Improvement of Vivarium Biodecontamination through Data-acquisition Systems and Automation

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Biodecontamination is important for eliminating pathogens at research animal facilities, thereby preventing contamination within barrier systems. We enhanced our facility’s standard biodecontamination method to replace the traditional foggers, and the new system was used effectively after creating bypass ducts in HVAC units so that individual rooms could be isolated. The entire system was controlled by inhouse-developed supervisory control and data-acquisition software that supported multiple cycles of decontamination by equipment, which had different decontamination capacities, operated in parallel, and used different agents, including H₂O₂ vapor and ClO₂ gas. The process was validated according to facility mapping, and effectiveness was assessed by using biologic (Geobacillus stearothermophilus) and chemical indicator strips, which were positioned before decontamination, and by sampling contact plates after the completion of each cycle. The results of biologic indicators showed 6-log reduction in microbial counts after successful decontamination cycles for both agents and found to be compatible with clean-room panels including commonly used materials in vivarium such as racks, cages, trolleys, cage changing stations, biosafety cabinets, refrigerators and other equipment in both procedure and animal rooms. In conclusion, the automated process enabled users to perform effective decontamination through multiple cycles with real-time documentation and provided additional capability to deal with potential outbreaks. Enabling software integration of automation improved quality-control systems in our vivarium.

Abbreviations: BI, biologic indicator; CI, chemical indicator; SCADA, supervisory control and data acquisition software; VHP, vaporized hydrogen peroxide

Biodecontamination is an imperative step to avoid cross-contamination, especially in functional animal facilities with high experimental turnover. Any contamination from animal rooms or outside sources can be transmitted to other areas that potentially affect animal health status, thus compromising research outcomes. A wide range of agents including quaternary ammonium compounds, phenol-based products, and alcohol are commonly applied for decontamination of animal rooms through various methods such as manual wiping, fogging, and vapor and gas exposure. In addition, H₂O₂ is commonly used as biocide agent; it decomposes readily to form water and oxygen, which are nontoxic and safe to use for effective biologic inactivation. In particular, vaporized hydrogen peroxide (VHP) has been recommended for the biodecontamination of a large variety of materials such as biologic safety cabinets, laboratories, and pharmaceutical contexts including production filling-line rooms, sterility testing environments, scalable enclosures, and lyophillizers. VHP-based decontamination methods are widely used as alternatives to formaldehyde because of their ease of use, higher levels of sterility assurance, and overall cost savings to a facility. Moreover, VHP is a broad-spectrum antimicrobial with virucidal, bactericidal, fungicidal, and sporicidal activity. Because the International Agency for Research on Cancer and Environmental Protection Agency have classified formaldehyde as carcinogenic for humans, alternative chemical liquids and vapors for use as decontamination agents are necessary in light of safety aspects. Consequently, research laboratories and hospitals must find a suitable decontamination method; VHP provides an alternative to formaldehyde fumigation because of VHP’s biologic efficacy against various microorganisms. Adding gaseous H₂O₂ to a standard low-temperature sterilization process may provide a useful method for prion inactivation; the gas is residue free, and can be released into the atmosphere.

Chlorine dioxide is a greenish yellow, single-electron-transfer oxidizing agent that has a chlorine-like odor. Pure ClO₂ is an unstable gas at room temperature and considered as true gas, therefore, it is generated as needed before decontamination and used in animal research facilities with appropriate sealing to avoid leaks from the enclosure. However, ClO₂ is sensitive to decomposition by light and must be stored and used in ways that prevent direct exposure to sunlight. Unlike H₂O₂, ClO₂ is a very selective oxidant, with 2.5 times oxidizing power of chlorine. Due to this strong oxidizing ability, the gas is effective against a wide variety of organisms; it has shown sporicidal activity against Bacillus subtilis spores and is effective against Bacillus thuringiensis, as well as Syphacia spp. ova.

In light of these considerations, VHP or ClO₂ might be especially effective for biodecontamination after the completion of animal experiments. In addition, both agents have excellent material compatibility and have been tested in diverse applications under laboratory conditions and used effectively in high-level containment facilities. To minimize contamination and achieve a 6-log reduction in microbial counts, a strategic plan was proposed, in which multiple H₂O₂- or ClO₂-based decontamination units replaced the existing traditional
SCADA is a software-application for process control that is customized to the needs of decontamination. Generally, SCADA gathers real-time data from remote locations to control equipment and its operational state. The interactive development process was carried out according to the documented design of facility’s operational needs. The ability to provide 61 cycles was developed initially, with further scope to add additional cycles as and when required. The software is password-protected at different levels, including facility engineers and user groups, so that the system can be operated and monitored from different floors of the facility. The software developer and facility engineering team optimized the system under test conditions and then the system operators and other users at the facility.

**Isolation of HVAC ducts and installation of bypass dampers.** Each HVAC unit in the facility serviced 4 to 6 clusters of rooms, and return air was conveyed through a single duct that had temperature sensors for capturing real-time data by the Building Monitoring System. However, an individual room could not be isolated for decontamination because shutting down the particular HVAC unit would affect adjacent rooms. Therefore, to isolate individual rooms and enclosures, bypass dampers were installed in the supply and exhaust ducts; actuators present in the supply and exhaust dampers control the bypass dampers. The online execution of damper opening and closing occurs within 1 min, regardless of the rooms planned for decontamination. All commissioning activities were carried out systematically through a documented verification process to ensure that all components operated according to specifications.

**Equipment validation and preparation of sealed enclosures.** Areas were constructed by using clean-room panels, and additional efforts to seal the areas included the replacement of light fixtures and other utilities to avoid potential leaks from the animal rooms by considering safety aspects. To supply air at adjustable pressure (0.5 to 3.0 psi) during decontamination by the Minncare dry-fog equipment, a system controlled by solenoid valves was designed to deliver compressed air from central cylinder banks. The decontamination equipment was procured in parallel with modifications and software creation, and subsequent validation was performed according to the facility set-up, which considered the room volume, agent used for decontamination, and calculated time. To achieve the desired concentration, oscillation fans (2 to 8) were placed for uniform distribution of agents to all corners and surfaces of a room. Portable sensors and indicators were used to monitor the relative humidity and concentration of decontaminant agent during the process.

**Minidox-M ClO₂ system.** The Minidox-M system (ClorDiSys Solutions, Somerville, NJ) generates pure ClO₂ gas which is injected into the sealed room or enclosed area. The decontamination phases for this unit comprise precondition, condition, charge, exposure, and aeration. The exposure concentration (360 ppm at 1 h, 720 ppm at 2 h, and 1080 ppm at 3 h) is calculated automatically according to the room volume, to a maximum of 70,000 ft³. The equipment holds cylinders containing 2% chlorine, 98% nitrogen at the back side of the unit, and the gas passes through a regulator and 3 sets of cartridges prior to delivery into rooms programmed for decontamination. All activities and parameters of the Minidox-M were monitored, and a portable gas leak detector (PortaSens II Model C16 - Analytical Technology) was used to measure potential leaks during decontamination and to verify the appropriate residual ClO₂ concentration at the end of each cycle. In addition, this system has an automated process for controlling humidity levels regardless of ClO₂ gas concentration during the entire decontamination cycle.

**VHP-Victory biodecontamination unit.** The VHP-Victory biodecontamination unit (STERIS, Mentor, OH) generates H₂O₂ vapor by using a stabilized aqueous solution of 35% H₂O₂, and can cover an area up to 20,000 ft² in a single cycle. The operator uses a programmable logic controller touchscreen to select a factory-programmed cycle, and the provided SmartPhase software...
automatically runs the cycle selected for biodecontamination. During dehumidification, the relative humidity was reduced to 50% to 60%, and the vapor generated from liquid H₂O₂ is introduced into the enclosure to achieve the desired concentration rapidly. The residual level was measured after the aeration phase at the end of the cycle, and data-log output was stored on a USB memory stick. VHP-Victory cycle phases include dehumidification, conditioning, decontamination, and aeration.

HaloFogger disinfection system. The HaloFogger system (Halosil International, New Castle, DE) uses a dry-mist dispensing device that delivers aerosolized disinfectant (5% H₂O₂ and 0.01% silver) and can cover a room volume up to 3700 ft³. This equipment was used for smaller areas and can support other high-capacity units when a large area (particularly corridors) is targeted for decontamination.

Minncare dry-fog system. The Minncare dry-fog system (MAR COR Purification, Plymouth, MN) was operated by air pressure and designed to produce ultrafine atomized dispersion of cold sterilant (22% H₂O₂ and 4.5% peracetic acid) for decontamination of up to 35,000 ft³. Manufacturer-provided software was used to calculate the amount of water and cold sterilant were required according to the room volume and time needed to achieve desired concentration; the recommended concentration was 1.5 mL/m³ (operating range, 0.5 to 3 mL/m³). The residual concentration was measured by using an Accuro gas-detection tube (Draeger Safety, Leubeck, Germany), which measures acetic acid (range, 5 to 80 ppm) and H₂O₂ (range, 0.1 to 3 ppm), to confirm completion of the decontamination cycle.

Quality-control methods. The effectiveness of decontamination process was assessed by using biologic indicator (BI) strips impregnated with a particularly hardy organism (Geobacillus stearothermophilus spores; Salesworth Synergies, Kamataka, India; Spordex [STERIS]; NAMS, Crosstex, Maumee, OH) and chemical indicator (CI) strips (Steraffirm [STERIS]; H₂O₂ and 4.5% peracetic acid) for decontamination of up to 35,000 ft³. Manufacturer-provided software was used to calculate the amount of water and cold sterilant were required according to the room volume and time needed to achieve desired concentration; the recommended concentration was 1.5 mL/m³ (operating range, 0.5 to 3 mL/m³). The residual concentration was measured by using an Accuro gas-detection tube (Draeger Safety, Leubeck, Germany), which measures acetic acid (range, 5 to 80 ppm) and H₂O₂ (range, 0.1 to 3 ppm), to confirm completion of the decontamination cycle.

Results
Before initiation of biodecontamination, the customized SCADA software system was configured for all cycle combinations, after which decontamination was executed for the scheduled areas as indicated on the facility maps (Figures 1 through 5). Relative humidity was maintained successfully at ≤70% during decontamination by the Minidox-M unit to achieve the desired CI and O₂ concentration (360 ppm at 1 h or 720 ppm at 2 h) for the exposure time based on the room volume. In addition, humidifier units were placed to raise humidity to the desired levels and were automatically controlled by Minidox-M system. For effective decontamination by using the Minncare dry-fog system and HaloFogger, a relative humidity of 65% to 85% and H₂O₂ contact time of 1 h were maintained regardless of the room volume. Conversely, dehumidifier units were used to decrease humidity levels and maintained between 50% to 60%, according to the VHP-Victory preset (H₂O₂ concentration, 150 to 300 ppm; Figure 6). The validation cycles performed in animal rooms, procedure rooms, and other areas demonstrated successful decontamination in 84%, where they achieved 6-log (10⁶) sporicidal reduction, based on the lack of growth from BI strips. The remaining 16% of decontamination cycles appeared to be unsuccessful, in light of growth from 1 or 2 of the 4 to 15 BI in several areas.

Similarly, none of the contact plates from rooms that were successfully decontaminated showed any growth, except for a few unsuccessful cycles for which sample enumeration showed colony growth at an average 1 or 2 cfu per plate. The unsuccessful cycles were rescheduled, and decontamination criteria were met thereafter (data not shown). During the validation process, a total of 265 BI strips, 195 CI strips, and 483 contact plates were used to assess decontamination of 86 different areas in the facility (Table 1). The results from BI strips and contact plates were compared and showed that no growth determined successful decontamination and cycle validation; in addition, 92 positive-control BI strips were inoculated concurrently, and all yielded appropriate turbidity within 24 h. In Figure 7, we note the pros and cons of each method we used. Overall, multiple cycles were performed for rooms with different dimensions and volumes of 1481 to 16,017 ft² and demonstrated successful decontamination associated with integral control of HVAC operation through the interactive process achieved by using SCADA software.

Discussion
The facility presented operational challenges regarding periodic decontamination as well as contact plate sampling, due to many ongoing experiments, which ranged from short-term to chronic studies, thus limiting free access and the ability to empty any particular room for decontamination. Given the complexity of the HVAC units, traditional foggers had previously been used in multiple units after covering doors and supply and return ducts. However, the entire current project was systematically implemented in a fully functional research facility, without compromise of regular operations, ongoing experiments, or cross-contamination. The SCADA system was controlled by trained operators who manipulated the HVAC units as needed to isolate the rooms for decontamination, and the system used equipment with built-in programmable logic controller monitoring to collect real-time data. Automation of the centralized system enabled the performance of as many as 4 independent decontamination cycles daily, in any chosen area and with options regarding using either H₂O₂ or CI.
with a variety of routine laboratory equipment and electronics. VHP was reported to be noncorrosive and compatible with electronic components, achieved a 4.5-log reduction in infectivity, and interpreted as an innovative decontamination approach recommended for prion inactivation, according to experiments designed to mimic decontamination of medical or surgical equipment, including fragile or inaccessible surfaces of complex instruments (endoscopes, laparoscopes).

The hamster bioassay model involved the use of VHP in a sealed container that was directly coupled to a VHP1000

Figure 1. (A) SCADA screenshot of the various units, which operate in parallel and perform different decontamination cycles. (B) SCADA screenshot of 61 programmed decontamination cycles identified according to area or room number (highlighted during operation).
biodecontamination system (STERIS) to maintain a dry (non-condensing) \( \text{H}_2\text{O}_2 \) gas (1.0 to 1.5 mg/L for 3 h at 25 °C); stainless steel wires were exposed to VHP with or without previous treatment with an enzymatic cleaner and implanted (prefrontal subcortical region) in anesthetized Syrian golden hamsters for bioassays.\(^9\) In a more realistic bioassay model based on transmissible spongiform encephalopathy, infectious brain materials (suspension or dried onto the surface of stainless steel wires) were or were not decontaminated in a \( \text{H}_2\text{O}_2 \) gas plasma sterilizer prior to implantation in hamsters.\(^44\) Another study demonstrated

**Figure 2.** (A) SCADA screenshot of Minncare (\( \text{H}_2\text{O}_2 \)) system, with independent supply and return dampers and bypass damper. (B) SCADA screenshot of Minncare (\( \text{H}_2\text{O}_2 \)) in operation, illustrating decontamination and the passage of return air directly through bypass damper when the supply and exhaust dampers are closed.
thorough decontamination of an entire class II biologic safety cabinet, including supply and exhaust filters; prior to decontamination, the cabinet was disassembled so that biologic indicators could be placed at various locations, and then the cabinet was prepared by sealing the front access, exhaust filter openings, supply diffuser, work area grills, to ensure that VHP reached all parts of the cabinet, including filters. VHP exposure of spores of *Clostridium botulinum* (dried spores spread over stainless steel slides and exposed to VHP in a sealed glove box) was found to be effective at deactivating spores of toxigenic *C.*

*Figure 3.* (A) SCADA screenshot of VHP Victory (H$_2$O$_2$) system, with independent supply and return dampers and bypass damper to exhaust return air to isolate the targeted area. (B) SCADA screenshot illustrating VHP Victory (H$_2$O$_2$) decontamination, when the supply air passes through the bypass damper and directly into the exhaust duct.
Biodecontamination in a laboratory animal facility

A set of 3 studies applied dry-mist H$_2$O$_2$ diffusion technology for air and surface decontamination of BSL3 areas where *M. tuberculosis* strain H37Ra was used and demonstrated that viable bacteria were reduced by 5 log and no BI yielded any growth, thus suggesting that this technology is an effective and safe alternative to formaldehyde. In addition, a report suggested that HPV was effective against bacterial spores including multidrug-resistant organisms on nonporous and porous surfaces of clinical areas and eliminated *M. tuberculosis* contamination from a room at a...
Furthermore, experimentally contaminated biologic safety cabinets in a room containing *M. tuberculosis* (3 log) and *Geobacillus stearothermophilus* (6 log) were decontaminated by using HPV (90-min exposure); the results revealed that all BI strips were deactivated for both organisms in all 10 locations. Another study similarly suggested that dried inocula of nosocomial organisms that had survived for as long as 5 wk in a 100-m² room were effectively inactivated by H₂O₂.³⁸

The current validation results of H₂O₂- and ClO₂-based decontamination were compiled from a large number of cycles (86 areas), showed 6-log reduction of BI strips in most cases (84%), and corroborated other experiments reported. All 213 BI strips, 195 CI strips, and 398 contact plates used to evaluate H₂O₂-based decontamination of our facility passed the test criteria. The few rooms that yielded positive BI results (16%), which appeared as mild turbidity in the media because of growth of the indicator organisms, might have been due to inadequate exposure concentration or time or to inappropriate placement of BI strips; however all CI strips, which were positioned next to the BI strips, passed the test criteria. During validation, appropriate residual concentration (0.1 ppm; acceptable level, 1 ppm) was ensured after completion of H₂O₂ decontamination and provided clearance for personnel re-entry. In one study of a dry-fog disinfectant system,²⁴ animal rooms were treated with cold sterilant solution consisting of a stable mixture of peracetic acid and H₂O₂. The dry-fog unit was positioned in the center of a room, after which the humidity was raised 80%; effective decontamination was achieved with no condensation of H₂O₂ in the room or on its components throughout the decontamination process using VHP and peracetic acid, either separately or together.²⁴ However, a separate experiment evaluated the use of VHP (32-min exposure) as a surface decontaminant and sterilant in a centrifuge application and examined its killing activity against spores of *Bacillus subtilis* subsp. *globigii* and *B. stearothermophilus*; the results revealed that VHP showed significant sporicidal capability.²⁵ Similarly, another study revealed that rodent bacterial species (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*) in the presence of 2% BSA on smooth surfaces were inactivated by VHP.²⁴ Although, a new facility biodecontamination demonstrated successful deactivation of all the BI exposed, and none of the lab materials, including electronic equipment, were affected adversely,¹⁶ thus corroborating our observations. Overall, the H₂O₂-based decontamination systems we evaluated were effective for large to medium enclosed areas (VHP Victory and Minncare systems) as well as small rooms, including ancillary areas (HaloFogger system), with an average cycle time of 3 h needed to complete the entire process.
After necessary conditions of humidity were met, ClO₂ decontamination (400 ppm/h) of large animal and neonatal intensive care units (4800 m³) contaminated with *Salmonella* was monitored by using 100 BI strips (*G. stearothermophilus* spores, *B. atrophaeus* spores, or *Salmonella newport* vegetative cells); the subsequent analysis indicated better than 6-log reductions in viability for *B. atrophaeus* and *S. newport* and greater than 5-log reduction for *G. stearothermophilus*, thus demonstrating that ClO₂ is an effective decontaminant under nonlaboratory conditions. Other studies reported that gaseous ClO₂ was effective against food-borne pathogens such as *E. coli*, *Listeria monocytogenes*, and *S. enterica* in the context of the food-processing industries. Gaseous ClO₂ has proven effective against viruses, even at relatively low concentrations with extended exposure time. In addition, a research facility performed ClO₂ decontamination for 65 rooms and noted complete killing of all BI; moreover, no physical residue or material degradation was noted on any of the metal-containing equipment in the building.

At our facility, a ClO₂ concentration of <0.1 ppm typically was present after cycle completion; in few cycles, ClO₂ at <0.3 ppm was detected during aeration phase, either in an adjacent area or at service floors (above the ceilings), where all the ducts for the rooms are connected. We therefore increased the aeration period of those particular cycles to reduce the concentration, especially that for chlorine odor (the threshold for odor from ClO₂ is 0.1 ppm). The 52 BI strips and 85 contact plates passed the test criteria, and ClO₂ (Minidox-M)-based decontamination was effective for large to medium areas of the sealed enclosure.

In a previous study, we investigated the efficacy of ClO₂ gas for environmental decontamination of *Syphacia spp.* ova by using perianal cellophane tape impression of pinworm infected mice; tapes with attached ova exposed to ClO₂ gas for 1, 2, 3, or 4 h and then incubated in hatching medium for 6 h to promote hatching. For controls, tapes with attached ova were maintained at room temperature for 1, 2, 3, and 4 h without exposure to ClO₂ gas and then similarly incubated in hatching medium ova viability after incubation was assessed by microscopic examination. Exposure to ClO₂ gas for 4 h inactivated 100% of *Syphacia* spp. and 17% of the ova on the control slides (unexposed to ClO₂) were nonviable; therefore, the results suggested that exposure of animals rooms to ClO₂ gas for at least 4 h was effective for surface decontamination of *Syphacia* ova. Similarly, an investigation of ClO₂ gas for inactivation of 8 β-lactams involved various concentrations and overall exposure lengths and showed that 5 of the 9 inactivation cycles passed the acceptance criteria of achieving a 3-log reduction (pharmaceutical manufacturer’s required 99.9%) of all 8 β-lactams. Overall, the performed cycles were free of residue by the end of decontamination, thus allowing access into the animal rooms. Together these observations provide support for repeating unsuccessful cycles after rectifying all issues of enclosure containment and refining equipment parameters during the validation process.

In conclusion, the improved engineering controls involving several types of low- to high-capacity equipment provided options for effective, low-temperature decontamination of vivarium facilities by using either H₂O₂ or ClO₂ gas. Implementation of a SCADA system enabled the operation of multiple decontamination cycles in different enclosures, improved process efficiency, and reduced the turnaround time for performing all of the planned cycles within the facility. VHP biodecontamination is a highly effective and safe alternative, because residual H₂O₂ vapor catalytically decomposes into oxygen and water as its final products, which can be directly released into the environment. Similarly, the beneficial properties of ClO₂ gas include excellent distribution and penetration, making it an effective biodecontamination agent for animal rooms that are proven sealed enclosures. Because both the agents have excellent material compatibility, decontamination cycles can be performed with a variety of materials generally used in laboratory animal rooms (racks, cages, trolleys, cage changing stations)
and procedure areas (centrifuge, biosafety cabinets, surgical equipment, anesthesia vaporizer, weighing balance, refrigerators and other equipment). There appeared to be no corrosion or any negative effects on material surfaces, and equipment continued to be functional after the successful decontamination. Nevertheless, each method has its advantages as well as limitations that must be considered, and a balance must be achieved that is based on facility requirements and the enclosure volumes to be decontaminated. Overall, the current automation process enabled users to perform successful decontamination with confidence, by saving time and resources, by generating real-time documentation of cycles, and by providing sufficient scope to deal with potential outbreaks, thus collectively improving the quality-control systems in laboratory animal facilities.

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**Figure 7:** Pros and cons of biodecontamination methods used.

<table>
<thead>
<tr>
<th>Biodecontamination</th>
<th>Minidox-M</th>
<th>VHP-Victory</th>
<th>Minncare</th>
<th>HaloFogger</th>
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<tbody>
<tr>
<td><strong>Pros</strong></td>
<td>a. Large-volume decontamination and real-time monitoring of exposure concentration</td>
<td>a. Software provides real-time monitoring of log reduction relative to exposure concentration</td>
<td>a. Works by air pressure</td>
<td>a. Easy to perform in smaller enclosure</td>
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<td></td>
<td>b. Compatible with vivarium materials and electronic components</td>
<td>b. Compatible for vivarium equipments including electronic items</td>
<td>b. Easy to operate in medium-size enclosure</td>
<td>b. Shorter duration of cycle time</td>
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<td>c. Pure gas uniformly distributes even into crevices</td>
<td>c. Dry process, no condensation after cycle</td>
<td>c. Options to use up to 4 nozzles, thereby reducing overall cycle time</td>
<td>c. Less expensive</td>
</tr>
<tr>
<td><strong>Cons</strong></td>
<td>a. Enclosure preparation needed to prevent gas leak</td>
<td>a. Enclosure must be sealed before use</td>
<td>a. No real-time monitoring of exposure concentration</td>
<td>a. Vapor condensation occurs after decontamination</td>
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<tr>
<td></td>
<td>b. Replacement of cartridges based on its usage</td>
<td>b. Maintenance of humidity is the critical factor for uninterrupted decontamination</td>
<td>b. Vapor condensation occurs when humidity levels are raised beyond set points</td>
<td>b. Unsuitable for electronic items</td>
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<td></td>
<td>c. Unstable in light and flammable</td>
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<td>c. Parameters cannot be monitored</td>
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