TEAGUE ENTERPRISES

Overseas Smoke Exposure System

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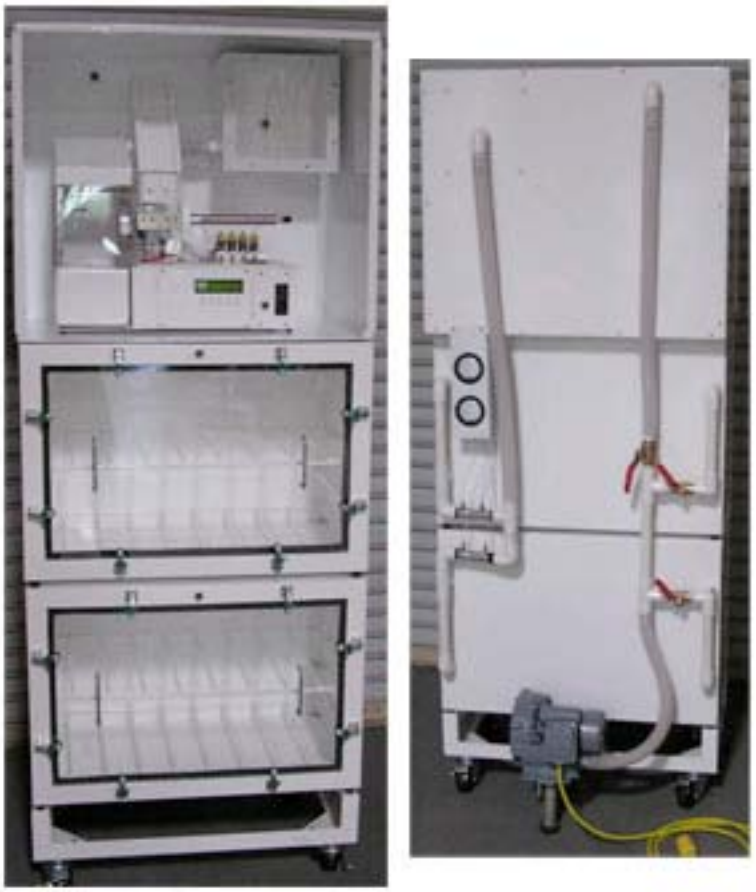
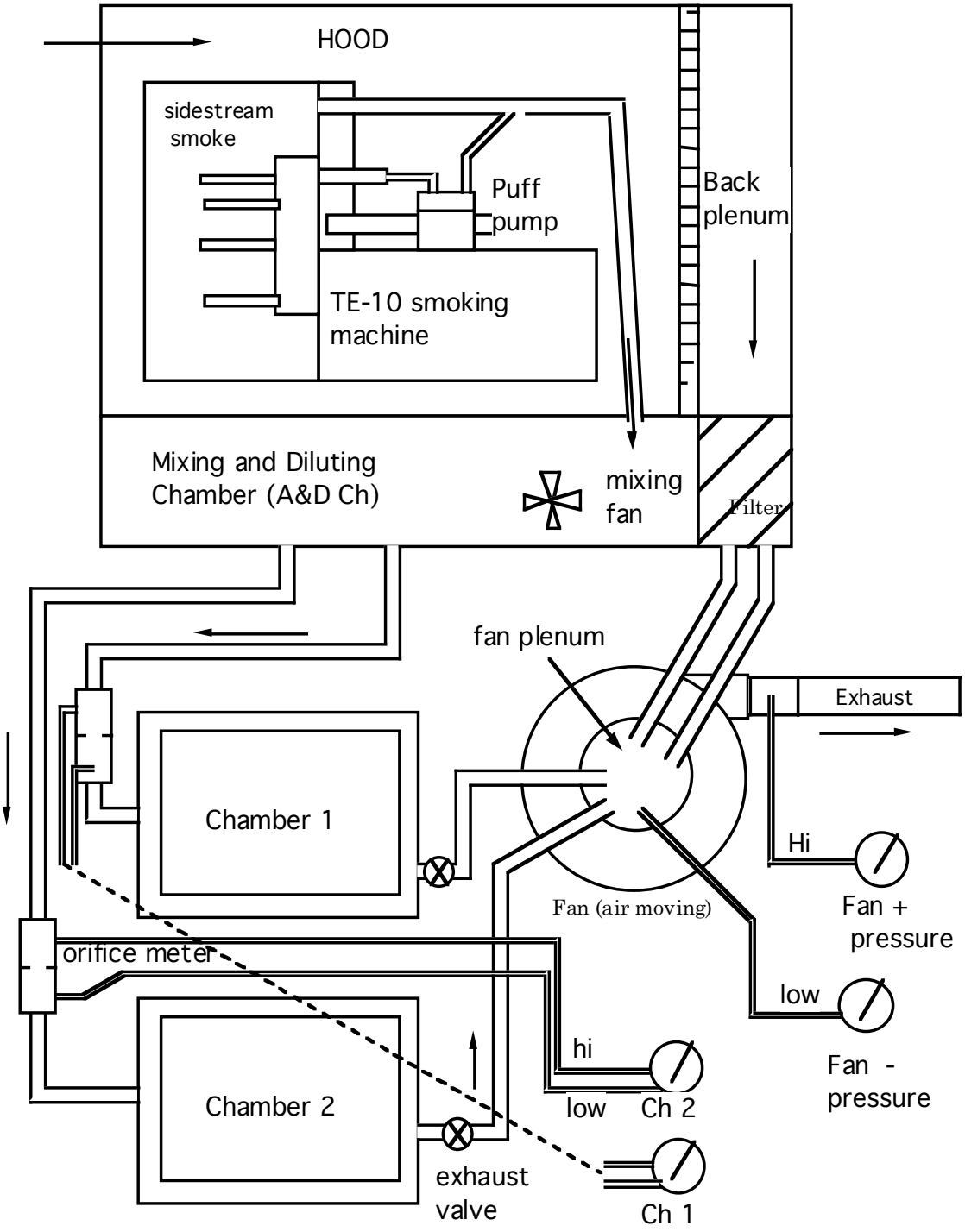
Smoke System Description

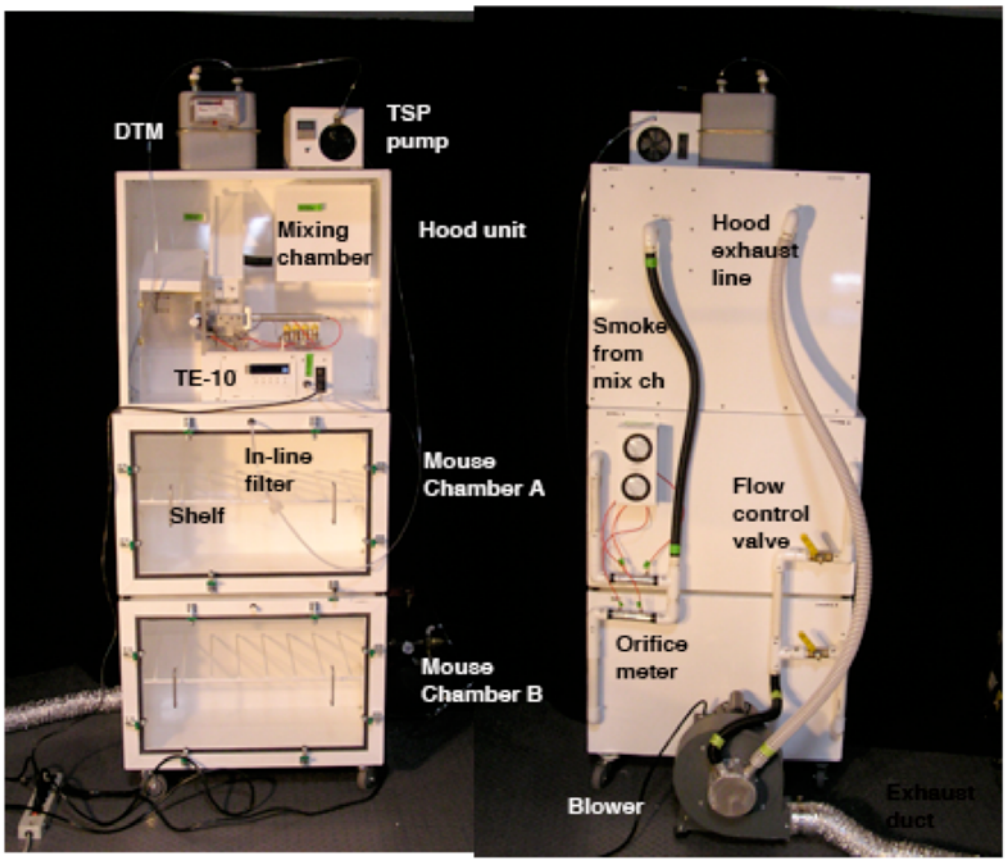
The cigarette smoking system consists of two animal exposure chambers and a hood unit on wheels. The unit is usually configured with the hood on top of the chambers but the hood unit can go anywhere near the exposure chambers. The unit is 71 cm wide 1.5 meters tall and 60 cm deep. The blower for the hood and chambers can be located in back or on the side of the chambers. The smoke machine, blower and TSP unit are plugged into 230-AC power (10A) and a 7.6 cm duct exhausts the smoke.



Smoke exposure system with two mouse exposure chambers.
Standard smoke system schematic showing air flow through the components.

CIGARETTE SMOKE SYSTEM - TWO CHAMBERS





The Teague Enterprises cigarette whole body exposure system consists of a TE-10 smoke machine for producing the smoke, a mixing and dilution chamber (M&D Ch) to stir and age the smoke and the exposure chambers. Smoke from the machine is pulled from the TE-10 and mixed with the mainstream smoke via a 2.5 cm tube. It is collected in the M&D Ch where it is mixed. The diluted smoke is then metered into the exposure chambers. The smoke from the chambers and the system hood is then exhausted. The exhaust may go into a laboratory hood, outside, or to the building exhaust. In most cases the waste smoke can just go into vent ducts but where complex air conditioning systems may be contaminated, a filter may be required. By using valves on the rear, the chambers can be used together or one can be used as a control and the other for smoke exposure. The smoking machine is located in a negative pressure hood that captures errant smoke to keep it out of the room area. The system hood contains the M&D chamber and a vent above the smoke machine . The system also includes a filter holder, timed vacuum pump and dry test meter for measuring the TSP (Total Suspended Particulate).

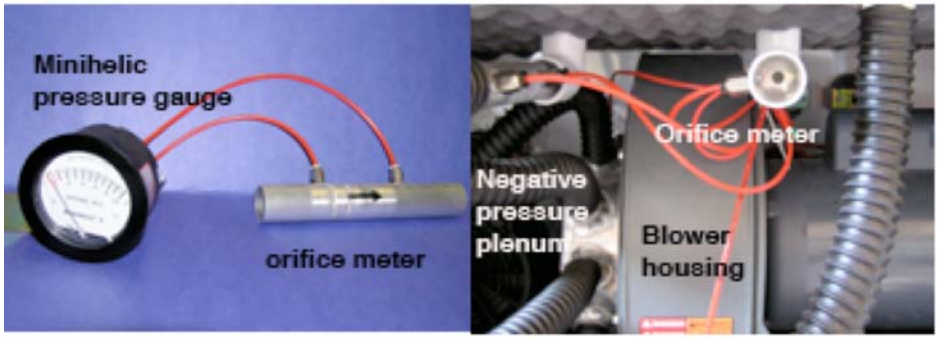
Mixing and Dilution Chamber

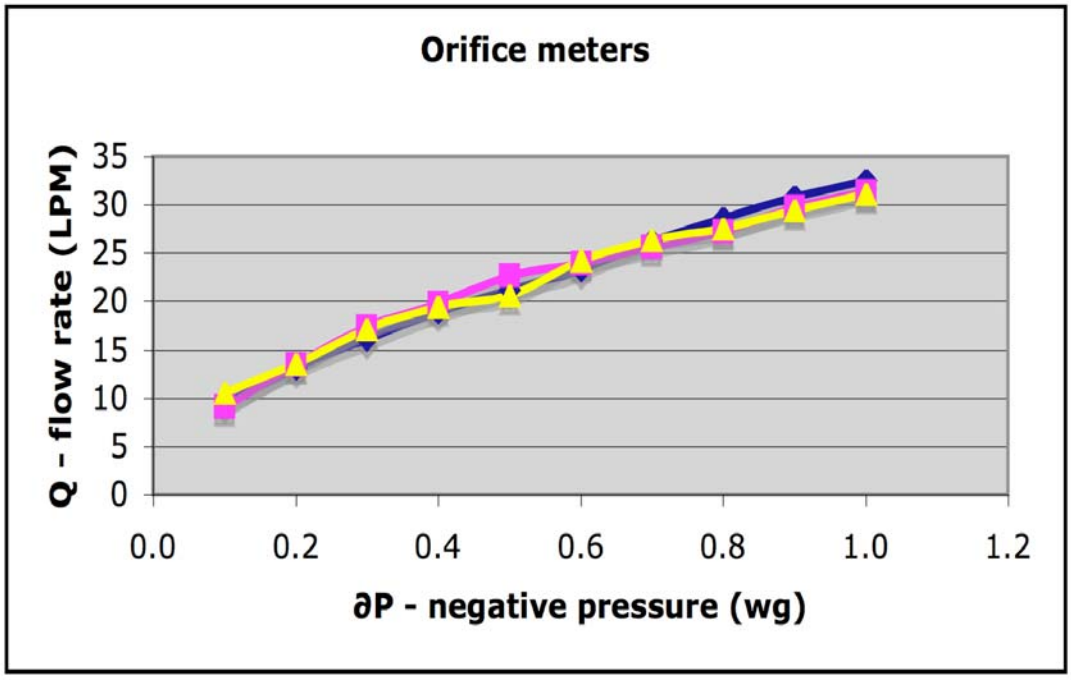
The M&D Ch is used to collect, dilute, age and mix the smoke from the TE-10. In the mixed smoke mode ambiant room air is mixed with the smoke generated in the chimney area of the smoking machine. Ambiant air is pulled out from the TE-10 chimney and mixed with the mainstream smoke. As the smoke is introduced into the M&D Ch a fan inside of the chamber provides a turbulent mixing to break up the cloud of smoke and stabilize the vapor and particulate suspension. The initial concentration depends on the amount of air pulled into the chimney and the number of cigarettes. More air can be added to dilute the concentrated smoke in the M&D Ch. The long term exposure concentrations being used by investigators to initate injury from smoking is in the range of 60 to 90 mg/m^3. Environmental tobacco smoke concentrations are in the range of less than 1 to 5 mg/m^3. The TE-10 needs an airflow of about 30 LPM to contain the smoke when 10 cigarettes are burning at one time. A lesser flow can be used when the machine is used for a smaller number of cigarette loading configurations. The M&D Ch also serves to dampen large fluctuations in smoke concentrations during the process of continuously collecting smoke from the smoking machine. If the smoke in the M&D Ch is used to directly connect to the exposure chambers the concentration will be the same as in the chamber (baring any leaks) minus any lost in the tubing.

Exposure Chambers.

The two sizes of exposure chambers used most often are the rat and mouse exposure chambers. The chambers are designed to house rat or mouse polycarbonate housing cages. The rat chambers are 71X60X60 cm (LHD) ((264L) with a shelf in the center. Using the standard polycarbonate rat cage size of 48X25X22 cm, two rat cages can be put on each shelf. The mouse cages are 30X15X19 cm and 5 mouse cages can be put on each shelf. For mouse cages using 4 mice per cage the number of mice that can be exposed at one time is then 160 mice. Using a standard housing number of 2 rats per cage and using the two exposure chambers, 16 rats can be exposed. For the typical exposure times periods of 6 hrs 3 rats per cage have also been used in which case the number of rats can be increased to 24. The mouse exposure chambers are 71X48X40 which allows 6 mouse cages to be exposed in this size chamber at one time.

ABS pipe or flexible ducting connect the conditioning chamber to each exposure chamber. An orifice meter monitors the flow of smoke from the conditioning chamber. Different concentrations in the exposure chambers can be achieved by introducing air by a valve before the mixed smoke is introduced into an exposure chamber to decrease the smoke concentration in that chamber. In practice when the same concentrations are used for a period of time the animals can be rotated between the exposures to allow the differences in chamber concentrations to be offset. Since the orifice meters are located right after the mixing and dilution chamber any leaks in the chambers (i.e. loose doors etc.) will be detected by a decreased flow as indicated on the pressure gauges. Orifice meters are made from 1" aluminum tubing with a orifice in the center. Pressure taps are placed before and after the orifice and the pressure drop is measured with a Minihelic pressure gauge.





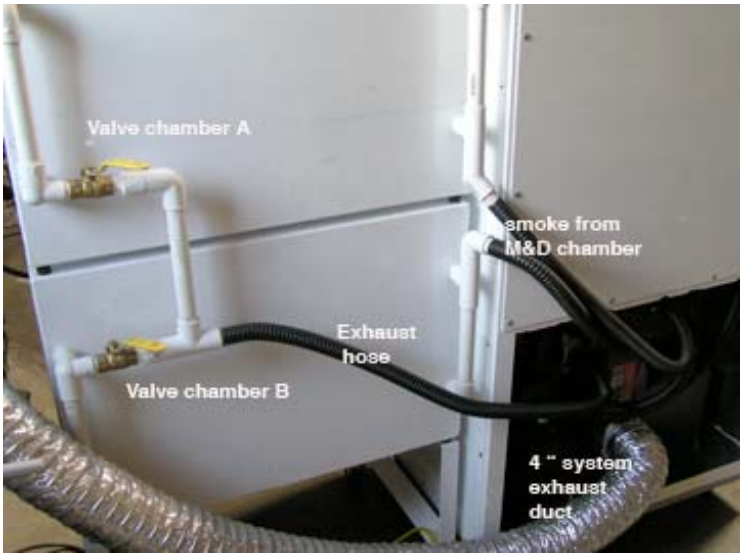
Because air is taken directly from the room and used for supply air, the relative humidity (RH) in the chamber depends on the ambient condition of the room. Standard exposure chambers have two entrance tubes and two exhaust tubes. Each tube has holes located along the length to insure air flow from one side to the other providing good mixing and uniform concentrations throughout the chambers. The animals can be exposed in standard polycarbonate cages with food and water provided in the wire tops. Tests have been done measuring TSP (total suspended particulate) concentrations in the cages and comparing them to the concentrations in the chamber and found to be comparable. This is due to the small particle MMAD (mass median aerodynamic diameter) of 0.3 to 0.1 μm, which is in the diffusion particle size region thereby and acting more like a gas.

Chamber doors are 3/8 acrylic Plexiglass with two handles for access. The doors are sealed with closed cell Neoprene (Pemko P388) placed on the chamber lips. Eight adjustable pressure clamps are located on the chamber to provide distributed force on the door for air tight seals. The clamps may be adjusted by moving the rubber pad using the nuts on either side of the clamp post. Care should be taken to keep the clamps properly adjusted, the gaskets clean and free of nicks.

In the center of each chamber a bulkhead union push to fit connector is installed to provide a sample port for taking TSP concentration levels. When the port is not being used for sampling it must have a plug inserted to prevent air from leaking into the chamber. The plug is sometimes hard to remove but by using a slotted tube removal tool or some other plate to push against the ring the plug can be removed.

System operation:

1. Inspect system to insure unit is connected to power, vent pipe is connected to room exhaust, door are sealed and all the tubes and ducts are properly connected.
2. Turn the compressor.
3. Turn on the main fan and observe the pressure gauges on the front of the hood. The chambers can be adjusted to the calibrated flow. Usually 0.5 ±.5 " wg on the gauge. By adjusting the valve on the back of the chambers the flow can be set. Also note there is some feed back between the chambers so turning up one will decrease the flow in the other.



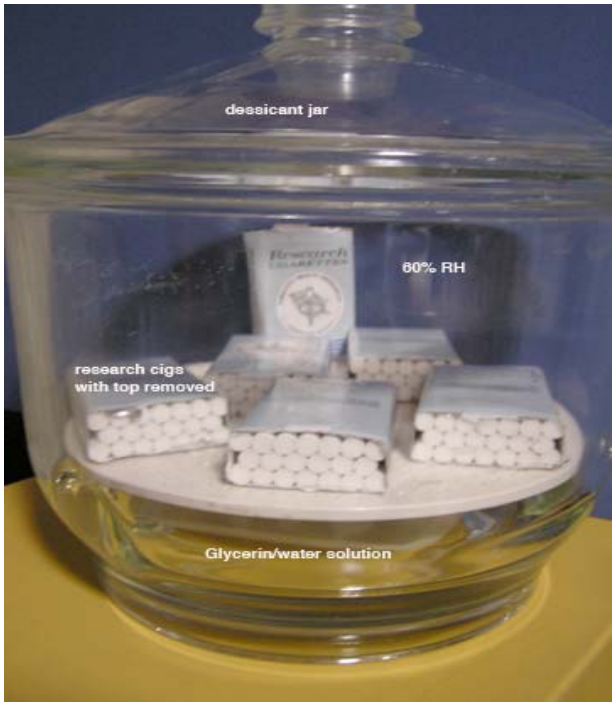
4. Perform a leak check by disconnecting the hose from the smoke chamber and placing hand or a stopper to block the flow. The flowmeters should go to 0

Research Cigarettes

Most of the smoking systems being used in the US are using standard reference cigarettes. There are a number of these cigarette types and are sold by College of Agriculture, Reference Cigarette Program, Reference Cigarette Program and is administered by Orlando Chambers, Ph.D. ochamb@uky.edu (University of Kentucky, Lexington, KY). The most widely used is the 3R4F cigarette (low tar modern cigarette). As part of the FTC method the cigarettes are stored at 4°C until needed. At least 48 h prior to use, the cigarettes are placed in a closed chamber at 23°C along with a solution of glycerin/water (mixed in a ratio of 0.76/0.26) to establish a relative humidity (RH) of 60%.

UK Research Cigarette Comparison **6/6/07**

	1R3F	1R4F	2R4F	3R4F
	1974	?	2001	2007
	mg / cig	mg / cig	mg / cig	mg / cig
TAR	15.0	9.2	9.9	9.4
NICOTINE	1.16	0.8	0.81	0.73
CARBON MONOXIDE	17.2	11.6	12.3	12.0
TOTAL PARTICULATE MATTER	18.1	10.8	11.6	11.0
PUFF COUNT	8.6	9.2	9.1	9.0



Flow check and leak check for animal chambers.

Prior to performing tests for flow through the chambers an examination of the gaskets and tension on the clamps should be conducted.

- Check for dirt on gaskets and clean with soft cloth and cleaning solution.
- Examine for gaps in corners and nicks in gasket material.
- Check for adhesion of gasket to box.

If gaskets are damaged replace with closed cell Neoprene (Pemko P388) 1/2 wide by 3/8 thick weatherstrip tape. This may be purchased at most hardware stores.

Using the Visi-float Flowmeter. (5-40 LPM)

(the stopper fitting is placed in the top back hole and screwed in securely for use in checking for vacuum flow)

Start by placing the flowmeter in the vacuum line that goes from the exposure chamber to the fan before the valve.

This is done by removing the line to the chamber by the valve. (a piece of flexible 1" Inch line may be needed to make the connection)

Set the flow with the valve to about 35 l/min AIR by observing the float ball.

Put the line back on the chamber and remove the tubing from the exit. Now with the flowmeter observe the flow at this location it should be the same. If there is a difference the chamber is leaking.

If the chamber is ok then put the tubing back on and remove the tubing at the location where it comes out of the M&D Ch. Again the flow should be the same. If the flow is different the leak is in the components after the chamber.

Do the same thing for the other exposure chamber.

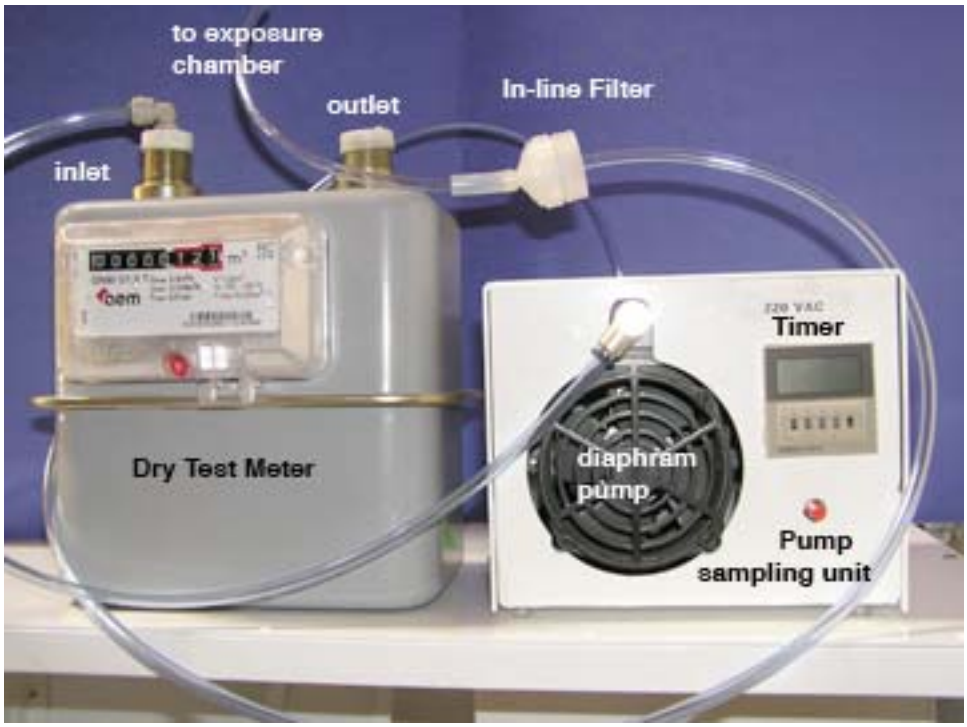
If the flows for the chambers are verified in this manner the smoke must get to the chambers.

Smoke Characterization

A calibration to characterize the cigarette smoke in the chambers is offered for each system. This is done at a set flow in the chambers using the calibrated flow meters for each chamber. For the standard rat/mouse exposure chamber size of 260 liters a flow of 25 liters per minute will provide an adequate flow rate. During the smoking of cigarettes, TSP, carbon monoxide and nicotine can be measured. TSP and carbon monoxide levels are the most frequently measured indicators of chamber concentrations.

1. TSP (total suspended partuculate)

TSP is measured the exposure chambers by filter samples. Teague Enterprises provides a sampler for determining TSP, which consists of a pump, flow control device (valve), timer, filter holder and a gas volume meter (dry test meter). This method samples smoke on a membrane filter for a length of time and an exact volume is measured with the dry test meter (DTM). The weight change of the filter is then just divided by the volume of air passing through the filter and the TSP in mg/cubic meter is determined. Another option available to laboratories that have a fluorometer is to wash a filter sample with methanol and measure the fluorescence of the solution. A more direct method is to use a bubblier or impinger to directly collect the smoke into methanol. This method, which measures the UVPM (ultraviolet particulate matter), can be used to collect a smaller sample on the filter or bubblier and appears to be a reliable measurement of the TSP.



TSP sampling:

1. Using 25 mm Pallflex membrane filters (EMFAB TX40H120-WW) weigh filter and record weight.
2. Using tweezers, place the filter in the filter holder and tighten the cap hand tight.
3. To set up sampler:
    a. Place filter holder inlet tube into chamber sample port.
    b. Tube from filter holder is put into the inlet of sampler unit. The other tube from the sampler exit is placed into entrance to dry gas meter.
    c. Sample time is set on sampler by pushing buttons on top or bottom of number.
    d. Flow rate is adjusted by a valve. To set flow-rate record dry gas meter, run for designated time on sampler, take sampled volume and divide by sample time to get flow in liters/ minute. Then adjust valve and test again until desired flow rate is achieved.
4. Record reading on AEM dry gas meter. e.g. 0013,020 is 13.020 cubic meters or 13,020 liters. The last digit on dial has horizontal lines to the right of the number. These are .2 liters per line and are read from the top. So, if the centerline on the far right is pointing to the second line from the top (zero is on top and one below it) the reading would be 13,020.2 liters or 13.0202 cubic meters. If you are sampling for a short time you could move the decimal point three places and read 20.2 liters as the starting value.
5. Turn on sampler by pushing button once and let it run until it stops.
6. Remove filter with tweezers and weigh recording the value.

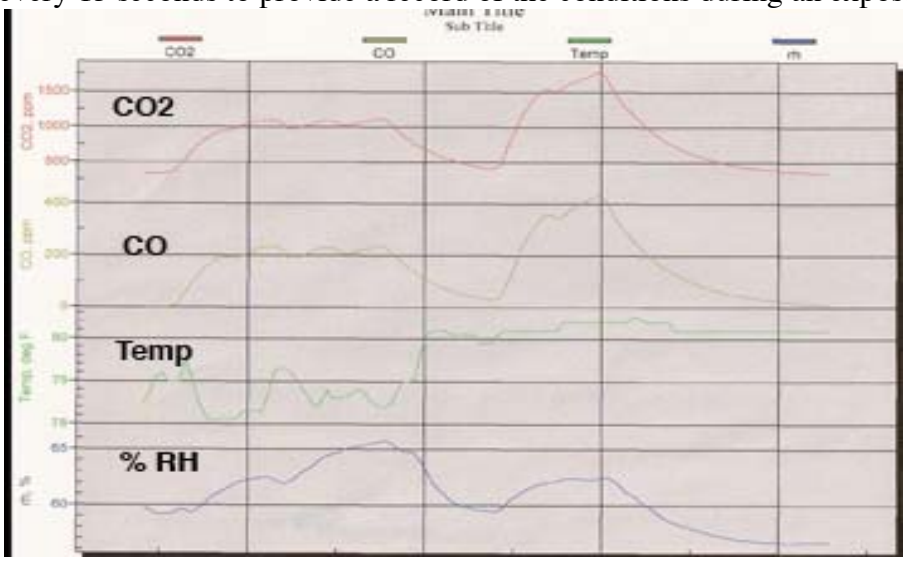
7. Record the ending dry gas meter reading.
8. Calculate the TSP by taking the difference in weight divided by the volume collected.
    a. A filter weight before sampling was 24.45 mg and after 25.58 mg. Amount of smoke collected is therefore 1.13 mg.
    b. A dry gas meter was 13,020.2 before sampling and 13,025.8. Difference is therefore .0056 cubic meters (5.6 liters)
    c. TSP is 1.13 mg divided by .0056 cubic meters or 201.8 mg/m^3.

2. Carbon monoxide

Carbon monoxide can be monitored in the M&D Ch and the exposure chambers with a model 880 non-dispersive infrared (NDIR) analyzer as described in the Teague paper. A less expensive alternative uses an electrochemical sensor, which can be used, for grab samples at various times. Monoxor II, Bacharach Instruments or Q-Trak Plus 8554 from TSI Incorporated, www.tsi.com.

3. Temperature & RH

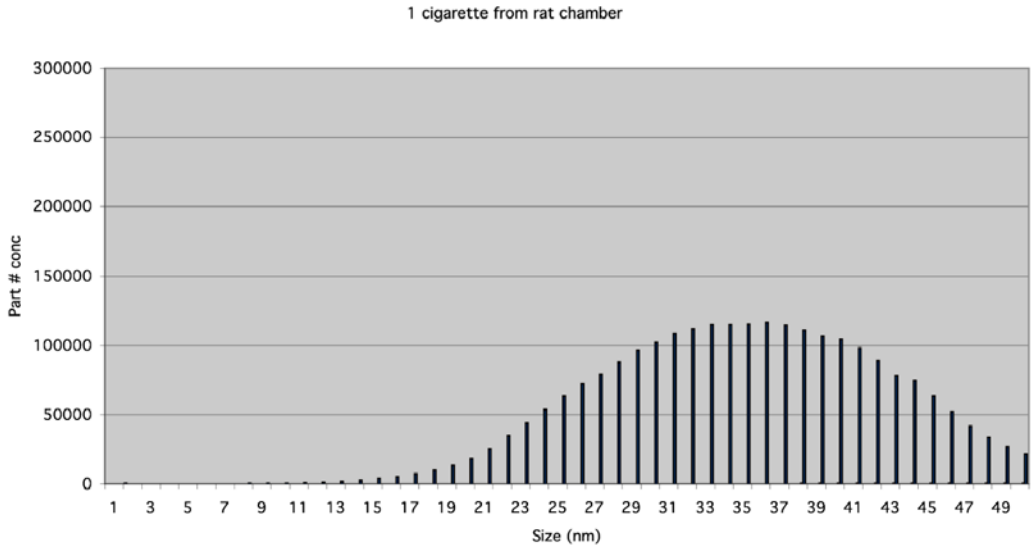
A temperature and relative humidity probe or Q-Trak Plus (not provided) is used to measure conditions in the exposure chamber. Output from the probe is sent to a data logger every 15 seconds to provide a record of the conditions during an exposure period.



4. Particle Size and Number concentration.

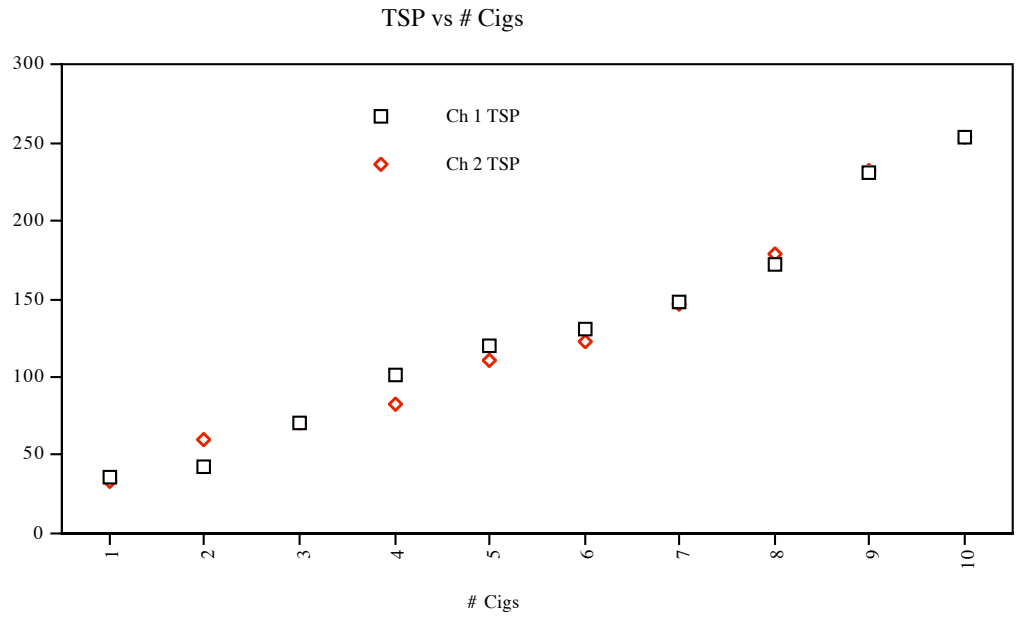
Particle size distribution was measured using a SMPS (scanning mobility particle sizer) from TSI. The method of counting the particles in the SMPS is a Condensation Particle Counter that counts all the small particles. The concentration is reported as particles per cc. The count median diameter equaled 0.228 ± 0.12 μm with a geometric standard deviation of 4.2 ±1.4. Mass median diameter can also be measured using a cascade impactor. The glass stages are removed and washed with methanol. This solution can be counted by fluorometry. (In-Tox Products, Albuquerque NM).

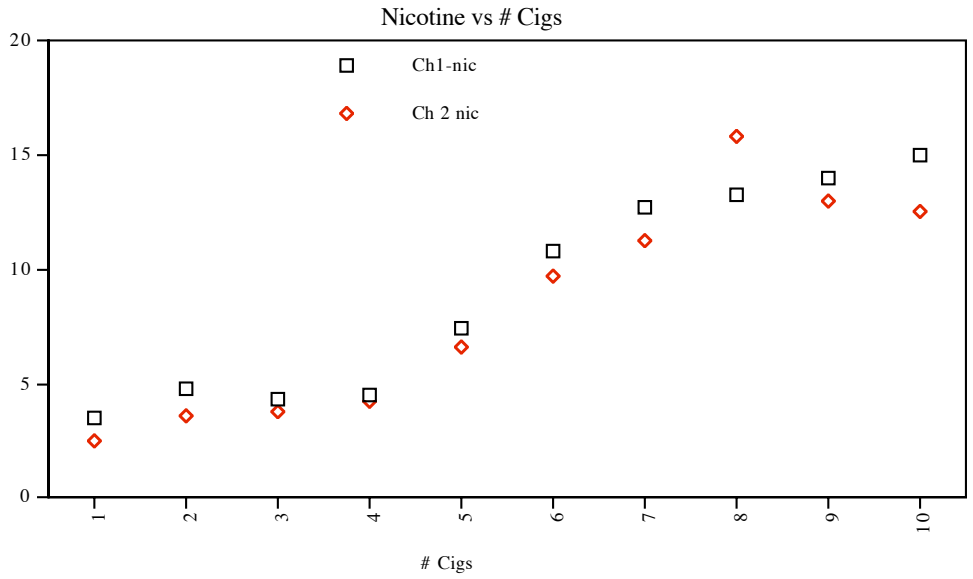
SMPS data taken from the exposure chamber with one cigarette burning.



5. Nicotine concentration.

Nicotine concentration was measured by drawing samples form the exposure chambers through sorbent tubes using a method modified from Ogden. Samples are extracted from the tubes and levels are determined using gas chromatography. Calibration curves of nicotine verses the number of cigarettes and TSP for each exposure chamber is provided.





Typical calibration of rat chambers using TSP and Nicotine methods for 1 to 10 cigarettes. Flow rate was set at 30 LPM for all measurements. TSP is in mg/m^3 and the Nicotine is in micrograms/m^3

DISCUSSION AND CONCLUSIONS

A variety of different options for using the TE-10 are available for producing a steady supply of cigarette smoke with minor deviations over an extended period. A

unique feature of the TE smoking systems is the conditioning and mixing chamber that sets primary concentration and provides a steady amount of “feed smoke” to the exposure components. Measurement and control of the flow rates from the TE-10 to the exposure chambers is essential for control of smoke levels. Some method or a combination of measurements for the continuous measurement of smoke concentration in the exposure component is highly desirable. Carbon monoxide measurement can be the anchor measurement and can be tracked as a record of exposure concentration over time. Measurement of TSP should be made on a regular schedule during the exposure.

In any experiment involving generation of smoke for extended periods of time, a well-defined and documented exposure needs to be established. Smoke exposures are man power intensive and require constant monitoring during the exposure period to insure a steady supply of smoke. Frequent cleaning to remove tar buildup is also important. The smoking machine will jam and get out of sync because bits of tobacco and paper get into the sliding parts and tar builds up. As the operator becomes more experienced with aligning the machine and keeping it clean, the performance will greatly improve. If performed correctly, an experiment with a known exposure level will be performed with good documentation of smoke levels that are very well controlled from day to day.