

# Japan's RIKEN BSI: Whole Facility Chlorine Dioxide Gas Decontamination Approach for a Barrier Facility—A Case Study

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## Abstract

*This article discusses one of Japan's newest research facilities—the RIKEN Brain Science Institute. The Brain Science Institute (BSI) conducts research that integrates multiple disciplines including medicine, biology, psychology, physics, computer science, and technology, and utilizes several different rodent models in a clean barrier facility. This article discusses the specific layout of the BSI barrier facility, the cleaning procedure used, and the decontamination process that uses chlorine dioxide (CD) gas as a replacement for formaldehyde gas. Diagrams show gas generator injection and sample locations, as well as fan locations. In addition, this article briefly compares the process of using CD gas to other methods of decontamination. Since CD was successful in the decontamination, the entire barrier facility now uses it to maintain the same level of cleanliness that was obtained in the initial process.*

## Keywords

Cleaning, Decontamination, Chlorine Dioxide (CD), Barrier Facility, and Specific Pathogen Free (SPF)

## Introduction

RIKEN is Japan's largest comprehensive research institution, renowned for its high-quality research in a diverse range of scientific disciplines. Founded in Tokyo in 1917 as a private research foundation, RIKEN has grown rapidly in size and scope, and today encompasses a network of world-class research centers and institutes across Japan. For nearly a century, RIKEN has fostered innovative research in fields spanning the entire range of the natural sciences, from developmental biology and neuroscience to quantum physics and computer science.

RIKEN supports research initiatives across Japan with its main campuses in Wako, Tsukuba, Yokohama, Kobe, and Harima offering state-of-the-art facilities that rank among the best in the world. This research environment, combined with a uniquely bottom-up approach to scientific innovation, has enabled RIKEN to foster an environment in which researchers are able to thrive.

BSI conducts cutting-edge research within the four strategic research core areas: mind and intelligence research; neural circuit function research; disease mechanism research; and advanced technology Development. BSI features an interdisciplinary collaborative research system encompassing biology, medical science, biophysics, informatics, mathematical science, psychology, and linguistics,

and is notable for its open, international organization and rigorous evaluations by international research review committees. To conduct this research, RIKEN designed and built its new facility as a barrier facility. This article discusses the new facility at RIKEN's BSI, its opening process of decontaminating with chlorine dioxide gas, and its continued procedures to maintain clean laboratory conditions using chlorine dioxide gas.

## What is a Barrier Facility?

A barrier facility is designed to house animals previously proven to be in good health and built in such a way that any source of contamination does not come into the facility accidentally. A barrier facility is typically part of a larger facility, but sometimes can be a stand-alone facility. Specific Pathogen Free (SPF) rodents (mice and rats) and genetically modified animals are the typical animals that reside in a barrier facility. A barrier facility commonly has an airlock or other appropriate entry system such as an air-shower or pass-through room for materials coming into the facility. All staff must wash hands properly and wear regulated clothing such as sterile and disposable gowns, caps, shoe covers, gloves, and masks prior to entry into a barrier facility (Figures 1, 2, and 3 showing RIKEN BSI proper gowning).

Consumable items, such as animal feed and cage-bedding, are likely contaminated, so they must be decontaminated by an autoclave system or irradiated with gamma at the manufacturing site before delivery. If this is done offsite, the outside of the feed or bedding bags must be decontaminated with an alcohol spray wipe down or other agent prior to entry into the barrier. Drinking water for the animals is decontaminated by autoclaving, chlorine disinfection, or reverse osmosis (RO). Equipment that comes into contact with the animals, such as cages, must be washed and autoclaved. All equipment that enters the facility should be decontaminated prior to entry into the barrier, either through the autoclaves or the CD gassing rooms. Supply air into the facility must pass through HEPA filters. A positive differential pressure is maintained between the barrier facility and its neighboring facilities to ensure that contaminants are kept out. A proper airflow must be controlled and secured between dirty areas and clean areas to reduce the risk of contaminated particles reaching the important barrier area where SPF mice and rats reside. For further protection of SPF mice and rats, isolator cages, individual ventilation cages, and cage exchange stations are used.

Access to conventional areas and SPF areas is limited to further prevent cross-contamination. Access to the facilities is monitored by security card access, and if a staff member enters the conventional areas (Figure 2), the staff member is then restricted from entering the SPF area (Figure 3). This limits the movements of staff from area to area, which in turn limits the potential for contamination.

Training is also part of the barrier facility process. Facility staff must be continually trained on procedures. Even perfectly written procedures are ineffective if people do not follow them. Training is one of the most important tasks for any facility trying to maintain cleanliness.

BSI's new research facility is a 3-story building. The Neural Circuit Genetics Research Building was completed in March 2011 with a total floor space of 9,500 m<sup>2</sup>. The facility houses approximately 20,000 mouse cages and 3,000 rat cages on the second and third floors. The building layout is as follows:

- 1st floor: Six laboratories and approximately 120 staff office area with desks. Equipment and supplies for the animal facility: the bedding supply system, disinfectant-producing system, and bedding waste disposal system
- 2nd floor (Figure 4): Mouse and rat behavior testing suites, imaging suite, automated guided vehicle (AGV) system, cage storage, and cage washing room
- 3rd floor (Figure 5): SPF mouse and rat holding suites (housing rooms), embryo manipulation laboratory, AGV system, cage storage, and staff rooms
- Husetop: RO water machines

## Research

The brain's integration of complex external and internal information is important to control animal behavior. Understanding how the networks of the brain interact to guide behavior is a fundamental goal of neuroscience. The networks are largely defined by genetic and environmental factors, and their differences among individuals underlie differences in personality and temperament. Abnormalities in the networks caused by various factors, including injury, infection, social and physical stress, and genetic factors, result in cognitive deficits and various mental disorders. To understand the networks at multiple levels of analyses, BSI laboratories use a number of rodent disease models and approaches including molecular manipulations, imaging, electrophysiology, and behavioral systems.

The research performed at RIKEN's BSI is done in the mouse and rat holding room and testing suites. The testing suites have a few special features; for example, the embryo manipulation laboratory on the 3rd floor is capable of culturing embryo and sperm (ES) cells to produce genetically modified animals and freezing/immobilizing these ES cells. The 3rd floor animal housing rooms are capable of producing/breeding mice and rats, as well as maintaining the current stock/populations. It has four suites for mice; one suite is approximately 230 m<sup>2</sup> with five holding rooms and one treatment room, and is capable of holding 4,410 cages. Additionally, there are two suites for rats; one suite is approxi-

mately 160 m<sup>2</sup> with four or five holding rooms and one treatment room capable of holding approximately 1,200 to 1,320 cages.

The four behavior testing suites on the 2nd floor consist of six testing rooms, one animal holding room, and one treatment room. All of the suites are soundproof and each testing room has an adjustable lighting system with a high-speed data-handling network. A small animal holding rack is placed so that highly sophisticated behavioral testing, including a maze task, can be performed in an ideal environment. Each behavior testing suite is capable of holding 420 mouse cages or 240 rat cages. The imaging suite on the 2nd floor is 125 m<sup>2</sup>, and consists of three testing rooms and one treatment room. Each testing room has two photon microscopes. The treatment room is capable of holding 70 mouse cages or 40 rat cages.

## Facility Cleaning and Decontamination

Prior to decontamination, cleaning is done to remove dirt and dust from the ceiling, walls, floors, and equipment. Cleaning was performed on all floors. Gaseous decontamination was performed on floors 2 and 3. Floor 2 had a gassing volume of 5,097 m<sup>3</sup> (180,000 ft<sup>3</sup>) and floor 3 had a gassing volume of 6,027 m<sup>3</sup> (212,841 ft<sup>3</sup>). Each floor was decontaminated individually. Since dirt and dust contain many microorganisms, a 100 ppm solution of sodium hypochlorite was sprayed over all surfaces using a spray gun. The dirt and dust were then wiped off. The sprayed solution on the floor was collected by Karcher Water Catcher Model NT-35/1 Tact TE (Singapore) and the dust in the water was filtered using HEPA filters. This cleaning cycle was repeated, conforming to the standardized protocols used by IKARI Corporation (Chuoku, Tokyo, Japan). For equipment that was difficult to spray, a hand cleaning was done first to remove dust and dirt from the equipment, followed by decontamination using an appropriate disinfectant solution such as alcohol (Figures 6, 7, 8, and 9). The equipment was then sealed. After drying, the whole animal facility was decontaminated with CD gas.

To avoid any potential damage to critical equipment such as smoke alarms and temperature/humidity sensors by CD gas, dirt and dust were wiped from the outer surfaces of all equipment, then cleaned by alcohol spray and dried. After drying, the equipment was sealed with masking tape. A mixture of humidity and gas sometimes appears as smoke to some smoke detectors, so this was another reason to seal them. To avoid CD gas leaking from the target chamber, all doors/shutters, elevators, and ducts were sealed with masking and sealing tape.

The relative humidity (RH) in the target chamber also needed to be increased to the set point of 65%, which was achieved by using electrical drive dry fog machines filled with 2 L of water. One or two dry fog machines were placed in each target chamber. Additionally, one circulator fan was placed in each room/chamber to ensure the even distribution of the CD gas in the target chamber. Since CD gas was injected into the hallways of each suite, circulator

**Figure 1**

Gowning procedure to enter the 2F procedure rooms or testing rooms in animal suites.

Please put on the things listed below.

- Shoe covers
  - Blue gown
  - Gloves
- (Only for the time when handling cages and animals)



**Figure 2**

Gowning procedure to enter the 2F animal holding rooms.

Please put on the things listed below.

- Shoe covers
- Blue gown
- Gloves
- Mask
- Hair cap



**Figure 3**

Gowning procedure to enter the 3F animal holding area.

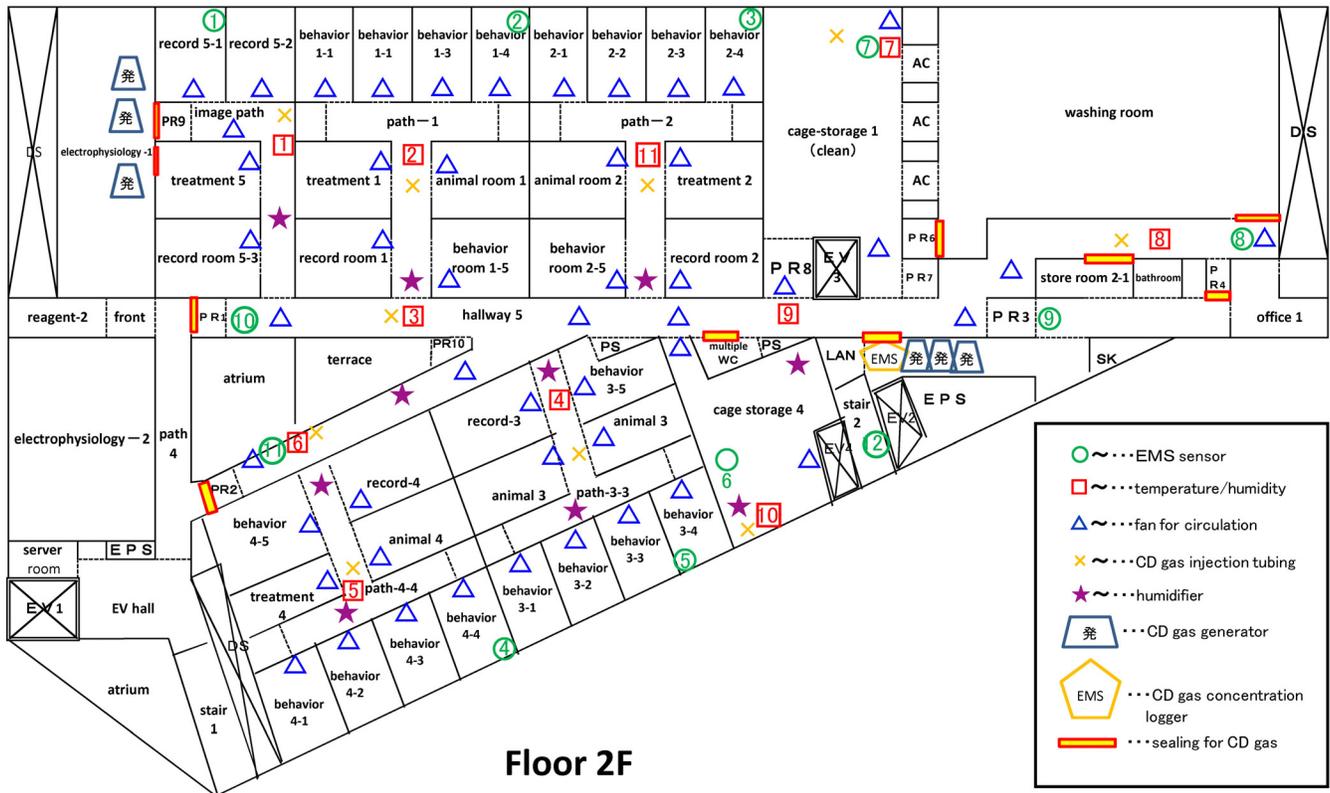
- 1) Please put on the things listed below in the gowning room and wear until you leave the vivarium area.

- Shoe covers
- Blue gown
- Mask
- Hair cap
- Gloves



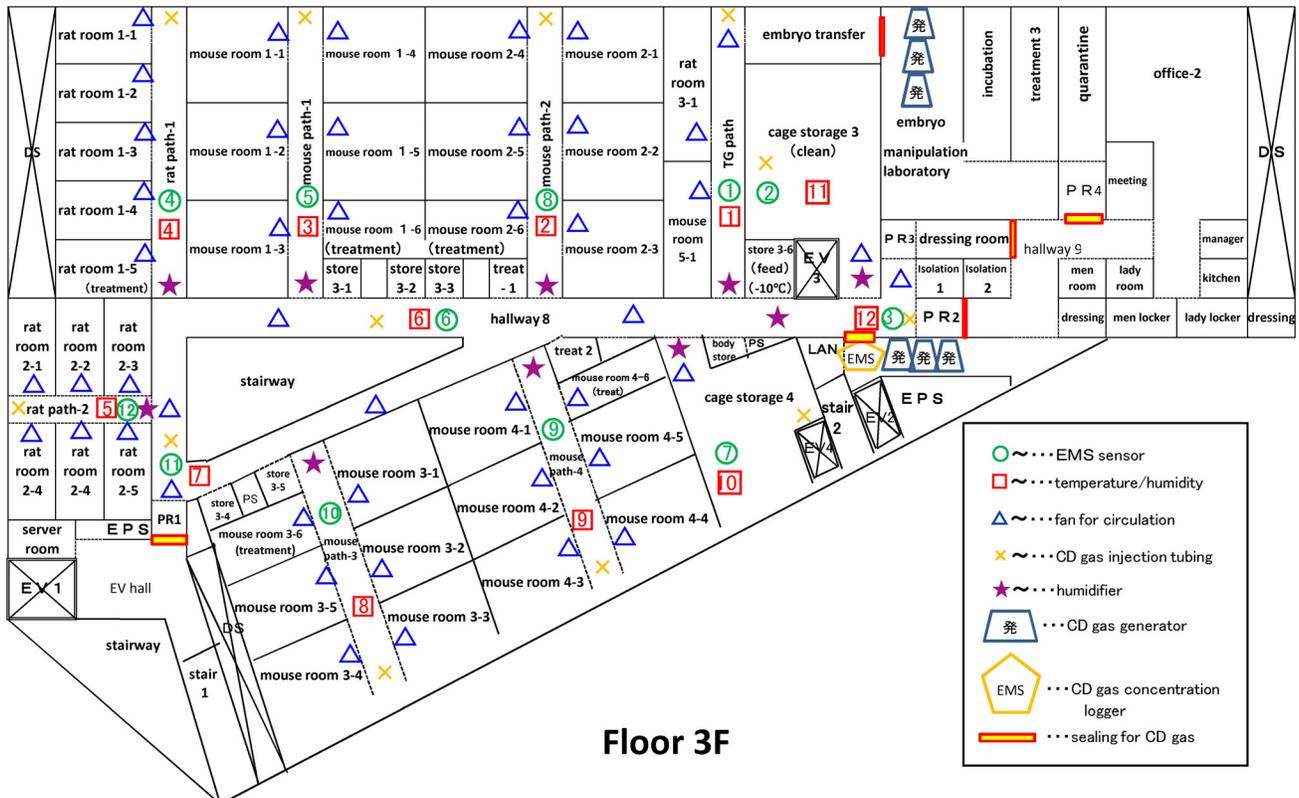
**Figure 4**

Floor 2 layout with sensor, gas injection, fan (circulator), etc., locations.



**Figure 5**

Floor 3 layout with sensor, gas injection, fan (circulator), etc., locations.



fans were placed at the front doorway of each room to assist with effective gas distribution in the target chamber. To test the process, 40 *Bacillus atrophaeus* spore strips containing  $10^6$  spores on a paper carrier wrapped in a Tyvek® envelope (ACD/6, Mesa Labs, Lakewood, CO) were placed around the facility. Twenty biological indicator (BI) strips were placed on each floor at the back of the rooms and farthest from the gas injection points.

Next, the air conditioning was turned off and the airtight damper in interstitial space (ISS) was manually closed. A temperature/humidity logger (Model RTR-53, T&D Corp., Burlington, NJ) was placed at each area and the humidity was raised to the set point of at least 65%. Tubing for CD gas sampling and monitoring was connected to the Environmental Monitoring System (EMS) (ClorDiSys Solutions, Inc., Lebanon, NJ) data logger for

**Figure 6**

Cleaning of walls and floors.



**Figure 7**

Cleaning of floors.



real-time measurement of the CD gas concentration, with one sampling tube for each monitoring point. At this time, tubing for CD gas injection was placed at each individual target point area. A BI and a chemical indicator (CI) to monitor gas distribution were placed in each room (see Figures 4 and 5 for injection, sample, and BI locations).

For this project, six manual CD gas generators (ClorDiSys Solutions, Inc., Lebanon, NJ) were used for the 3rd floor and five were used for the 2nd floor. The manual

gas-generation system was used in conjunction with the EMS. The EMS is a real-time CD gas sensor/monitor with data-log capability that is able to log data from 12 different monitoring points. The CD gas generators and EMS were placed outside the target chamber, and gas injection tubing and sensor tubing was run to the targeted areas (Figures 4 and 5). For even and effective distribution of gas to all areas in the target chamber, all cabinet doors and drawers were opened and circulator fans were switched on. Once all tub-

**Figure 8**  
Spraying walls.



**Figure 9**  
Wiping walls.



ing, fans, and humidifiers were checked and in order, the decontamination-gassing cycle was ready to start. The final step was to ensure all areas were properly sealed, equipment was in order, lights were off, exit doors were closed, and no staff members were present in the target decontamination area. Once the last exit door was sealed and warning signs were placed around the doors to prohibit entry to the restricted areas and after confirming that the target chamber area was clear of all personnel, the gassing of the target chamber began.

Next, gas generation began. Chlorine dioxide gas is generated by passing a very low level of chlorine gas (2% chlorine/98% nitrogen) over solid sodium chlorite, producing pure CD gas and leaving solid sodium chloride in the generator. When the generators were turned on by opening the chlorine cylinder valve, the pressure regulator was checked and the pressure was adjusted to 25 PSI (172 KPa) and the flow meter to 20 LPM. When this was set, the cylinder valve was opened fully to allow the flow of chlorine gas for the production of pure CD gas. To monitor the CD gas concentration, sampling tubing (numbers 1-12) was manually connected. Tubing was switched one tube to the next every 20-30 seconds to log the gas concentration data onto the EMS w/USB memory. When the reading at any sampling data point reached 1 mg/L or 360 ppm, that gas-supply tubing valve was closed to stop the gas supply to that injection tubing point. The exposure was maintained until it reached the set point of a minimum of 720 accumulated ppm hours at all sampling points. The accumulated exposure was calculated by multiplying the concentration by time; for example, holding the 360 ppm for 2 hours is equivalent to  $360 * 2 = 720$  ppm-hrs. When all sampling point readings reached greater than 720 ppm-hours, the exposure cycle was complete.

When exposure was complete in all areas, the aeration cycle began. At this point, a staff member wearing a uniformed gown with a multi-purpose respirator cartridge mask designed for use against organic vapor and acid gases entered the mechanical area to manually open the air-tight damper in the ISS duct. Once the damper was opened, the air conditioning was started to discharge CD gas to the outside environment. Masks were worn in case any leakage occurred in the ISS area. During aeration, the security staff was standing by to avoid anyone coming close to a gas discharge area. The EMS continued to sample the gas until it reached 0.0 mg/L. The aeration continued until the ppm was less than a OSHA safety time weighted average (TWA) of 0.1 ppm. Then, staff wearing gowns and masks (following the procedure outlined in Figures 1, 2, and 3) entered the target chamber, removed all sealing, and removed the equipment (fans and tubing). All BI strips were removed for incubation analysis. The following day, the settling plate sampling and swab tests took place. The results of the settling plates, swab tests, and 7-day BI incubation, following CD gas exposure, have shown successful decontamination by chlorine dioxide gas.

CD gas decontamination requires well-organized prep-

aration but saves a significant amount of time compared to traditional decontamination methods which require the cleaning of residues.

### **Maintaining a Clean Environment and Continued Decontamination Efforts**

To maintain the achieved level of cleanliness and decontamination, the Minidox-M automated CD gas system (ClorDiSys Solutions, Inc., Lebanon, NJ) was purchased. It has been kept in constant use for decontaminating incoming items that are difficult to decontaminate/sterilize by autoclave. Pass-through rooms were built on floors 2 and 3 (Figures 10 and 11) to decontaminate cleaning tools, consumables, behavioral equipment, anesthesia equipment, dissection tools, and many other items. Cycles in the room are run at least once per week, and the time for each run is about 4 hours in total. This time includes about 1 hour for preparation (loading materials in the room), 2 hours for the decontamination/CD gassing cycle, and then about 1 hour more for unloading of the materials.

The CD cycle to decontaminate a chamber is basically the same as the procedure used to decontaminate the entire facility; the main difference is the equipment used. To decontaminate the entire facility, manual equipment is used, but for routine decontamination, automated equipment is used. The automated equipment provides a level of security (password protection), safety (programmable logic controller (PLC) control and alarms), and repeatability (real-time concentration monitoring with feedback) that is not possible with manual equipment. The decontamination cycle used for incoming equipment raises the RH to 65% and holds it for 5 minutes, allowing the RH to stabilize. After the 5-minute conditioning time, CD gas is injected (charge step) to reach a concentration of 1 mg/L. This concentration is maintained for 2 hours. After exposure is complete, aeration is started by allowing fresh air to enter and exhausting the gas outside, a procedure that takes approximately 10-15 minutes.

### **Choices for Decontamination: Vapor Phase Hydrogen Peroxide, Formaldehyde, or Chlorine Dioxide Gas**

The latest-generation fumigant is CD gas. One of the benefits of CD gas is that it is a true gas at room temperature. It has been used for a long time in studies (Han, 2000; Jeng, 1990), isolators (Czarneski, 2008; Eylath, 2003), processing vessels (Eylath, 2003b), juice tanks (Han, 1999), HEPA housings with small tubing (Devine, 2009), biological safety cabinets (BSCs) (Luftman, 2008a; NSF International, 2008), rooms (Leo, 2005; Sawyer, 2010), and large facilities (Czarneski, 2007; Luftman, 2008b). It is a gas at room temperature (boiling point 11°C) like formaldehyde, and is not considered to be carcinogenic by the International Agency for Research on Cancer (IARC), Network Time Protocol (NTP), the Occupational Safety and Health Association (OSHA), or the American Council of Governmental Industrial Hygiene (ACGIH).

With barrier facilities maintaining cleanliness in all

**Figure 10**

Vivarium B pass-through room with the Minidox-M chlorine gas generator connected.



**Figure 11**

Shelving inside the decontamination pass-through room.



areas is very important. A variety of methods can accomplish this; the most common method, but the least effective, is spray and wipe. This method relies on the user to spray and wipe a disinfectant or sterilant on every surface. The inherent problem with this method is that most rooms or facilities have many pieces of equipment (lab benches, cabinets, sinks, animal racks, cages, etc.) with a variety of surfaces (horizontal, vertical, round, flat, etc.), and to ensure that every surface is sprayed and kept wet for the prescribed amount of time is practically impossible. Because of the problem with reaching every surface, gas is commonly used for sterilization. For example, formaldehyde reaches all surfaces and fills the volume of the space as true gases do. However, while formaldehyde is very effective at achieving the necessary kill to maintain cleanliness, it creates residues. Residues are formed when the gas contacts cooler surfaces and causes a repolymerization back to paraformaldehyde, and additional residues are formed at the end of the exposure when the gas is neutralized (Luftman, 2005). Another drawback of this method is that formaldehyde is classified as a known human carcinogen (IARC, 2004).

Because of the issues with formaldehyde, vapor phase hydrogen peroxide (VPHP) was developed. This method typically starts with a 30%-35% solution of hydrogen peroxide that is heated or vaporized to the boiling point. The VPHP is then delivered through hoses to the target chamber. Some generators can be placed inside the target chamber. Since it is a liquid at room temperature (boiling point 108°C), the VPHP will return to its normal liquid state. Because of this, the distribution and penetration are not as efficient as a true gas (Devine, 2009; Herd, 2005; Sawyer, 2010; Shearrer, 2006; Steris Corp., 2000). When it returns to a liquid state, it does so as condensation, which is one of the issues with VPHP. Vapor phase hydrogen peroxide condensate has led to corrosion and damage to painted surfaces and floors (Feldman, 1997; Hultman, 2007; Malmborg, 2001; Sawyer 2010).

Limitations with other forms of sterilization led to the next-generation decontamination method, CD gas. Chlorine dioxide, like formaldehyde, is a true gas. Chlorine dioxide gas does not have the issue of residues and is not carcinogenic like formaldehyde. Chlorine dioxide will not repolymerize on surfaces and it does not have the condensation issues associated with VPHP, so it combines the best of both processes with high efficacy and fewer drawbacks.

## Conclusions

Much research at BSI has been devoted to the investigation of the mechanisms of cognitive and emotional function, and the use of experimental animal models to study pathogenic mechanisms and potential therapeutic methodologies. It is worth special mention that the Neural Circuit Genetics Research Building, with its laboratory animal facility, is quite a large-scale facility, capable of holding 20,000 mouse cages and 3,000 rat cages. Its research and testing facility is equally as large. These facilities are ex-

pected to facilitate research in behavior testing and are expected to yield exciting results. However, to obtain consistent, repeatable outcomes, many genetically-modified laboratory animal strains must be housed and maintained under well-controlled, clean, or pathogen-free conditions. Any bacteria and/or parasitic worms from the outside must not be introduced as they will cause disease to the laboratory animals that can be spread from animal-to-animal or from personnel-to-animal. To avoid such bacteria and parasitic worms from entering the facility, all materials used for the laboratory animals, including testing equipment, must be sterilized and decontaminated before coming into the facility. The BSI Neural Circuit Genetics Research Facility has chosen to use CD, and this method of decontaminating the facility has been performed safely and successfully with shorter cycle times compared to formaldehyde. After decontamination, no noticeable corrosion or damage to the facility's materials, to workers' clothing, or to animal equipment in the facility has been observed. Also, no damage or malfunction to the computers or to the precision analytical equipment has been observed after decontamination by CD. Chlorine dioxide is a good alternative to formaldehyde for decontamination because it is not a carcinogen, it requires shorter cycle times, it is safe to operate under proper conditions, and its decontamination results are thorough, consistent, and repeatable. CD has also helped the facility reduce labor costs as well as improve compliance to the required regulations for facility management.

## Acknowledgments

The authors would like to thank Takayuki Kuroda for his help with English to Japanese and Japanese to English translations. \*Correspondence should be addressed to Mark A. Czarneski at [markczarneski@clordisys.com](mailto:markczarneski@clordisys.com).

## Disclosure

Financial Disclosures: Eiki Takahashi is employed by RIKEN Brain Science Institute, Wako, Saitama, Japan; Mark A. Czarneski is employed by ClorDiSys Solutions, Inc., Lebanon, New Jersey; and Akihoki Sugiura is employed by IKARI Corp., Chuoku, Tokyo, Japan.

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