Design of Scent Separating Conditioned Place Preference Apparatus and Rat Head Restraint

A Major Qualifying Project Report:

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by

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Abstract

Current conditioned place preference testing procedures expose test subjects (rats) to various stimuli to measure their motivational effects. The test subjects are imaged using MRI to delineate various regions of the brain that are affected by the training and/or stimuli. This MQP project developed MRI head restraint harnesses; created CAD models of them, and printed them using 3D printers and verified their effectiveness. The project built an odor testing chamber with three unique compartments. Ventilation was designed to keep two odors in separate compartments. The middle section of the test facility was the neutral zone that the test subject enters initially. The flow characteristics in all sections was analyzed and documented to ensure that odors presented in either chamber did not drift into other chambers.

Acknowledgements

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A special thanks goes to Dr. Raffaelle Potami and Dr. Adriana Hera, who helped us conduct the CFD analysis concerning the airflow in the CPP box.

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Last but not least, many thanks go to Dr. Jean King, Kelly Tam and the rest of the CCNI at UMMS for allowing us to complete our research for construction and designing of the CPP box as well as the head restraints on their premises.

Introduction

University of Massachusetts Medical School (UMMS) has been conducting various projects with Worcester Polytechnic Institute (WPI) in the past years. The Center for Comparative NueroImaging (CCNI) at UMMS has been working on understanding the psychiatric effects of drug addiction since 2001, doing their testing mainly on rats. The facility has a 4.7 Tesla ultra-high field magnetic resonance imaging (MRI) machine, which they use to image the rat's brain and analyze its activity when exposed to certain stimuli. Since MRIs only produce images of the tomography of the brain and not function, the CCNI must take functional MR images (fMRI) which track the blood flow to and from the brain by utilizing the Blood-oxygen-level dependent (BOLD) contrast. If a region of the brain is in use, it requires more oxygen; therefore blood flow to this area is increased. Similar to an MRI, an fMRI measures the change in the magnetic field produced by oxygen-poor and oxygen-rich blood, and in this way tracks what areas of the brain are currently undergoing the highest oxygen exchange. In order to get a 3 dimensional model of the entire brain, the MRI machine takes its images in different slices, the thinner the slices, the more detailed the model will be. This is a quite lengthy process and since the blood flows quickly, the slices cannot be too small or part of the hemodynamic response will be missed. In order to make the image as clear as possible, the brain must be completely still for the entire process. The test subject is introduced into the MRI machine with a custom-sized head restraint, which holds the head in place by securing the ears and nose. These test subjects require training and exposure to the head restraint prior to being imaged within the MRI machine. The subjects are allowed to acclimate in the environment that they would experience inside the MRI machine starting from their juvenile days (15 days old). Thus, our project involves developing a new model of the head restraint, a model that would be more compatible with the age and size of the test subject.

Conditioned place preference is used to measure the motivational effects of certain stimuli. In the past, the CCNI at UMMS has used tactile and optic stimuli in their CPP experiments; however, they would like to incorporate scent as stimuli as well. Odor is a fundamental sense that rats use extensively. We designed a test facility that would introduce two different odors in separate compartments with an intermediate neutral zone from which the test subject could select a chamber. The rat is trained to identify scent or other stimuli with receiving a drug. Once a rat has been acclimated to the box and conditioned, two scents are introduced to opposite sides of the chamber, and a camera tracks the rat's motion. After the experiments are completed, fMR images are taken while the rat is being exposed to the same stimuli. In this manner, UMMS staff can study the effects certain stimuli have on conditioned subjects. Therefore, the primary goal of our project was to design and construct an apparatus which would allow the CCNI staff to conduct the decision making experiments for the rat using odor as a stimulus. This apparatus has three chambers, with odors being introduced in opposite chambers and an intermediate neutral zone for the initial introduction of the test subject. The apparatus had to ensure that the odors would not leave their respective chambers while still allowing the test subject to traverse freely between all three chambers.

1. Background

This project worked with the CCNI at UMMS to observe the behavioral changes of rats that are habituated to conditioned stimuli using primarily an MRI machine and other techniques. In order to conduct these experiments we designed a number of instruments and apparatuses. Our first objective was to make a prototype (head restraint) of the instrument that would go into the MRI machine. This prototype had to be made in two sizes, one for the adult rat (~350 grams) and one for the adolescent rat (250-350 grams).

To accomplish our second objective we designed an apparatus that we can use to assess the behavior of the rat with reference to scent. After assessing all of our options we succeeded in developing an ideal instrument for the MRI machine, and a working apparatus for the scent experiment. In this report we will be focusing primarily on the operation and analysis for the apparatus built for the scent experiment.

1.1. University of Massachusetts Medical School Center for Comparative NueroImaging

Our project was conducted at CCNI which is part of the Department of Psychiatry. The CCNI founded in 2001, its primary goal is to conduct research to develop the methods of treatment for mental disorders by utilizing animal models to better understand changes in the brain. Specifically, methods used by the CCNI to achieve these goals include innovative multi-modal imaging methodologies, neural networks involved in mental health disorders (such as addiction, depression, and anxiety) and potential usefulness of complimentary/alternative medicines (CAM) in order to alter cognitive and emotional networks. The CCNI also houses an ultra-high-field magnetic resonance imaging (MRI) spectrometer, which is used to study mental illness. In terms of being a health provider, the University of Massachusetts Department of Psychiatry is one of the largest psychiatric departments in the United States with 330 faculty members and 2000 staff members. They are the largest psychiatric care provider in central Massachusetts, and are currently working to improve psychiatric healthcare globally with project groups working in Brazil, China, Finland, Germany, Russia, and other countries (Souza, 2014) (Ragaev, 2014). The primary goal of the CCNI's research portfolio is to understand the complex interactions among the brain, body and mind to unlock the mysteries of the causes, prevention and treatment of mental illness and addiction across the life span. They are also internationally known for their neurobiological research taking place in the Brudnick Neuropsychiatric Research

Institute where their focus is on molecular mechanisms of psychiatric diseases (Center for Comparative NeuroImaging, 2014).

1.2. Head Restraint

The CCNI had developed an adult rat test harness that was successful. However, original designs of it were lost. One of our tasks was to re-engineer the adult test harness and then alter its size to accommodate adolescent and juvenile test subjects.

1.2.1. Establishing a Need

The first objective was to re-engineer the adult rat restraint holder used in subject acclimation conditioning. As these instruments were to be used for acclimation, the need for additional instruments arose as the staff wished to acclimate numerous subjects simultaneously. Previous head restraints being used for acclimation were also damaged and unsuitable for use anymore. Furthermore, due to the size difference of the subjects in their adolescent and adult stages, problems were arising in the orientation of the subject in the head restraint. Due to this the department required two separate sizes for the instruments to fit the adolescent and adult subjects.

The sizing of the adult head restraint was to be precisely the same as the current model being used by the CCNI staff. For the adult model on the other hand, the bite bar for the instrument was to be lowered eighty percent of the original size. Similarly, the ear plug slots were also to be lowered by a similar margin in order to avoid unnecessary discomfort that the subject was experiencing in the current model.

1.2.2. Materials Research

The three criteria that had to be met were as follows; the material had to be transparent so that the test subject could be viewed when it was being acclimated as well as when it was inside the MRI machine; secondly the material had to be non-toxic and had to be strong enough to withstand the force of a rat bite (48.26 MPa) (Rat Genotype); and lastly, it has to be MRI compatible, so it does not interfere with the MR imaging. As the rat was to be biting on the bar continuously during the MRI scan as well as the acclimation period, we had to make sure biting this material would not cause them any health defects. The material also had to be transparent as so the rat could be viewed during the MRI scan period. We found two materials which met all our requirements, MED610 and Veroclear. We decided to go with the Veroclear material due to its higher strength, and availability.

1.3. Conditioned Place Preference Apparatus

Conditioned place preference (CPP) apparatuses are used in clinical studies to analyze animal behavior under distinct conditions (Prus, James, & Rosecrans, 2009); in our case, it is being used to study the effects different scents have on a conditioned test subject's want for drugs. A typical design of a CPP has three different chambers, the outer two being designed to have noticeably different characteristics while the middle chamber has no distinct characteristics.

1.3.1. Establishing a Need

In order to study the effects that scent has on drug addiction, a three compartment CPP that would keep two scents separated needed to be designed. At first, the apparatus was designed to be a single chamber, and the subjects would choose left or right depending on what they smelled on either side. However, rats need a more clear-cut choice, so the box was separated into three unique sections: the outer compartments are black or white, and the middle chamber is gray.

1.3.2. Material Research

A major part of the initial research conducted on construction and material choices involved finding materials and products to be used for the exhaust system (connecting the box to the fan). In order to ensure even airflow on either side of the CPP box, the two ducts running from the fan to each outlet of the box had to cause equal friction loss. It was already ensured that each side of the box would create the same losses, due to their symmetry, so as long as the exhaust system caused equal losses, each side of the box would theoretically have the same airflow. While longer lengths of straight duct cause slightly higher losses, these are negligible compared to the losses caused by the 90 degree turns and other changes of direction, especially in a system as small as the one needed to connect the fan to the outlets of the box (friction losses in straight duct is generally given in loss per 100 feet of duct). As long as the same number of turns are made (using the same fittings) for each side of the exhaust system, the only other cause of uneven airflow would be due to the method of splitting the airflow from the fan into two even streams.

A variety of fittings and methods were considered as possible methods of splitting the airflow. The initial idea was to simply have the two runs of duct go from their respective outlets of the box and meet at the inlet to the fan. Then a faceplate would be used to adapt the inlet of the fan to the ends of the ducts, as shown in Figure 1.**Error! Reference source not found.** This is known as a straight reduction, and while it may create even airflow if manufactured correctly, some concerns arose in researching the pressure losses and airflow through these types of fittings. The pressure loss for an abrupt reduction of the duct is caused primarily due to turbulence in flow, rather than friction. This caused some uncertainty as to whether such an abrupt change in the airflow would cause an uneven flow in the two sides, or cause extreme losses of airflow due to turbulence.

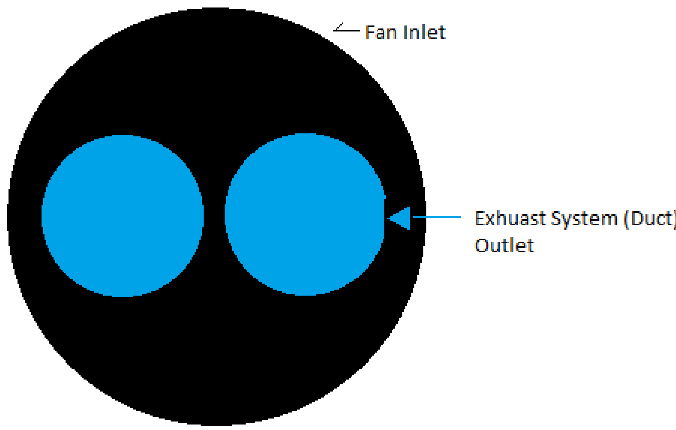


Figure 1: Straight Reduction from Fan to Both Ducts

The recommended method of splitting flow in most guides to HVAC design is to add a straight length of duct, before adding a fitting that splits the flow evenly. One such fitting is a tee, illustrated in Figure 2, which is normally used to split airflow from one of the two concentric ports into a smaller "secondary" airflow at a 90 degree angle, and a continuing "primary" path of air in the same direction. Fittings better suited for splitting a single flow into two even flows are "wyes", which come in a variety of angles and arrangements as shown in Figure 3-Figure 5. While all of these are effective in splitting the stream of flow, the sweep tee and true wyes both result in the two streams of flow moving in different directions, unlike the manifold type wye. This could become problematic depending on the location and orientation of the fan. For instance if it were decided that the fan should be mounted on top of one of the "scent chambers" on the side of the box, the sweep tee and true wyes would cause the split airflows to be traveling in different directions (at different orientations with respect to the outlets of the box). This would mean that one of the two paths would have to make a larger degree of turns than the other. As discussed above, any difference in the number of turns or degree of turns made (between the two ducts) will cause a difference in the flows on either side of the box. The manifold–wye on the other hand would be a possible choice for any configuration of the fan, because the two ducts will come out of this wye oriented in the same direction, meaning that both will have to make the same number and type of turns in order to be connected to the outlets on either side of the box at the correct orientation.

Figure 2: Duct Tee

Figure 4: Sweep Tee Wye

Figure 3: Manifold-Wye

Figure 5: "True" (120 degree) Wye

For material selection of the duct system, research was conducted on whether or not different types of duct would pose an advantage for the system. While galvanized steel duct is generally used in ventilation situations, the products offered were generally found to come in much larger sizes than were appropriate for the small airflows used. Due to the fact that many plumbing fittings and pipe are able to be arranged similarly to duct, and the fact that these come in much smaller diameters than duct, research was done to determine whether there would be drawbacks or limitations to using a system of PVC pipe and fittings rather than duct in the exhaust system. While there are small differences in friction coefficients of PVC and steel, both are relatively low, and neither produce enough friction to be a problem, especially when flow is laminar (as it will be in this system). Furthermore, piping (schedule 40) is pressure rated, as it has to carry a compressible substance (water) rather than an incompressible one (air); while unnecessary, this means that there are no safety concerns when using PVC pipe in place of traditional duct. One problem is that combining the use of PVC (or other plastics) and metal duct can make it difficult to create an air-tight seal, as those sealants used in PVC piping are generally only effective in connecting two pieces of PVC. Therefore it would be best to use the same material/type of duct or pipe throughout the system, to avoid problems with air loss and fitting (ducts and pipes are built using different standard sizes).

For the selection of the fan, there were a few considerations that we had to take into account. First, as this will be used in a lab setting, the fan motor would ideally be powered by a simple wall outlet (120V 60Hz AC power). It was also desirable to have a fan motor that was speed controllable, to allow for variation and adjustment of fan speed if testing proved this to be necessary. Alternating current motors utilize windings to control the speed at which the fan moves. These windings are only designed to run at a certain voltage, current, and frequency of power, meaning that if the voltage input is changed, the current and frequency will automatically adjust to maintain a constant speed. This makes use of a traditional voltage divider (or rheostat) control ineffective in controlling the fan, and even if the speed can be altered using one, the motor will have problems with over-heating, making operation unsafe, and possibly causing damage to the motor (most manufacturers state that the warranty is voided if speed controllers are used with the fan). The only safe method of controlling the speed of a normal (single speed) alternating current fan is to use a variable frequency drive (VFD) that changes not just the voltage and current, but also the frequency of power supplied. These proved to be extremely expensive for a project of this scope, as they are generally used to improve the efficiency of large fan motors used in commercial HVAC applications. From research of a number of manufacturers and suppliers, it is clear that a speed controllable AC fan would be much too expensive for a project of this scope, and that such speed controllable fans are extremely rare, as the airflows needed are very low in comparison to those purchased for commercial and residential applications. While speed controllable DC fans are much more affordable and available (especially those used for computer cooling), they cause the problem of requiring a power transformer in order to be plugged in to a normal wall outlet. From this research it is apparent that if the speed of airflow is to be altered, it would be best accomplished by increasing the pressure loss in the duct system (resistance to flow), especially because energy efficiency of the system is not a major concern.

1.3.3. Airflow

If two scents were simply introduced (from opposite sides) in a CPP box, each would diffuse (in a random manner) until they were equally distributed, with the same concentration at each point throughout the entire box (Philibert, 2005). In order to keep the scents separated, with one on each side of the test subject, and have them travel in a controlled manner, an airflow pattern must be established to allow for control of their movement.

Due to the fact that the rats used are raised in a lab environment rather than the wild, it was important to ensure that there were not unusually high air-speeds through the box, as these could affect the behavior of the rat, influencing the results of the experiments. Rats are accustomed to airflow caused by normal ventilation for laboratory buildings, which are designed based on the number of air changes per hour, also known as the air change rate. For laboratories, there is no single standard for air changes per hour; however, chapter 14 of 2007 ASHRAE HVAC Applications lists a guideline of a minimum of 10 to 15 air changes per hour for secondary animal enclosures in laboratories (AIRCUITY, 2012). While the airflow based on these guidelines should not be exceeded during normal operation, it may be necessary to induce higher airflows to draw in or remove scents before and after testing.

It was also important to research possible obstacles to producing an airflow that is not only moving at a speed that is comfortable to the rat, but also carrying the scent to all parts of the box. Although the scent will diffuse throughout the box evenly given enough time, any “dead-spots” that do not contain traces of scent due to the induced airflow will cause the process of filling and emptying the box of scent to take longer. One phenomenon observed in a similar situation, the ventilation of rooms, is called the “vortex effect”. This effect describes the re-circulation of air in certain areas of a room, especially when the air is brought in near the ground at one side of the room (or box), and removed higher up (near the ceiling) on the other side. This phenomenon would prevent some of the scent from being removed from the box as quickly as would be anticipated based on the volumetric flow induced in the box.

It is evident from research that regardless of system design, any CPP device that uses airflow as a method of distributing scent must be validated to ensure that the airflow pattern induced will carry the scent throughout the majority of the box, while not causing discomfort to the rats that will be present. While analysis of the volumetric flow rate out of the box will be sufficient in approximating the time needed to clear the scent, Computational Fluid Dynamics (CFD) analysis is necessary to predict the characteristics of airflow through geometry given point in the box. In addition, a scientifically valid test of the presence of scent at a point is not available to us, so research was carried out to determine a suitable test for confirming the results of any CFD analysis.

1.3.4. Testing

One test to ensure that the CPP device was airtight was to apply soap to the crevices of the box. If bubbles become visible, the surface is not air-tight, and needs to be re-sealed.

In order to test that the scents remained separate, equal flow rate had to be established on either side of the middle chamber. This experiment was conducting using an anemometer, which is used to measure velocity of air across a given cross-section. The anemometer used was the Omega HHF-SD1 Hot Wire Anemometer/Thermometer.

Another possible method of testing was the use of gas analyzers in tandem with concentrated gasses in order to mimic the mixing of scent with the airflow at the inlets. In this method of testing a concentrated gas would be mixed with atmospheric air, which has known concentrations of nitrogen (78.08%), oxygen (20.95%), 0.93% argon, 0.038% carbon dioxide, and trace amounts of other gasses. The addition of the concentrated gas alters the concentration of gasses in the air, which can be detected by a gas analyzer. The concentrated gas could act as an impurity that reduces the concentration of a gas present in atmospheric air which can be measured using a gas analyzer. Another possibility is the introduction of a gas of high concentration, such as carbon dioxide, oxygen, or carbon monoxide, into the box that will create a net increase in the concentration of that gas (when compared to atmospheric air), which would be measured by a gas analyzer. Fortunately WPI's Fire Protection Engineering (FPE) labs, located in Gateway Park, use carbon monoxide (CO), carbon dioxide (CO_2), and oxygen (O_2) gas analyzers to evaluate air produced by burning various materials. The FPE lab also has access to a variety of concentrated gasses, such as carbon dioxide, carbon monoxide, and nitrogen; making either of the methods of testing using gasses a possibility.

2. Conditioned Place Preference Apparatus

2.1. Goal Statement

The primary objective of this project was to design an apparatus that can be used to assess the behavior of a pre-conditioned rat when exposed to various scents. Design Specifications

2.2. Design Specifications

- Must keep scents from crossing the center line when air is flowing
- Must provide enough space for the rat to move around
- Must create equal airflow on each side of the box
- Must provide significant sensory variance on either side of the entry chamber
- Holes on either side of the box must be small enough that a rat cannot pass through
- Lid must be transparent to allow for motion tracking
    - Lid must be removable to allow for cleaning
- Airflow through box must be equivalent to (or less than) that of normal animal enclosures (10-15 air changes per hour).
- Airflow must be controllable
- Parts of the box other than inlets and outlets must be airtight

2.3. Final Design

We placed three guillotine style sliding doors, on one side of each chamber so the test subject can be introduced in any of the three sub chambers as and when required, as seen in Figure 6 (part a). Furthermore, we created a hole of dimensions 4"X 6" on each separation wall, so the test animal can move from one chamber to another freely. The side chambers are 9" long and 12" wide, while the middle chamber is 6" long and 12" wide; the entire apparatus is 24" long 12" wide and 9" tall. These openings also come with sliding doors, which can be opened after the rat has been introduced into one of the chambers, as seen in part b of Figure 6. The ability to confine a subject in one chamber might facilitate specific conditioning. There are two scent boxes that are attached to the right and left end of the system with holes on the side facing the main chamber. These holes will be used to cycle the scent from the scent box into the main chamber and out the exhaust system. The scent will be introduced using filter screen paper which will be attached to the wall along the holes. The effectiveness of these filter papers is reasonable, as they have been functional in the past to conduct previous experiments by the UMMS Staff.

The exhaust system is designed with the main focus being to avoid the interference of either of the smells. The ventilation will be continuous during the experiment to recycle the air constantly.

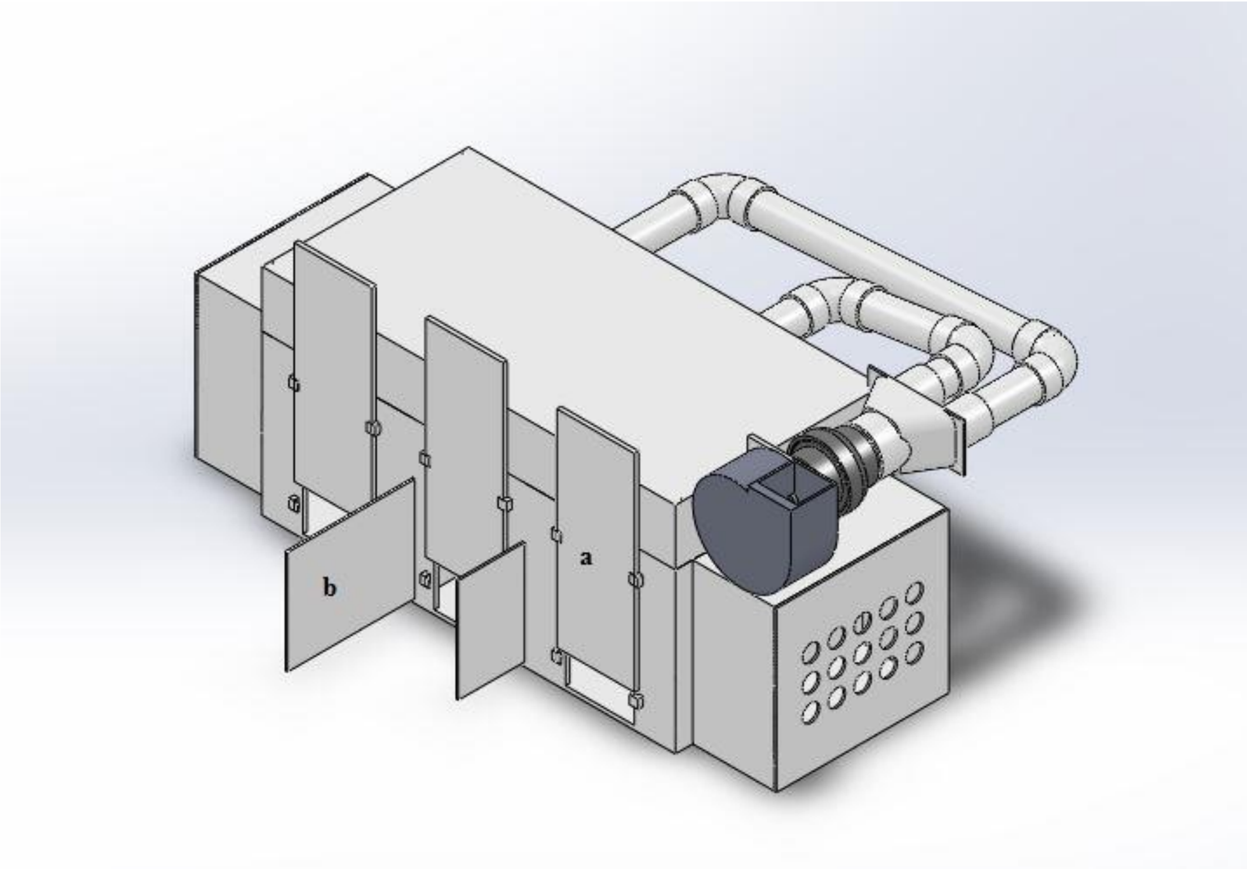


Figure 6: CPP apparatus

a. Guillotine Door

b. Sliding Door

2.4. Methodology

2.4.1. Construction

After conducting widespread research, and based on the suggestions presented to us by the CCNI staff to pick an ideal material for the construction of the box, the decision was made that PVC would be the best material to use. PVC, or Polyvinyl Chloride, is a plastic with the following chemical formula: $CH_2=CHCl$. PVC is a thermoplastic material which comes in various colors and mechanical properties depending on the added compounds and the final application that it is required for. As we required the apparatus to have three different colors, PVC's attributes would be ideal for our construction. Some other properties of PVC that made it an ideal construction material were its ability to absorb shock without damage. As we had an exhaust system that was supported by a fan of airspeed 49 cfm, which would be mounted on the scent box next to the black chamber, we had to make sure the vibrations of the plastic due to the fan would not interfere with the scent experiment at any time. Furthermore, PVC in general is a very inexpensive because it is created using inert materials that simply add bulk to plastic; hence it made the construction process much more economical.

We began construction of the CPP with the main chamber. Before putting the apparatus together, all of the sheets of PVC and acrylic had to be ordered. The sheets were already cut to the correct dimensions when purchased, but the more detailed patterns had to be cut manually. First, a pattern (3 rows by 5 columns) of holes of 1 inch diameter had to be cut into the east and west walls of the apparatus using a drill press. Next, it was necessary to cut 4 by 6 inch doorways out of the chamber dividers. Two 1/8 inch thick slits for sliding doors had to be cut into the components of the front wall as well. These two cuts were made using a band saw. Finally, the pattern of holes in the lid and holes for the exhaust from the main chamber were cut out of the acrylic using a laser cutter as seen in Figure 7.

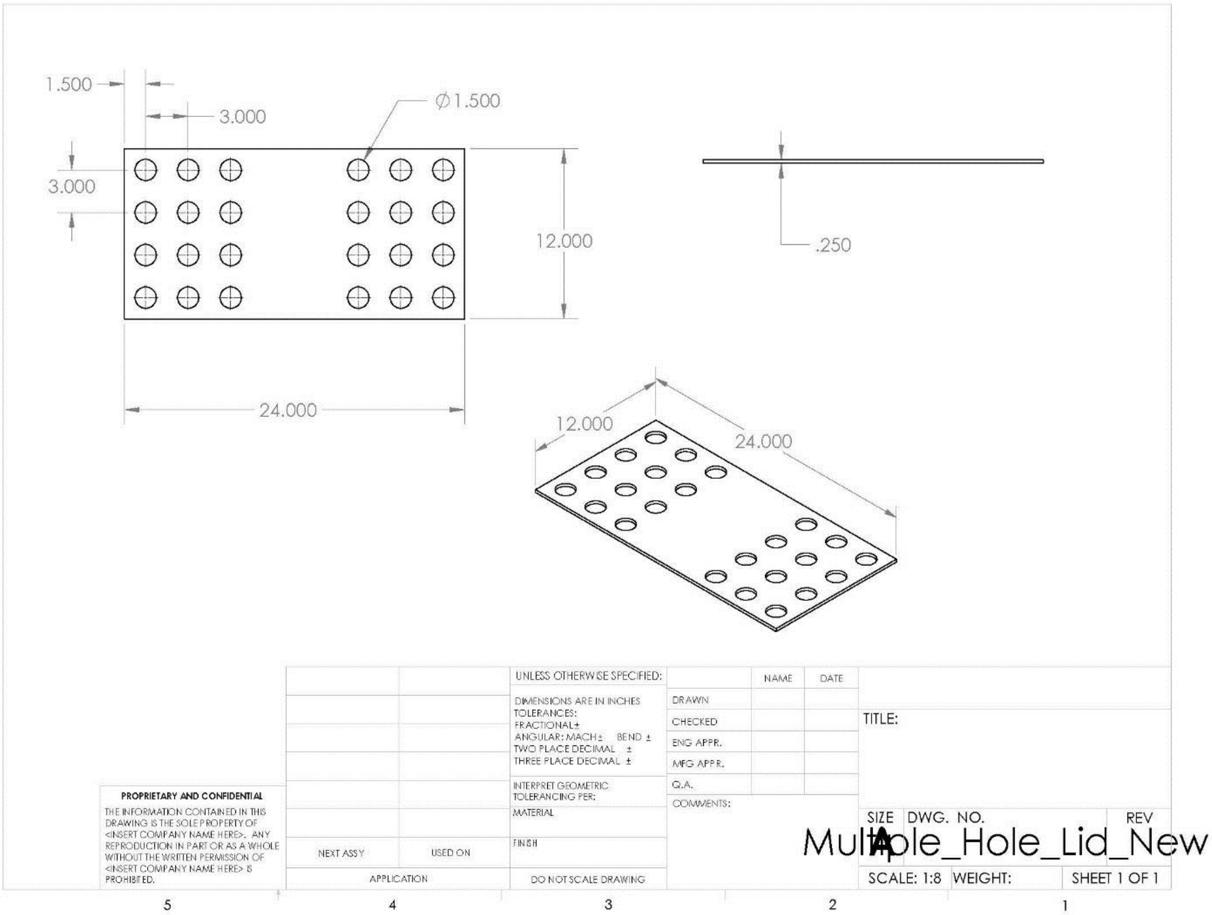


Figure 7: Hole Pattern Cut into Acrylic Lid

The PVC could not be cut using the laser cutter because this would create a high quantity of chlorine gas. Also, it was not possible to cut the PVC using a CNC machine because the sheets were too thin, and the vibration caused by the CNC machine would cause the plastic sheets to fracture.

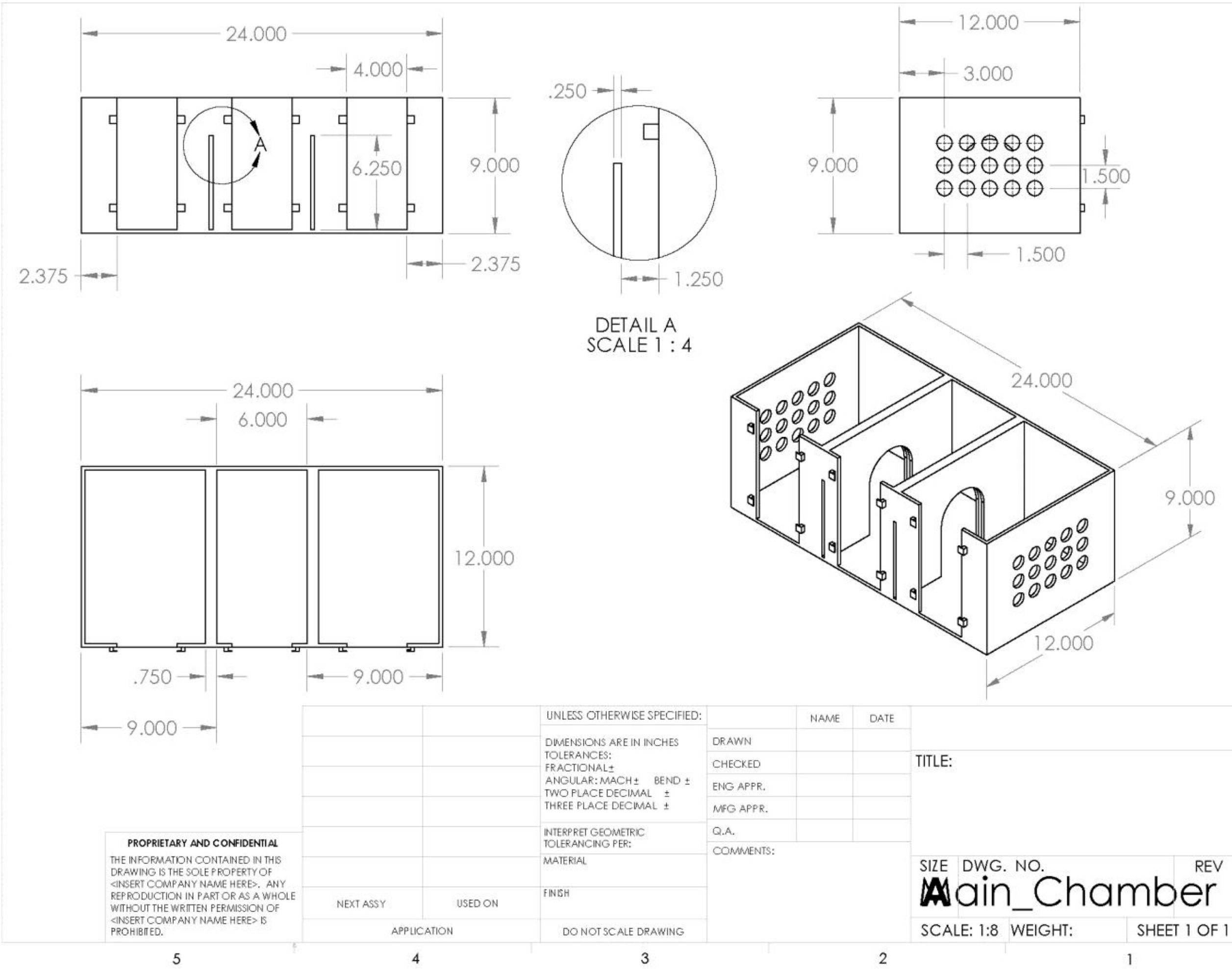


Figure 8: Engineering Drawing of Main Chamber of the CPP Apparatus

In order to construct the box out of PVC, an adhesive that bonds PVC together was needed. AZEK ® is a liquid solvent cement that is water based and water soluble with a mixture of plastic resin dissolved in solvents. When the adhesive is applied to two sheets of PVC, a chemical reaction starts and causes the PVC to melt and fuse the two sheets together. The new bond is stronger than the PVC itself, making this the perfect adhesive for our purposes. However, it was also necessary to glue acrylic to acrylic, acrylic to PVC, rubber to acrylic, and metal to acrylic/PVC for which the liquid solvent cement would not work. Since these bonds only needed to be made less than three times each, Gorilla ® Super Glue was used. There are specific adhesives for each of these connections, but purchasing a bottle of adhesive for each of these was not economical. To minimize the amount of air that is entering the system from sources other than the holes in the side of the apparatus, the guillotine style doors had to maintain a near airtight seal with the apparatus when closed. Since the doors are a quarter of an inch thick, ¼ inch mirror hangers were glued around the entry ways, allowing the guillotine doors to slide up and down, while maintaining contact with the wall at all times. A detailed view of the assembled main chamber can be seen in Figure 8.

To assemble the exhaust lid system, the left and right outer walls of the lid assembly, made of PVC, and the front and back walls, made of acrylic, were glued to the acrylic lid. Then, in order to ensure that the scents and gases did not mix in the exhaust lid, two dividers were glued to the lid as well. In order to allow the air to exit the exhaust lid system through two pipes, two holes were cut into the back wall of the assembly using a laser cutter before assembly. The entire exhaust system assembly can be seen in Figure 9.

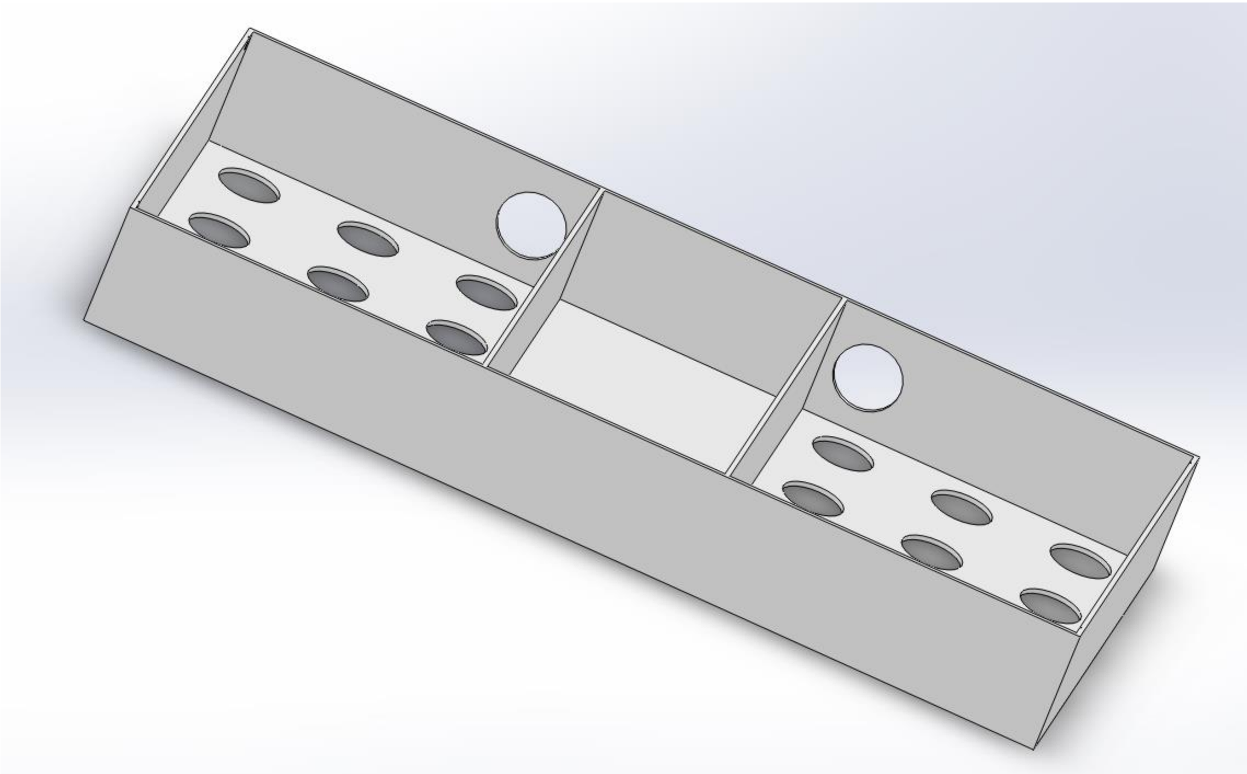


Figure 9: Exhaust System Assembly

The exhaust system lid is attached to the CPP in two ways, with magnets and locking latches. In order to ensure that no excess air is entering the system, rubber strips were glued around the perimeter of the CPP. The magnets help with primary orientation of the lid, and the latches clamp it down, compressing the rubber strips and creating a near to airtight seal between the lid and the main box. The fasteners also help prevent the lid from falling off when the piping system is detached from the fan, which causes a moment about the edge of the box (on the side where the piping is attached).

The clear acrylic lid of the exhaust system was screwed down into the PVC walls of the system. The lid was fastened in a quick removable/attachable manner to provide for easy maintenance and cleaning after use of the apparatus. Lastly, two scent boxes were glued to the sides of the main chamber in order to ensure scent containment. These scent boxes had hole patterns matching the main chamber. The entire CPP apparatus assembly with guillotine and sliding doors can be seen in Figure 10.

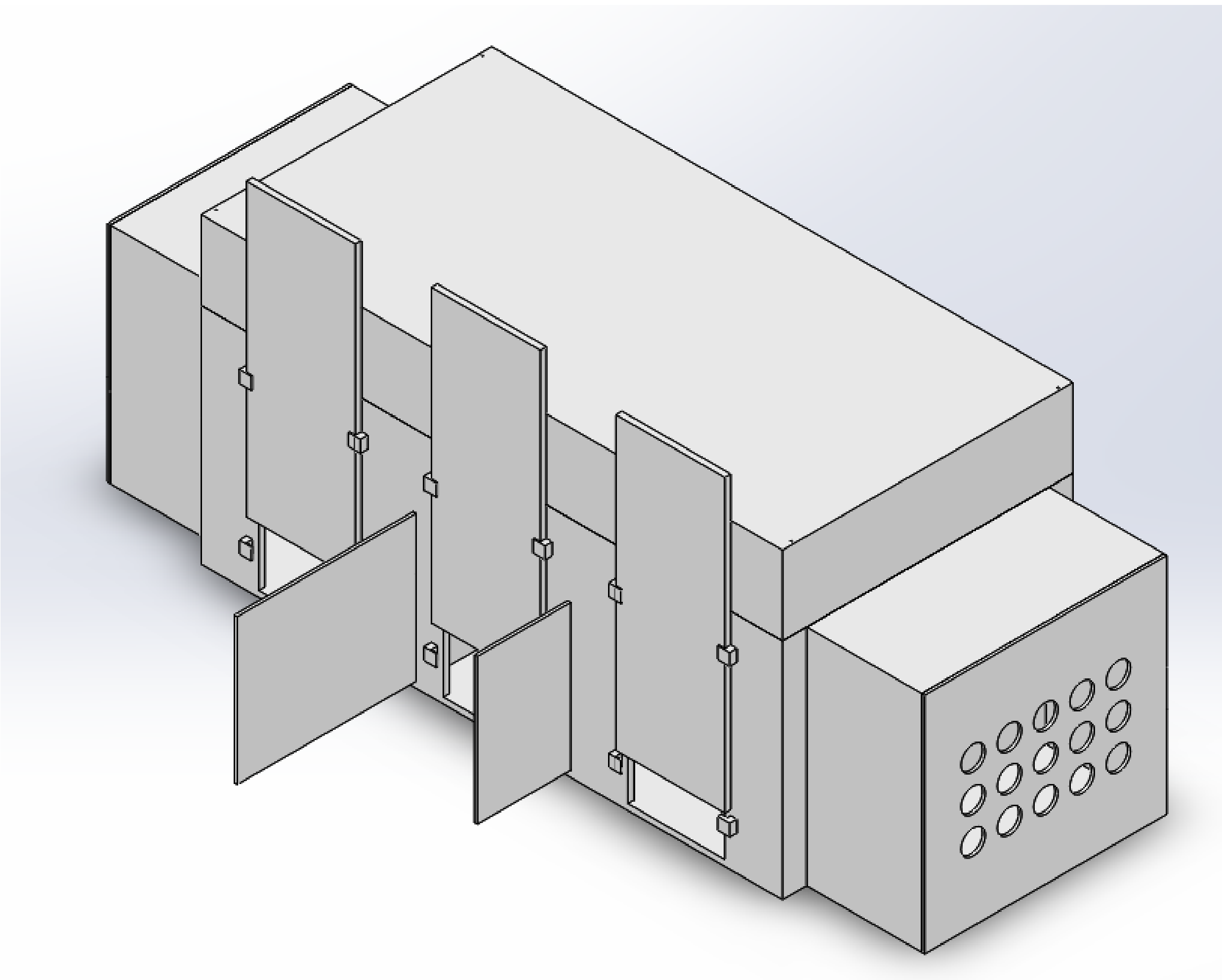


Figure 10: Conditioned Place Preference Apparatus

Once the main box and lid were assembled, the ventilation system had to be fabricated and attached. This consisted of the fan, PVC piping, fittings (elbows and manifold wye-splitter), as well as the faceplate used to attach one end of the pipe to the inlet of the fan.

First, the faceplate, which provides the reduction from the diameter of the fan inlet to the outer diameter of the PVC pipe was fabricated by laser cutting a hole (the size of the outer diameter of the threaded pipe) in a small piece of black acrylic sheet. This faceplate was then attached to the fan by applying a continuous circle of adhesive to the plastic, and then pressing the plate against the fan, while ensuring that the circle of adhesive created a seal between the plate and the fan. The threaded pipe was then cut in half, to produce two shorter pipes, each with a threaded and unthreaded end. The unthreaded end of one of these pipes was attached to the faceplate (and fan) using super glue, while the unthreaded end of the other was inserted into the single inlet end of the manifold-wye. The threaded ends were then inserted into each end of the threaded couple, allowing the fan to be disconnected from the rest of the piping without removing the fan from the box. The fan was secured to the rubber vibration isolation pad and the top of the scent box by using two screws, which ran through existing holes at the base of the fan and then through manually drilled holes in the pad and box. With the ventilation system from the fan to the manifold wye complete, the lengths of the schedule 40 PVC pipe and 90 degree elbows that connected the wye to the outlets of the box were re-measured to account for human error in manufacturing as well as unknown dimensions and tolerances in the manufacturing of the fittings.

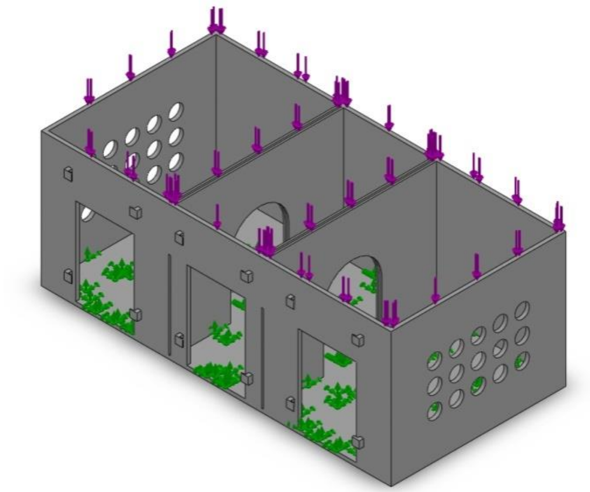
With the lengths of PVC sections confirmed, each section was cut from the 5 foot length of PVC using a hacksaw; and then sealed into elbows as appropriate, using Azek PVC liquid solvent cement**.** Then this assembly was fit into the two inlet side of the manifold wye (which was attached to the fan as shown in the design drawings) on one end, and the two outlets of the box on the other. The PVC adhesive was then used to attach one end to the manifold wye, and allowed to set while resting in the outlets of the box, and being supported vertically (in the middle) at a fixed height. This ensured that the pipes attached to the manifold wye at an angle that allowed the other end to easily insert into the outlets of the box (when all components including the fan were secured to the box).

The final aspect of manufacturing the ventilation system was assembling the L-brackets that would provide vertical support for the piping. First a bracket was placed with one end attached to the (removable) top of the box using two bolts, and the other end running out over the section of pipe directly upstream (close to the fan) of the manifold-wye. Each end of a steel wire was then fed, up through two holes in the "top" of the L-bracket, creating a "saddle" or loop on which the pipe rested on. This wire was tightened slightly (to ensure that it would bear the load of the piping at all times) and tied off over the top of the bracket to keep it in place. Two, larger brackets were assembled similarly, but placed near the two outlets of the box, primarily to prevent the piping from rotating about the first support when it was detached from the fan using the couple.

2.4.2. Stress Analysis

Stress analysis was conducted on a model of the CPP device in order to determine if it would be able to withstand the expected forces applied during its normal operation. This analysis was carried out using the SolidWorks "simulation express" feature, with the material defined as solid, linear elastic, isotropic, rigid PVC. Analysis was carried out on the main chamber (the three chambers that the rat will travel through) and the scent box bearing the weight of the fan, in order to ensure that the thickness of the PVC sheet used would not result in unsafe deformations or failure. Each body was analyzed individually, with glued walls treated as mated surfaces (due to the fact that the bond created by the glue is stronger than PVC). Loading for each was estimated conservatively by including the weights of all other components of the device that could possibly be supported by each section at any given time.

For the simulation of the main chamber, the floor was made a fixed point, as it is reasonable to assume that the box will not be moved during normal operation, and that any movement will be done with the lid and other loads removed from this portion of the box. The load used for the main chamber included those due to the weight of the lid assembly and the PVC duct, as well as the fan (in case the fan was ever placed on the box itself rather than the scent box) with a total load of 153 lbf. This load was applied uniformly, normal to the top surface of the box, which while not completely realistic, is sufficient in predicting the general magnitude of deformations to be expected.



Body Properties	
Mass	5.3 kg
Volume	00.0041 m^3
Density	1300 kg/(m^3)
Weight	52 N
Material Name	
Material Name	PVC Rigid
Model Type	Linear Elastic Isotropic
Tensile Strength	4.07 e7 N/(m^2)
Loading Properties	
Type	Normal Force
Value	153.8 lbf

Figure 11: Setup of Stress Analysis on Main Box

The scent box was modeled similarly, with the floor fixed, and the weight of the fan and duct system being the cause of loading. With a load of 85.5 lbf applied uniformly (normal) to the 4"x4" rubber pad that the fan is mounted on. The rubber pad had to be modeled as a PVC surface, due to the limitations of the SolidWorks stress analysis tools, but the distribution of loading applied to the scent box should not be significantly different, as both would transfer the uniform compressive load similarly. Because the load was capable of being moved around, this test was run for four different locations of the fan (more specifically the rubber pad it is mounted on). First the surface representing the rubber pad was placed offset (by 1/4") from the wall of the main box and the side of the scent box (either side will produce the same result due to symmetry) as seen in Figure 12. To determine the effect of mounting the fan closer to the "open" end of the scent box (where the door will be placed), the second model was placed the same distance from the side of the scent box, but offset ½" from the wall of the main box Figure 13. The final two models were run at the same distances from the wall of the main box as above, but offset 0.75" from the side of the scent box as seen in Figure 14 & Figure 15. As in the analysis of the main box, the box was treated as a solid body, with the material properties of rigid PVC, and the default mesh generated by SolidWorks (Figure 16) was sufficiently accurate.

Body Properties	
Mass	0.945 kg
Volume	0.00073 m^3
Density	1300 kg/(m^3)
Weight	9.257 N
Material Name	
Material Name	PVC Rigid
Model Type	Linear Elastic Isotropic
Tensile Strength	4.07 e7 N/(m^2)
Loading Properties	
Type	Normal Force
Value	8.6 lbf

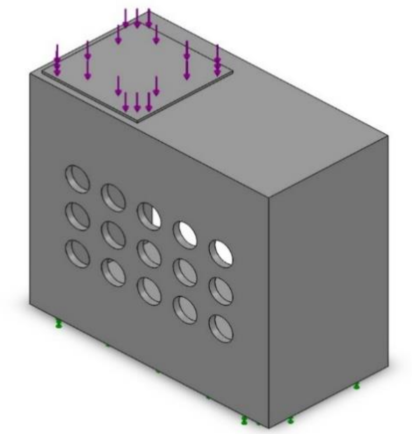


Figure 12: Setup of Stress Analysis - 1/4" from wall and side

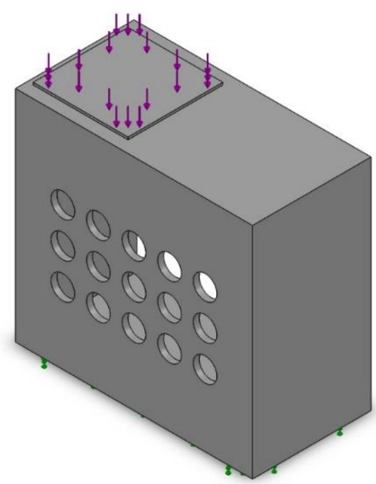


Figure 13: Setup of Stress Analysis - 1/4" from side and 1/2" from wall

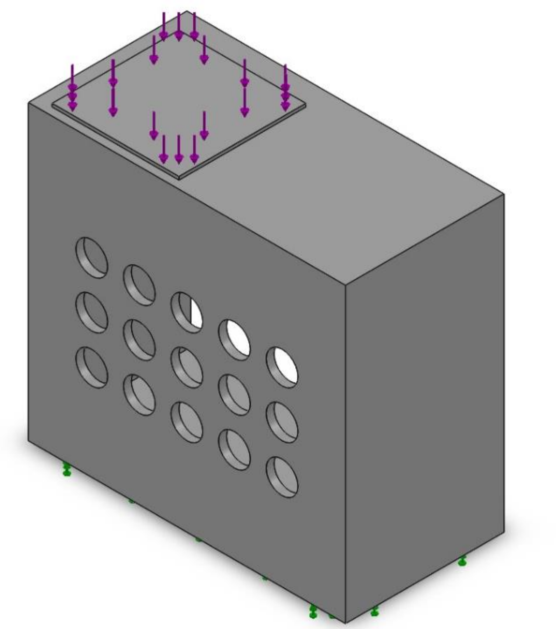


Figure 14: Setup of Stress Analysis - 1/2" from side and 1/4" from wall

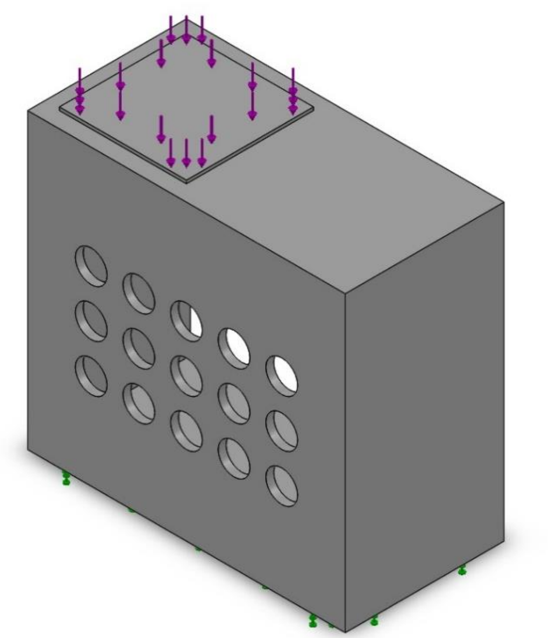


Figure 15: Setup of Stress Analysis - 1/2" from side and wall

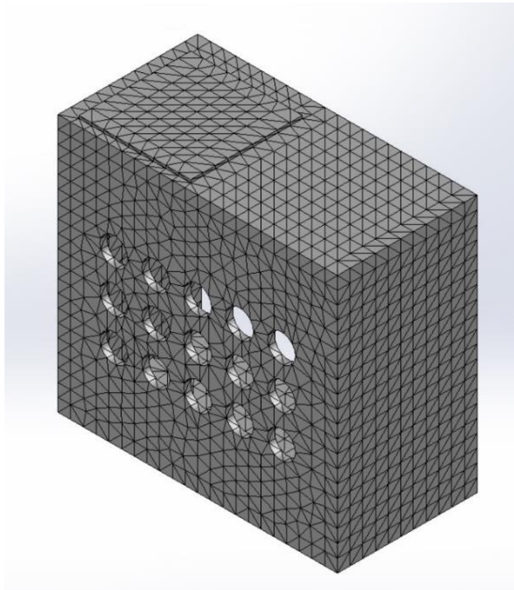


Figure 16: Example of Meshing For Stress Analysis of Scent Box

2.4.3. Testing

After the system had been modeled using computational fluid analysis software, physical testing could begin. The primary purpose of the CPP apparatus is to keep two different scents on opposing sides of the box, since there is no scent detection device, nitrogen was pumped into the box and changes in oxygen concentration were measured. The exhaust fan was running throughout all of these tests. The nitrogen that was used was 99.99% pure and the oxygen analyzer used was (Servomex 1400B4 Gas Analyzer). The first hypothesis that needed to be proved was that the ventilation system was bringing new air to all locations in the box. In order to do this, nitrogen was introduced from the east side of the apparatus and measurements were taken on the floor and at an elevation of 3 inches at the locations shown in Figure 17.

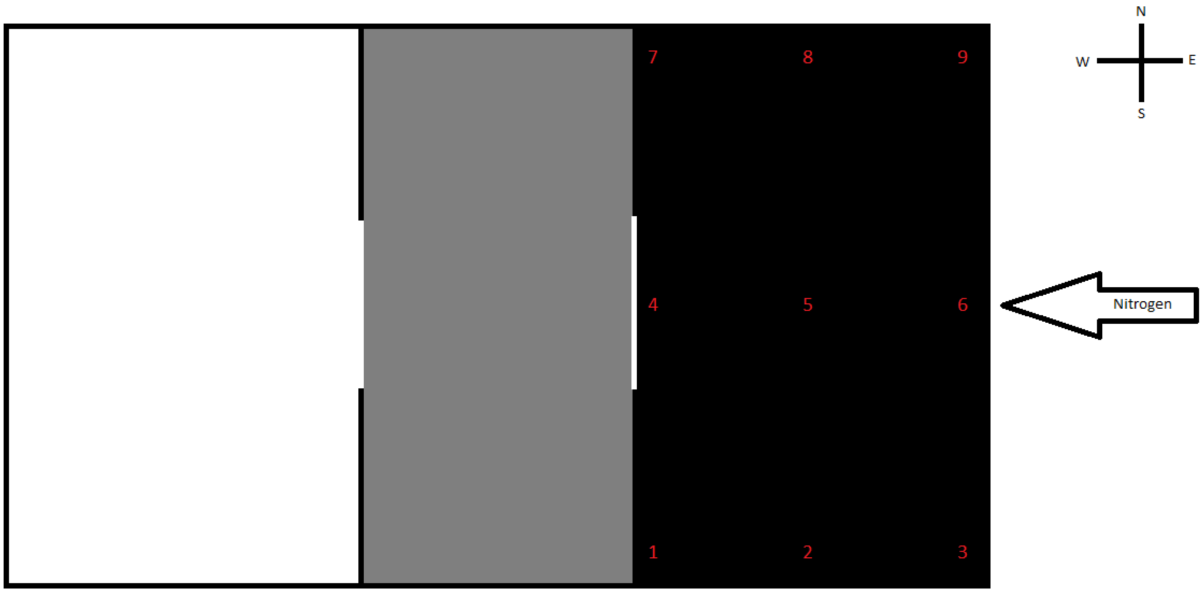


Figure 17: Locations Where Outer Chamber Measurements were Taken

Next, it was necessary to check the distribution of nitrogen in the middle chamber; measurements were taken according to the locations shown in Figure 18. This region needed to stay relatively neutral so that the rat would not be confused by two different scents from the beginning. Initially testing was conducted with the guillotine door separating the east chamber from the center one open, however additional tests were done to analyze the effect of keeping the door closed while the east chamber filled with the gas (or scent). To do this the fan and nitrogen were run for as long as necessary to allow the east side to have scent located at all points (determined from the results of the outer chamber measurements) with the door closed. Once sufficient time had passed the door was opened, and measurements were taken at point 4 (Figure 18) at the floor and an elevated location for at least a minute to determine if gasses crossed the mid-line, and if so how long it would take for them to do so.

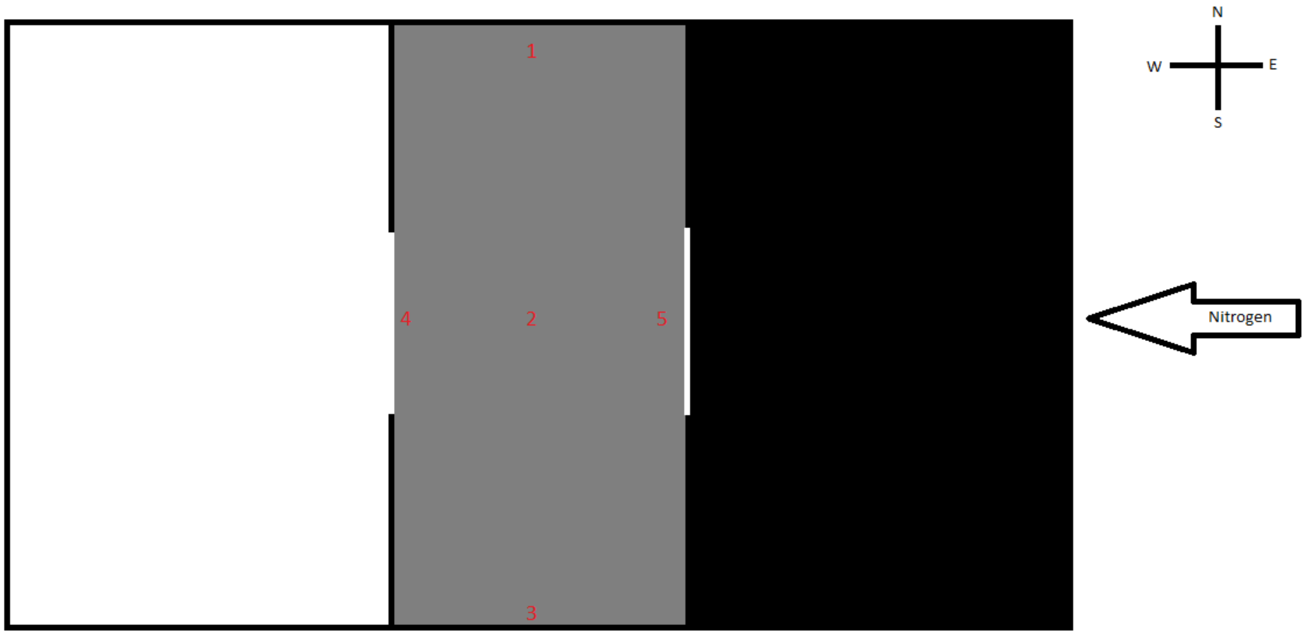


Figure 18: Locations Where Middle Chamber Measurements were Taken

To prove that the gases did not cross over to the other half of the apparatus, the sensor was placed at the exhaust pipe of the opposite outer chamber, as shown in Figure 19. If any nitrogen were to have crossed into this section, it would be exhausted through this pipe and the O_2 concentration would change.

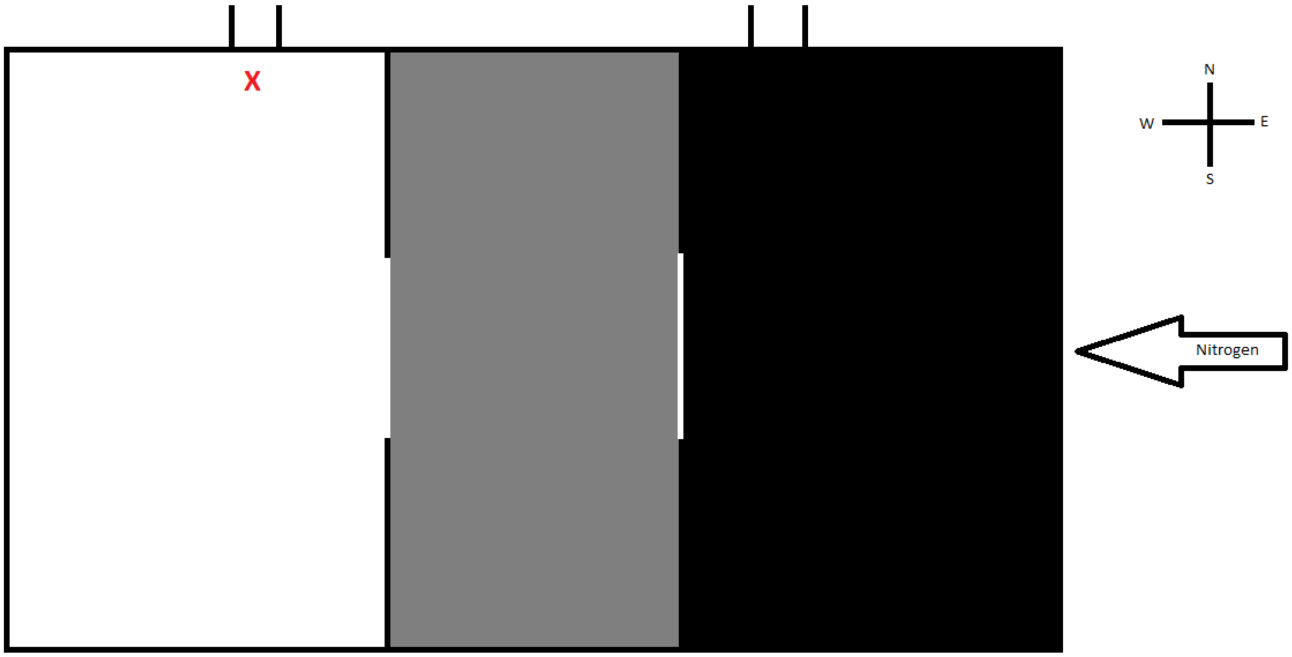


Figure 19: Opposite Outer Chamber Measurement Location

It was also necessary to measure how much time it takes for the apparatus to completely recycle the air in the box. This clearing test was run as a two part test and the analyzer was placed at the exhaust pipe for the east (Figure 20) chamber since all of the gas would exit through this pipe. Part one required us to run the nitrogen and exhaust fan until the maximum change in oxygen concentration was reached. Then, the nitrogen was turned off and the exhaust fan was run until normal oxygen concentrations were reached.

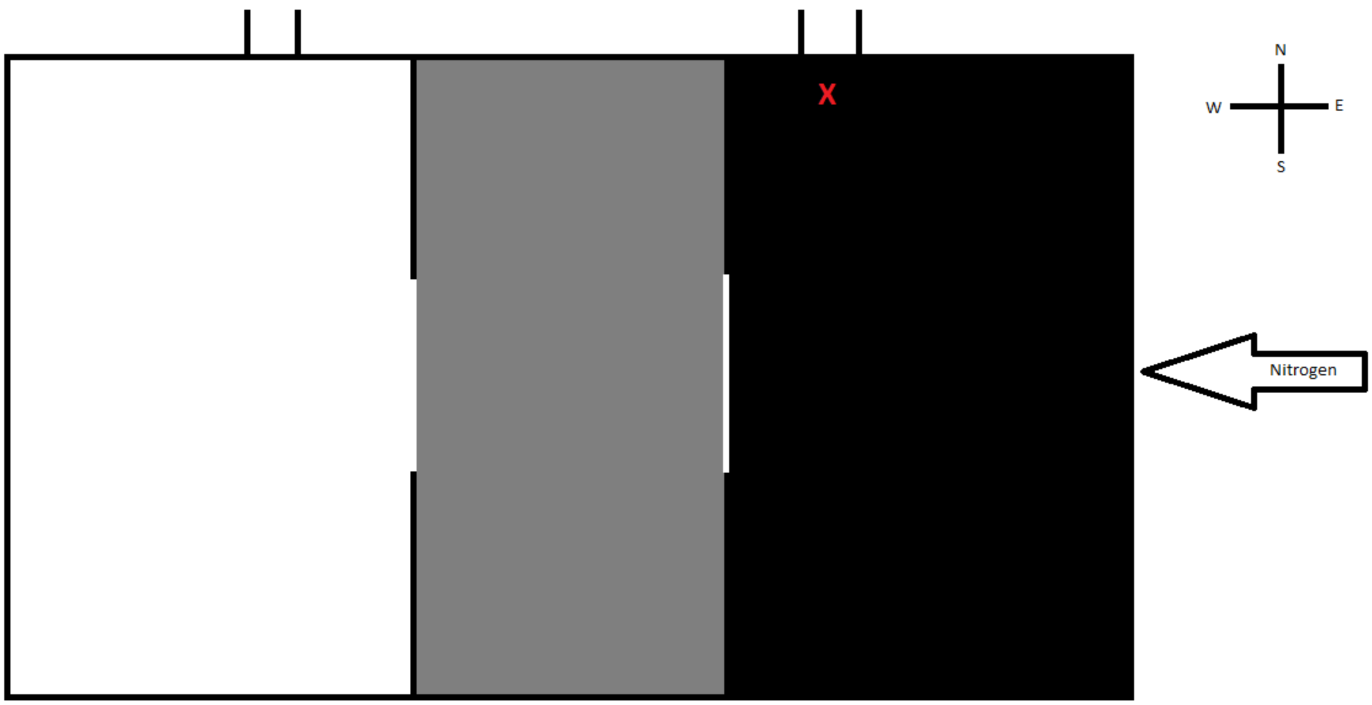


Figure 20: Clearing Test Measurement Location

The data was run through a data acquisition (DAQ) box and analyzed using LabView and Microsoft Excel. Raw data was given in voltages, so a correlation between voltage and oxygen concentration had to be established. This was done by running only nitrogen into the analyzer, recording values, and then running atmospheric air with normal oxygen concentrations through the analyzer while recording values. This gave two different known concentrations of oxygen, 0.01% and 20.9%, to compare to voltages recorded; a third set of points was taken from the voltages at the minimum concentration (19.89%) measured at location two of the floor level tests of the black side of the box (see Figure 41). These three sets of points were plotted (concentration vs. voltage) and a linear fit was performed, as the relation of the voltages output by the analyzer were known to be linearly related to the oxygen concentration, to allow for the voltages recorded to be converted to oxygen concentration. In addition to allowing the voltages to be scaled to oxygen concentrations, the two tests run at constant oxygen concentrations allowed for validation that the test results indicated significant changes in oxygen concentration (indicated by a change in voltage well beyond those measured at constant concentration). To do this, the standard deviation of the two constant concentration results (left in voltage) was found using the Microsoft Excel “stdev.p” function to determine the precision of each measurement, and the range of voltages measured at each was recorded.

The anemometer testing was conducted in order to prove that we established even airflows in the black and white chambers of the CPP box. In order to do so we had to set up the probe facing the inlet of the piping, this was connected to the upper chamber of the box. In order to get the probe concentric with the inlet we first unscrewed the top of the box, and then introduced the two probes from the black and white chambers. Using electrical tape we fastened the analyzers to the acrylic lid, and set them up in position. As the tubing of the analyzers was going through the guillotine doors, it left a small gap when we would close it. Therefore, we used silicone seal to seal this gap and left it overnight to dry. We also screwed the acrylic lid back on so the airflow in the lab wouldn’t affect the experimental data.

When we were ready to begin testing, we set up the anemometer to read cubic feet per minute, so it would give us precise readings. Our first test to be conducted was the airflow tests on both sides simultaneously. We started the fan and took measurements when the fan was at its peak exhaust speed. Later, as we wished to present the UMMS staff with a model that would be able to produce variable exhaust speed, we wished to conduct the experiment when a portion of the inlet piping system was to be blocked off. This experiment was conducted only on one inlet of the apparatus. We conducted our experiments using cardboard to block off sections of the inlet. First, we blocked off one fourth of the right half of the inlet, started the fan and took measurements. We then repeated the process with half of the inlet blocked off, and one-fourth section blocked off from the left side of the inlet.

Once we had completed all the experimental processes for this test, we used excel to plot the change in air flow rate in all cases. From this, we calculated the time that it would take to cycle out all the air from the box in each case so we could get an estimate of how long it would take the UMMS staff to cycle out one of the scents and then introduce another.

2.4.4. Valve Damping

Once we got conclusive results from our damping test, we decided to install a ball valve into the piping system so we could regulate the volumetric flow rate, which is representative of the rate at which air is recycled through the apparatus. The ball valve has a spherical disc inside it, which controls the fluid flow through it. The sphere has a hole, or port going through the middle so when the port is in line with both ends of the valve, maximum flow will occur. When the valve is closed, the hole is perpendicular to the ends of the valve, and flow is blocked (Figure 21). When between the open and closed positions, it is possible to regulate the airflow. The ball valve was attached 1.5 inches away from the first 90 degree turn from the inlet of the piping. Once it was attached, we glued the valve to the piping system and let it sit for a day so it would be permanently fixed. Once the new piping system was ready, we attached it back to exhaust fan and the CPP apparatus and started the testing procedures.

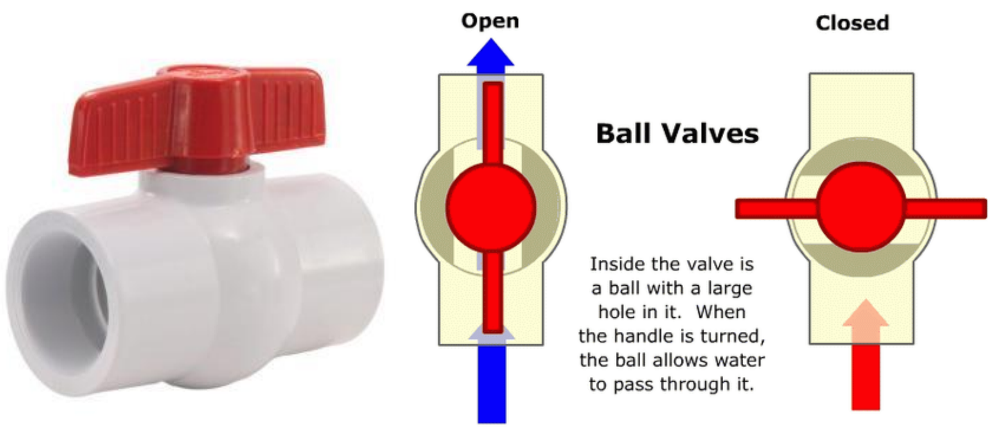


Figure 21: Ball Valve and its Function

Similar to the fan damping test, we introduced the anemometers inside the CPP apparatus and set up the analyzers with their open ends facing the inlet of the piping system. Then, we unscrewed the acrylic top, and taped the analyzers in place so their orientation wouldn't alter during the testing process. Once the analyzers were set up, we screwed the acrylic lid back on, and were ready to start up the fan and begin taking recordings.

We set the anemometer to record at cubic feet per minute so it would give us accurate results. The first recording we took was with the ball valve completely open, so we would have an idea of how much the wind speed was altered just by having a valve attached to the system. Next, we turned the fan off and made a quarter turn on the valve. We turned the fan back on and waited until it reached its maximum speed, and then recorded the data for the quarter closed valve. We repeated this process with the half closed as well as three-quarter closed valve.

2.4.5. Computational Fluid Dynamics

In order to establish that the final design would in fact produce a desirable airflow (with reasonable airspeed and coverage of all parts of the left and right compartments of the box); a 3 dimensional Computational Fluid Dynamics (CFD) analysis was performed. First, a geometry was made in SolidWorks, in which the free space (air) in the box was modeled, rather than the solid features of the box. Only half of the box was modeled (from one inlet to the midline of the middle compartment) in order to limit the number of cells needed for analysis. This could be done because the mid-plane of the box could be labeled as a “symmetry” boundary condition, so that the analysis accounted for the identical airflow on the other half of the box, while reducing the computational domain. The model was imported into an ANSYS Workbench 14.5 (Fluent) CFD analysis setup, and a mesh was generated, which can be seen below in Figure 22.

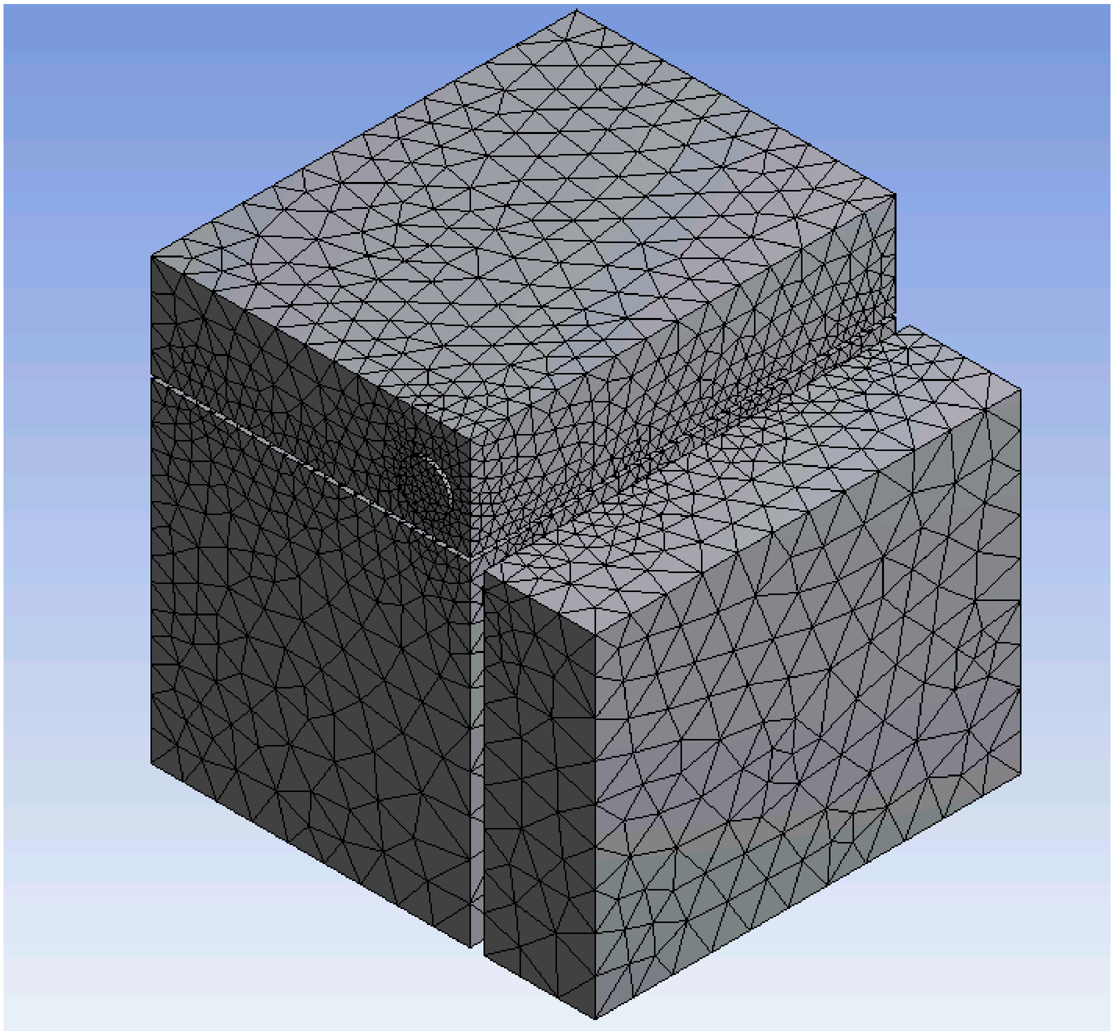


Figure 22: Mesh Used for CFD Analysis

We ran a pressure based, laminar flow analysis based on the airflow results of our valve damping tests. This test gave us data for the velocities at which the air was exiting the box, which can be used to calculate the mass flow leaving the box using Equation 1 below.

$$\dot{m} = \rho v A$$

Equation 1: Mass Flow Rate

Since the mass exiting the box is the same as the amount of mass entering the box, the inlets were grouped together and modelled as a mass-flow-inlet boundary, while the outlet was modelled as a pressure-outlet boundary. The initial conditions for the inlets were changed depending on the velocities of the valve damping test, and the initial condition for the outlet was always 0 gauge pressure. The CFD analysis was run for 300 iterations and ANSYS was used to generate streamline and contour plots of the airflow through the box.

2.5. Results

2.5.1. Stress Analysis

The analysis of the three chambers of the box resulted in stresses ranging from 0.11 N/m^2 – 177e^3 N/m^2 (1.6e^{-5} to 25.67 psi), which produced displacements ranging from 0 to 9.3e^{-3} mm. The displacements throughout the box are shown in Figure 23. From this figure it is evident that the largest displacements occurred at the tops of the doorways, where there was no material below for support, as shown by the regions colored red. Smaller displacements occurred above the inlet holes on each side of the box, with even smaller displacements in the solid walls. There was little to no displacement in the floor, as would be expected for a fixed region. The graphic shown in Figure 23 seems extreme, because it is an exaggeration of the actual displacements that the apparatus will experience. From this analysis, this part of the CPP apparatus would not be expected to experience significant deformations, even under higher loads than are expected during normal operation. While there was initial concern due to the fact that the ventilation system (specifically the ducting from the outlets of the box to the fan) will cause an uneven distribution of applied stress, this would cause more of the load to be distributed in the area which experienced the smallest displacement (the solid walls). In fact, because the total load in the analysis accounted for the weight of the ventilation system, the actual loading situation will cause smaller displacements at the areas of greatest concern (the doors) as some of the load will be shifted to the areas of least concern (the solid walls) in the actual device.

Table 1: Resulting Stresses of the Analysis on the Main Box

Name	Type	Min	Max
Stress	VON: von Mises Stress	0.110741 N/m^2 Node: 8795	177123 N/m^2 Node: 18415
Main_Chamber-SimulationXpress Study-Stress-Stress			

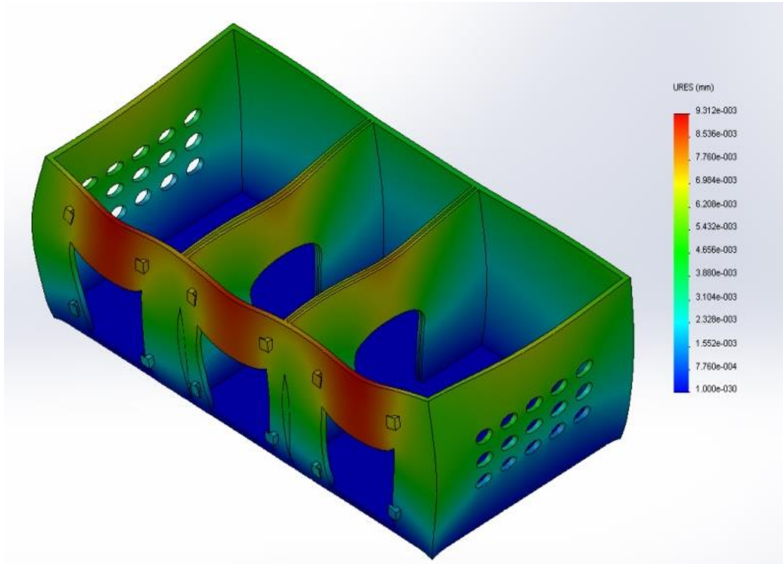


Figure 23: Displacement of the Main Box

The first loading situation for the scent box, in which the 4”x4” rubber pad is placed ¼” from the wall of the main box as well as from the side of the scent box, resulted in the smallest deformation (0.7 mm). This is expected, as in this case the stress was applied closest to the supporting walls. As can be seen in Figure 24 through Figure 27, the displacement was distributed the same regardless of loading situation, with the largest displacements taking place at the point farthest from any support (the center of the box, on the side without a wall). The largest displacement took place in the case where the load was applied ½” away from both the wall of the main box and the side of the scent box, with a maximum displacement of 9 mm occurring at the point described above. Moving the loading farther from the side of the scent box had a greater effect on the displacement than moving it away from the wall of the main box, as can be observed by comparing Figure 25 & Figure 26 to Figure 24.

Table 2: Stresses in Scent Box - 1/4" from wall and side

Name	Type	Min	Max
Stress	VON: von Mises Stress	0.361492 N/m^2 Node: 11171	1.01415e+006 N/m^2 Node: 14211

Table 3: Displacement of Scent Box - 1/4" from wall and side

Name	Type	Min	Max
Displacement	URES: Resultant Displacement	0 mm Node: 461	0.730114 mm Node: 14799

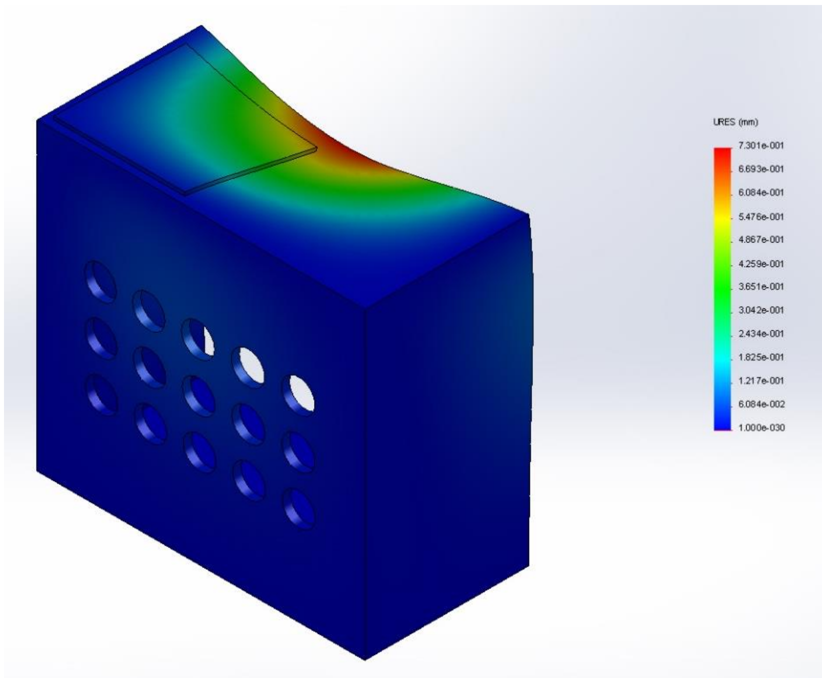


Figure 24: Displacement of Scent Box - 1/4" from wall and side

Table 4: Stresses in Scent Box - 1/4" from wall 1/2" from side

Name	Type	Min	Max
Stress	VON: von Mises Stress	7.73073 N/m^2 Node: 11018	1.06635e+007 N/m^2 Node: 14205

Table 5: Displacement in Scent Box - 1/4" from wall 1/2" from side

Name	Type	Min	Max
Displacement	URES: Resultant Displacement	0 mm Node: 452	8.41807 mm Node: 223

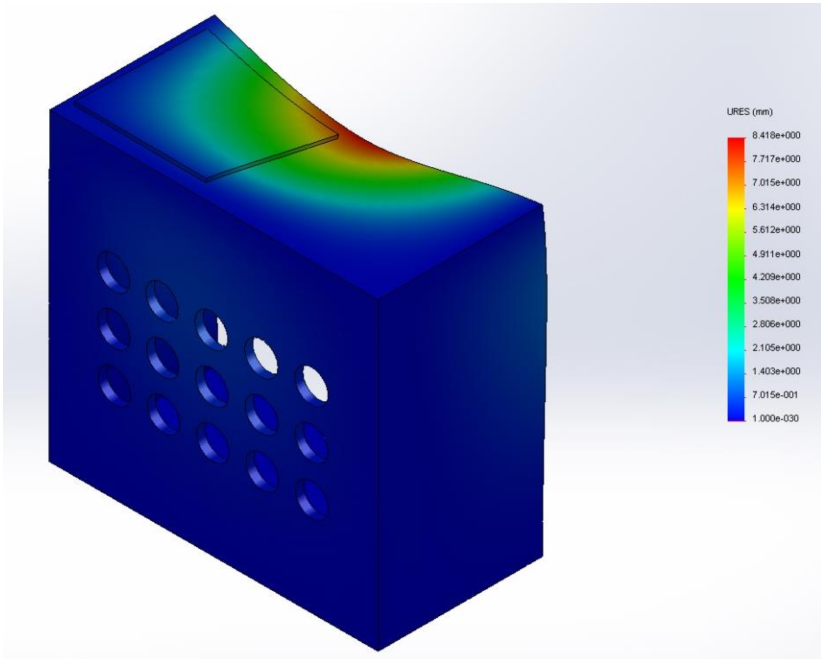


Figure 25: Mapping of Displacement in Scent Box - 1/2" from wall 1/4" from side

Table 6: Stresses in Scent Box - 1/4" from wall 1/2" from side

Name	Type	Min	Max
Stress	VON: von Mises Stress	0.911774 N/m^2 Node: 11147	1.38186e+006 N/m^2 Node: 14157

Table 7: Displacement in Scent Box - 1/4" from wall 1/2" from side

Name	Type	Min	Max
Displacement	URES: Resultant Displacement	0 mm Node: 452	0.805028 mm Node: 223

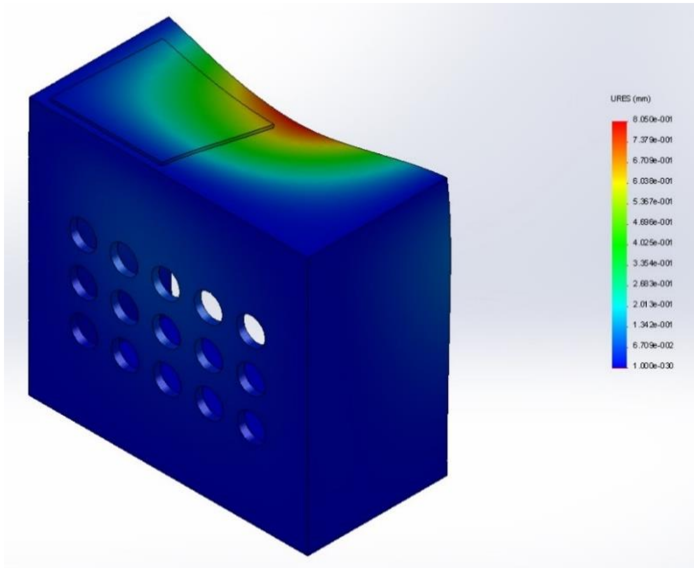


Figure 26: Displacement distribution of Scent Box - 1/4" form wall 1/2" from side

Table 8: Stresses in Scent Box - 1/2" from wall 1/2" from side

Name	Type	Min	Max
Stress	VON: von Mises Stress	6.83076 N/m^2 Node: 11066	1.15614e+007 N/m^2 Node: 14075

Table 9: Displacement of Scent Box - 1/2" from wall and side

Name	Type	Min	Max
Displacement	URES: Resultant Displacement	0 mm Node: 442	9.25472 mm Node: 14668

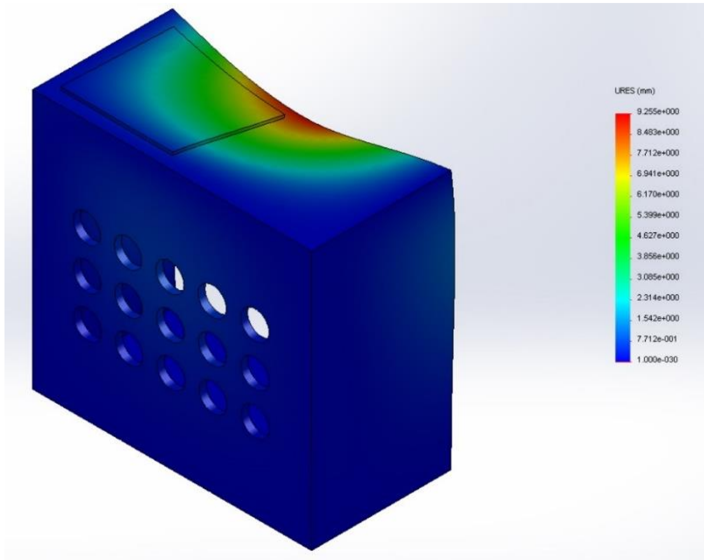


Figure 27: Displacement Distribution in Scent Box - 1/2" from wall and side

2.5.2. Anemometer Test

First, for the anemometer tests to determine the speed of airflow, we compared the air velocities at the outlets of the black and white sides. For the white side we observed a slowly increasing curve for the air velocity, ranging from the 200-250 fpm range. Whereas, the air velocity for the black side was steadier and ranged just about halfway in the 200-250 fpm region in the whole time period.

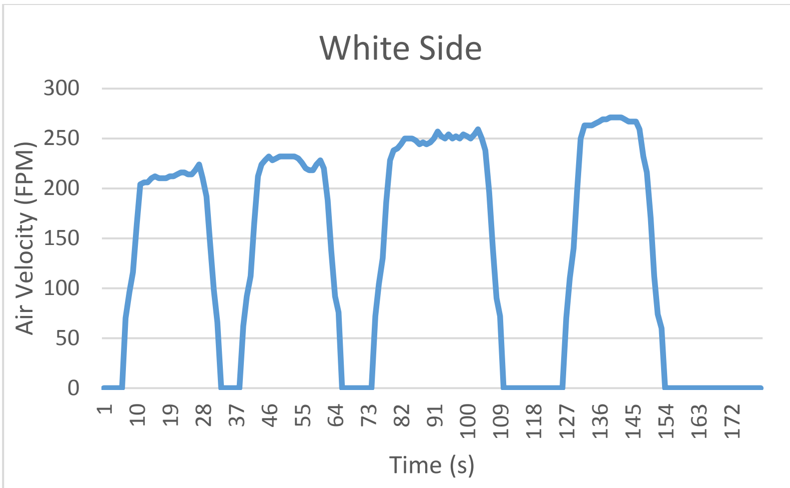


Figure 28: Change in Velocities for White Side

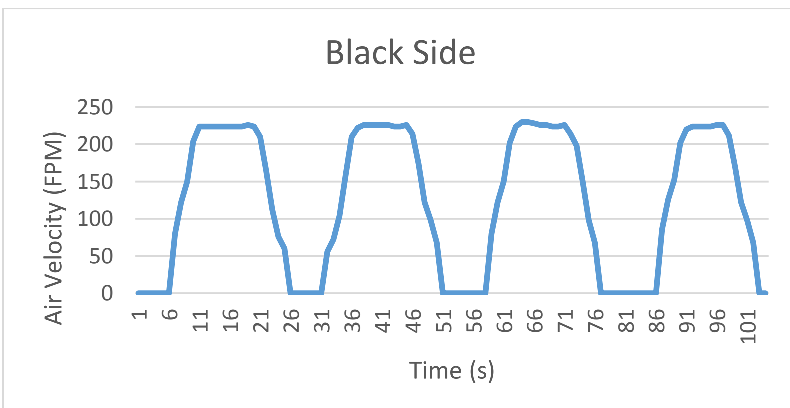
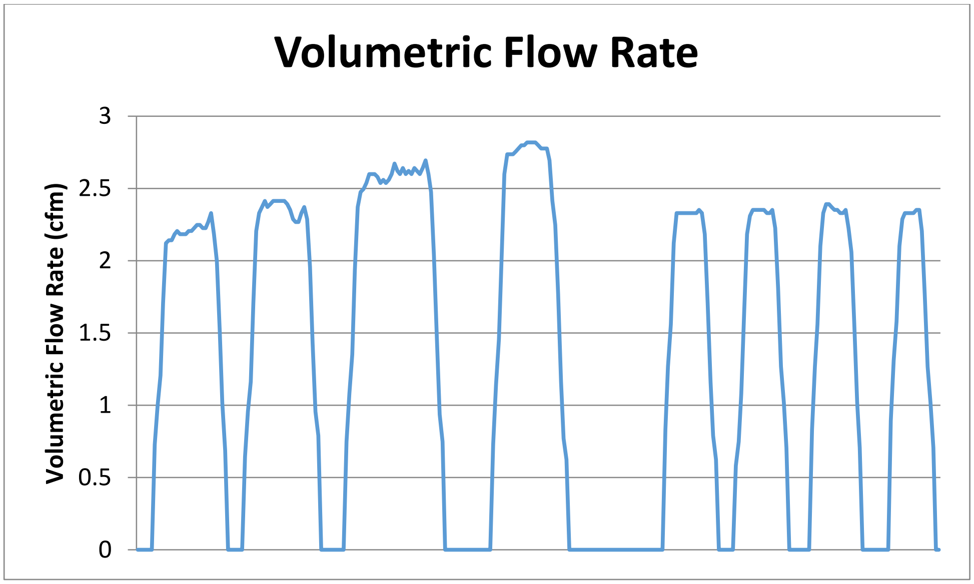


Figure 29: Change in Velocities for White Side



White Side

Black Side

Figure 30: Calculated Volumetric Flow Rates for White and Black Sides

Similarly, the time to clear out the air from the white side reduces gradually ranging from the 19 seconds to 17 seconds in the whole time period. Whereas, we see a much more constant average time of about 18 seconds to clear out the air from the box in the case of the black chamber. These time values are estimated from the volumetric flow rates that we calculated, corresponding to the volume of air in each compartment of the box.

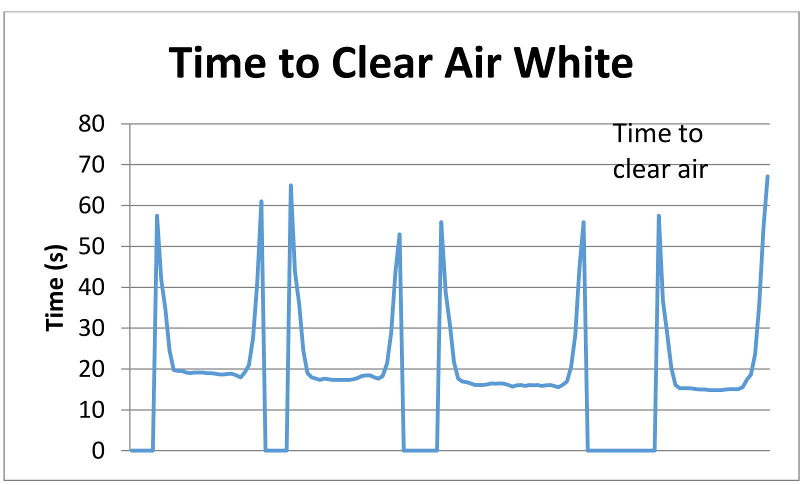


Figure 31: Time to Clear Air White Side of Chamber

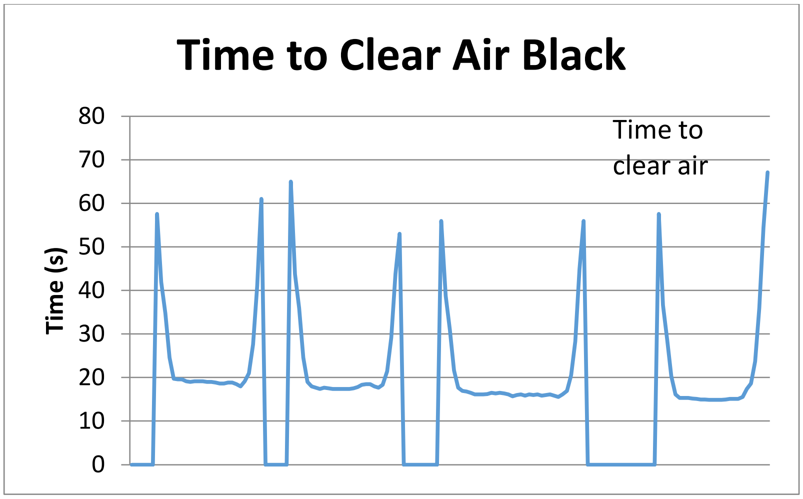


Figure 32: Time to Clear Air Black Side of Chamber

Fan Damping

In the case when we use cardboard to cover up a portion of the white piping inlet, we got three different variations in volumetric flow rate. Firstly, when a quarter of the inlet is covered up from the right side, we see the volumetric flow rate drops to .75 cubic feet per minute. Thereafter, when we covered up half of the inlet, the volumetric flow rate drops down lower, to about .6 - .65 cubic feet per minute. When we cover up the inlet about a quarter of the original size it increases back up to the .75 cubic feet per minute range. Lastly, to give an estimate of how this compares to the regular reading of the exhaust system, we took a measurement of the air velocity with the inlet 100 percent open, which gives us a value between 2-2.5 cubic feet per minute once again. The time to clear out the air also varies inversely to the volumetric flow rate.

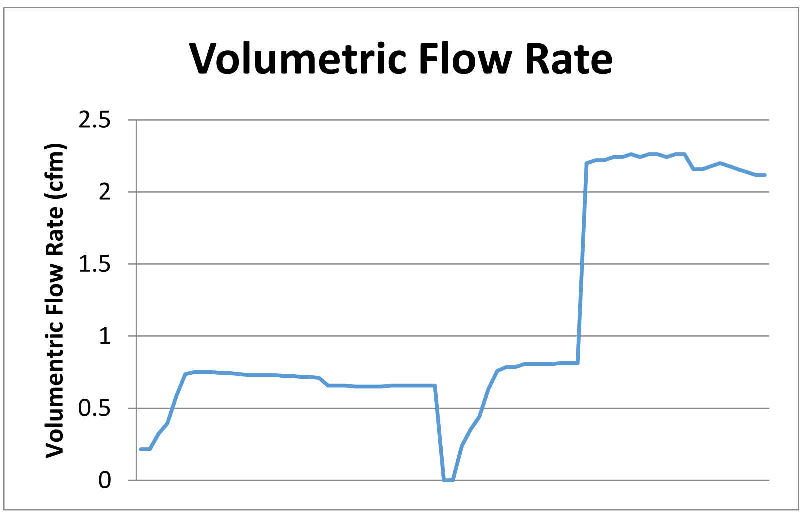


Figure 33: Volumetric Flow Rate on Black Side - Fan Damping Test

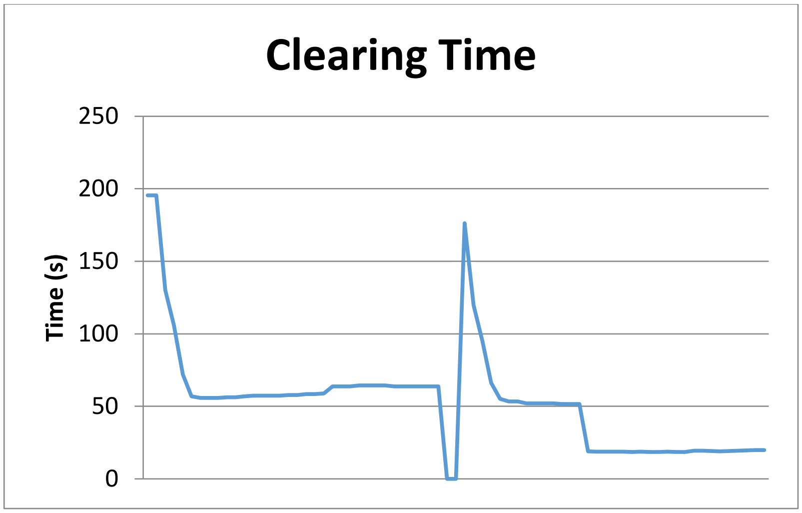


Figure 34: Clearing Time For Fan Damping Test

Valve Damping

Our valve test gave us much more conclusive results regarding the wind flow restrictions as compared to the damping test. The average of the wind speeds at the different restriction quadrants are as follows:

Fourth Closed: 228.45 fpm

Half Closed: 222.035 fpm

Three Fourths Closed: 181.437 fpm

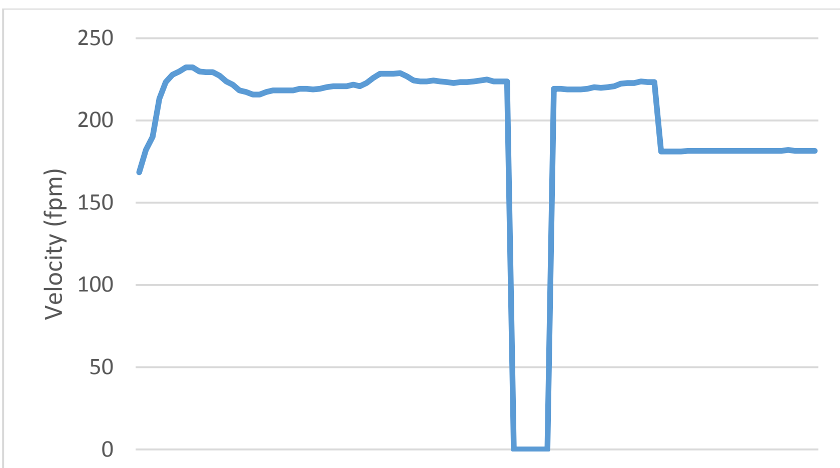


Figure 35: Velocity Change due to Valve Damping

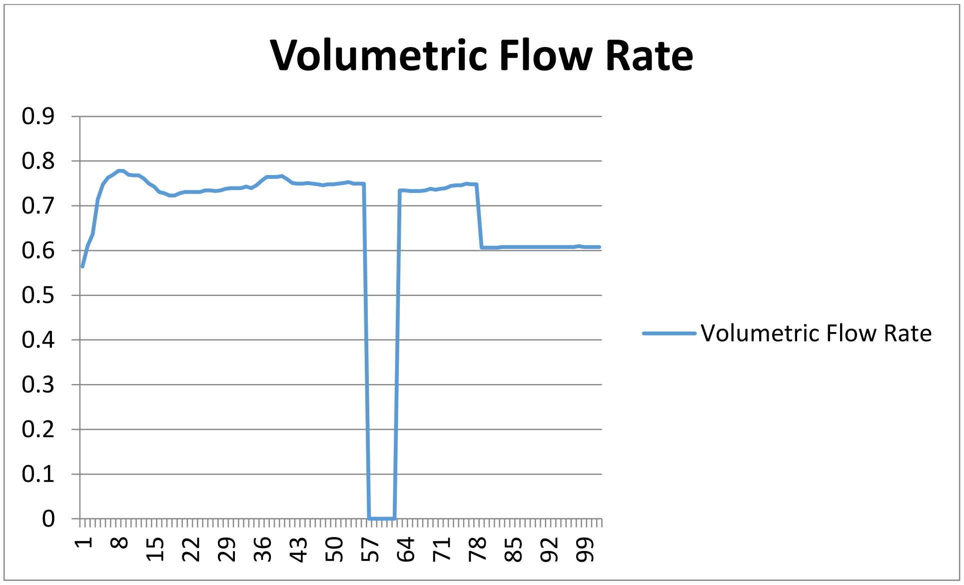
Therefore, our results show progressive decreases of airflow through the ball valve, as predicted by the damping test. We however see a higher drop from the fully open to the fourth closed air speeds, as well as the half to three fourths air speeds.

The corresponding volumetric flow rates that we found at the three different valve damping positions were as follows:

Fourth Closed: 0.76531 cfm

Half Closed: 0.74382 cfm

Three Fourths Closed: 0.60782 cfm

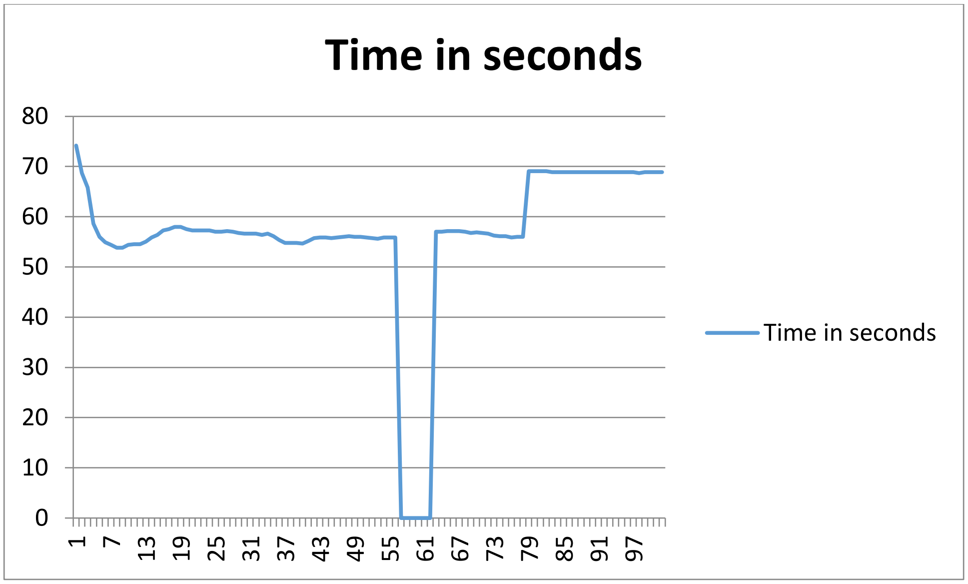


The estimated time to recycle the air out of the main chamber of the CPP box according to the given air speeds is as follows:

Fourth Closed: 54.7399 seconds

Half Closed: 56.3252 seconds

Three Fourths Closed: 68.9124 seconds



2.5.3. Oxygen Tests

The relation between the oxygen concentration and the voltages recorded is described by the equation shown in Figure 36. This equation had a R^2 value of 1, also seen in Figure 36, which indicates that the fit performed was extremely accurate, and that the relation between the oxygen concentration and voltage was in fact linear. With this information, the data collected was able to be converted from voltages to oxygen concentrations.

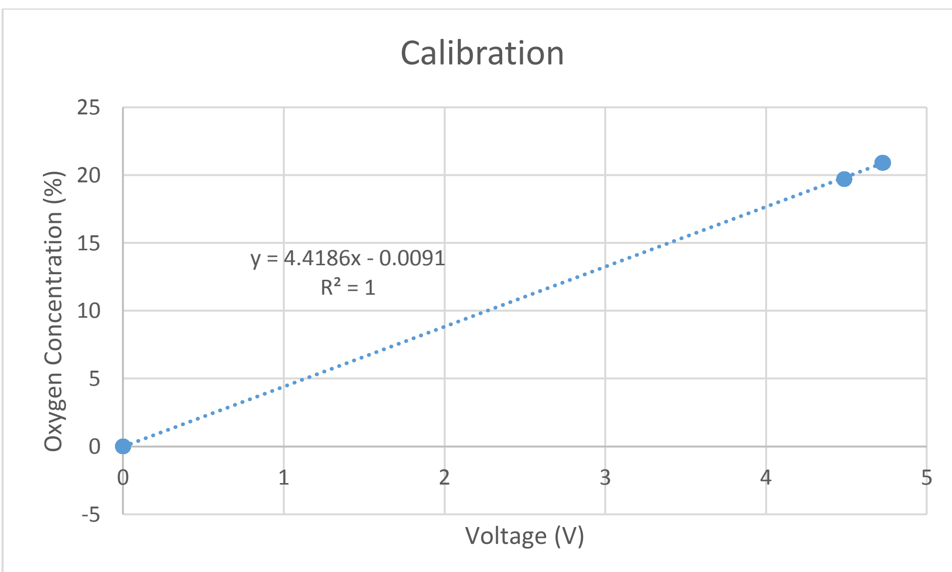


Figure 36: Calibration - Voltage vs. Oxygen Concentration

The voltages for the known oxygen concentrations (0% and 20.9%) are plotted in Figure 37 & Figure 39. Using the equation derived above, this data was used to create a similar dataset of oxygen concentration versus time, as shown in Figure 38 & Figure 40. In both datasets, there are well-defined "jumps" in voltage that correspond to small changes in oxygen concentration, which are due to error, as the oxygen concentration input to the analyzer was constant. The magnitude of this step was found to be the same for both data sets (as would be expected, since this is due to the precision of the analyzer), with a value of 0.328 mV, which is equivalent to a 0.001449% change in oxygen concentration. While the relative error of the 0% O_2 tests cannot be calculated using Equation 2, however this can be used to determine the error in the 20.9% O_2 test, which is 0.103%. This shows that the measured values are very accurate to the true results.

Equation 2: Percent Relative Error

$$\%Relative\ Error = \frac{\%O2\ Known - \%O2\ Measured}{\%O2\ Known} * 100$$

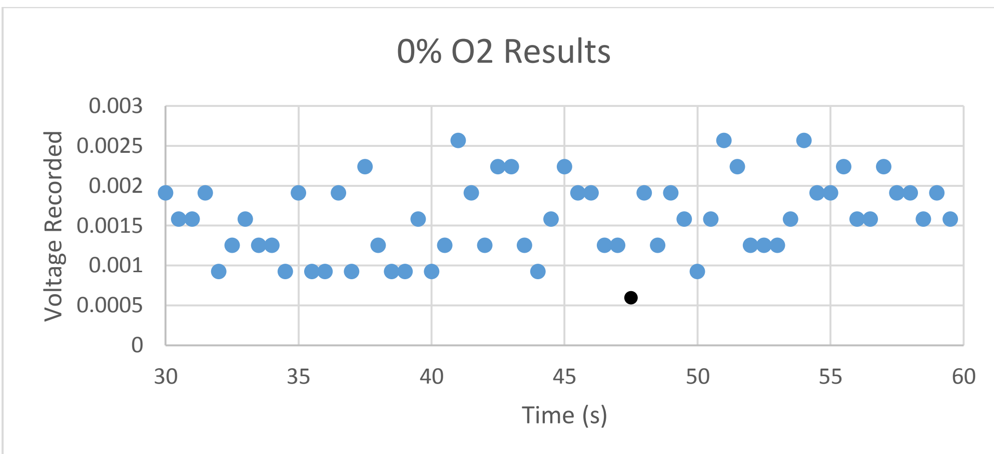


Figure 37: Plot of Voltage vs. Time recorded at O percent Oxygen

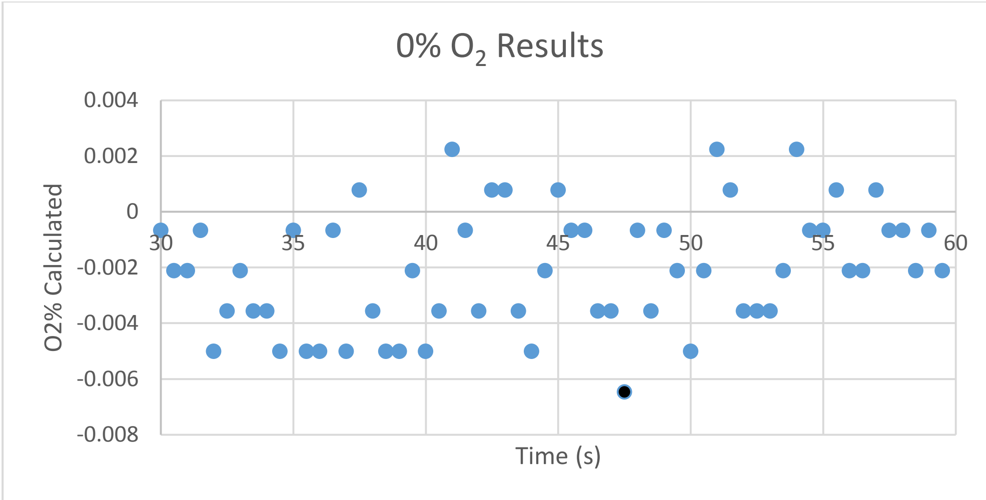


Figure 38: Plot of Oxygen Concentration vs. Time Calculated at 0 percent Oxygen

Table 10: Data Calculated for 0 percent Oxygen

Volts		$\%O_2$	
stdev	0.000484625	stdev	0.002141
max	0.002565	max	0.002234
min	0.000597	min	-0.00646
avg	0.001586467	avg	-0.00209
max-min	0.001968	max-min	0.008696
largest Δ	**0.001462476**	**largest Δ**	**0.006462**
min+1	0.000925	min+1	-0.00501
step size	0.000328	step size	0.001449

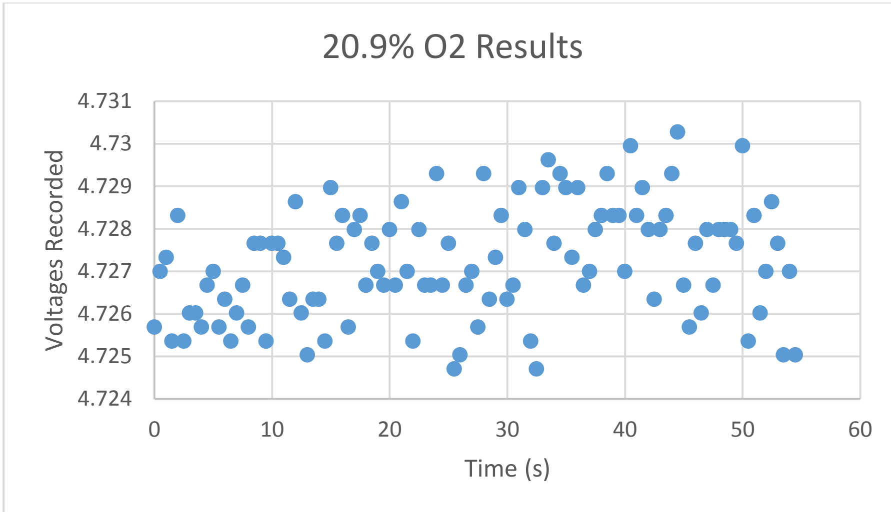


Figure 39: Plot of Voltage vs. Time for Ambient Air

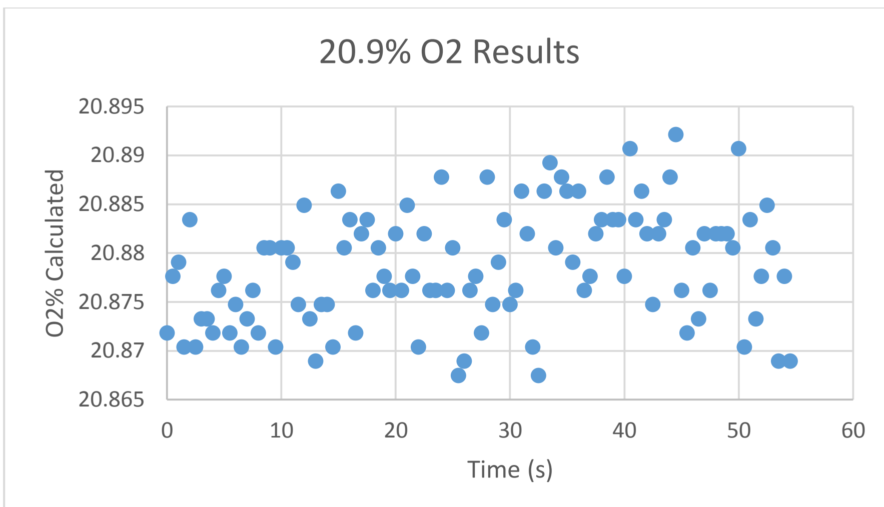


Figure 40: Plot of Oxygen Concentration vs. Time for Ambient Air

Table 11: Data for Ambient Air Test

Volts		$\%O_2$	
stdev	0.001317	stdev	0.00582
max	4.730278	max	20.89211
min	4.724701	min	20.86746
avg	4.727206	avg	20.87853

max-min	0.005577	max-min	0.024643
largest Δ	**0.007363**	**largest Δ**	**0.032536**
min+1	4.725029	min+1	20.86891
step size	0.000328	step size	0.001449
		Avg %Error	0.102719

While percent relative error is useful in assessing the accuracy of the measurements, it is more important to assess the maximum deviation of the values from the actual value, to ensure that any changes in voltage recorded are significant enough to be considered an indicator of a change in oxygen concentration. The maximum deviation from the expected value for the 0% oxygen concentration is approximately 1.463 mV (or 0.00646% O_2), and for the 20.9% oxygen concentration it is 7.363 mV (or 0.03254% O_2). This means that any analysis of the test results should take into consideration that any change in concentration greater than 0.033% O_2 is statistically significant, and any smaller than that cannot be considered significant.

As expected, a significant change in oxygen concentration was observed at all locations, meaning the exhaust system will carry scent to all of the locations we measured. The changes in oxygen concentrations in the eastern chamber for both elevations are mapped below in Figure 41. The steady state (maximum) concentration is 20.9% oxygen. The largest change in concentrations on the floor was reached at location 9 and location 7, and the smallest change in concentrations was reached at location 4 and location 2.

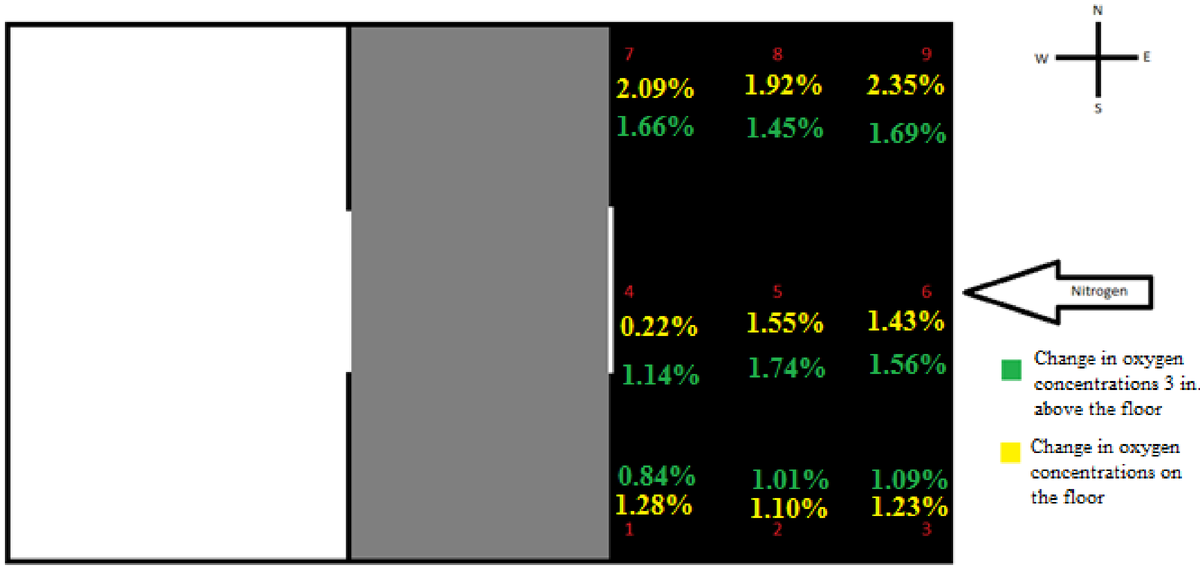


Figure 41: Change in Oxygen Concentrations in East Chamber

The change in oxygen concentrations in the middle (grey) chamber for both elevations (when the guillotine door was left open throughout the test) are mapped out in Figure 42 below. The largest change in oxygen concentration was at location 4, and the smallest change in concentrations happened at location 1. As expected from the CFD analysis there was some flow across the mid-line of the grey chamber when the door was left open.

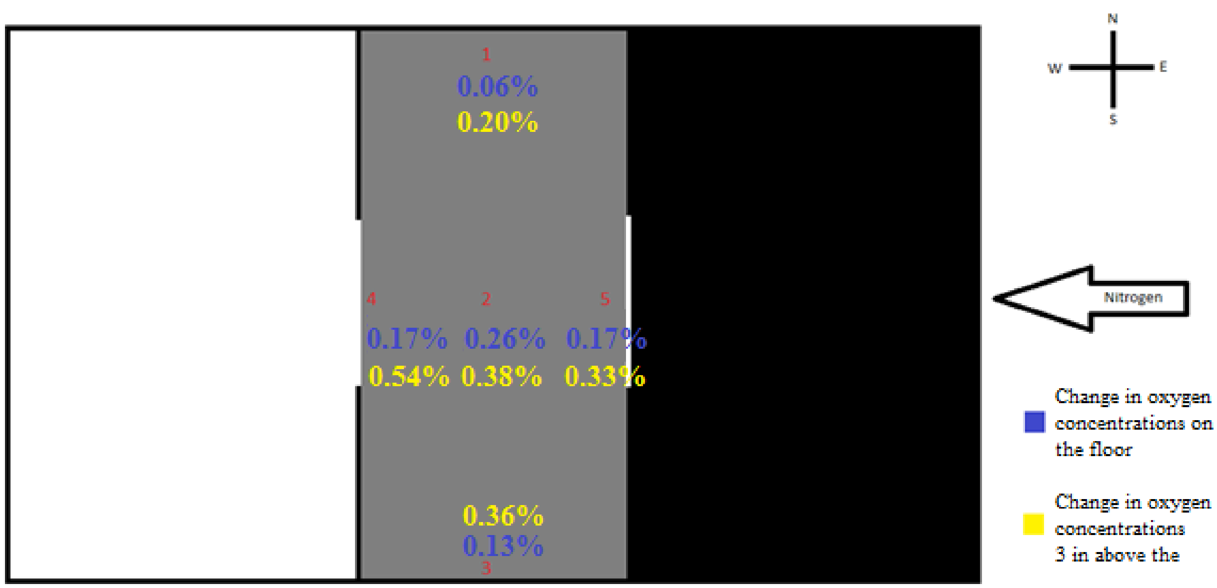


Figure 42: Change in Oxygen Concentrations in Middle Chamber

For the closed door tests of point 4 of the grey compartment of the box, as expected, there was no significant change in concentration when the doors were closed. As seen in Figure 43 & Figure 44, there is a change in oxygen concentration that begins approximately 20 seconds after the door is opened. The total changes are somewhat significant, with the difference between the average concentration for 0-20 s and 40-60 s (at the floor readings) being 0.058%. The change in average concentration between 0-20 s and 70-105 s is 0.336%, a much more significant change than that measured at floor level (as would be expected from the open door results and CFD analysis).

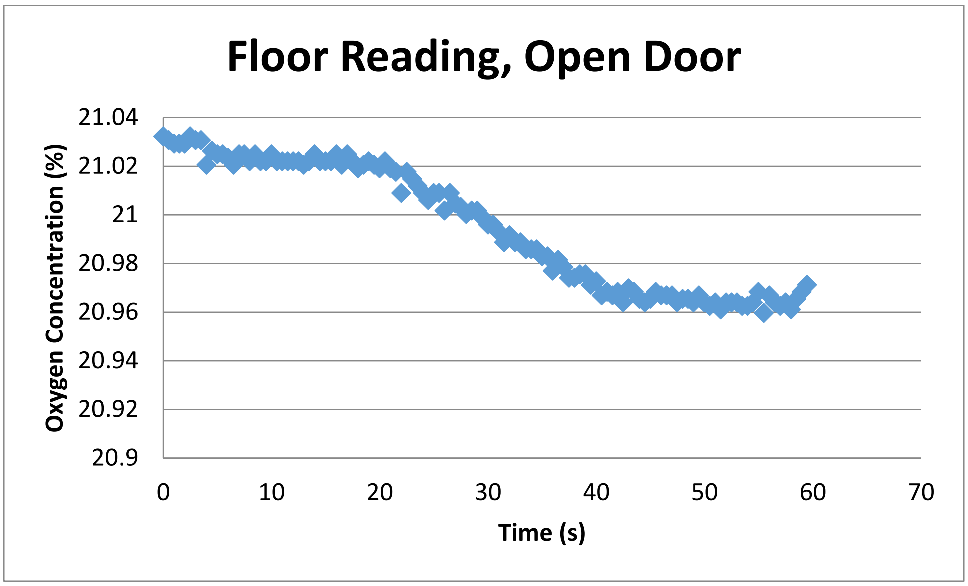


Figure 43: Oxygen Concentration vs. Time for Floor Reading - Door Test

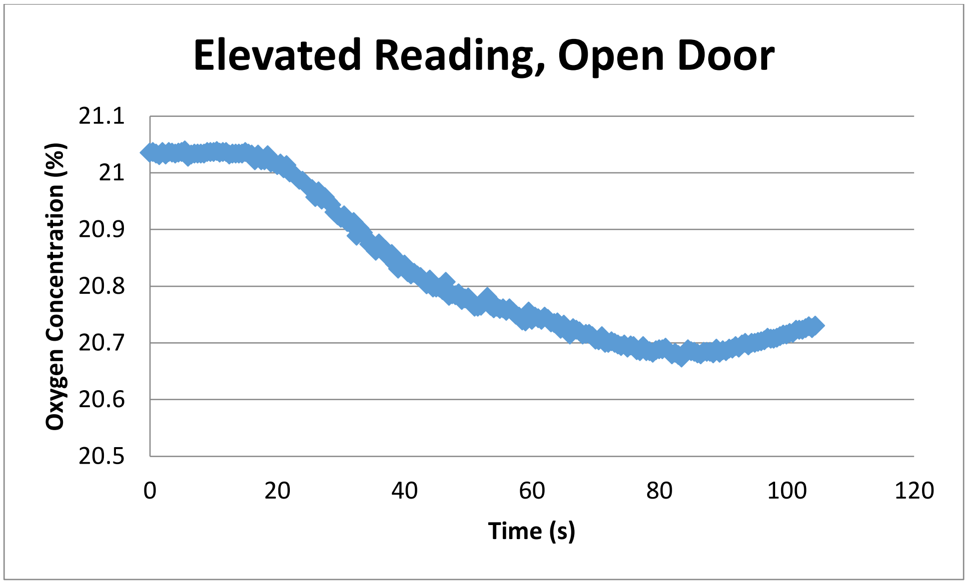


Figure 44: Oxygen Concentration vs. Time at 3 inches - Door Test

As seen in Figure 45, when it was tested whether or not the nitrogen crossed over into the east chamber, the oxygen concentrations showed some variation, likely due to small errors in the measurements. These could be caused by a variety of factors that influence the gas analyzer including: changes in the magnetic field surrounding it, changes in the flow rate input into it (due to the oscillating nature of flow from the pump), and small traces of nitrogen or other impurities entering the air stream of the gas analyzer. The clearing test showed that the levels returned to normal atmospheric conditions after approximately 30s of running the fan after the nitrogen flow was stopped.

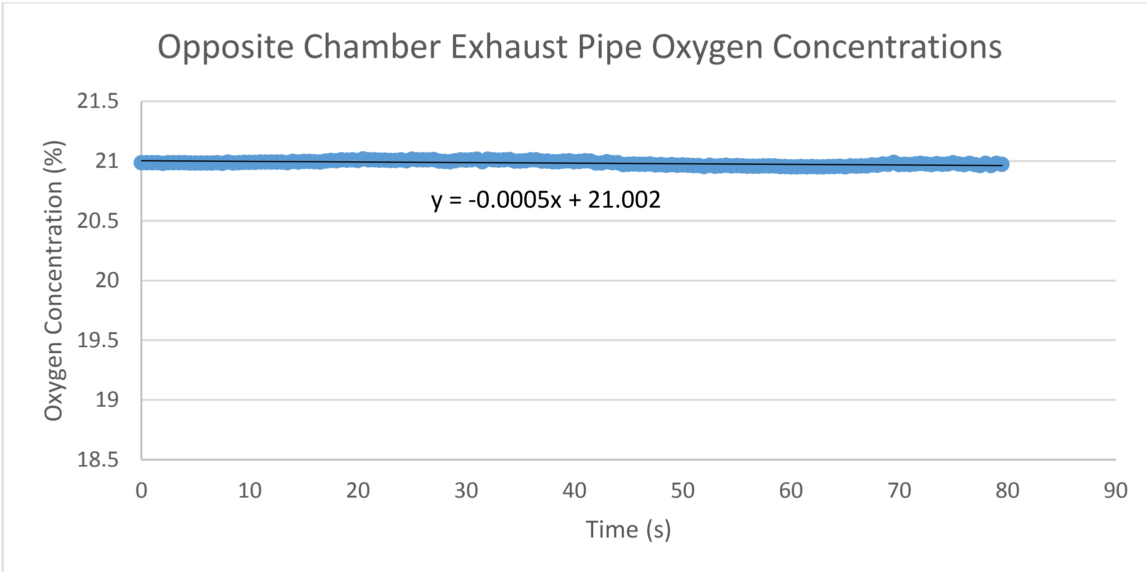


Figure 45: Opposite Chamber Exhaust Pipe Oxygen Concentrations

2.5.4. Computational Fluid Dynamics

The results of the CFD analysis are shown in Figure 46 to Figure 53; Figure 46 & Figure 47 show the flow when the outlet is fully open (un-damped), Figure 48 & Figure 49 show the flow when the outlets are three-quarters open, Figure 50 & Figure 51 show it when half open, and Figure 52 & Figure 53 show flow when the outlet is one-quarter open. As can be seen from the top-views there is airflow throughout all parts of the (with slightly more airflow through the center and to the side of the box on which the outlet is located). The side view shows that there is in-fact re-circulation of air through the bottom of the box, with the rest of the flow going directly up after entering the box, as was expected due to the location of the outlet. One troubling trend was the flow across the center (right wall in the side view), which indicates that scent could be carried to the opposite side of the box. Airflow was found to be reasonably small (<1ft/s) throughout the majority of the box, with the fastest regions of flow located in the ventilation portion of the box (the lid) and near the inlets with upward trajectory

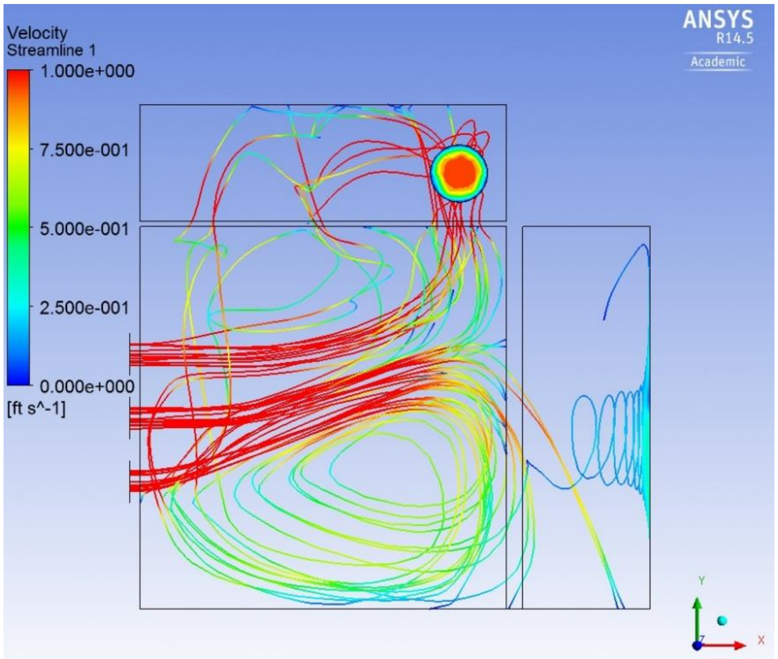


Figure 46: Side View - Fully Open CFD Results

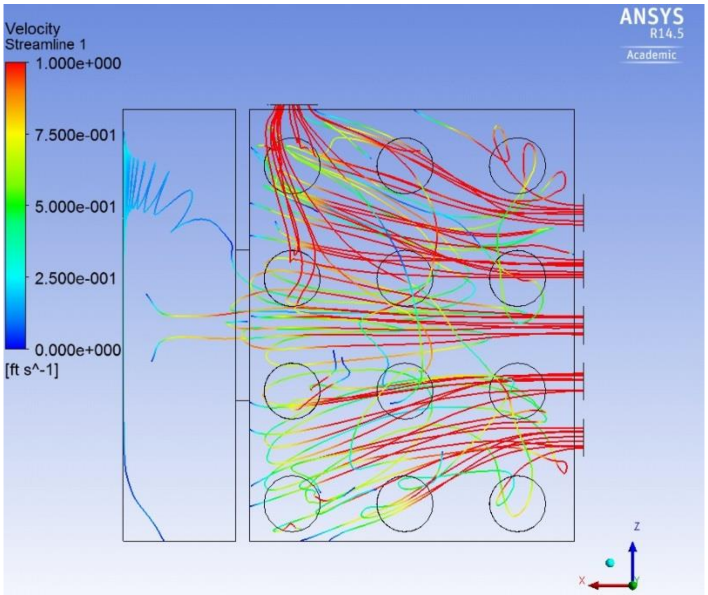


Figure 47: Top View - Fully Open CFD Results

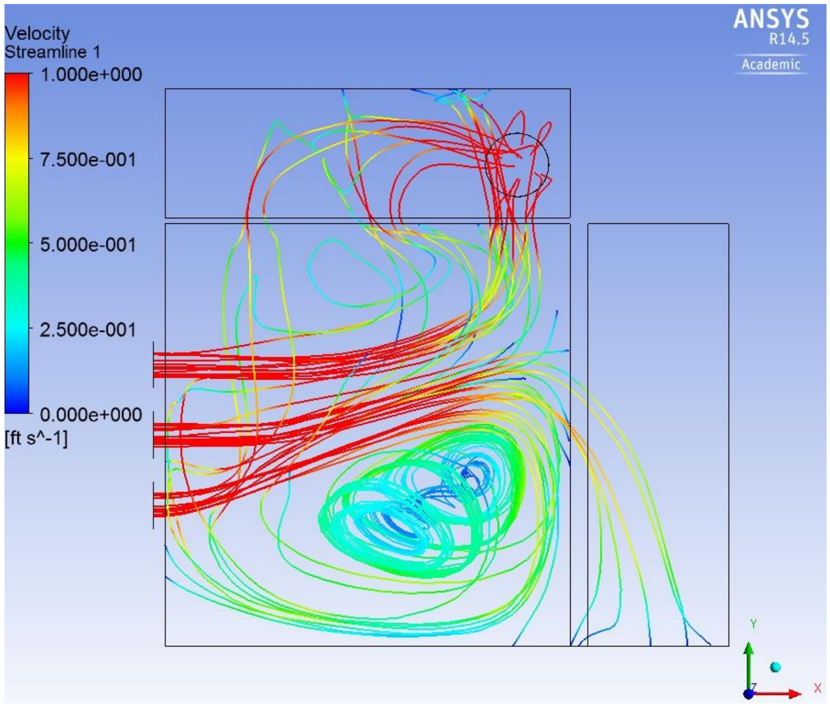


Figure 48: Side View - Three-Quarters Open CFD Results

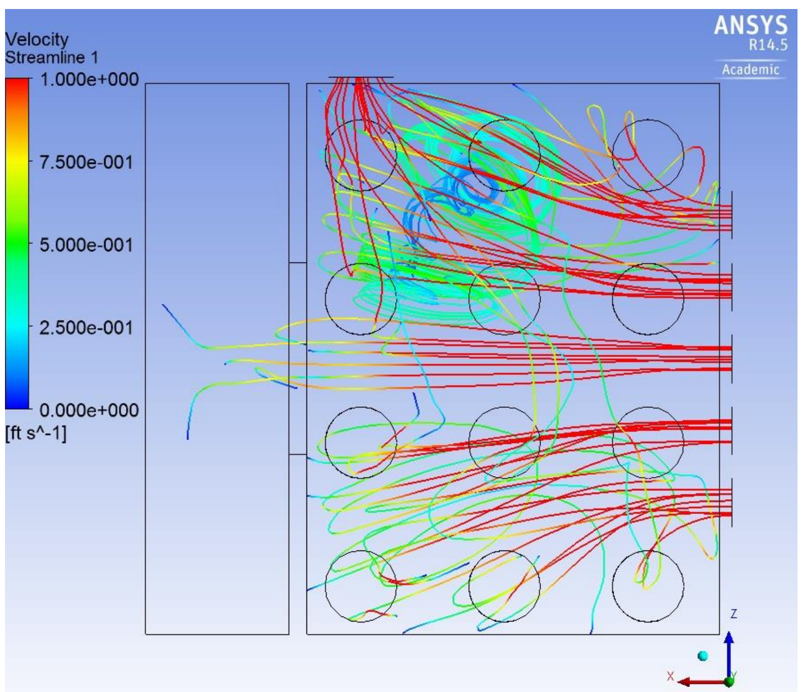


Figure 49: Top View - Three-Quarter Open CFD Results

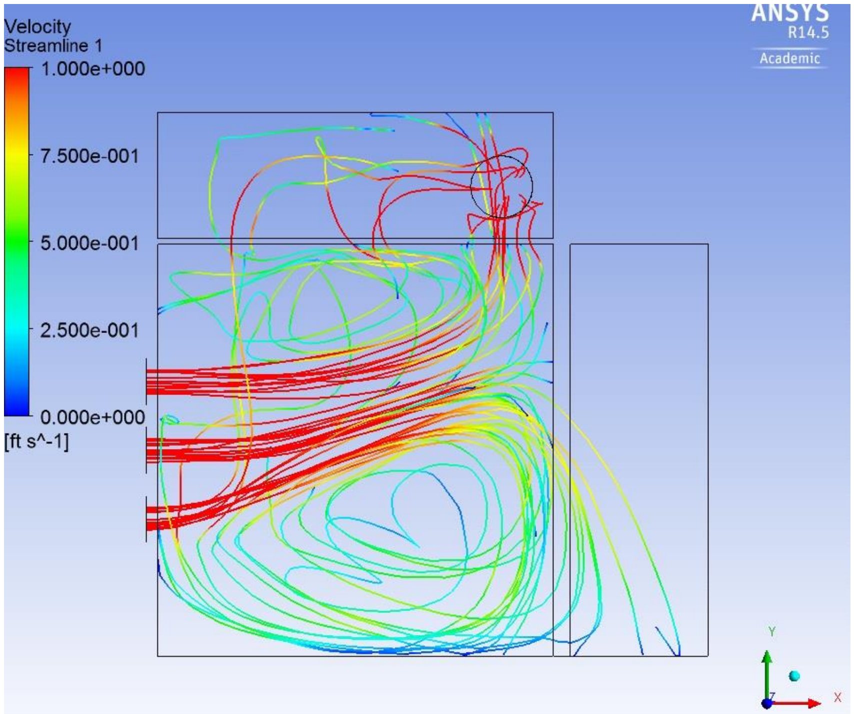


Figure 50: Side View - Half Open CFD Results

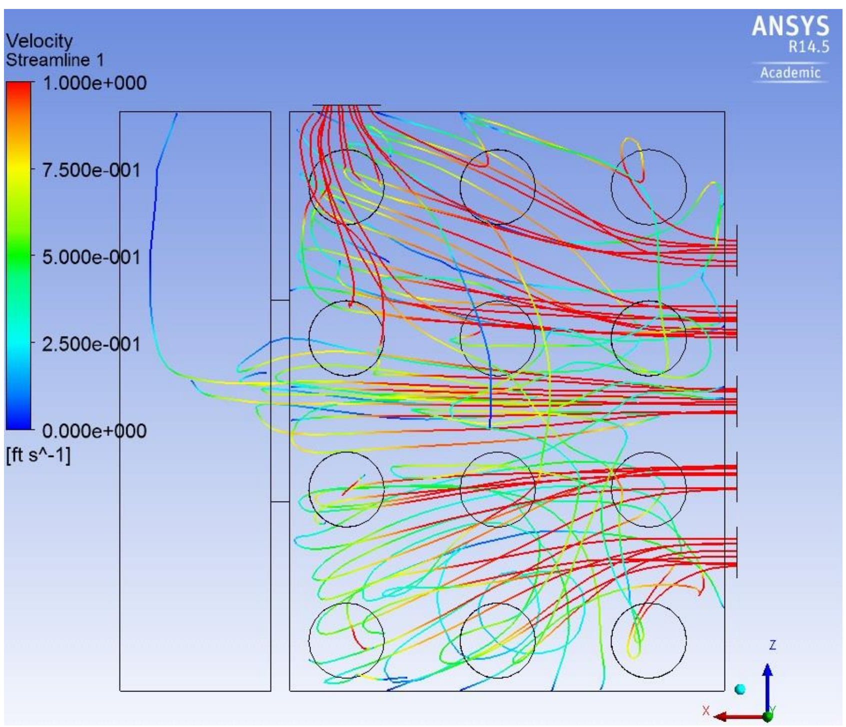


Figure 51: Top View - Half Open CFD Results

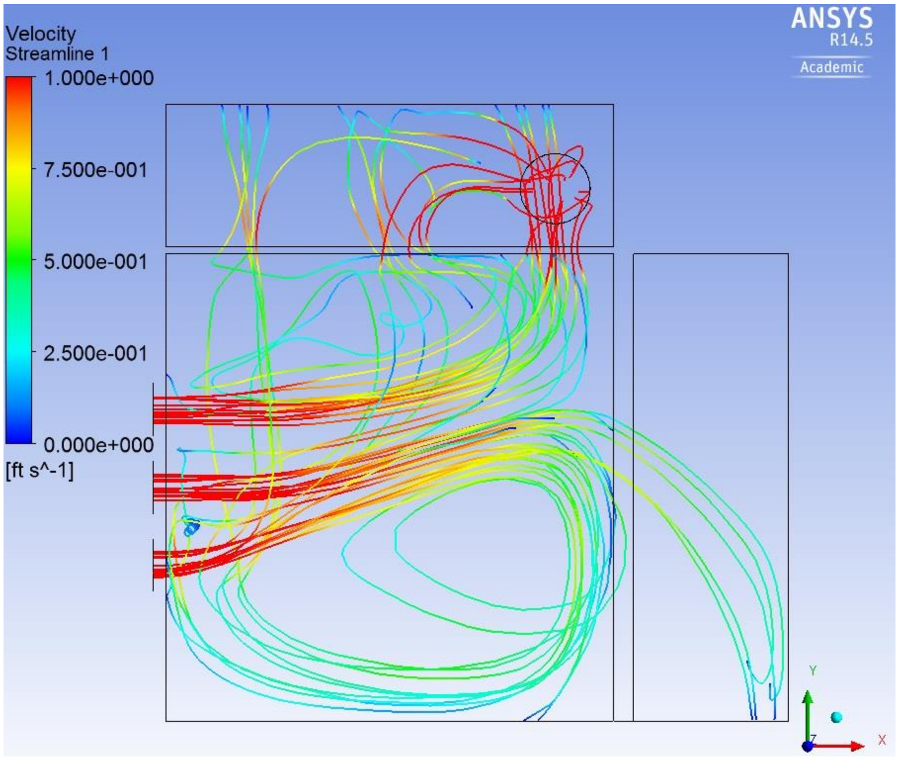


Figure 52: Side View - Quarter Open CFD Results

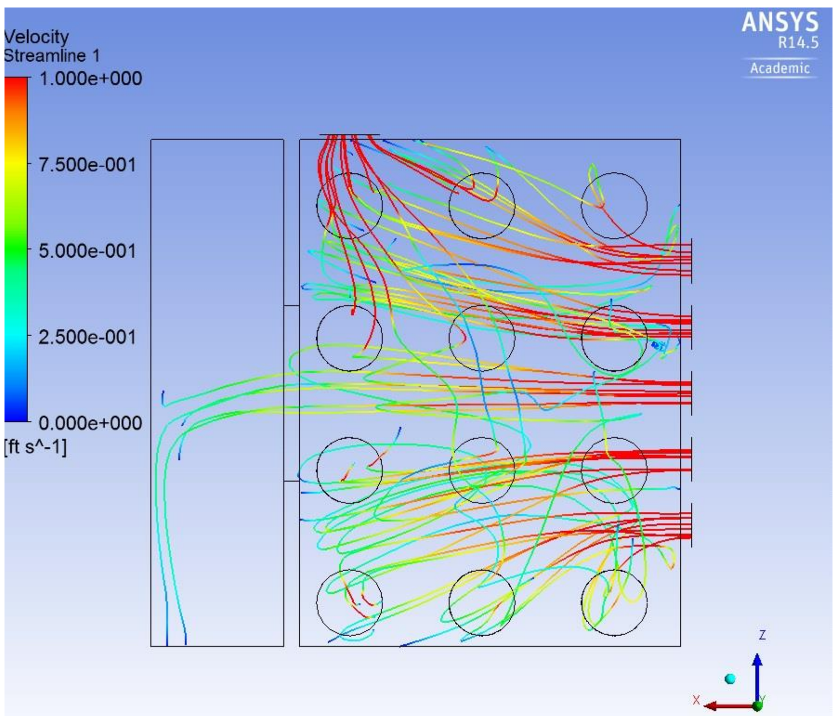


Figure 53: Top View - Quarter Open CFD Results

2.6. Discussion

2.6.1. CO_2/CO Test

The CO_2/CO test was the first test conducted in order to determine if the flow of the scents being introduced in the main chamber of the CPP box met the goals set. In this test we introduced a mixed CO_2/CO gas into one side of the CPP box using a tube connected to CO_2/CO gas tank. The gas was introduced through one of the scent boxes, and the CO_2/CO analyzer was set up on the opposite side of the main chamber. The analyzer was connected to a CO_2/CO detector which would read the concentration of the gas in the air. The test was run in 9 different parts of the right chamber of the box, at 3 different heights for every position (see Figure 17 & Figure 18). However, the validity of this test was uncertain as we were not sure if the amount of CO_2/CO gas that was introduced into the main chamber was enough to make alterations in the readings made by the detector, as it was only accurate up to one decimal point. The test did not give us any readings for the all regions of the box, hence we concluded it as a failed test, and used the O_2 test to determine the concentration and intermixing of the scents in the different chambers of the box.

2.6.2. O_2 Test

The results of the oxygen tests proved to be conclusive, with a very small error (~0.1%), and provided a valid methodology for accomplishing the goals set at the beginning of the project. The airflow pattern caused by the ventilation system did not create even concentration of gas at all points in the side compartment of the box, however a significant (>0.03% O_2) change in oxygen concentration was observed at all points in less than 30 seconds. While leaving the door between the middle and east compartments open resulted in a drop in concentration at some points across the midline of the box, the test leaving the doors closed until the side compartment filled with gas gave a 20 second period in which no gas crossed the midline. Under the assumption that any scent will be carried similarly to the gas used, the following procedure could be carried out. The user of the device would turn on the fan with the sliding doors closed; allowing the side chambers to fill with scent, then the user would place the test subject in the desired compartment, and finally open the doors to the side chambers. This way the subject will have 20 seconds in which there will be no mixing of the scents (from each side) in the center chamber. This should provide ample time for the rat to be able to decide which side to walk to. To determine whether it is in fact necessary to dampen the airflow, it will be up to the users of the CPP device to observe whether tests run using the box show inconsistent results, and whether it takes longer than 20 seconds for the test subjects to choose a side/scent. If the rat seems to be effected by the airspeed in the box (acting differently than is normal in a CPP experiment that does not use scent), an experiment's results are inconsistent (a large number of decisions made in the same experiment contradict each other), or the motion tracking (via overhead camera) reveals that the subjects take longer than 20 seconds, then the experiment should be re-run with the valve set to ¼, ½, or possibly ¾ closed before the doors are opened. Tests could even be run using the fan at full speed (valve fully open) to fill the side chambers, and then turned off when the doors are opened and throughout the course of the experiment. The fan should always be run at full speed after the experiment is completed for a minimum of 30 seconds without the scent being allowed to enter, to allow for the scent in the box to be removed.

2.6.3. Stress Analysis

The stress analysis conducted on the main box showed that the design more than supported the necessary loading for normal operation of the CPP device. The scent box analysis helped in understanding the effect of moving the fan with respect to the side walls of the scent box and the wall of the main box that the scent box was fixed to (using glue). In the final product produced, significant deformations in the lid of the scent box were observed when the door was opened. This was due to the fact that the ventilation system was somewhat shorter than in the CAD files, because some of the parts did not have existing CAD files when the model was created. In addition, the lack of a more accurate method for cutting lengths of PVC pipe (in the Washburn machine shop) forced the team to cut the sections manually using a hacksaw. This meant that the fan had to be positioned based on the lengths of pipe cut, and was therefore moved farther from the walls than initially intended. Regardless, this arrangement did not cause major deformation (or any plastic deformation), so this error will not affect the operation of the final device. If a new device were built more precisely, the ventilation system could be designed with the position of the fan being a high priority, and the deflection of the lid of the scent box could be minimized.

2.6.4. Comparison of Fan Damping and Valve Test

As we were uncertain about the functionality of the valve in the piping system, we ran the fan damping test prior to it, in order to be assured about creating varying air flow. Once we got varying wind velocities from different levels of damping of the exhaust system inlet, we could use this data to compare with the results we obtained from the valve damping test. However, there were evidently some errors cause due to the rotating of the valve as it was impossible to close the valve accurately at a quarter or a half turn. Whereas, in the case of the fan damping test, we made sure we had precisely quarter, half, and three-quarters of the inlet covered.

In terms of volumetric flow rate, we see that the values from both tests were quite similar, and vary between the regions of 0.6-0.9 cfm. The graph obtained from the valve testing gives us a much more precise graph as we only considered the three cases of quarter, half, and three fourths closed, whereas the fan damping test also gives us the fully open volumetric flow rate as well. We observe that the values obtained from either test gives us conclusive results that the volumetric flow rate is decreasing as the opening of the piping system gets smaller. We also observe that there is a slight jump in the volumetric flow rate in the three fourths covered case in fan damping experiment; however this was error mainly due to the placement of the anemometer, which was not aligned completely parallel to the opening of the piping system.

Similarly we observe in the time taken to clear out the box, there is an increase in the clearing time as we block more off the inlet off. The time taken to clear the air out of the box stays between the ranges of 50 seconds to 70 seconds, increasing progressively as we move from fourth closed to three fourths closed inlets. In comparison we see quite similar results, with fourth closed inlet giving us close to 55 seconds clearing time in both cases, half closed giving us about 60 seconds and three fourths closed giving us 68 seconds in the valve damping case, and close to 50 seconds in the fan damping case. This alteration is once again due to experimental error due to the placement of the anemometer.

2.6.5. Comparison of CFD Analysis and Test Results

As can be seen from Figure 46Figure 53, distribution throughout the entire box was achieved. The vortex that can be seen in the side views indicate that the scented air would be circulated throughout the bottom of the box where the rat's nose is most likely to be. With the scale limited at 1 ft/s, anywhere where the streamlines are red, the air is moving faster than that. According to the National Climatic Data Center, the average wind speeds in the US range between 4.92 ft/s and 26.25 ft/s, and since streamlines in the region where the test subject will be all indicate wind speeds less than 1 ft/s, the wind should not affect the behavior of the subject. The side and top views also indicate that some of the "new" air enters the middle chamber at very low velocities. This would be mirrored by the other side of the box since the same airflow characteristics are occurring. This could confuse the rat, since it is getting both scents at the same time. For this reason, the sliding doors would be kept closed while the scent is diffusing through the left and right chambers.

2.7. Recommended Improvements to Design

2.7.1. Construction

One of the things we could have improved about the CPP apparatus is the types of glue we used. There are many different types of adhesives, and there are specific glues that have been developed for each different type of connection. We used the correct adhesive for the PVC to PVC connection, however, for the remaining bonds; we simply used Gorilla™ Super Glue. This is a very versatile adhesive and can be used on almost any surface, but it undergoes a chemical reaction with acrylic which can cause the acrylic to become cloudy or foggy and this is unfixable, as seen in Figure 54. A clear lid is important for UMMS staff to easily track the movements in the box, however, most acrylic adhesives do not dry clear. In the future, an adhesive called Weld-On® IPS #40 which is made for bonding acrylic to itself and various other plastics. Another type of bond we had to connect was acrylic to metal. Although super glue works to bond these two surfaces together, again there are adhesives that have been developed specifically for this. An epoxy, like J-B Weld, would have been suited for this purpose; however, all of these different types of adhesive were not in our budget. Super glue works for all of these bonds, and since these materials only had to be glued together less than three times, we decided to simply use super glue (except for the PVC-PVC bonds which had to be done many times and therefore merited a specific adhesive).

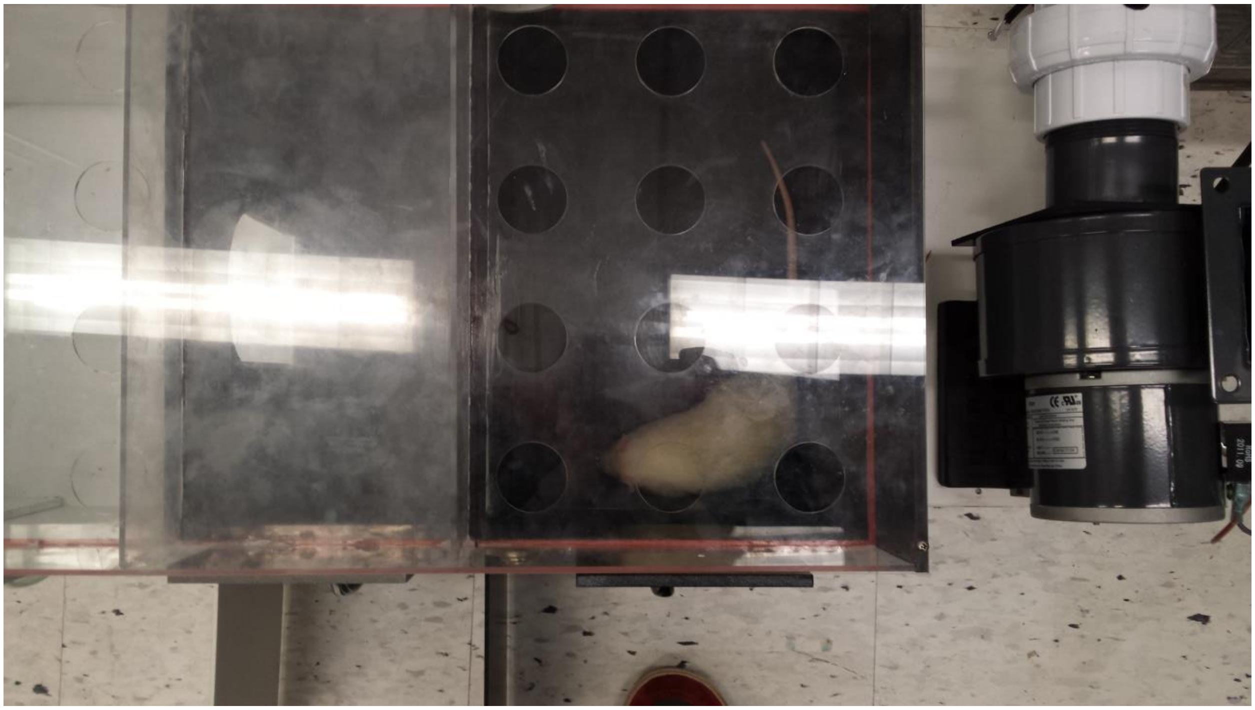


Figure 54: Rat in Final CPP Device

For the construction process, in order to have sliding doors, we had to cut 1/8th inch thick slits into the white and black sections of the front wall. These slits had to be cut 1/8th of an inch from the edge in order to ensure symmetry of the sides. However, in this case, aesthetics should have been sacrificed for practicality, and the sheets of PVC that make up the front wall should have been ordered in such a manner that the 1/8th inch thick slits could simply be shaved off of the PVC slits instead. This would have created a more reliable airtight seal between the sliding doors and the front wall, and the manufacturing process would have been greatly simplified.

Because one problem observed in the final design of the CPP device was that the scent will be carried up, leaving lower concentrations at the floor of the box, the team revisited some of the ideas for forcing the airflow to pass through the bottom parts of the device. One idea was the inclusion of a downward slanted grille to be placed over the inlet holes on each side of the box. This would force the airflow to start with a downward directionality, before being pulled up by the ventilation system, and would be affordable, since it only requires some additional plastic sheet to be implemented.

Another improvement could be to use a more precise method for damping the airflow. The ball valve used is difficult to set to an exact position, and there are (more expensive) alternatives that are designed to be much more precise. A valve that has settings to which it can be locked into would be ideal, as this would allow the user to ensure that both valves were set to the exact same position, limiting the chance that the airflow becomes uneven (on either side of the main compartment).

2.7.2. Testing

If a second prototype of the CPP device were developed, it would have to undergo similar testing to that run for this project. Error in testing could be reduced in a few areas, using knowledge gained through the teams testing efforts.

One observation was that, in the CFD analysis, the local velocity at the outlets differed based on the radial distance from the center of the opening. If a more precise measuring device was used, the velocity at multiple locations (at different points of the inlet) could be measured, and this could be used to better model the airflow out of the box. If these measurements were then converted to an average velocity at the inlet, the volume flow rate would be much more accurate than the one measured in our tests. One product used in the HVAC industry is the linear averaging sensor used by companies in variable air volume terminal applications. While this would improve accuracy, it would likely be an expensive solution. A less expensive method would be to measure the airflow at a specific point, and then produce CFD models using a similar range of airflows. These models could then be examined using the probe tool (placed at the same point measurements were taken from) to determine what overall airflow produces the air velocity at that point that was observed. This method was too time-consuming for the team to carry out, but would be a more economical solution to better estimating the flows that occur at different levels of damping. Using the results of this would allow for better models of the overall system to be made using CFD, and more accurately predict the airflow.

One possible issue with the "O_2" tests was that the volume flow pulled out of the box to analyze the properties of the air could cause a change in the airflow from the normal operation. While this is not a major concern, since the subject will similarly have to draw in a small amount of air to be able to smell it, the two volume flow rates are not guaranteed to be the same. If research into the amount of air drawn into the rat's nose were conducted, the instrumentation could be adjusted so that the volumetric flow rate caused by the gas analyzer was closer to the expected flow caused by the subject's nose. If this proved to be too difficult, or impossible (due to limits on the airflows that the sensor operates accurately at), a CFD analysis could be done in which an outflow was modeled at each of the points being measured, the flow could be set to the flow caused by the rat in one set of models, and the flow caused by the gas analyzer in another set. This was much too time consuming to be carried out by the current team, and would likely show that there were very small effects caused by the differences in outflow at the various points.

Another aspect of the project that did not end up as planned was the method for releasing the scent. Initially the team planned to use a device that would release a controlled volume of scent into the airstream at the inlet of the box, similar to the method used to introduce the gas in testing. After discussion with UMMS staff, it was determined that they planned to introduce scent utilizing porous paper infused with scent, which would be placed over the inlets. By inducing airflow through this porous media, the scent within is released, and mixes with the air. This means that the gas introduced at a controlled volume flow rate, may cause the airflow to be slightly different than it will be when scent is introduced using the porous scented paper. If this paper were obtained, and placed over the inlets during testing, the effect that it has on the pressure on the fan (and therefore the airflow caused by it) could be accounted for. In addition, if possible the gas could be infused in these pieces of paper to make a testing model that matches the actual use of the device even better. As shown in the results of the CFD analysis, the characteristic of (path taken by) the airflow is not influenced greatly by the total airflow, meaning that this effect on the device's performance would likely be very small. Therefore, this would only be necessary if issues with performance are observed in the existing device. If the device seems to operate properly, these time intensive and somewhat costly changes to the testing procedure would likely not be necessary.

3. Head Restraint

3.1. Goal Statement

The secondary objective was to design a head restraint to be used for acclimation of the rat before being introduced to the MRI machine.

3.2. Design Specifications

- Must be non-toxic to the rat
- Must have adequate tensile strength to withstand a rat bite
- Must be transparent
- Must be easily to modify and manufacture

3.3. Final Design

Out of the five models presented to the CCNI staff, two were picked based on their requirements for adult and adolescent sizes.

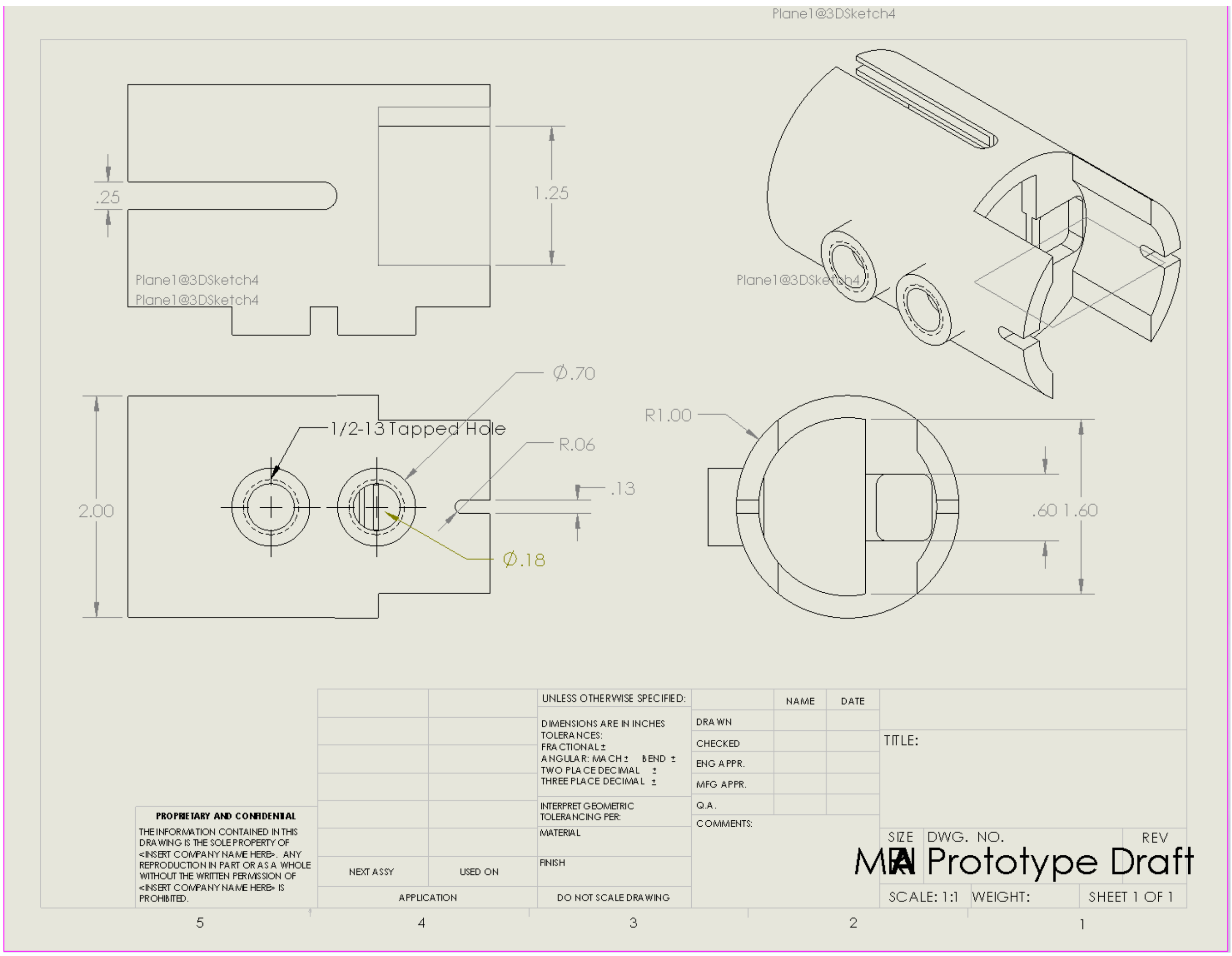


Figure 55: Adult Head Restraint Final Solidworks Drawing

The adult head restraint prototype size was specified by the CCNI staff for adult rats. The outer diameter of this model was 2.00 inches, with a wall thickness of .2 inches. The length of the model in this case was 3.00 inches.

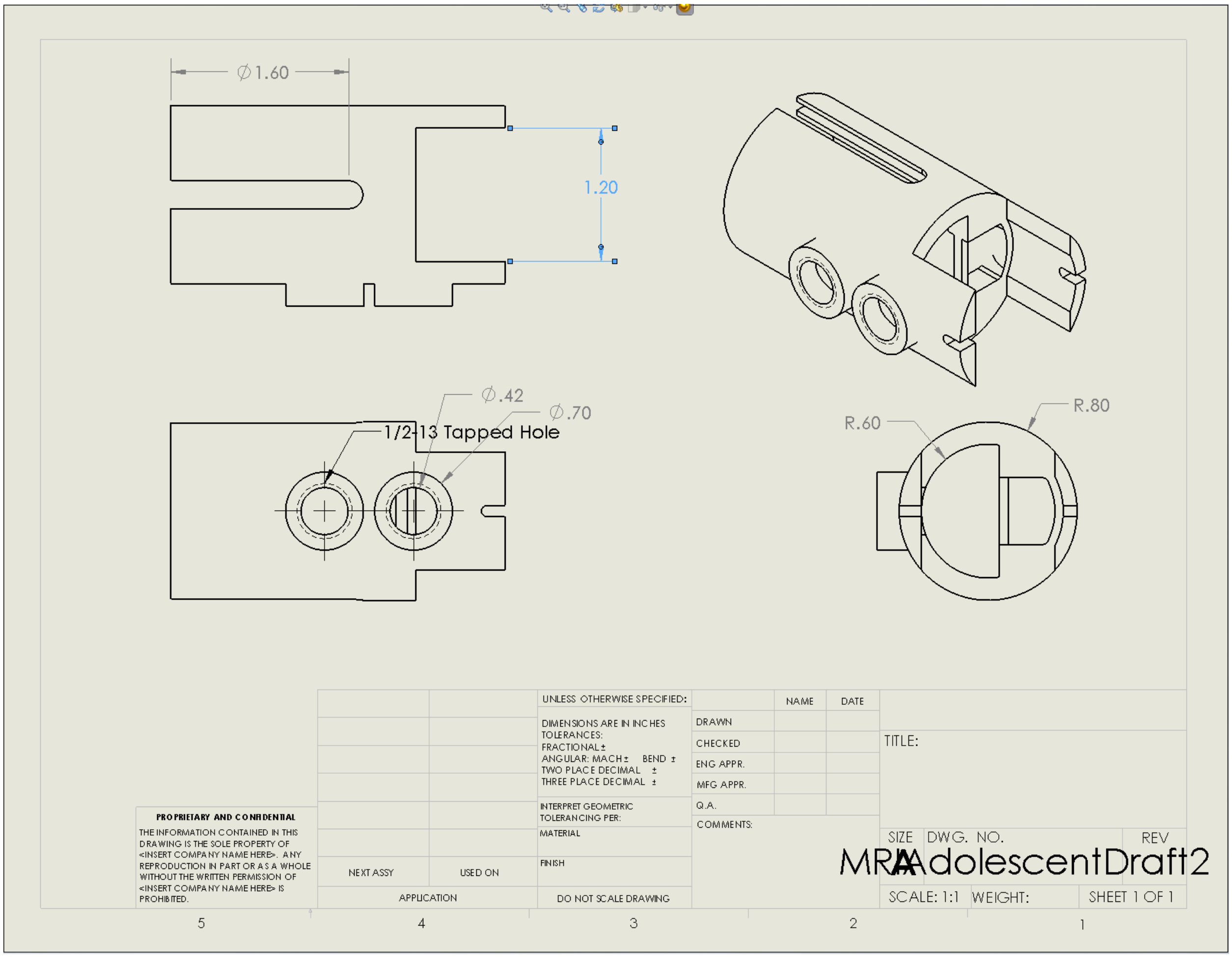


Figure 56: Adolescent Head Restraint Final Solidworks Drawing

The adolescent sized model that specified by the CCNI staff had an outer diameter of 1.60 inches and a wall thickness of .2 inches. The length of the head restraint remained the same, as it would not affect the positioning of the rat in the MRI machine. The radius of the hole for the nose plug remained the same in each case as well, to allow the usage of the same screw for each model. These holes had an outer diameter of 0.70 inches and an inner diameter of 0.42 inches. They were then tapped manually with a 13 threads-per-inch tap. The length and positioning of the ear plug slit is also the same for both models, as the positioning of the rat would not change in reference to the bite bar. We verified this before printing the final model by presenting draft prototypes and examining the positioning of the rat inside the head restraint along with the CCNI staff.

3.4. Methodology

In order to design the head restraint, we used the program Solidworks as it would allow the UMMS staff to have access to a digital file of the head restraint design so they could make changes to the file easily if required in the future.

One of the major changes that we made in the new apparatus for both the adult and the adolescent was in the nose plug that was being used to hold the rats in place. Previously, the nose plug being used was a sliding assembly that was manually lowered and held the rat stayed in place. We believed that this plug could be regulated in a much more controlled way rather than forcing it down onto the rats nose. Instead of using a plug straight to the nose of the rat, we used a screw that would gradually lower down onto the nose of the rat, rather than making its forceful stop on the rat's nose. The bolt we used for this purpose was 13 threads per inch, and was about almost three inches in height. We intend to reduce the height of the screw; however the new bolt idea gives us a much more controlled motion to adjust the nose plug onto the rat.

We managed to retrieve the 3d printed adult model, analyze it and make the required changes in the prototype of the adolescent instrument. One of the major flaws in the design that we made was the wedge slits for the ear plugs being too small, as well as the width of path through which the actual screw would go in was too small as well. After making all the required changes the adolescent model was submitted, and delivered to the UMMS staff to re-check the sizes. We presented them with four different sizes for the different stages for acclimation of the rat. They picked two of the four sizes, specifically the ones with 2.0 inches diameter for the adult prototype and 1.6 inches diameter for the adolescent prototype.

Figure 57: Adult Rat in Final Head Restraint

3.5. Recommendations and Improvements

One of the major challenges we faced with the final design of the head restraint was with the lowering of the nose plug. The lowering of the nose plug using a screw gave it less leverage when it was placed on the rodent’s nose. The rodent would move around when placed in the acclimation head restraint, causing the nose plug to move upwards and not rest on the rodent’s nose. In that case, we recommend that the nose pin be lowered manually instead of using a screw to lower it. This will allow the nose pin to rest on the rodent’s nose keeping it in place. Another modification that would have to be made is the holes which are used to introduce the nose pin would be smaller in order to make the motion of lowering the pin restricted.

4. Conclusion

This project involved two designs, the first was to design, model, construct, and test a conditioned place preference apparatus. We achieved the design specifications set for the device; most importantly, the airflow in the box successfully kept two scents on opposite sides of the apparatus. The other objective of the project was to design a head restraint for acclimation of adult and adolescent rats. The head restraints met the major design specifications, most importantly, restraining the head of both adult and adolescent rats during acclimation in a manner that successfully models the device used for restraining the test subjects in the MRI machine.

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4. Appendices

4.1. First CPP Apparatus Design

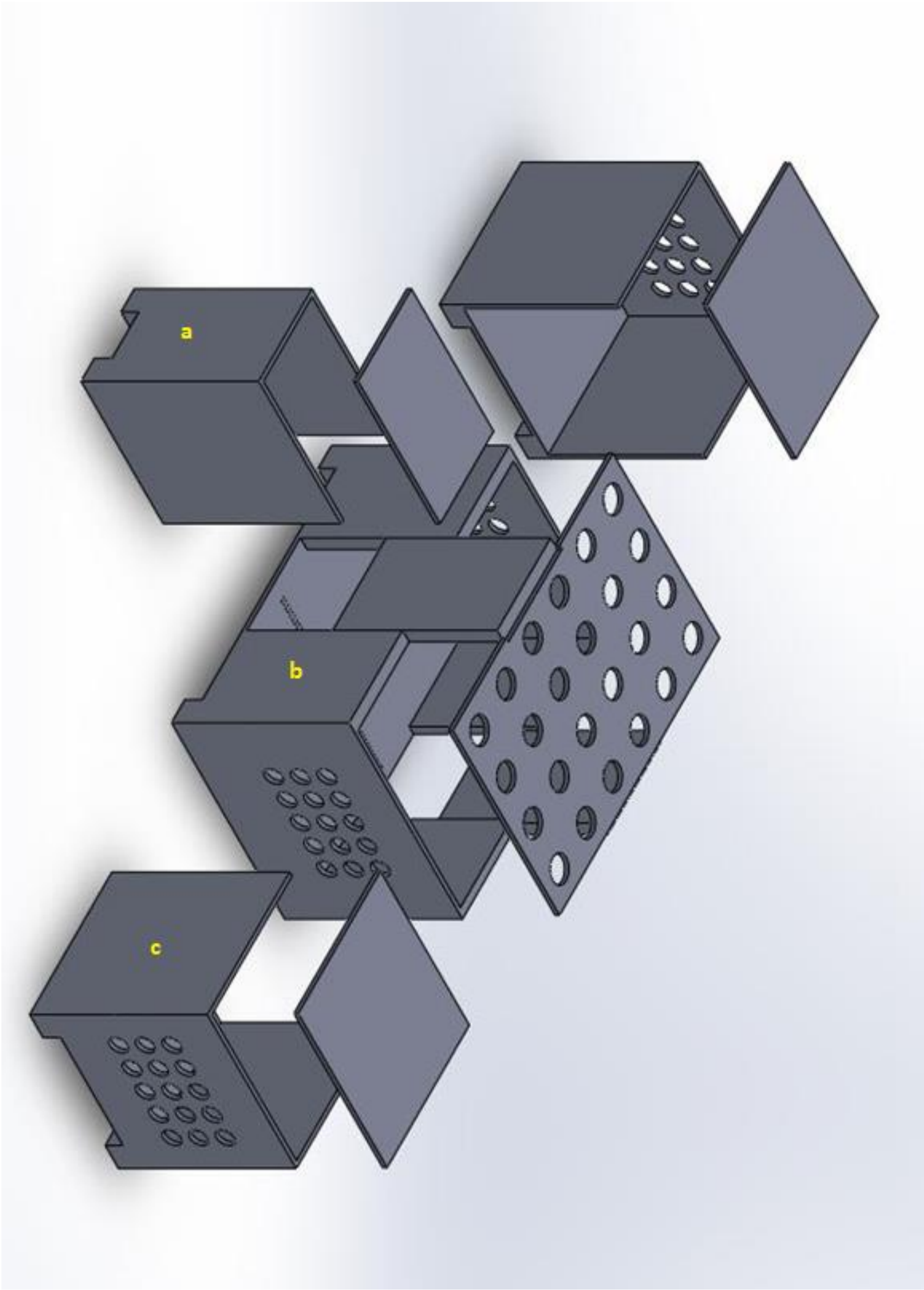

Figure 58: Exploded View of First CPP Apparatus Concept

a) Entry Chamber

b) Main Chamber

c) Scent Box

An exploded view of the first CPP apparatus design can be seen in Figure 58. In this concept, the rat was placed in an entry chamber while the scents diffused through the main chamber. In order to keep the scents contained and to make switching scents easier for UMMS staff, the scent boxes were kept detached from the main chamber with lids. This would also make cleaning the scent boxes easier in case of a spill and to avoid cross contamination.

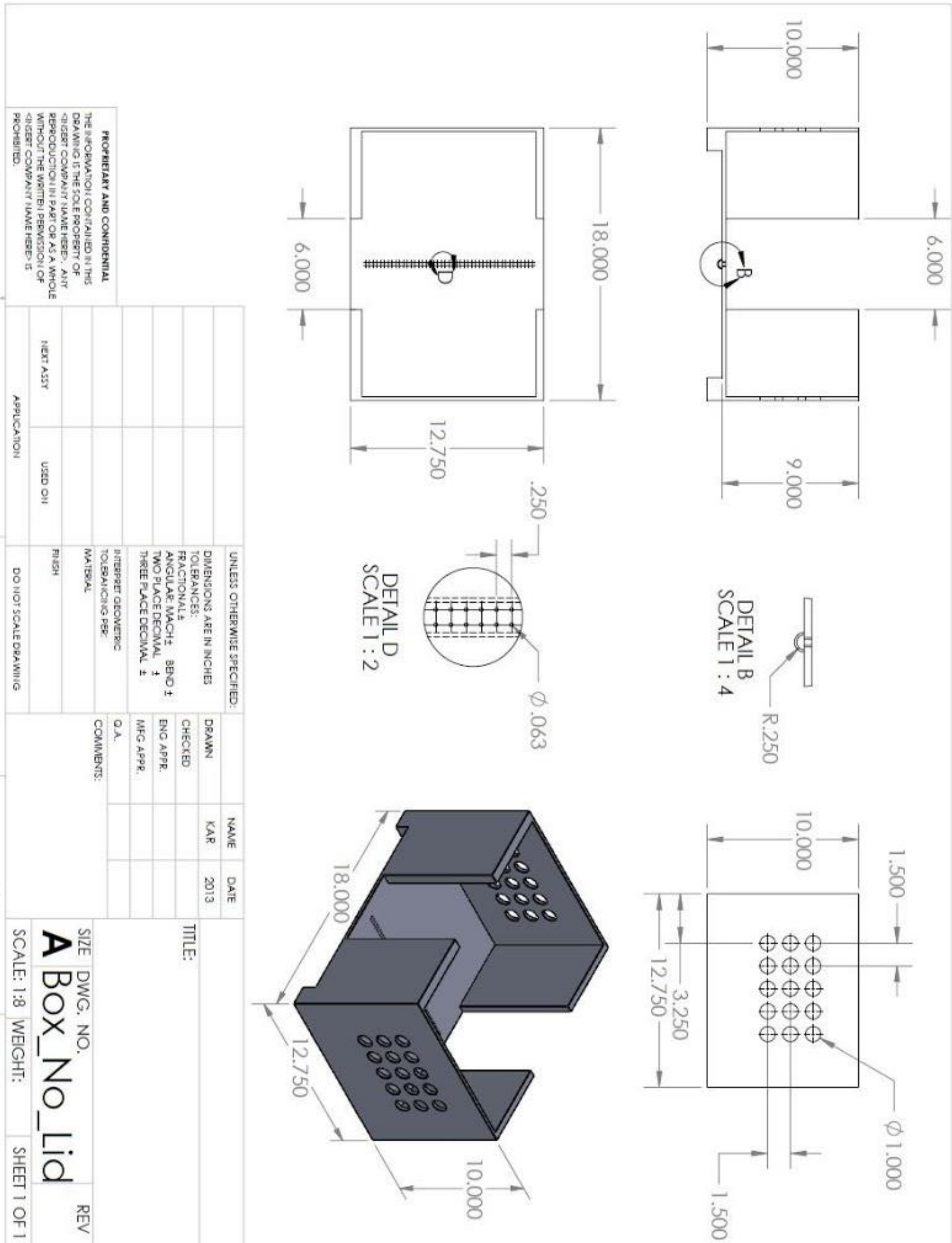

Figure 59: Engineering Drawing of Original Box Concept

The first iteration of the CPP box was designed without dividing walls in order to allow the rat to roam freely in the box. To keep the scents on opposite sides of the chamber, the apparatus was designed with two rows of 1/16 inch diameter holes down the middle of the main chamber. Underneath the rows of holes was a separate, flexible hose (as can be seen in Figure 59 Detail B) which would be connected to an air tank. The air tank would supply a steady stream of air through the holes, creating a wall of upward moving air that would prevent any gas from crossing over into the other half. In order to make room for the tube on the bottom of the box, the entire assembly was raised 1 inch off the ground. This box was also 6 inches shorter. There were two entry ways, one on the front and the other on the back, so that the UMMS staff would not have to clear the box of scent before trying the same scents on different sides of the box. The hole pattern on the sides was the same for both designs.

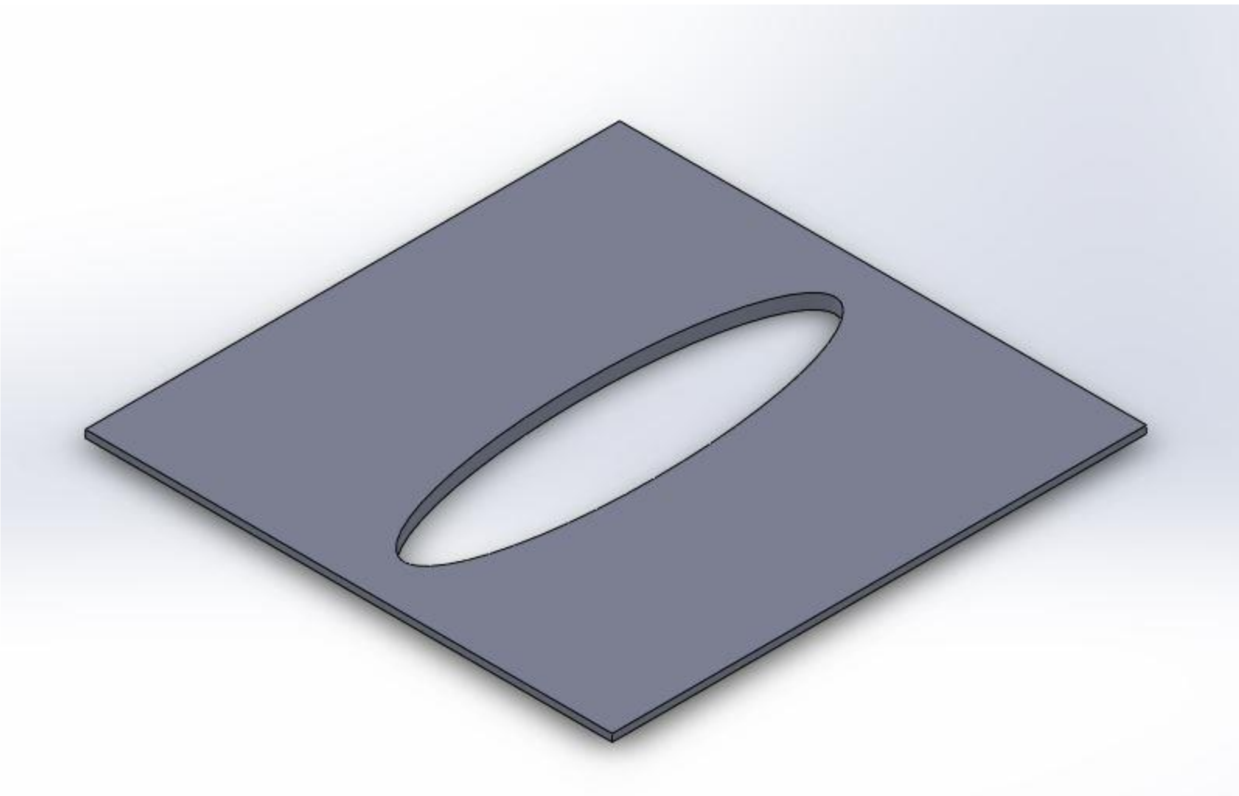

Figure 60: Lid for First Concept with Single Hole in the Middle

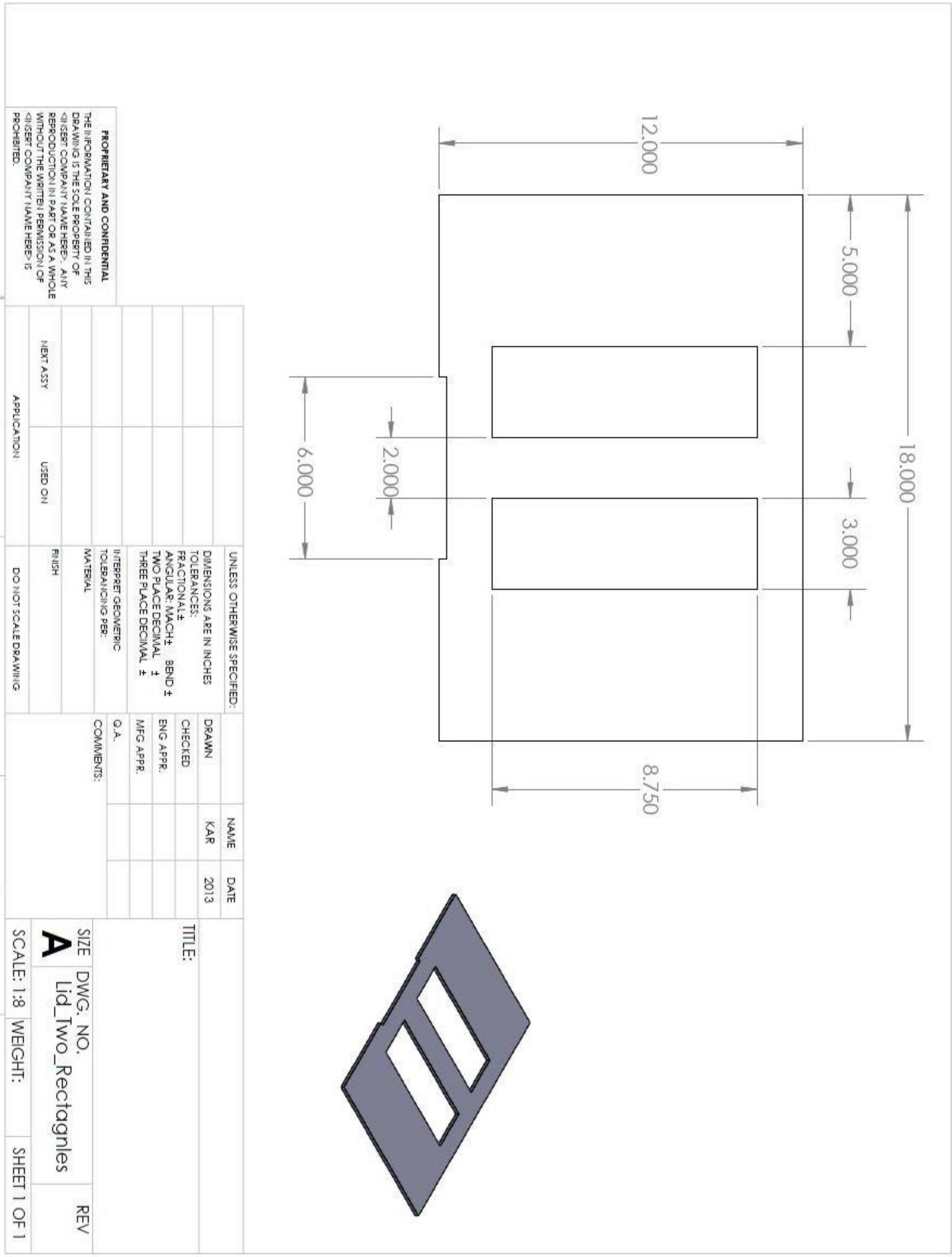

Figure 61: Lid for First Concept with Two Rectangular Holes

In order to ensure that the scents go all the way to the middle of the main chamber, where the rat enters, we had designed a couple of lids through which the air would be exhausted. The first idea that we had was to put a single hole over the middle of the chamber which can be seen in Figure 60. This idea, however, was quickly dismissed when we decided that having a hole over the middle would allow the scents to mix in the middle, where the rat will start out. Another idea that we had was to make a lid with two large rectangles in the middle (Figure 61). This would force the air to go all the way through the main chamber before being exhausted out the top, and in combination with the strip of holes on the floor of the main chamber, would keep the scents from crossing over onto opposite sides.

4.2. Head Restraints

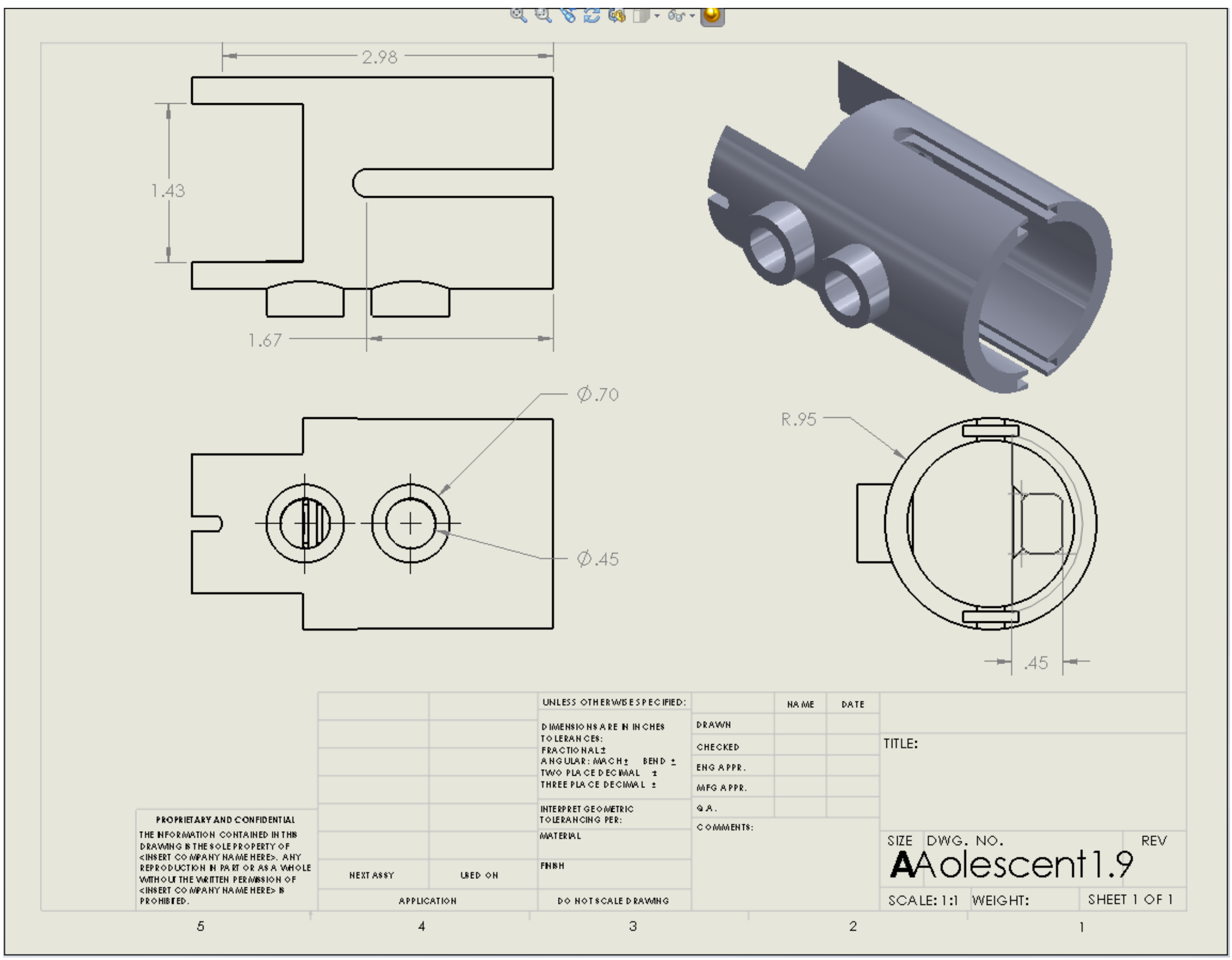

Figure 62: Unused 1.9" Outer Diameter Head Restraint

This head restraint design was originally designed for the adolescent rat. The outer diameter of this design was 1.9 inches, and the inner diameter was 1.7 inches. This design was declined due to the size being too big for the adolescent rat. The final design that was used was partially smaller than the one described above.

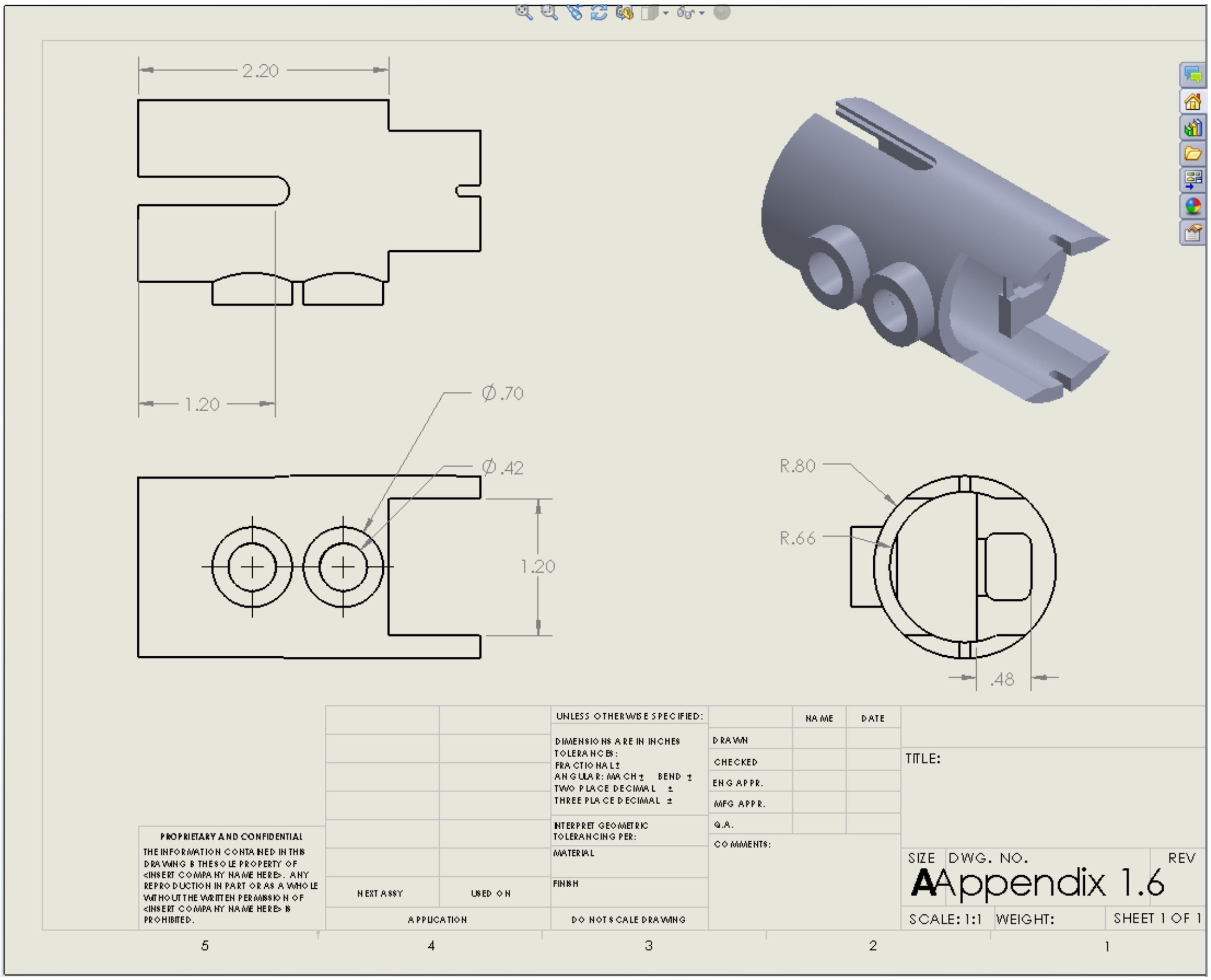

Figure 63: Unused 1.6" Outer Diameter Head Restraint

This head restraint has an outer diameter of 1.6 inches (which is the same as the final adolescent head restraint) and an inner diameter of 1.32 inches. This design was declined as the inner diameter needed to be moderately smaller than 1.32 inches (1.20 inches used in final design).