $O_2$ concentrations ≤5% during the exposure periods used here.

This trial demonstrated that it is feasible to reduce $O_2$ concentrations within sheeted enclosures efficiently to ≤5.0% and to hold them at <2.5% for periods extending to 6 h. In the work reported here, the objective was to attain low $O_2$ levels and maintain them

TABLE 3
Ambient temperature, and temperatures in sheeted equipment during treatment

| Treat ment no. | Equipment | Ambient temperature (°C) | | | Temperature inside enclosure (°C) | | |
|---|---|---|---|---|---|---|---|
| | | Average | Average maximum | Average minimum | Average | Average maximum | Average minimum |
| 1 | Pipe racks 1 | 22.1 | 30.0 | 19.2 | 24.4 | 30.4 | 21.5 |
| 2 | Pipe racks 2 | 22.5 | 30.1 | 19.2 | 23.8 | 29.6 | 21.2 |
| 3 | Container 1; Toolshed | 22.2 | 27.3 | 18.8 | 24.6 | 31.3 | 20.4 |
| 4 | Container 2; Kumo shack | 22.5 | 29.6 | 19.3 | 25.9 | 32.0 | 20.2 |
| 5 | Container 3; Transportable office on wheels | 21.9 | 25.9 | 20.1 | 25.2 | 29.2 | 18.4 |
| 6 | Container 4; Jan's office rooms | 20.0 | 27.3 | 17.4 | 20.8 | 29.3 | 18.7 |
| 7 | Container 5; Transportable office on wheels | 21.5 | 27.8 | 18.4 | 23.8 | 31.2 | 19.3 |

sufficiently long to kill any infestations of mice in the equipment treated. Levin *et al.* (1987) reported 100% mortality in 42 rats exposed for 30 min to atmospheres containing 7.5% $O_2$, while Pryor *et al.* (1974, cited in Levin *et al.*, 1987) reported that mice were killed at the same $O_2$ concentration in 4 h experiments. Thus, with the $O_2$ concentrations achieved and the exposure periods used in this trial, it can be expected that at least all adult animals would have been killed. Similarly, we believe that the prolonged exposures to the low-$O_2$ atmospheres attained during this work, well in excess of those used for euthanasia, would have been lethal to neonate rodents.

With respect to a dosage and exposure regime, laboratory studies will be undertaken to confirm the results obtained during this trial and establish recommendations for atmospheric $O_2$ concentrations and exposure periods. In the absence of detailed mortality dosage data, no recommendation is made here. However, we expect that the required exposure periods will be substantially shorter than those in these trials.

It may be important for toxicological or logistical reasons to reduce the time needed for pull-down to low $O_2$. This can be achieved by using a generator with a greater output than that used in this work. The modelling that was undertaken above allows for appropriate sizing of $N_2$ generators for a particular, specified pull-down.

Very low leakage as calculated by ventilation rates were observed in the three enclosures which were allowed to stand after purging. Two had a rate of about 0.04 per day, while the third admitted air at a rate of only only 0.008 per day (Fig. 5). The latter rate is very low, and this is perhaps attributable to the better seal afforded by the PVC and floor sheet.

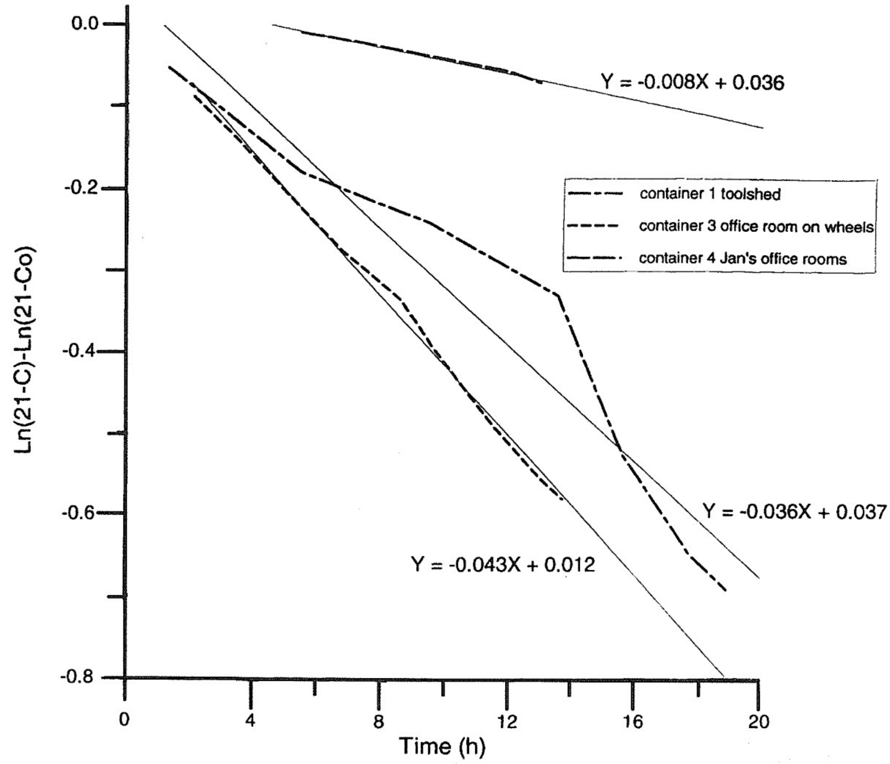


Fig. 5. Ventilation rate ($O_2$ decay) in enclosed equipment. Container 1 sheeted with Valeron without a floorsheet; container 3 with floorsheet; container 4 with PVC sheeting and a floorsheet.

Direct comparison of the cost of disinfestation with low-$O_2$ atmospheres to that of using MB is complex in this situation, where environmental and worker-safety concerns are difficult to quantify. However, the direct material costs involved in this use of low-$O_2$ atmospheres can be compared to those when MB is used. The cost of hiring the membrane system was AU$ 500 as compared to the material cost of AU$ 100 for MB, had it been used. Costs for electricity to run the membrane system are estimated at AU$ 0.15 per $m^3$ $N_2$. However, when MB is used, a licensed fumigator and protective equipment for the workforce engaged in this work are required. This would offset the cost difference, particularly in a remote region such as that in this trial series. Labour requirements for enclosing the equipment under gastight sheeting are the same in both cases.

MB treatments with a 4-h exposure period (Bond, 1984) followed by about 2-h airing time are typically rapid. Low-$O_2$ atmospheres are dependent on the capacity of the equipment available, which determines the rate of pull-down and the duration of the required exposure period. For the latter, we have not yet obtained definitive data, but we expect low-$O_2$ treatments to be at least as rapid as those with MB, given the appropriately sized generator and the mortality data reviewed above.

The time required for airing after treatment with low-$O_2$ atmospheres was shorter than for MB. The maximum time required with low-$O_2$ atmospheres was 0.5 h.

There are particular benefits to be gained by adopting the low-$O_2$ atmosphere technique in the application described here. Firstly, emissions of MB, a powerful ozone depletor (UNEP, 1992; WMO, 1995), would be reduced. Secondly, it would eliminate a worker-safety hazard from the oil and gas exploration and production workplace.

The technique may have wider application as a replacement for MB in such other situations as aircraft and other equipment already constructed to standards of gastightness. Furthermore, in situations where time constraints are critical, as with aircraft, there appears to be a case for adding $CO_2$ or carbon monoxide to increase the speed of action against rodents (Levin *et al.*, 1987; Pryor *et al.*, 1974).

## CONCLUSION

The work described here demonstrated the feasibility of using low-$O_2$ atmospheres for rodent control in machinery and equipment which is under gastight fumigation sheeting. The technique using $N_2$ delivered from a membrane system to generate low-$O_2$ atmospheres is environmentally acceptable and safer than is the use of MB (Fig. 6). In operation, it is compatible with oil and gas exploration and production operations. It is also suitable for use at remote locations. The logistics of on-site gas generation provide environmental and

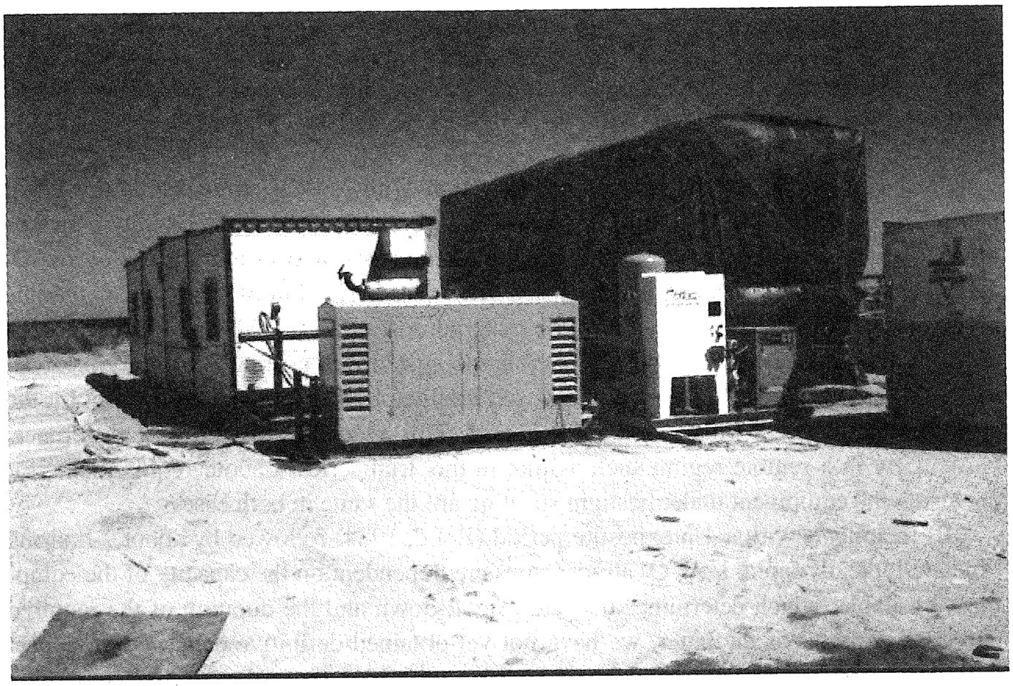

Fig. 6. View of membrane $N_2$ system and enclosed transportable office on wheels under treatment.

worker-safety advantages that make it an attractive alternative to MB. The technique may have wider application beyond the oil industry.

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