Volatile Chemicals and Class II Type A2 "Recirculated" BSCs: How Much is Safe?

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Abstract

Volatile chemicals, while typically used in a fume hood, may sometimes be used in conjunction with biological experiments which often require the use of a biosafety cabinet (BSC). While a 100% exhausted Class II Type B2 cabinet is most often mentioned as the safest option, this is not always possible or may not always be necessary. International safety standards have recommended "minute" amounts of volatiles can safely be used in an internally exhausted Class II Type A2 BSC, but what constitutes "minute" can be left up for interpretation (NSF/ANSI Standard 49, 2014). We have derived a series of equations to calculate the internal concentration, maximum amount allowed safely and the time required for the evacuation of any volatile chemical, shown both by calculation and experimentation. Since each chemical has its own safety limits, it is imperative to perform this calculation for each specific volatile in question. These same principles may be applied to many situations, such as the use of alcohols in BSCs, anesthetics for animal work, or air exchanges within a clean room. While no universal volume of any volatile chemical should be deemed safe to work with in a Type A2 BSC, here we present a tool to find the safe working concentration of each specific chemical that can be used to aid in the most accurate risk assessment.

Introduction

Biosafety Cabinets (BSCs) are ventilated enclosures for containing biohazard work assigned to Biosafety Levels 1 through 4 as described in the BMBL (CDC, 2009). These enclosures or Primary Engineering Controls (PECs) are categorized into three Classes depending on the type of protection offered. Class II offers three types of protection: Personnel, Product, and Environmental. Within this classification are four types of BSCs. Type A cabinets are internally recirculating cabinets, with Type A1 and Type A2 differing on their minimum intake velocity of air. Type B2 cabinets exhaust 100% of all the air passing through the cabinet to the outdoors. Type B1 cabinets have both recirculation and direct exhausting capabilities. The different styles of BSCs create their protection through the use of HEPA (High Efficiency Particulate Air) filters. These filters will remove 99.97% or better of 0.3µm mass median diameter particles. What is critical to note is that HEPA filters cannot filter out gasses and vapors.

Which PEC or ventilated enclosure to use can be confusing. It is important to do a proper risk assessment and evaluate which is the most appropriate cabinet for your specific needs. While fume hoods are for chemical work and BSCs are reserved for biohazard work, sometimes volatile chemicals are needed as an adjunct to biological work. The standard solution for this predicament is to recommend using a 100% exhausted Class II Type B2 cabinet since volatile chemicals will permeate all the air within the cabinet if recirculated. Exhausting all contaminated air makes intuitive sense. However, is this really the best option? Are there other options that are still safe?

The most common cabinet found in laboratories worldwide is the Class II Type A2 BSC. It has a partial recirculation system and a front intake velocity of 100 fpm. The Type A2 cabinet may be installed with the cabinet ventilated to the outdoors utilizing a canopy connection, so that the exhaust air is not returned to the laboratory. The concern with using a Type A2 cabinet with volatile chemicals is the buildup of chemical within the cabinet due to the constant recirculation (around 70% of the air) which could reach levels close to the Lower Explosion Limit (LEL) or OSHA exposure limitations. Is there a way to determine if a desired chemical could be safely used within an exhausted Type A2 BSC?

Equation derivation

Concentration of chemical in the downflow air. To determine whether a chemical can safely be used in a specific BSC, some calculations of airflow and chemical concentration need to be determined. Using the basic principles of airflow is the product of velocity and area ($Q = V \times A$), and mass flow rates are the product of airflow and concentration ($\dot{m} = Q \times C$), we can express the amount of chemical within the downflow air, or the unidirectional air coming directly from the HEPA filter down onto the work surface in characteristics specific to the Type A2 BSC being used. A while back, one manufacturer of BSC technology authored a paper used to assist in determining the vapor handling characteristics of Class II BSCs (Stuart et al., 1983). In this paper, we learn that continual release of a volatile chemical will reach a steady state concentration within a recirculating BSC, such as a Type A2. This can be calculated in two ways. First, as described in the paper:

Equation 1: $C_d = C_i + \dfrac{X}{Q_i}$

where C_d is the concentration of chemical in the downflow air, C_i is the concentration of chemical in the intake air coming through the front access opening, X is the rate of chemical release, and Q_i is the intake airflow. **Figure 1** shows the variable denotations used to describe BSC airflow.

C_i is often 0, as there is no chemical in the lab room air, or it is so low that it is negligible. Therefore **Equation 1** becomes:

Equation 2: $C_d = \dfrac{X}{Q_i}$

The accuracy of these equations had been confirmed empirically through the release and measurement of toluene into the airstream (Stuart et al., 1983).

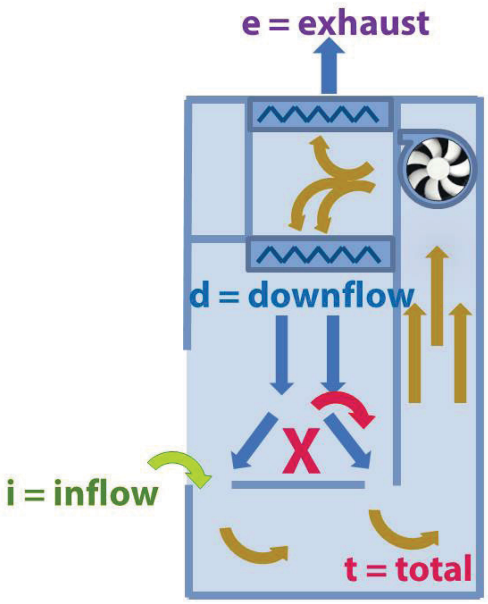

Figure 1. Airflow within a Class II Type A2 BSC with abbreviations.

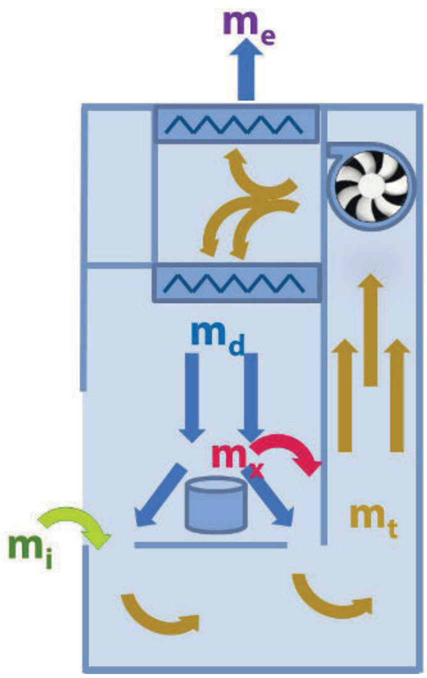

Figure 2. Mass flow rates for specific airflows within a Class II Type A2 BSC diagram.

It would appear, however, that this study did not take into consideration multiple recirculation of the air pattern, specifically within **Equations 1 and 2**, to reflect the amount of recirculated air characteristic of a Type A2 BSC. In order to account for this characteristic, the air volumes can instead be expressed as mass flow rate ($\dot{m}$). Given $\dot{m} = Q \times C$, each segment of air can be written in terms of each mass flow rate. For example: intake air flow would have a $\dot{m}_i = Q_i C_i$. Also, the mass flow rate of the total air in the back plenum of the cabinet is equal to the sum of the individual mass flow rates (**Figure 2**):

Equation 3: $\dot{m}_t = \dot{m}_d + \dot{m}_i + \dot{m}_x$

Similarly, $\dot{m}_d$ is a proportion of the total amount of air dependent on the recirculation rate (R):

Equation 4: $\dot{m}_d = R\dot{m}_t$

If this were solved as an infinite series, the mass flow rate of the downflow air at that infinite cycle can be expressed as:

Equation 5: $\dot{m}_{d^{\circ}} = \left(\frac{R}{1-R}\right)(\dot{m}_i + \dot{m}_x)$

When empirical data is used to solve this, **Equation 5** yields the same result as **Equation 2**, demonstrating that both equations may be implemented to accurately describe the amount of chemical in the downflow air (C_d).

A critical point to mention is that this is the steady state concentration of chemical mixed in the downflow air that is reached upon a prolonged release. Small pockets of air directly adjacent to the source would contain much higher concentrations of the chemical, and good practice needs to be followed to avoid accidental exposures.

Allowable Emission Rate. Equally important as the concentration of chemical in the downflow (C_d) is the amount of chemical that can be released into the airstream and stay below the Lower Explosion or Exposure Limit (LEL). A Dilution Ventilation equation for explosion limitations in chemical fume hoods (Caravanos, 1991) may be modified to describe a recirculating Type A2 BSC using metric units, as shown below:

Equation 6: $ER = \frac{Q_i \text{ x } MW \text{ x } LEL \text{ x } 473}{403 \text{ x } SG \text{ x } S_F \text{ x } 100}$

Where ER is the emission rate, MW and SG are the molecular weight and specific gravity of the chemical, respectively, the numerical values are conversion factors, and S_F is a safety factor. The Safety Factor can be determined by the end user or Biosafety Professional. A S_F of 4, where the chemical concentration will not exceed 25% of the LEL is commonly used. More cautious users may prefer to use S_F=10, thereby staying within 10% of the LEL, as recommended by NIOSH (NIOSH Pocket Guide to Chemical Hazards, CDC Department of Health and Human Services, 2007). See **Table 1** for some commonly used volatile chemicals and their properties needed to solve **Equation 6**.

Chemical	Molecular Weight (g/mol)	Specific Gravity	LEL (%)
Ethanol	46.07	0.787	3.5
Isopropanol	60.1	0.786	2.0
Toluene	92.14	0.865	1.2
Diethyl Ether	74.12	0.714	1.7
Hexane	86.18	0.657	1.1
Chloroform	119.38	1.48	0.0002*
Acetone	58.1	0.79	2.1
Dichloromethane	84.93	1.32	13
BME	78.13	1.14	2.3

Table 1. Some commonly used volatile chemicals and their properties.

A practical example was performed to measure how much chemical can be volatilized upon a spill or spraying a surface. First, 100mL of 70% isopropanol was spilled on the work surface of a 6 ft. nominal width, Class II Type A2 BSC from The Baker Company (a SterilGARD®). The spill covered 0.313 ft2 of the surface and took 1 hour to dry. A spill covering the entire work surface would take 250mL of 70% isopropanol, and still require 1 hour to dry. Similarly, 50mL was sprayed on the entire interior of a 4 ft. nominal width SterilGARD®, which took 5.5 min to dry. It was noted that larger drops of alcohol took longer to evaporate than the smaller droplets. This led to varying rates of chemical volatilization as shown in **Table 2**.

Amount of 70% Isopropanol	Method of Application	Time to Dry	Average Evaporation Rates
100mL	Spill	60 min	4.2mL/min
50mL	Spray	5.5 min	9.1mL/min

Table 2. Evaporation rates of isopropanol in a Class II Type A2 BSC based on application method.

Likewise, if the allowable limit for isopropanol is calculated using **Equation 6**, the emission rate with a S_F = 4 is 171 mL of 70% isopropanol per minute. At the most vulnerable, spraying an entire interior of a BSC work surface may be almost 20 times safer than the predicted maximum amount safe to use in a Type A2 BSC.

Purge rate within a Type A2 cabinet. Once the allowable amount of chemical is determined and the cabinet has reached its steady-state concentration in the downflow air, it will be critical to know when the BSC is clean again. How long does it take for the airstream to reach a nominal or background level of chemical again? This would be useful if there was a spill and you want to know when it is safe to work in the cabinet again. Once the chemical spill has been cleaned up, or the chemical recapped or removed, the amount of circulations of the air pattern required to reduce the concentration of chemical back to a nominal amount can be determined by:

Equation 7: $C_X \; x \; R^n = C_{X,low}$

where C_x is the starting amount of chemical in the cabinet, (this may be the same as C_d), R is the recirculation rate (often ~0.70), n is the number of circulations, and $C_{x,low}$ is the background or nominal amount you would like to reduce the chemical to.

In a practical example, a 4 ft. SterilGARD® was injected with helium to reach 73,000 ppm. It took 60-90 seconds to reach the background level of 10 ppm. Using this information we can apply it to **Equation 7** and determine that n=25 circulations. Given that one circulation takes ~4 s, time to reach background is 100 s, confirming the empirical data.

Conclusions

Since volatile chemicals are not removed from the airstream by a HEPA filter, conventional thought has led us to believe that the majority of volatile chemical use, when used as an adjunct to biological studies, should necessitate the use of a 100% exhausted, Type B2 BSC. There has always been a concern that the cumulative amount of chemical building up in a recirculating BSC, would exceed the exposure and/or explosive limits. However, all volatile chemicals are not created equal, nor do they necessarily need to be treated equally. Instead, shouldn't the safety measures applied differ and/or be dependent on the type and amount of chemical as well as the work being conducted in order to provide an option which is both safe and practical?

We have developed a series of equations to aid in a risk assessment, as a tool to help the biosafety professional to determine whether a specific experiment will be safe to conduct in a Class II Type A2 recirculating BSC. This takes into account the manufacturer's specifications of your BSC as well as the characteristics of the chemical in question. Here, we can determine the amount of chemical in the downflow air (**Equations 2 and 5**), the maximum amount of chemical allowed within the cabinet (**Equation 6**), and how long it takes for the contaminated air to reduce to a nominal background amount (**Equation 7**). From this information we can help to define the "minute" amount of chemical that can be allowed in a Type A2 BSC: the least amount of chemical needed to conduct your experiment, which is below the Exposure and Explosion limitations for that chemical, and should be placed in an appropriate container designed to minimize spillage. If calculations meet all these requirements, then a biosafety professional may use this information to aid in determining whether or not this research could be conducted within an exhausted Type A2 BSC. If not, then the benefits of a Class II Type B2 100% exhausted BSC may apply.

It has long been discussed whether Type A2 BSCs could be safe for use with volatile chemicals. Here we provide the means for you to determine whether this is the case for a specific chemical and amount thereof. By applying our knowledge of ventilated enclosures to what we know about the properties of certain chemicals, we have been able to develop a tool to help the biosafety professional determine whether or not a chemical can be used safely within a externally vented, recirculating, Type A2 BSC. With this tool set, we aim to shed light on the wide safety margin associated with BSC technology, while assisting the industry in conducting an appropriate risk assessment. Of course, a complete risk assessment must be conducted before beginning any new procedure, and these equations are only one tool to aid in that assessment.

References

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Appendix A

Equation 1: $\dot{m} = Q \times C$

Mass flow rate using airflow and the concentration of chemical.

Equation 2: $C_d = \frac{X}{Q_i}$

Calculates the rate of chemical in the downflow air, taking into consideration recirculation by mass flow rates.

Equation 3: $\dot{m}_{d^\circ} = \left(\frac{R}{1-R}\right)(\dot{m}_i + \dot{m}_x)$

Concentration of chemical in the downward flowing air using the chemical emission rate and the inflow air.

Equation 4:

$$ER = \frac{Q_i \times MW \times LEL \times 473}{403 \times SG \times S_F \times 100}$$

Emission rate of chemical allowed within a safety factor of the explosion limit, using characteristics of the chemicals.

Equation 5: $C_X \times R^n = C_{X,low}$

Calculates the number of recirculations needed to dilute a chemical within a recirculating A2 BSC to a background or lower level. Multiply this by the recirculation flow rate (e.g. ~4s for time to clear.

Key for Equations	
m	Mass flow rate, kg/s or mg/s
Q	Airflow, ft^3/min, m^3/min, cfm
C	Concentration (e.g. mg/m3)
X	Chemical, mg/min
d	Downflow
i	Intake
R	Recirculation (e.g. 0.70)
ER	Emission Rate, mL/min
MW	Molecular Weight
LEL	Lower Explosion/Exposure Limit
SG	Specific Gravity
S_F	Safety Factor (4 = 25% of LEL)
n	Number of recirculations

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Dichloromethane	84.93	1.32	13
BME	78.13	1.14	2.3

*NIOSH REL (2 ppm) = 13 µL/min in 4' A2 BSC

Note: These calculations are intended to be a tool and a risk assessment should always be conducted for every experiment involving hazards.

To learn more about Class II, Type A2 Cabinets, please visit

bakerco.com/products/sterilgard