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Project Summary

HERL Biological Exposure Chamber Conceptual Design: Technical Note

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Because of the current interest in biotesting of potentially hazardous air pollutants, the Health Effects Research Laboratory (HERL) of EPA/RTP has contracted Radian to design biological exposure chambers that can be used to expose test organisms to the secondary aerosol effluent of the MARC (Mobile Aerosol Reaction Chamber). The purpose of this technical note is to describe the conceptual design of the biological exposure chambers.

The three organisms HERL desires to use for bioassays will be exposed in four different ways. They are (1) *Salmonella* in Petri dishes, (2) *Salmonella* in Erlenmeyer flasks (3) *Drosophila* in nylon mesh cages, and (4) *Tradescantia* cuttings in pots and/or beakers. The physical environment within the four exposure chambers will be controlled to expose the organisms with minimum stress and within published tolerance limits. The four streams that HERL would like to test for biological activity are: (1) the diluted source stream; (2) the filtered MARC exit stream; (3) the unfiltered MARC exit stream; and (4) the clean air supply.

The report describes the chamber design and rationale behind the design. The report also discusses the connecting of the biochambers to the MARC.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

Radian Corporation is currently under contract to the Environmental Protection Agency (EPA) to design, build, and test a Mobile Aerosol Reaction Chamber (MARC) to generate secondary pollutants from advanced fossil fuel technologies.

HERL desires to expose several organisms to the MARC effluent, including *Salmonella, Tradescantia,* and *Drosophila*. Radian has designed the HERL Chamber in such a way that the organisms can be suitably exposed and the MARC is unaffected by the chamber operation. The process of designing the chamber required consideration of the following:

- Insuring that the conditions within the chamber are conducive to the growth and survival of the organisms and consistent with established experimental protocols,
- matching the HERL Chamber to the MARC so that the aerosols are introduced into the chamber with minimum impact on the MARC, and
- designing a sampling system so that certain specimens in the

chambers can be treated in such a way that their exposure to the chamber environment is stopped while the remainder of the specimens are continuously exposed.

Sections 2 and 3 of the technical note are concerned with the conditions within the chambers. Section 4 is concerned with connecting the biochambers to the MARC, and Section 5 is concerned with sampling the organisms in the chambers.

Size, Shape and Mixing

The size and shape of the HERL biological exposure chamber will be determined mainly by the constraints of the trailer presently housing the MARC. While the chamber must be designed to perform the functions HERL desires, the exposure chamber must fit into the available space in the trailer and not perturb the operation of the MARC.

Radian recommends a rectangular configuration for the chamber shape. While this shape will not be the best for mixing the contents of the chambers, it will make the best use of the available trailer space. A cylindrical vessel that would fit in the same space and give better mixing performance would cut the number of Petri dish samples by half and would not offer any advantages over the rectangular shape. Also, there will be shelves and containers in the chambers which will have more of an effect on the mixing characteristics than the vessel shape. Although Radian does not anticipate any major mixing problems, thoroughly testing and documenting of the mixing characteristics of each chamber is recommended before biotesting is initiated.

The three organisms HERL desires to use for bioassays will be exposed in four different ways. They are:

Salmonella	Petri dishes Erlenmeyer flasks
Drosophila	Nylon mesh cages
Tradescantia	Pots or beakers

Since the chambers will be used to expose three different types of organisms, the internal configuration of the chambers will vary for each organism.

When *Salmonella* are exposed in Erlenmeyer flasks, appropriate fittings can be used to introduce the aerosols directly from the MARC to the liquid medium in the flasks. Small rotary shakers will mix the contents of the flasks. These shakers can be placed directly in the biochambers if temperature control is a problem, or they can be left out of the chambers if temperature control is not a problem.

To expose the *Drosophila* to the MARC effluent, the top shelf of the biochambers can be removed to allow stacking of cages containing the organism in the chambers. The number of cages that can be located in the chambers for a given experiment can be predetermined by HERL when they size cages. This allows HERL a greater degree of flexibility in experimental design.

Cuttings of *Tradescantia* can be exposed in a chamber of configuration similar to the ones used for *Drosophila*. Cuttings can be placed in a small pot or beaker and the beakers inserted in the chambers to obtain multiple exposures. This method of exposure allows for large amounts of data collection which would aid HERL in obtaining statistical valid results.

For both the *Drosophila* and *Tradescantia* exposures, a feed stream for each biochamber will be introduced in the upper part of the vessel and the output will be in the lower part of the chamber. These requirements can be readily met with the chambers as described above for *Salmonella*.

Temperature, Lighting and Humidity

The physical environment within each chamber will be controlled to expose the organisms with minimum stress and within published tolerance limits.

Temperature

The temperature that should be maintained in the biochambers while exposing the *Drosophila* and *Tradescantia* can be the ambient trailer temperature with no damage to the organisms. For runs utilizing these organisms, as well as experiments exposing *Salmonella* at ambient conditions, the heating or cooling of the chambers will be no problem. The trailer housing the MARC is sufficiently conditioned to hold the MARC as well as the biochambers at the desired temperatures. However, when running *Salmonella* at the higher temperatures, $37 \pm 0.5°C$, auxiliary heating equipment will be used to elevate the chamber temperature. Also, adequate insulation will be provided around the chambers to reduce heat loss.

Humidity

The relative humidity in the biochambers needs to be kept at 60 to 80 percent for all the organisms. When the MARC is operated at the high relative humidity condition, there will be little problem maintaining the necessary moisture in the biochambers when they are operated at ambient temperatures. However, for the high temperature *Salmonella* runs, as well as the MARC runs at low humidity levels, moisture will have to be added to the biochambers to raise the relative humidity to the desired level.

Lighting

Both *Drosophila* and *Salmonella* can be exposed to the secondary aerosols in the dark. However, *Tradescantia* should be exposed in a lighted environment for a maximum response to the aerosols. The amount of light will not be critical, but should be photosynthetically active radiation. By exposing the *Tradescantia* system under lighted conditions, the stamens of the organism remain open and diffusion of the aerosols into them will be maximized.

Interfacing the Biochambers to the MARC

Since the biochambers are considered auxiliary testing devices for the aerosols produced in the MARC, they must be attached to the MARC in such a way that they do not disturb the operation of the aerosol reaction chamber. Although there are to be four compartments in the biochamber module, only two of these will directly utilize the MARC effluent.

Flow Patterns for Chamber Interface

Connecting the HERL biochambers to the MARC will be done in such a way as to supply the biochambers with the desired aerosol effluent while not causing any adverse effects on the MARC. Two of these MARC streams can be sampled with no perturbation to the system.

1. The clean air supply.

2. The diluted source stream (the MARC feed stream).

Since the clean air system is capable of delivering up to 10 cubic feet per minute (10 cfm) and the MARC will only require 1-3 cfm, there will be enough excess clean air to feed the clean air section of the biochamber. The flow rate of this stream to the biochamber will be in the range of 0.25 to 0.5 acfm. Since the chamber volume is 6.75 ft^3 the residence time (t) in the clean air chamber will be 13.5 to 27 minutes. Provisions will be made to humidify this chamber since the clean air is dry (a 0% relative humidity). The feed stream to this chamber will be tapped from the clean air feed just downstream of the clean air generator. This will cause the clean air chamber to operate at a positive pressure. The exit from this chamber can be routed to the atmosphere with no further treatment.

The diluted source stream is the second stream which should be in plentiful supply and should pose no sampling problems. This stream will be sampled after all dilutions have occurred and will be transported to the appropriate biochamber by a line large enough to keep the pressure drop across the system to a minimum. The stream will be tapped off the MARC feed stream between the inlet manifold and the inlet sampling pump. This arrangement will mean that the biochamber is operating at a pressure slightly below atmospheric. The flow rate of this stream will be between 0.25 and 0.5 acfm to give an average residence time (t) of 13.5 to 27 minutes for the 6.75 ft^3 chamber. Also, because the stream will be sampled before it is humidified, provisions for humidifying this chamber will be necessary to provide the exposure conditions discussed in Section 3 of the report. The exit stream from this reactor can be routed to the atmosphere.

Two samples remain that HERL would like to test for biological activity.

1. A filtered MARC exit stream.

2. An unfiltered MARC exit stream.

Both of these streams will come directly from the MARC.

Of the two samples, the filtered effluent sample can be most easily obtained. One of these streams goes to a sampling manifold where periodic samples are taken for chemical characterization. The excess from this manifold is pumped through a final filter and exhausted through a dry test meter to the atmosphere. Since the flow rate of this line will be on the order of 1 cfm, the biochamber can be inserted between the final outlet filter and the outlet sampling pump. The feed to the biochamber will be the stream coming from the outlet filter and the exit stream from the biochamber would be returned to the outlet pump. The pump exhaust could be dumped to the atmosphere as originally planned or filtered again for sample collection. Since the feed to the biochambers is taken upstream of the pump intake, the biochamber will operate at a negative pressure. The pressure drop through the biochamber is expected to be small and there should be little disturbance to the MARC system.

The final sample HERL would like to bioassay is the unfiltered MARC effluent. Since this sample will contain aerosols, it will be best not to transport the sample any great distance. A separate output port will be placed in the MARC at the rear of the trailer in the area where the biochambers are located. While this separate output line from the MARC will represent an ideal feed for the biochamber, several things have to be considered before a successful interface can be achieved. At present, the total flow rate of material through the MARC has not been determined and since a mass balance has to be satisfied around the MARC (Input to MARC = Output from MARC) only the extra aerosol beyond that needed for chemical characterization will be available for the biochamber feed. If baseline chemical characterization tests show that enough aerosols can be collected with a high throughput of feed materials, then there will be enough aerosols remaining for the biochamber feed. The final decision on this matter will have to await the completion of the chemical characterization of the MARC output.

As in all the other cases, the line taking the effluent sample to the biochamber will be sized to prevent significant pressure drop across the system. Depending on the final outcome of the chemical test, the flow rate to this chamber could be 0.25 to 0.5 acfm, giving a residence time (t) of 13.5 to 27 minutes. Also, water will have to be added to this chamber to bring the relative humidity up to the desired level for the high temperature runs.

The biochamber will need to have a filter and pump downstream of the exit and will operate at a slight negative pressure. There should be only a slight pressure drop associated with passing the effluent through the biochamber and connecting lines and should cause no problems in the MARC operation. At present, a restricting orifice is used to adjust the back pressure in the chamber by controlling the flow in the vent line. The size of this orifice will have to be adjusted to allow the proper pressures and flows in the MARC allowing the two reaction vessels to operate at almost the same pressure.

Since three of the chambers are operating at negative pressures, the output of the pumps will vent to the atmosphere and are not anticipated to cause any more problem than venting the associated MARC process stream itself.

A separate flow system will be provided to flush all the chamber with clean, sterile air before the start of each experiment. This stream will be provided by filtering air from the existing MARC compressor, or a separate compressor may be used. Filters will have to be installed in line to provide the required quality of air. This arrangement will insure that the experiments are started with sterile air in the chambers.

Sampling Systems

To determine the effect of dosage, samples must be periodically isolated from the exposure stream. This type of operating procedure will enable HERL personnel to develop dose-responses information on the organisms exposed to the aerosols. The dosage variable can in this way be expressed in units of time. To do this, the exposure chambers have to be filled with the appropriate number of test organisms, and certain ones isolated at given intervals. For *Salmonella* exposed in Petri dishes, it will be possible to cover the Petri dish at the appropriate time and limit exposure. This will be the easiest and least disturbing method of sample control for the chamber. For *Tradescantia* and *Drosophila,* the organisms also can be isolated from the aerosol environment of the biochambers. The provisions for handling the samples in this way will have to be designed into the chamber sampling system.

Several techniques can be employed to isolate the organisms, *Tradescantia* and *Drosophila*, from the reactive aerosols. The simplest system is to insert the organisms into an isolation vessel contained inside the biochambers. The isolation vessel can be purged of the aerosol reactants and the biological exposures effectively quenched. The biochambers have been designed to accommodate this type of isolation procedure. The glove/glove box design of the front panel of the biochambers will allow easy access into all areas of the chambers and facilitate the isolation of selected samples. The isolation vessel will be a flexible-walled container that can be flushed with clean air from the clean air system. Appropriate converters will be used to pass the clean air from the generating system outside the chambers to the isolation vessel inside the biochambers. When the entire experiment is completed, the front panel of the chambers can be removed and the specimens recovered.

While each of the three types of organisms will require their own type of sampling protocol, the glove arrangement will offer the maximum protection and flexibility to all the samples.

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The complete report, entitled "HERL Biological Exposure Chamber Conceptual Design: Technical Note," (Order No. PB 82-114 646; Cost: $7.50, subject to change) will be available only from:
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