Operation and Performance of the Gas In-Vitro Exposure System (GIVES)

Supplement to manuscript APC-2012-1:

Gaseous VOCs Rapidly Modify Particulate Matter and Its Biological Effects: Part 1, Simple VOCs and Model PM

GIVES Components

The Gas In Vitro Exposure System (GIVES) uses commercially available components. The modular incubator chamber (MIC; Billups-Rothenberg, MIC-101, Del Mar, CA) is an air-tight, eight-litre, two-part, polycarbonate chamber (31 cm in diameter, and 11 cm high) with a removable top and a gas-tight clamping ring. Removing the top permits open access to the plastic lattice mid-floor over the bottom portion of the chamber. Cell culture plates are placed on this grid during exposure (Fig. S1a). This unit was adopted because it has been commonly used in gas-cell exposure studies (Billups-Rothenberg, 2012). The chamber is operated in a temperature controlled incubator, as shown in Fig. S1b (maintained at 37°C). Air flows out of the MIC at the same rate that it enters. Below the plastic lattice mid-floor is a 2-cm deep cavity for air mixing from the 0.6 cm inlet and outlet tubes that extend partially across the diameter of the chamber bottom and open into the cavity space below the mesh (Fig. S1a). Air then flows up though the lattice into the top part of the chamber and randomly circulates over the open cell culture plates.

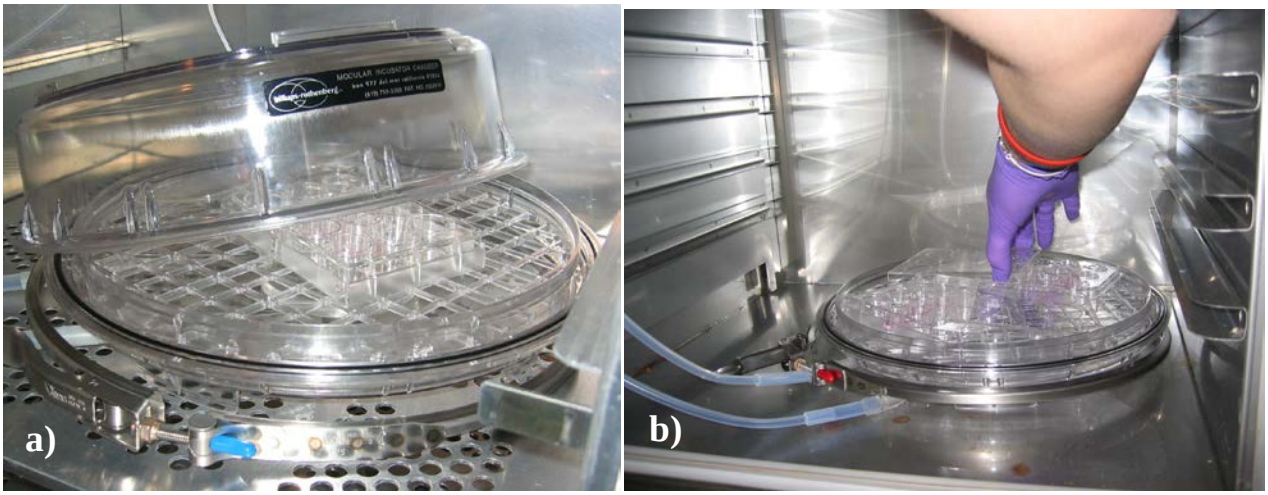

Figure S1. Billups-Rothenberg modular gas exposure chamber housed in a tissue culture incubator.

For cellular exposure, one or two multi-well tissue culture plates are placed into the eight-litre chamber, on the mid-floor (Fig. S1). Due to the open design of the MIC, plates with any number of cell culture membranes can be used, thereby providing flexibility in meeting the needs of any experimental design. Typically in the GIVES, six- or twelve-well plates are used with Transwell (Costar, Cambridge, MA) cell culture membranes (similar in design to those shown in Fig. S2a). This allows cells to be exposed at air-liquid interface while the bottom volume of each cell culture well (below the membrane) is filled with

appropriate media for the cells. Each Transwell has cells plated on its porous membrane (Fig. S2b). The Transwells used in the work described in the manuscript associated with this supplement were 12 mm in diameter, with the membrane on which cells are grown 17 mm below the top of the well. The membrane area was 1.12 cm^2.

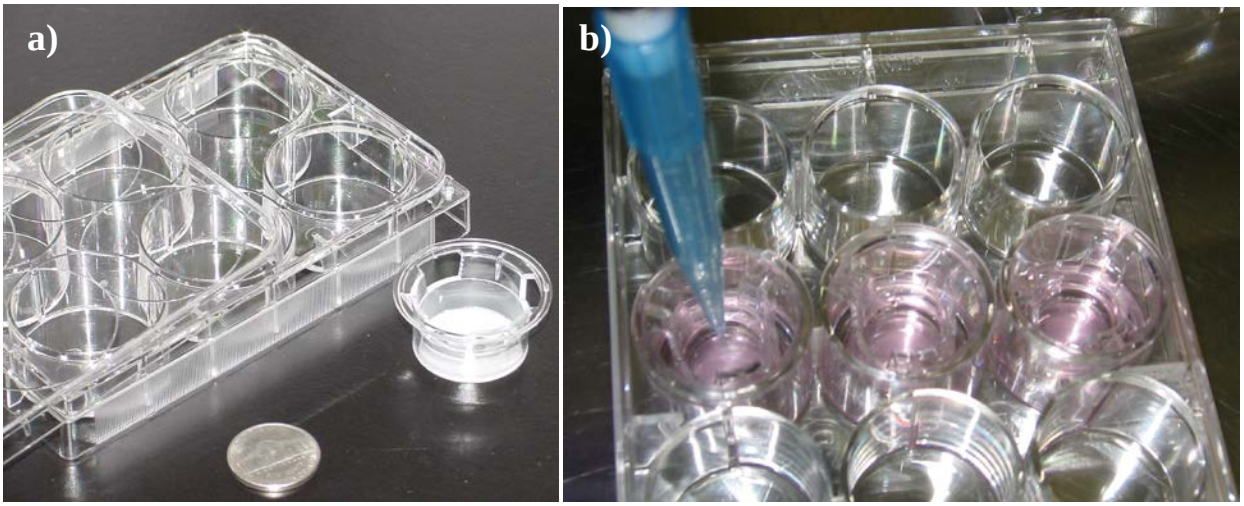

Figure S2. a) Costar Transwell cell culture membranes (24 mm diameter, 6-well tissue culture plate); and b) Adding cells to 12 mm diameter membranes (in a 12-well tissue culture plate).

GIVES Flow Regime

The inlet of the MIC is connected via Teflon tubing to a sampling manifold coupled directly to the roof-top smog chamber just above the laboratory (Fig. S3 and Fig. 1 of the associated manuscript).

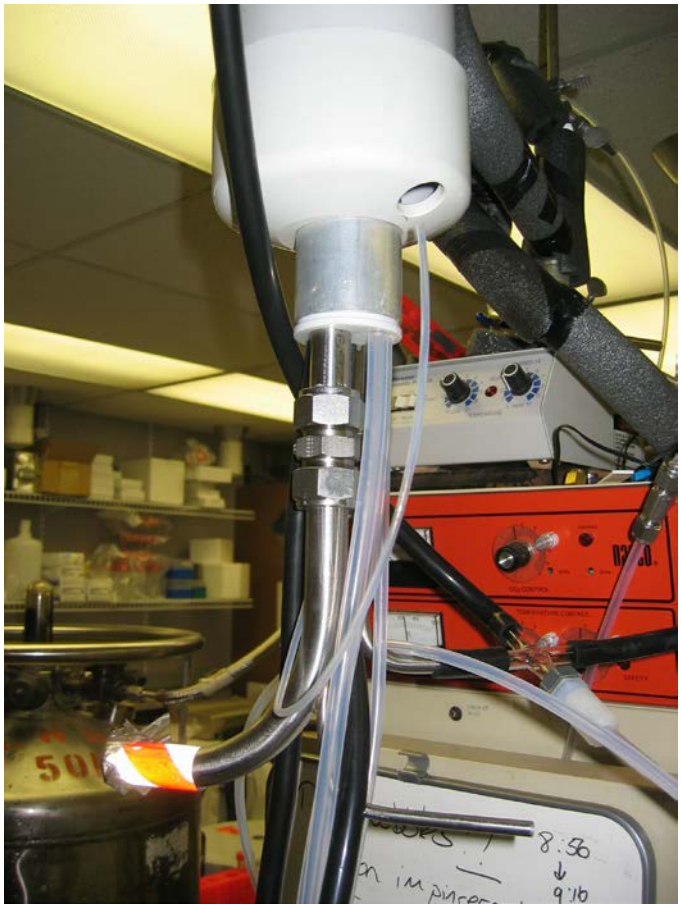

Figure S3. Multiple conditioned sample lines from the roof as they enter the laboratory immediately below the chamber.

An idealized model of the interior of the MIC for our operating conditions is a Continuous Stirred Tank Reactor (CSTR; mass balance described below). In this model: a) gases and particles flow in the reactor volume and are mixed with previous gases and particles; b) gases undergo chemical reaction, surface loss, or uptake by cells; and c) well-mixed gases and particles flow out of the reactor. In an ideal reactor, the mixing is assumed to be instantaneous. In practice, a generally accepted approximation of this condition is the mixing is complete after flowing once around the reactor under turbulent conditions. In the MIC, the lattice mid-floor that separates the inlet/outlet area from the cell exposure area assures that sufficient turbulence or shear is introduced to give random mixing. As a result of the (near) ideal mixing, the composition of the gases and particles is the same everywhere in the reactor and in the exhaust.

A mass balance on the modular incubator chamber is

$$\text{accumulation} = \text{flow in} - \text{flow out} - \text{reaction loss} \tag{1}$$

$$V\frac{\mathrm{d}\,C}{\mathrm{d}\,t} = fC_{in} - fC_{out} - Vr \tag{2}$$

where

V	is the volume of the chamber, liters
$\mathrm{d}\,C/\mathrm{d}\,t$	is rate of concentration change, (mass/liter)/min
f	is the flow rate of gas and particles into and out of the chamber, liters/min
C_{in}	is the concentration of gas and particles in inlet flow stream
C_{out}	is the concentration of gas and particles in outlet flow stream and everywhere inside the chamber due to ideal mixing
r	is the rate of loss by deposition or reaction of C inside chamber

Rearranging and letting $Q = f/V = 1/\tau$, where τ is the average residence time of a molecule of C in V,

$$\frac{\mathrm{d}\,C_{out}}{\mathrm{d}\,t} = Q(C_{in} - C_{out}) - r \tag{3}$$

After approximately 7τ, $\mathrm{d}\,C/\mathrm{d}\,t = 0$ and a steady-state in which flow exactly balances loss by deposition or reaction will be achieved. For a simple, first-order loss process, with a rate constant of k, equation 3 can be integrated to give

$$C_{out} = \left(\frac{Q}{Q+k}\right)C_{in}(1 + e^{-(Q+k)t}) \tag{4}$$

The exponential term will have decayed away after $t > 7\tau$ and

$$C_{out} = \left(\frac{Q}{Q+k}\right)C_{in} \tag{5}$$

A mass balance on the chamber is described by equation (2) and leads to a definition of a time constant, $\boldsymbol{\tau}$. For our system (with f = 1.0 L min^{-1} and V = 8.0 L) has a $\boldsymbol{\tau}$ value of 8 min and, with steady concentrations in the inlet flow, leads to stead-state concentrations in the MIC for times greater than 56 min. Exposures in the MIC are 3–5 hours in duration with nearly constant chamber conditions during exposures. As stated in the Methods section of the manuscript associated with this supplement, exposures in this work lasted 4 hours.

GIVES Replicate Exposure Results for Clean Air and for Gas Mixtures

To account for variability in each new cell passage, which might have a slightly different absolute response, for each exposure test there are paired 'incubator' and 'clean air' control exposures that are processed in parallel with the pollutant-exposed cells. Cytokine analyses produce quantitative measures of the cytokines expressed due to exposure, and always include results from the unexposed cells. Results are then reported as a ratio of the test exposure response to the clean air exposure response (pollutant exposures are normalized by their associated clean air exposure). This is often referred to as a "fold increase over controls" in the toxicological literature. In Figs. S4 and S5, this "fold increase" is plotted for inflammatory response (IL-8 expression) for each individual cell membrane exposed in plates for two different gas-phase exposures. Each time new cells are acquired from a cell culture supplier, they undergo standard exposure tests to clean air (‘zero effect’ exposure) and to 400 ppb ozone (‘positive response’ exposure) for 4 hours before being used in real exposure experiments. The white bars in Fig. S4a show IL-8 expression as fold increases for a 4-hour exposure to ‘clean’ chamber air flowing through the GIVES (refer to Methods section of the associated manuscript for details on ‘clean air’ preparation). The blue bars in Fig. S4b show IL-8 expression for fold increases for exposure to photochemically aged SynUrb54 VOC/NOx mixture with no PM (ozone, NO_x, and secondary VOCs including aldehydes) flowing through the GIVES (see condition E in Table 2 of Ebersviller, et. al, 2012a). Figure S5 shows well-by-well results for a 12-well plate holder for one such an ozone test. The replicate numbering scheme in Figs. S4 and S5 follow the layout shown in Fig. S6.

Another way to interpret the results shown in Figs. S4 and S5 is that these data illustrate a combination of the uniformity in cellular response and gas conditions over the exposure plate. In the tests included in Fig. S4, only half of the plate contained membranes (the remaining wells were left empty), while the test shown in Fig. S5 had membranes in all 12 wells. Results over these and many other tests with filled and partially filled 12-well plates show no discernible systematic differences in response associated with the location of a cell membrane on the tissue culture plate.

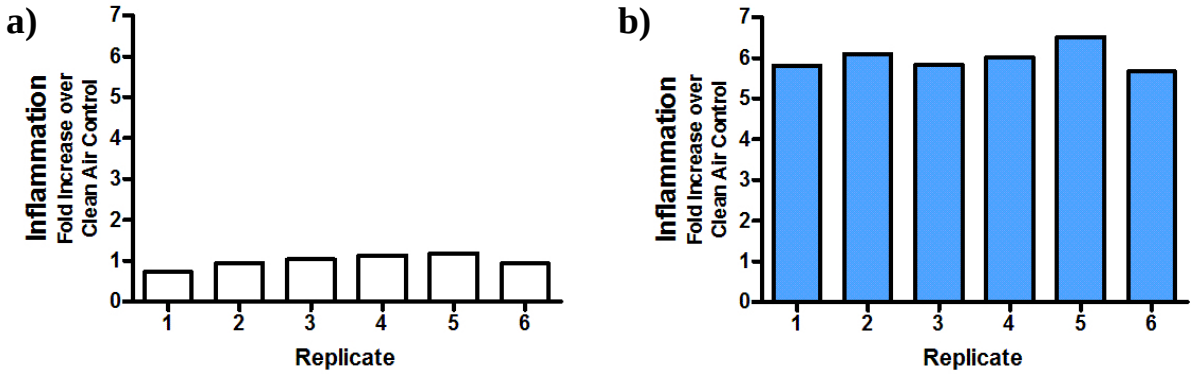

Figure S4. IL-8 (inflammation) individual well relative responses for 4-hour GIVES exposures to: a) 'clean' chamber air exposure, Standard Error (S.E.) = 6.6%; and b): Aged SynUrb54 VOC/NOx mixture, S.E. = 12.3%.

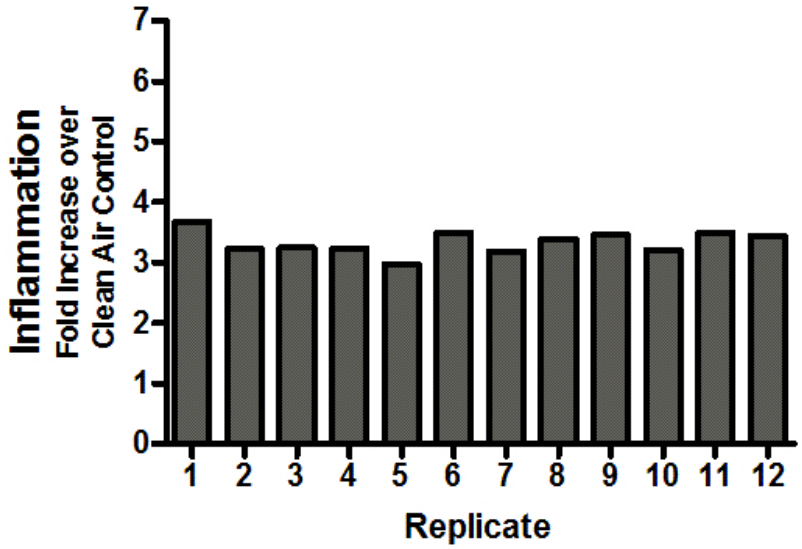

Figure S5. IL-8 (inflammation) individual well relative responses for 4-hour GIVES exposures to 400 ppb ozone in clean air, S.E. = 5.5%

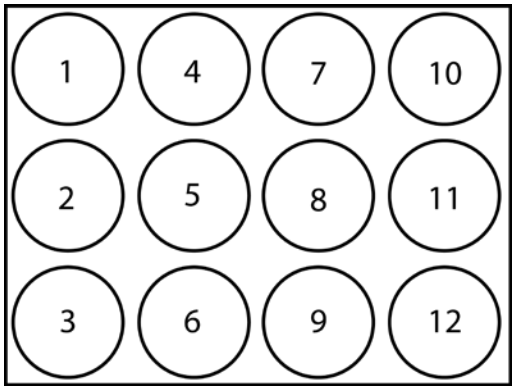

Figure S6. Physical location of replicates on a 12-well tissue culture plate (by ID number)

Exposure of cells in GIVES to PM

As described in the associated manuscript, particles were present in the sample stream that went to both the GIVES and the EAVES. PM with diameters less than 1000 nm are hard to collect on cell-culture membranes without external forces being applied. The mineral oil aerosol (MOA) used in this study was typically at a mass concentration of 1.4 mg m^{-3} in the chamber, and the measured number-mode diameter was ca. 200 nm, which is similar to measured distributions of diesel exhaust particles in previously reported work (that range from 200-500 nm; de Bruijne, 2009). By employing particle charging and a high voltage electrostatic field, the EAVES sampler deposited ca. 1.5 μg of PM (per well) from the sample stream in 1 hour. The only significant forces acting in the GIVES, however, were gravity and the mixing of new and old air volumes caused by the 1 L/min sample flow. As was explained in the section above, there is no organized flow over the cell wells. The measured cellular responses imply that the conditions above each well were similar (on average).

Moreover, gravitational settling velocity for the mass-mode-diameter particles in the test conditions (ca. 1000 nm, at 82 particles per cm^3) is 2.86 x 10^{-3} cm-s^{-1} (Hinds, 1999). Turbulent air mixing length exceeds this distance. Nevertheless, *if we make the counterfactual assumption that air in the MIC was motionless*, we could estimate the time required to achieve the same PM mass deposition in the GIVES that we obtained in the EAVES in 1 hour. If there were no flow and the air column were maintained above the cells, it would require ca. 150 days to obtain the same mass deposition in GIVES as was collected in the EAVES in 1 hour.

At the other extreme, *if we make a counterfactual assumption that every particle entering the GIVES were deposited uniformly over the entire sampler floor surface (including the cell wells),* we would still need 12 hours to collect the same mass as was collected in the EAVES in 1 hour. Of course, there is no force or mechanism in the GIVES that would result in all or even a significant fraction of PM being deposited. Thus, cells in the GIVES *do* have some (likely very small) exposure to PM in the air stream, but the extent is so limited that it cannot be detected by biomarker changes.

These conditions in the GIVES make this sampler ‘virtually’ a gas-only sampler (when the PM present is smaller than 1000 nm).

References for Supplement

Billups-Rothenberg: http://www.brincubator.com/faqs.htm, last access: 8 December 2012.

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