

Exposure Core

Purpose: To expose mice, rats, and epithelial cells to chlorine (Cl_2) gas under tightly controlled conditions.

A.1. The Chlorine Exposure System: This approach will be used when experimental protocols call for exposure of a small number of animals (1-2 rats or up to 5 mice) to chlorine gas. Animals are placed inside glass chambers (Specialty Glass, Inc. Houston Texas) (**Figures 1A and 1B**) and exposed to Cl_2 gas as shown in **Figure 2**. Two mass flow controllers (MFC) with Kalrez seals (Scott Specialty Gases, part # 05236A1V5K) and a microprocessor control unit (Scott Specialty Gases, part # 05236E4) are used to control the volume of compressed air and Cl_2 delivered to the exposure chamber. Communication between each MFC and the control unit is established with an RS-232 cable. Cylinders of medical grade air and 1000 ppm Cl_2 (in air) are purchased from Airgas, Birmingham, AL and delivered directly to the facility. The Cl_2 and air gas cylinders are connected to the MFCs via stainless steel and teflon tubing, respectively. MFCs accuracy is checked against a bubble flow meter weekly. Gas flow from each MFC is first directed through a 3-way mixing device (which provides turbulence and eliminates any laminar streaming into the chamber) and subsequently delivered to the chamber via heavy walled Teflon tubing as shown in **Figures 1A, 1B, 1C**.

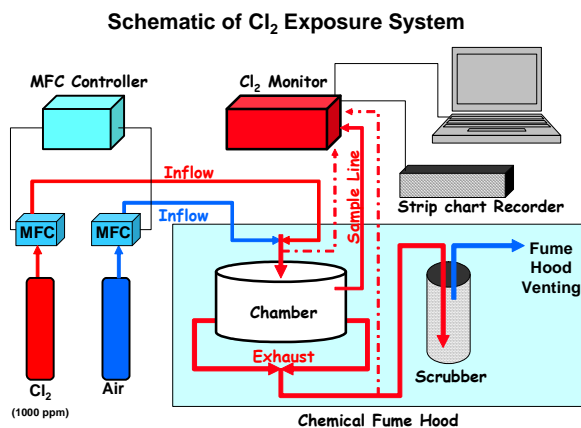


Figure 1. (A) Schematic diagram showing important features of the Cl_2 exposure chamber. Air and Cl_2 mix before entering the chamber; further mixing is achieved inside the chamber while they pass through a diffuser. Total gas flow rate in the chamber is 5 L/min which allows for about 1 full exchange of the chamber atmosphere per min. **(B)** Photograph of a chamber housing mice. Water bottle is removed during exposure to Cl_2 and the port used for continuous monitoring of Cl_2 concentration (see text for details). The gas diffuser is seen on the top lid of the chamber. **(C)** Chamber inside a chemical hood, gas cylinders on right.

Figure A: Schematic of Cl_2 Exposure System. See text for details



B



C

Upon entering the chamber, air and Cl_2 flow through a specially designed "diffuser" (see **Figures 1A, 1B and 1C**) which provides further mixing and assures equivalent gas phase concentrations throughout the chamber interior. All gas fittings are routinely leak tested. The Cl_2 concentrations delivered to the chamber, inside the chamber, and exiting the chamber, are monitored with an Interscan Corporation (model # RM34-1000m) Cl_2 detector, connected to an IBM computer for real time display of the concentration and data storage. The

exposure chamber and the detector are located inside a chemical hood. Gases exit the chamber via two ports located at the chamber bottom (which further enhances even gas distribution inside the chamber).

The exit ports are connected to equal length, heavy walled Tygon tubing joined by a 3-way connector with the then single exit flow proceeding through an activated charcoal scrubber before being vented to the roof of the building. The chemical hood is located inside one of the Exposure Facility rooms, which are maintained at lower ("negative") pressure relative to the Facility suite. The concentration of Cl_2 in the room is monitored continuously with another Interscan LD series, mounted on the wall, adjacent to fume hood. Data are electronically captured and stored in a portable computer and are also collected via a strip chart recorder. An additional probe for the continuous recordings of temperature and humidity inside chamber will be installed. Stored data is downloaded from the analyzer to a data acquisition computer where it is statistically analyzed and archived to CD.

The Director and senior staff personnel have off-site secured access to the data acquisition computers to permit remote assessment of exposure conditions. Via this experimental approach we have been able to conduct whole animal exposures with essentially a square wave pattern. Due to the intra-chamber flow patterns and the rapid turnover rates, both the rise time and chamber washout occur rapidly, thus exposures are conducted under relatively steady-state conditions with respect to the gas phase Cl_2 concentration. Displayed above is a typical exposure pattern with animals in the chamber. Cl_2 gas was initiated at time 0 and stopped at the time indicated by the blue arrow. Gas phase sampling was conducting by direct withdrawal from the chamber interior by the analyzer and values recorded every 10 sec. we are also constructing two small stainless steel chambers, similar to the Hinners chambers described above, that will permit exposure of 3-5 rats or up to 10 mice at once. The chambers will be operated under negative pressure and exhaust gases scrubbed before venting.

A.2. Exposure of cells to chlorine: The *In vitro* Exposure Facility. This exposure system permits controlled exposures of cells to Cl_2 and mimics the animal exposure system as closely as possible. We utilize a water jacketed CO_2 incubator equipped with a glass exposure chambers (\approx 4 liters internal volume) to expose confluent mono-layers of human, rat and mouse lung epithelial cells to Cl_2 . The glass exposure chamber is mounted on rocking tables to permit controlled tilting of cultures dishes, if needed. Cell cultures can either be exposed on a level plane or with cyclic tilting which produces an intermittent thin film over the elevated half of the cells. Because the lung surface is not quiescent due to ventilation induced anatomic changes and mucociliary activity, the tilting protocol is not dissimilar from what occurs *in vivo* and allows a volume of apical media that provides a sufficiently large pool of reactive substrates to mimic the extracellular cellular reactions that occur within the ELF. Reactive gases such as Cl_2 likely undergo appreciable extracellular reactions so that it is important to consider the chemical species that directly contact the underlying epithelium. However, polarized cells cultured under conditions that facilitate differentiation (gas/liquid interface), as is the case for studies proposed in this application are exposed without tilting. Using mass flow controllers, 95% air / 5% CO_2 is metered through a gas dryer and molecular sieve, followed by a humidifier (containing sterile water) that is mounted inside the incubator, wrapped with heat tape, and heated to 40°C (**Figure 2**). Just prior to entry into the glass chamber, Cl_2 gas (Scott Specialty Gases; \sim 1000 ppm in air) is injected countercurrent to the inflowing 95% air / 5% CO_2 gas mixture after which it flows through a diffuser mounted on the inside of the chamber lid. Flow proceeds from the upper portion of the chamber to dual exit ports that are located at the bottom. Culture dishes are secured onto a stainless steel perforated shelf located above the chamber floor. Culture dishes receive the same amount of Cl_2 regardless of their position within the chamber. Flow rates are adjusted so the chamber volume is exchanged approximately 30 times per hour. Temperature inside the chamber is maintained at 37°C and approximately 95% humidity. We have determined that cultures only minimally dehydrate during extended ($>$ 12 hr) exposure periods. The Cl_2 concentrations are measured prior to entering and after exiting the glass chamber, as described for the animal exposure facility (see above). Exposures are initiated only after the downstream concentration is equivalent to the upstream, indicating that the chamber system has equilibrated (conditioned). Samples are actively removed either by the analyzers or manual sampling but are passed through a countercurrent drying tube to prevent water build up and interference within the analyzer. This device (MD Gas Dryer™, constructed from Nafion® polymer by Perma Pure Inc, Toms River, NJ) utilizes a countercurrent flow of dry N_2 gas to remove water vapor through a selectively permeable membrane. We have detected no loss of reactive gases (e.g., O_3 or NO_2) during transit through this device. Exhaust gas from the chamber is passed through a bleach bath (to inactivate aerosolized biological agents) and a charcoal scrubber before being ducted to the fume hood. During exposures, the chambers are monitored for pollutant concentration, temperature, and humidity. Post

exposure, the chambers are removed and sterilized. Data are acquired, analyzed, and stored similar to the animal chamber system.



Figure2. Photograph of facility for exposing cell to Cl₂. A glass exposure chamber is sitting on a platform rocker (bottom) and can be tilted during exposure. The heating mantle and air/gas humidifier are shown in the left of the glass chamber. The glass door is closed during exposure. The two tygon tubes from the sides of the chamber vent to the inside of a nearby Biosafety Level Two hood.

A.3. Safety and Security Precautions: Facility Safety - The facility air handling incorporates 100% exhaust to prevent toxicant release into the building in the event of a system failure. Building conditioned air flows into all four rooms but within the facility, the pressure differential is from the suite hall to the chamber rooms, where it is exhausted. The procedure room also contains a fume hood that provides additional exhausting. The facility contains an electrical “safety” circuit that monitors the pressures in both the input and exhaust ducts to the chambers. In the event of a system failure (e.g., a blower fails, a power outage, etc.) and pressure falls below a set criteria, all electrical systems automatically shut down and any electrical device plugged into the safety circuit will be inactivated. The Cl₂ mass flow controllers, and high-pressure pneumatic-actuated valves (installed in the delivery lines), which automatically shut during power loss, are all controlled via this mechanism. Furthermore, a smoke detector in the suite exhaust duct is also linked to the safety circuit and initiates a shutdown if activated. The safety circuit is permanently connected to an alarm system that is monitored at all times by UAB Physical Security. Upon shutdown, the facility Director and manager are called and paged to alert them of a problem. The system requires a manual restart so that designated personnel must physically enter, inspect the facility, and analyze the problem before toxicant generation can be re-initiated. During system shutdown, animals remain essentially unaffected for at least 12 hours.

Access to the Facility is tightly controlled. The door to the hallway is locked at all times and only approved personnel are permitted access. A separate access card is needed to enter the facility. Furthermore, BMR II doors are locked after business hours and access is limited to those with card keys. For safety reasons two persons will be present in the room during each exposure. All personnel will be trained and certified by the UAB office of Occupational Health & Safety. We have exposed more than 200 rats and 100 mice to Cl₂ concentrations ranging from 50-400 ppm for 30-45 min during the last year with no adverse incidents.