ADVISORY NO. 8.4: GUIDELINES FOR THE SELECTION, INSTALLATION AND USE OF BIOSAFETY CABINETS (INCLUDING ISSUES REGARDING NATURAL GAS USE)

BACKGROUND

Hospitals and private industries have specific requirements regarding the uses and limitations of biosafety cabinets. One such requirement is the annual inspection and certification of those units. Biosafety cabinet manufacturers have limited warranty attached to the use of such cabinets. Facility audits and conversations with manufacturers, researchers and different agencies conducted by the Environmental Health & Safety have prompted the development of university-wide guidelines which both inform and alert users to the limitations and appropriate uses of such cabinets.

WHAT IS A BIOSAFETY CABINET?

Biological safety cabinets[1] are the most commonly used primary containment devices in laboratories working with biological agents. They are sometimes called by manufacturers as safety cabinets, laminar flow biological safety cabinets, laminar flow hood, etc.

Biosafety cabinets are categorized into different classes and types (**see Appendix A**) and recommended to be used for different risk levels of agents. An important difference in biosafety cabinets is their ability to properly handle chemical vapors. The classifications of biosafety cabinets are based on air inflow velocities, percentages of exhausted or recirculated air, directions of airflows inside the cabinets, and pressure differentials between contaminated duct or plenum[2] to the room. Class II biosafety cabinets are most commonly used in laboratories, and have air partially recirculated in the units (except for Class II Type B2 cabinets).

SELECTION OF BIOSAFETY CABINETS

Administrative Memo #110 requires program design discussion among laboratory users, the Construction Management Department (Architect/Engineering), and Environmental Health & Safety. The discussion regarding biosafety cabinets should include a description of the processes that are to occur in these cabinets. Special emphasis should be placed on:

- The intended use of the biosafety cabinets: what the use of these cabinets is meant to protect (the process, the operator, the environment, or all the above);
- The substances to be used;
- The likely disaster scenarios that can be expected to occur within these units over a five-year time frame.

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[1] Clean benches with horizontal or vertical laminar flow which discharge air out of the front opening and into the room are different from biosafety cabinets. Clean benches provide product protection but may expose the worker to potentially hazardous materials.

[2] An enclosure that forms part of an air distribution system for flowing gases in which the static pressure at all points is relatively uniform.

From this discussion, the proper type of cabinet can be specified if it is not currently owned by the occupants, and provisions can be made to properly install the units (**see Appendix B** for the applications of different types of biosafety cabinets).

INSTALLATION OF BIOSAFETY CABINETS

New Design

A team approach consisting of users, Construction Management, Facility Planning, Facilities Management and Environmental Health & Safety should be used to develop a safe system for research using a biosafety cabinet.

Cabinet Location

Biosafety cabinets should be located out of the traffic pattern, and away from room air currents that could disrupt the containment provided by the work access opening air barrier.

Venting

The venting methods for biosafety cabinets depend upon cabinet types. Class I and Class III biosafety cabinets must be attached to an exhaust system and air is 100% exhausted to the outside atmosphere after passage through a High Efficiency Particulate Air (HEPA) filter. Class II Type A cabinet can be installed to discharge exhaust air to the room. It is preferable to have the cabinet discharge the exhaust air to the laboratory exhaust system via a special canopy hood or "thimble unit" available from cabinet manufacturers. Class II Type B (including Type B1, B2 and B3) cabinets are to be vented outside the building. Class II Types B1 and B2 units must be physically connected (hard-ducted) to the exhaust system; the thimble connection cannot be used. It is recommended by the National Sanitation Foundation (NSF) Standard 49 and American Society of Heating, Refrigerating and Air Conditioning Engineering (ASHRAE) Handbook that each Type B cabinet have its own exhaust system, designed and field balanced for the individual unit. Type B cabinets "hard connected" to any exhaust system may require an anti-backflow device to prevent reverse airflow through the HEPA filter.

CERTIFICATION OF BIOSAFETY CABINETS

It is recommended by the Centers for Disease Control, the National Institutes of Health, and the National Sanitation Foundation Standard 49, that biosafety cabinets are tested and certified onsite at the time of installation within the laboratory, at any time the cabinets are relocated, HEPA filters are changed, or maintenance repairs are required, and at least annually thereafter for assuring that all physical containment criteria are met.

ISSUES REGARDING USE OF NATURAL GAS IN BIOSAFETY CABINETS

Requests by researchers for the installation of natural gas to biosafety cabinets have raised several issues concerning safety, liabilities and cabinet manufacturer warranty. The following recommendations were formulated after researching this subject and consulting with biosafety cabinet manufacturers and other agencies.

- Natural gas is generally used to fuel a bunsen burner or other flame source for purposes of sterilization. The use of gas in biosafety cabinets presents several potential safety hazards.
- Use of gas in a biosafety cabinet presents a potential fire or explosion hazard. The cabinets are not constructed to be explosion-proof. Electrical components such as the fan motor, lights, and electrical outlets are not designed to operate in flammable atmospheres. Since most biosafety cabinets have air recirculated in the units[3], gas leaks or petcocks left open can produce a flammable atmosphere in the cabinet, which could be ignited by a spark. Because of this manufacturers post their cabinets with warning labels stating that flammable materials should not be used in the cabinet. The warning label is required for UL listing.
- Use of a burner in a biosafety cabinet can distort the normal air flow patterns. The flow patterns in the cabinet are designed to provide a sterile working area and protection to the user. Normal flow in the

cabinet is from the top down across the working surface. Addition of a burner will produce turbulent flow due to the heated air rising countercurrent to the normal downward flow. This condition may contribute to the spread of contamination within the cabinet and could cause user exposure.

- Additionally, the heat generated by the burner can damage the HEPA filter and/or the filter's adhesive. This can produce leaks in the filter, adverse flow patterns in the cabinet, as well as potential user exposure.

By using gas in a biosafety cabinet labeled with the warning sign **"DO NOT USE TOXIC EXPLOSIVE OR FLAMMABLE SUBSTANCES IN THIS CABINET"**, the user will be voiding the cabinet manufacturer's warranty. The UL approval will automatically be void. The cabinet manufacturer will assume no liability in the event of fire, explosion, or worker exposure due to the use of a flammable gas in the cabinet. This information was confirmed by major biosafety cabinet manufacturers.

RECOMMENDATIONS

Based on the above information, it is the recommendation of Environmental Health & Safety that the use of natural gas or any other flammable materials in Class II biosafety cabinets be prohibited (except for Class II Type B2 when an explosion-proof roof fan is present). This is also the recommendation of the World Health Organization and the British Standards Institute as well as the major biosafety cabinet manufacturers.

If a researcher feels flame sterilization in a biosafety cabinet is necessary, the situation should be brought to the attention of the Environmental Health & Safety for review. A Class I, or Class II Type B2, or Class III biosafety cabinet which includes exhaust ductwork to the outside and an explosion-proof roof fan is suitable for using natural gas or other flammable substances (although the problem of distorting the air flow pattern may still exist). An appropriate biosafety cabinet can be selected and properly installed based on material usage (biological, chemical, etc.) and purposes of protection, if it is not currently owned by the laboratory.

Current cabinet users should have their areas inspected for safe operating procedures and work towards implementing safe engineering design practices.

Electric burners and microincinerators may be a workable alternative to using gas. These devices eliminate the danger of working with a flammable material. Thus the manufacturer's warranty and the cabinet's UL listing are not affected. It must be noted that these devices may still create heat and turbulent airflow in the cabinet. Excessive heat may damage the HEPA filter. To minimize these effects, the electric burner or microincinerator should only be used in the rear of the workspace.

[3] Class II biosafety cabinets are most commonly used in laboratories. Except Class I, Class II Type B2, and Class III biosafety cabinets, all other types of biosafety cabinets have air recirculated in the units. Flammable materials may be used in Class I, Class II Type B2 and Class III biosafety cabinets if explosion-proof roof fan is present, since those units have 100% exhausted air and the exhausted air does not pass any non-explosion-proof internal blowers.

For existing Class II Types A, B1, or B3 biosafety cabinets, if one of the following alternate methods using gas is implemented, the researcher must understand that the cabinet's UL listing and warranty will be voided and that the researcher may be assuming personal liability in the event of a fire, explosion or personal exposure. The University General Counsel may not permit an individual to assume personal liability.

The following procedures are risk-reduction methods for existing Class II Type A, B1, or B3 biosafety cabinets if natural gas or other flammable materials are used:

- Use bottled gas or gas cylinders as the fuel supply for the burner to limit the supply of fuel. An automatic burner with a foot or hand switch to operate the flame must be used.
- Plumbed gas in contrast to bottled gas, provides an inexhaustible supply of fuel. Plumbed gas may be used if the following design criteria and operator procedures below are implemented.
  - ① The plumbed natural gas line must have a shutoff valve outside the cabinet. An automatic burner with a foot switch or hand switch to operate the flame must be used.
  - ② Excess flow check valves or flow limit valves designed to shut off gas flow if a pre-set limit is exceeded should be installed. These devices could prevent the flow of flammable or toxic gases into an area when other conditions have resulted in failure of point-of-use control systems. Use of these valves must be considered early in the design of the piping system.
- Regardless of gas source, users must be trained to visually inspect the gas petcock or valve and check for the odor of gas before turning on the biosafety cabinet blowers. The burner should only be used in the rear of the cabinet to minimize the effects of air turbulence. At the end of burner operation, the user must turn the gas petcock or valve off and check for the smell of gas.
- The exhaust for the biosafety cabinet should be properly vented through an exhaust system to the outside of the building.

Please contact Environmental Health & Safety at 556-4968, if you have any questions.

APPENDIX A

Classifications Of Biosafety Cabinets
From
National Sanitation Foundation
Standard 49

This Standard only covers Class II biohazard cabinets. However, the following classes are currently known to be available:

CLASS I: A ventilated cabinet for personnel and environmental protection with an unrecirculated inward airflow away from the operator.

NOTE: The cabinet exhaust air is treated to protect the environment before it is discharged to the outside atmosphere. This cabinet is suitable for work with low to moderate risk biological agents where no product protection is required.

CLASS II: A ventilated cabinet for personnel, product, and environmental protection having an open front with inward airflow for personnel protection, HEPA filtered laminar airflow for product protection, and HEPA filtered exhausted air for environmental protection.

NOTE: When toxic chemicals or radionuclides are used as adjuncts to biological studies or pharmaceutical work, Class II cabinets designed and constructed for this purpose should be used.

CLASS II, TYPE A: Cabinets that (1) maintain minimum calculated average inflow velocity of 75 fpm through the work area access opening; (2) have HEPA filtered downflow air from a common plenum (i.e., plenum from which a portion of the air is exhausted from the cabinet and the remainder supplied to the work area); (3) may exhaust HEPA filtered air back into the laboratory; and (4) may have positive pressure contaminated ducts and plenums. Type A cabinets are suitable for work with low to moderate risk biological agents in the absence of volatile toxic chemicals and volatile radionuclides.

CLASS II, TYPE B1: Cabinets that (1) maintain a minimum (calculated or measured) average inflow velocity of 100 fpm through the work area access opening; (2) have HEPA filtered downflow air composed largely of uncontaminated recirculated inflow air; (3) exhaust most of the contaminated downflow air through a dedicated duct exhausted to the atmosphere after passing through a HEPA filter; and (4) have all biologically contaminated ducts and plenums under negative pressure or surrounded by negative pressure ducts and plenums. Type B1 cabinets are suitable for work with low to moderate risk biological agents. They may also be used with biological agents treated with minute quantities of toxic chemicals and trace amounts of radionuclides required as an adjunct to microbiological studies if work is done in the direct exhausted portion of the cabinet, or if the chemicals or radionuclides will not interfere with the work when recirculated in the downflow air.

CLASS II, TYPE B2: (Sometimes referred to as "Total Exhaust") Cabinets that (1) maintain a minimum (calculated or measured) average inflow velocity of 100 fpm through the work area access opening; (2) have HEPA filtered down flow air drawn from the laboratory or the outside air (i.e., downflow air is not recirculated from the cabinet exhaust air; (3) exhaust all inflow and downflow air to the atmosphere after filtration through a HEPA filter without recirculation in the cabinet or return to the laboratory room air; and (4) have all contaminated ducts and plenums under negative pressure, or surrounded by directly exhausted (not recirculated through the work area) negative pressure ducts and plenums. Type B2 cabinets are suitable for work with low to moderate risk biological agents. They may also be used with biological agents treated with toxic chemicals and radionuclides required as an adjunct to microbiological studies.

CLASS II, TYPE B3 (Sometimes referred to as "Convertible Cabinets"): Cabinets that (1) maintain a minimum (calculated or measured) average inflow velocity of 100 fpm through the work area access

opening; (2) have HEPA filtered down flow air that is a portion of the mixed downflow and inflow air from a common exhaust plenum; (3) discharge all exhaust air to the outdoor atmosphere after HEPA filtration; and (4) have all biologically-contaminated ducts and plenums under negative pressure, or surrounded by negative pressure ducts and plenums. Type B3 cabinets are suitable for work with low to moderate risk biological agents treated with minute quantities of toxic chemicals and trace quantities of toxic chemicals and trace quantities radionuclides that will not interfere with the work if recirculated in the downflow air.

OTHER TYPES: Other cabinets may be considered Class II if they meet these requirements for performance, durability, reliability, safety, operational integrity, and cleanability.

CLASS III: A totally enclosed, ventilated cabinet of gas-tight construction. Operations in the cabinet are conducted through attached rubber gloves. The cabinet is maintained under negative air pressure of at least 0.5 inches (12.7 mm) water gauge (wg). Supply air is drawn into the cabinet through HEPA filters. The exhaust air is treated by double HEPA filtration, or by HEPA filtration and incineration as described in Laboratory Safety monograph, January 1979, NIH Supplement to Guidelines).

Illustrated Figures of Different Classes or Types of Biosafety Cabinets (see Appendix C)

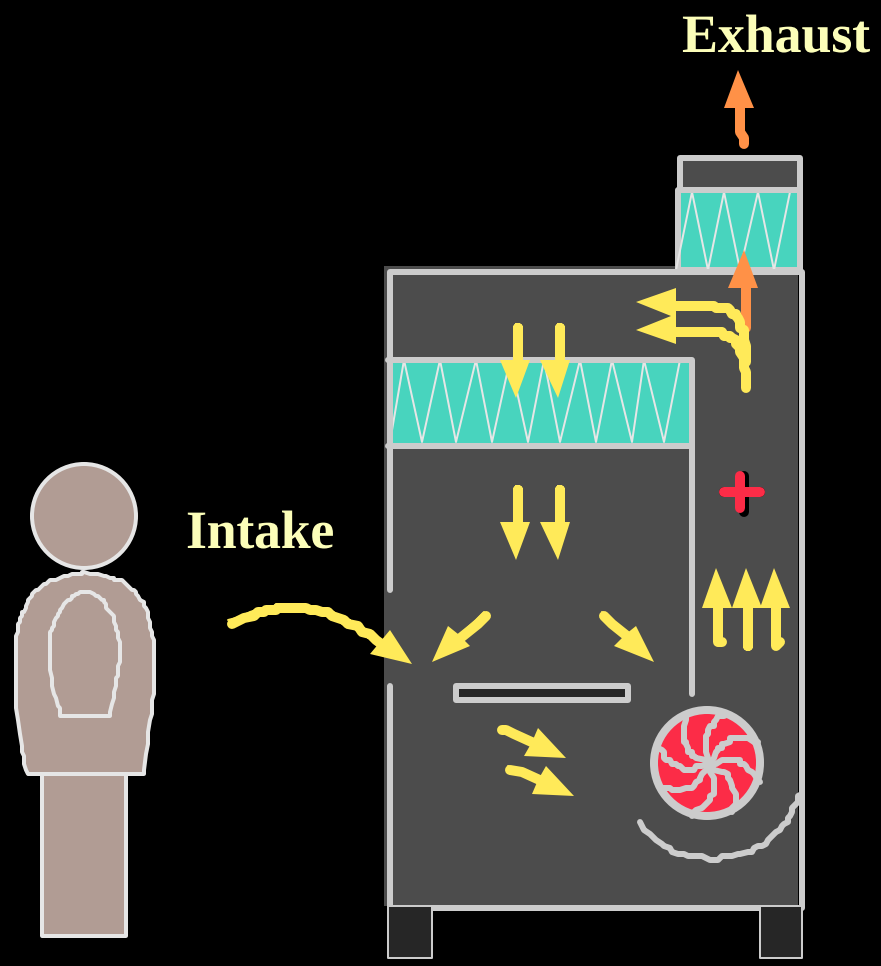
Venting Methods and Exhaust Transitions for Biosafety Cabinets (see Appendix C pgs. 4-5)

APPENDIX B

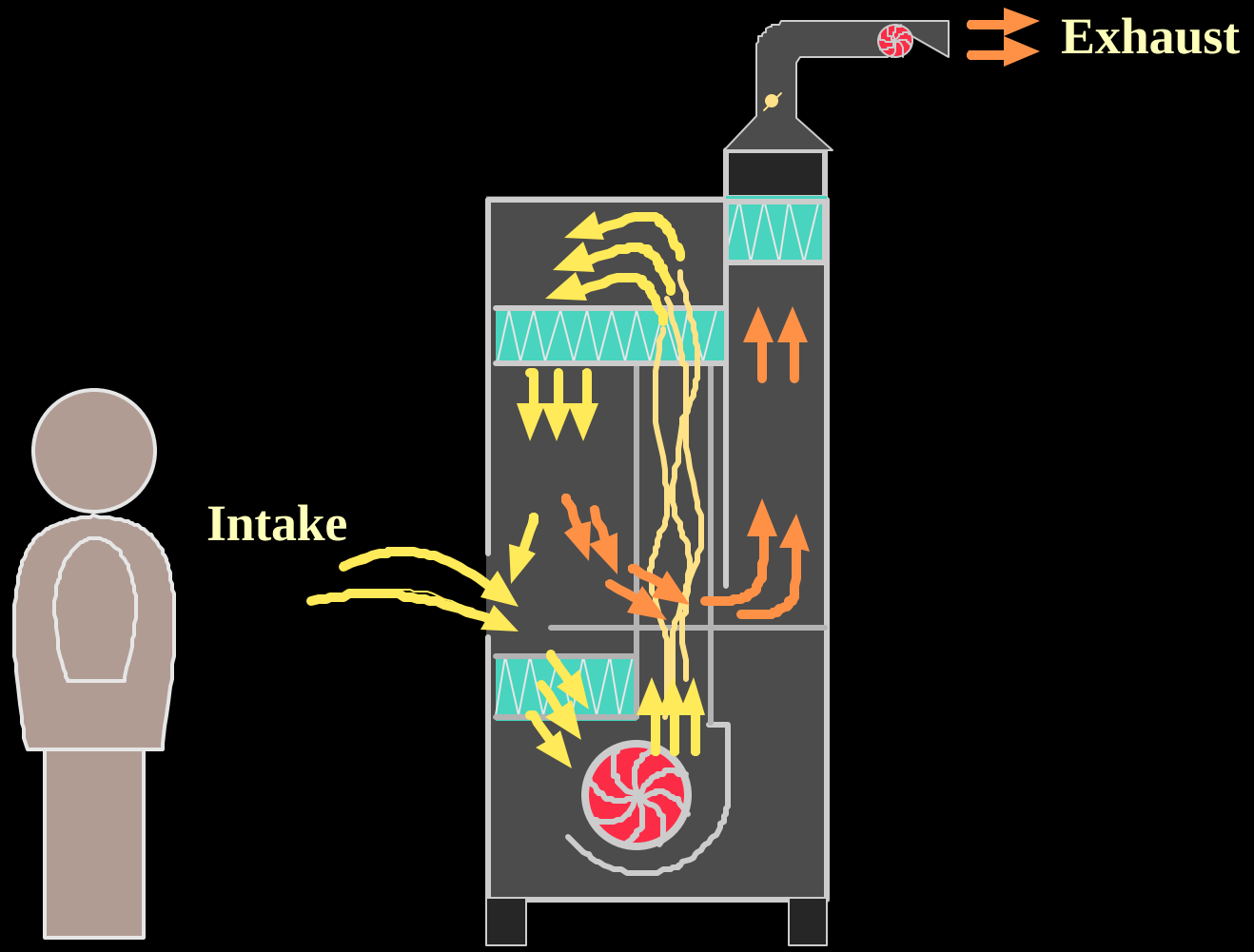
Applications Of Biological Safety Cabinets

Type Of Unit	For Use With	Not Recommended For Use With
Clean Benches	- Non-hazardous drugs - Sterile apparatus - Filling procedures for non-hazardous materials - Sterility testing - QC inspection in industrial applications (to check for absence of particles) - Similar risk free procedures where product protection is important	- Antineoplastic or other hazardous drugs - Hazardous and flammable chemicals - Tissue culture procedures with animal cells - Microbial agents - Radioactive materials - Carcinogens - Hazardous (even mildly) procedures where personnel protection is required
Class I Biological Safety Cabinets	- Low to moderate risk microbial agents - Low levels of radioisotope - Low levels of flammable chemicals if explosion-proof roof blower on roof - Low levels of chemical carcinogens	- High risk microbial agents - High levels of hazardous chemicals or carcinogens - Tissue cultures - Drugs - Procedures where product protection is important
Class II-Type A Biological Safety Cabinets	- Low to moderate risk microbial agents - Tissue cultures - Antineoplastic drugs - Low levels of radioactive materials in particulate state only - Anything a clean bench can used for	- High-risk microbial agents - Flammable or volatile chemicals - Gaseous or potentially gaseous radioactive materials - Chemical carcinogens
Class II-Type B1 Biological Safety Cabinets	- Low to moderate risk microbial agents - Tissue cultures - Antineoplastic drugs - Low levels of radioactive materials in particulate state only - Anything a clean bench can be used for - Low levels of chemical carcinogens - Low levels of volatile (non-flammable chemicals)	-High risk microbial agents - Flammable chemicals - Gaseous or potentially gaseous radioactive materials - Moderate to high levels of chemical carcinogens
Class II-Type B2 (Total) Exhaust Biological Safety Cabinets	- Low to moderate risk microbial agents - Tissue cultures - Antineoplastic drugs - Low levels of radioactive material - Chemical carcinogens - Volatile and flammable chemicals (if explosion-proof blower on roof) - Anything a clean bench can be used for	- High risk microbial agents - High levels of hazardous chemicals or carcinogens - High risk chemical agents where slight exposure could be deadly
Class III Biological Safety Cabinets	- Any microbial agent (high risk included) - Low levels of radioactive material - Chemical carcinogens - Volatile & flammable chemicals (if explosion-proof blower on roof) - Procedures where highest level of personnel protection is necessary	- Procedures where a high level of product protection is necessary (tissue cultures, drugs)

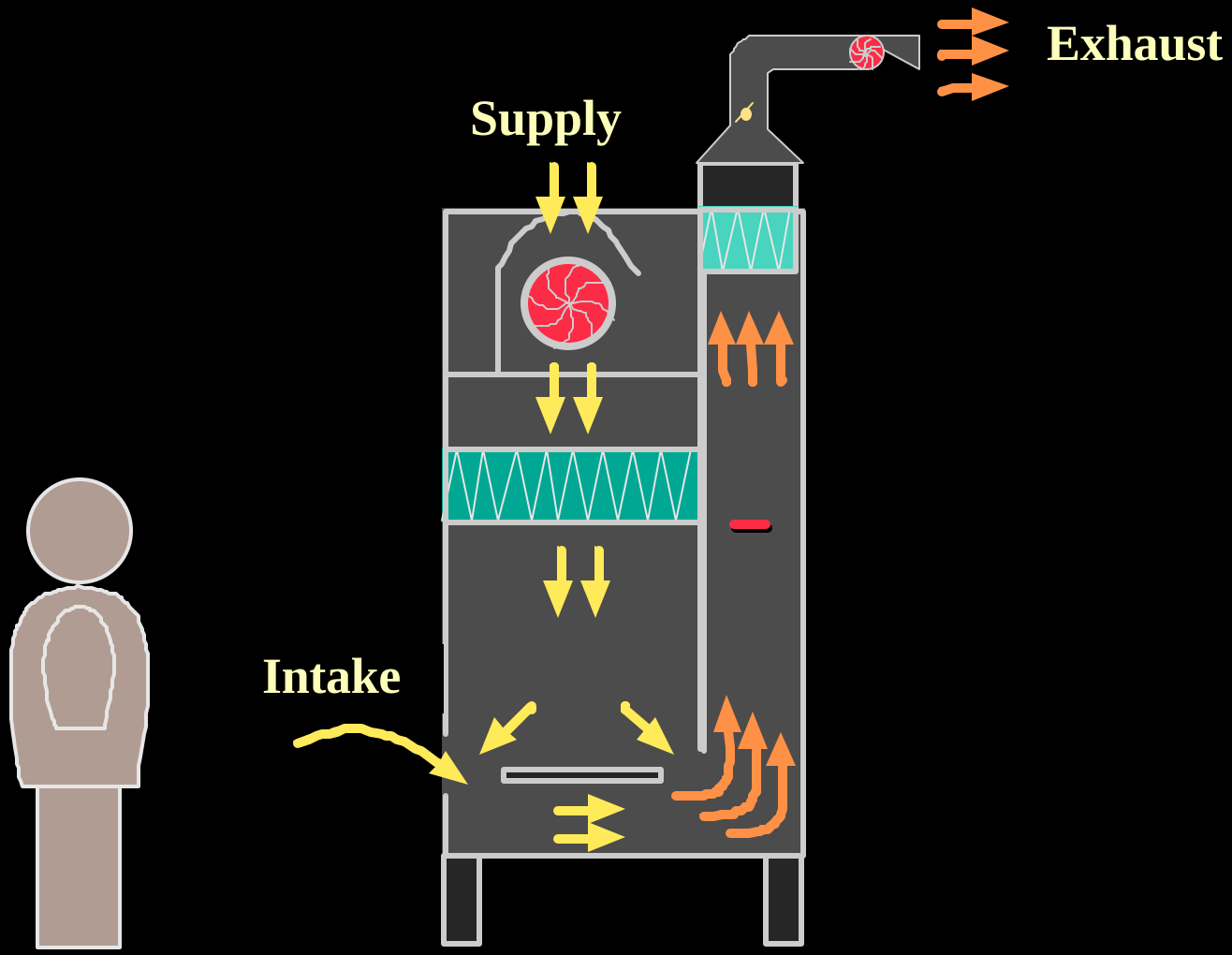
Class II Type A Airflow Schematic



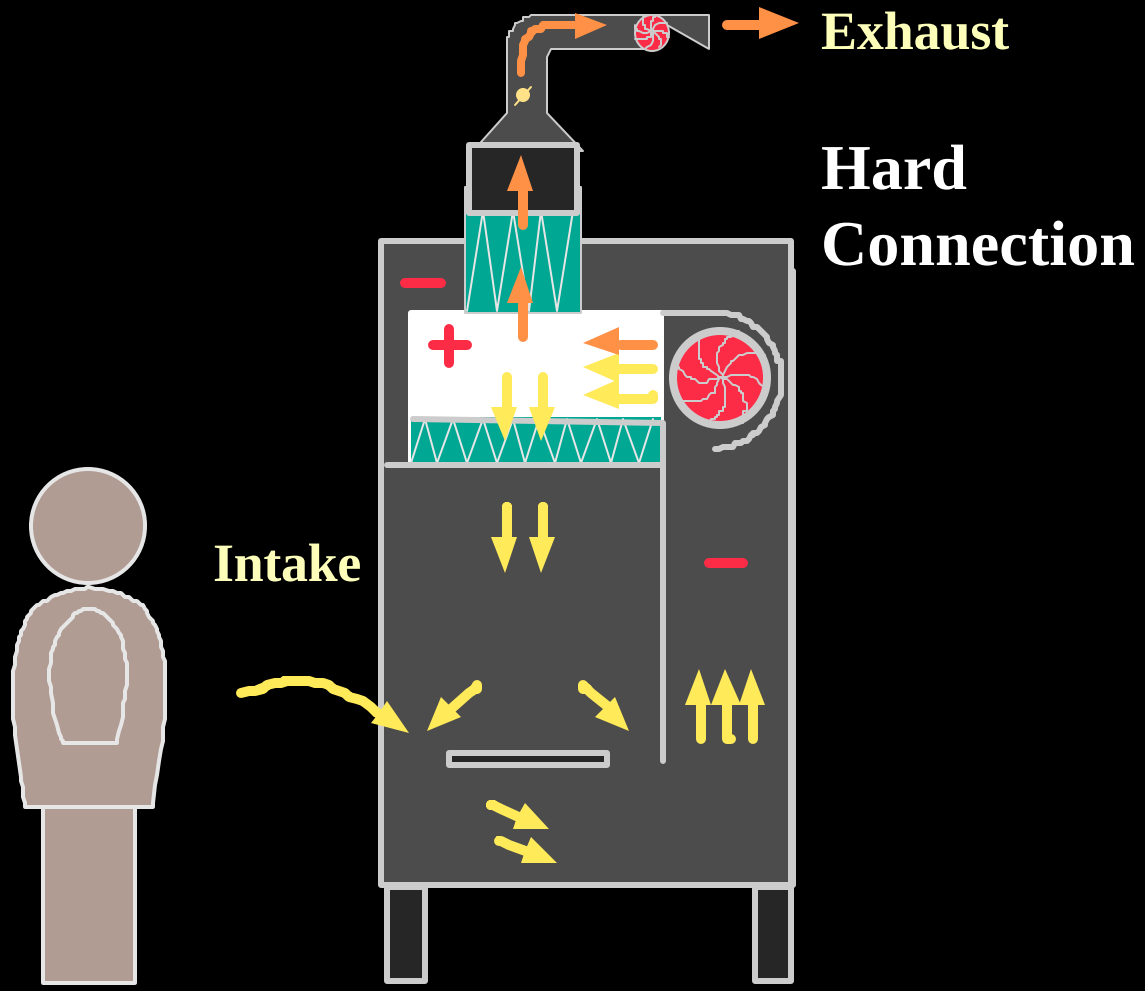
Class II Type B1 Airflow Schematic



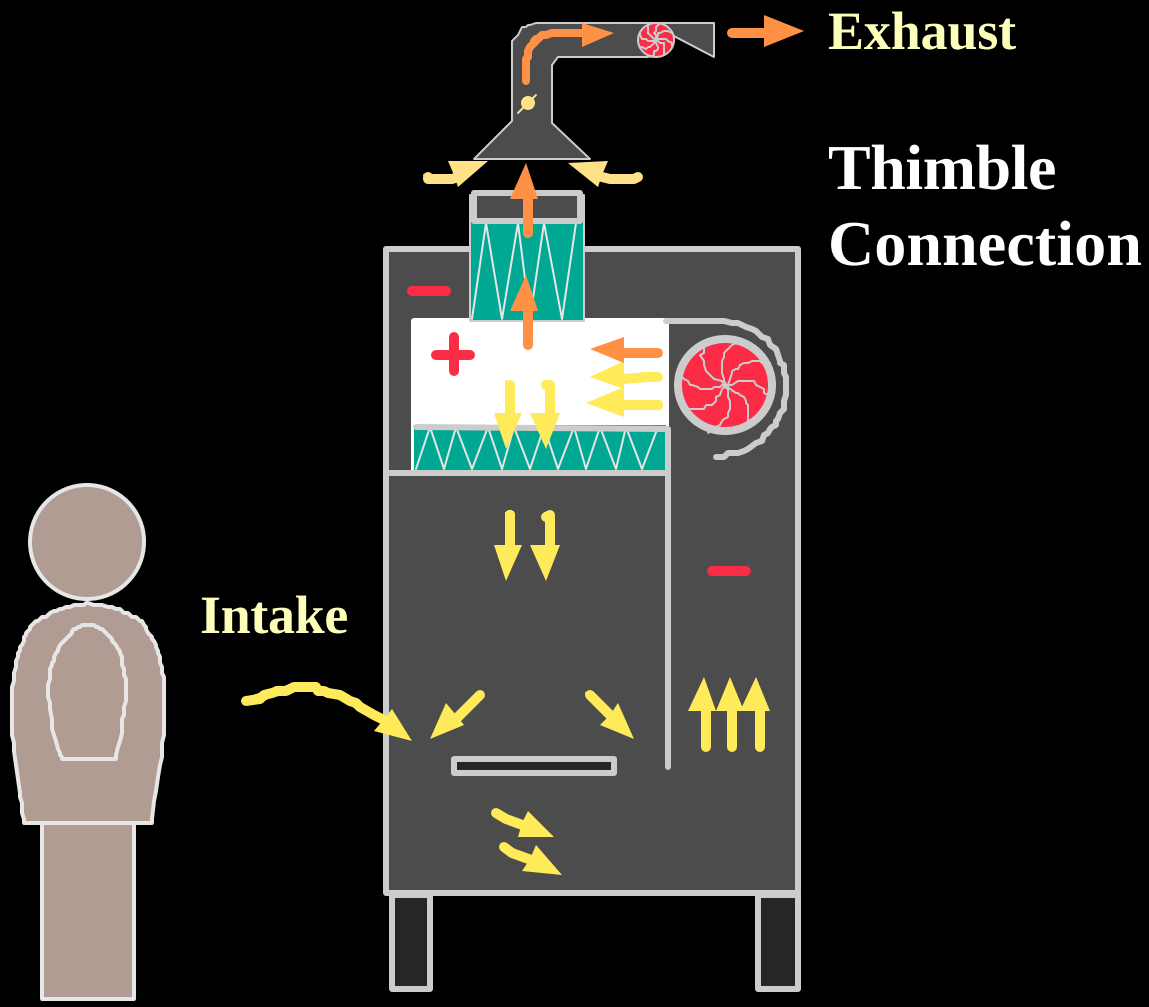
Class II Type B2 Airflow Schematic



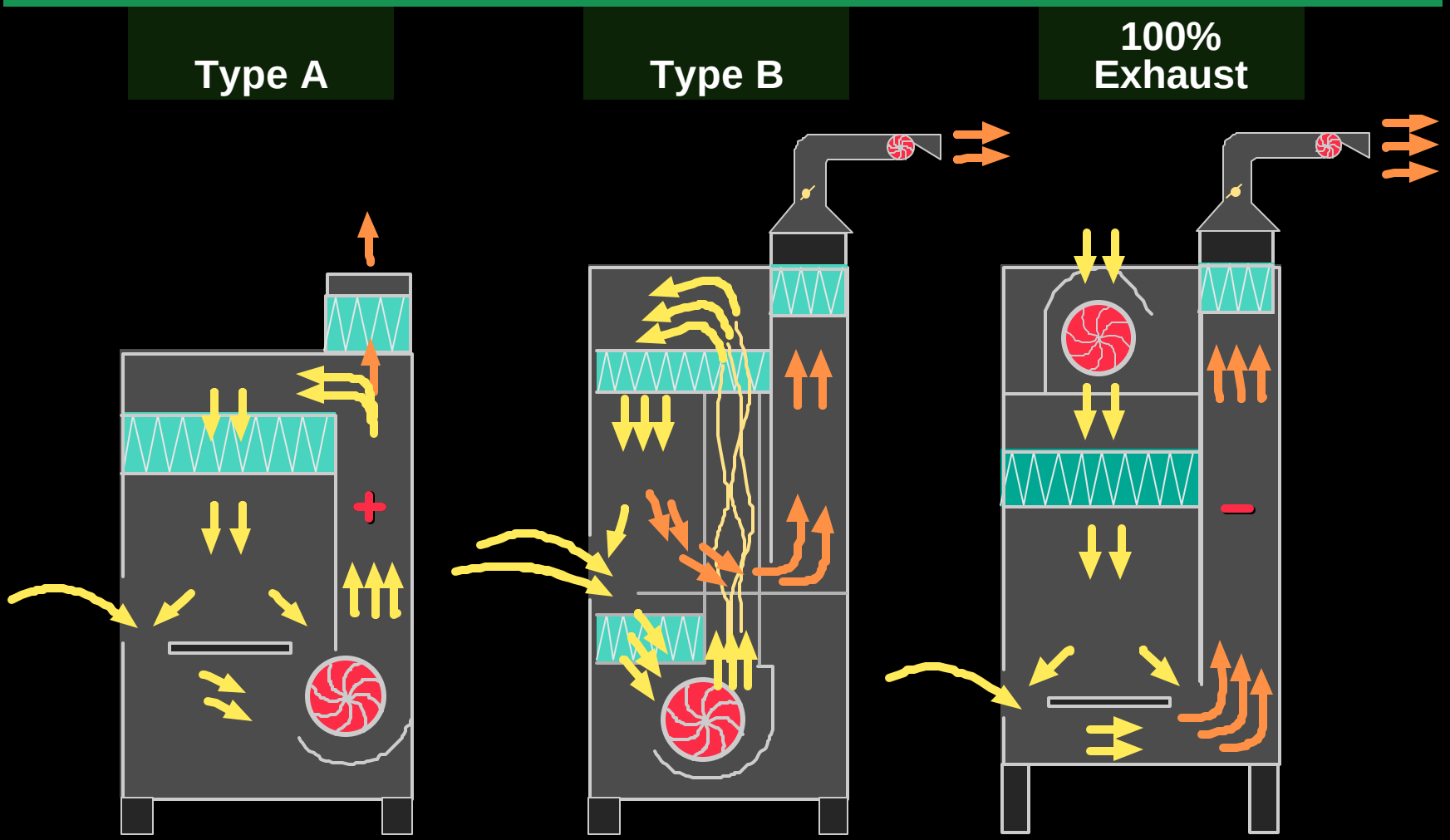
Class II Type B3 Airflow Schematic



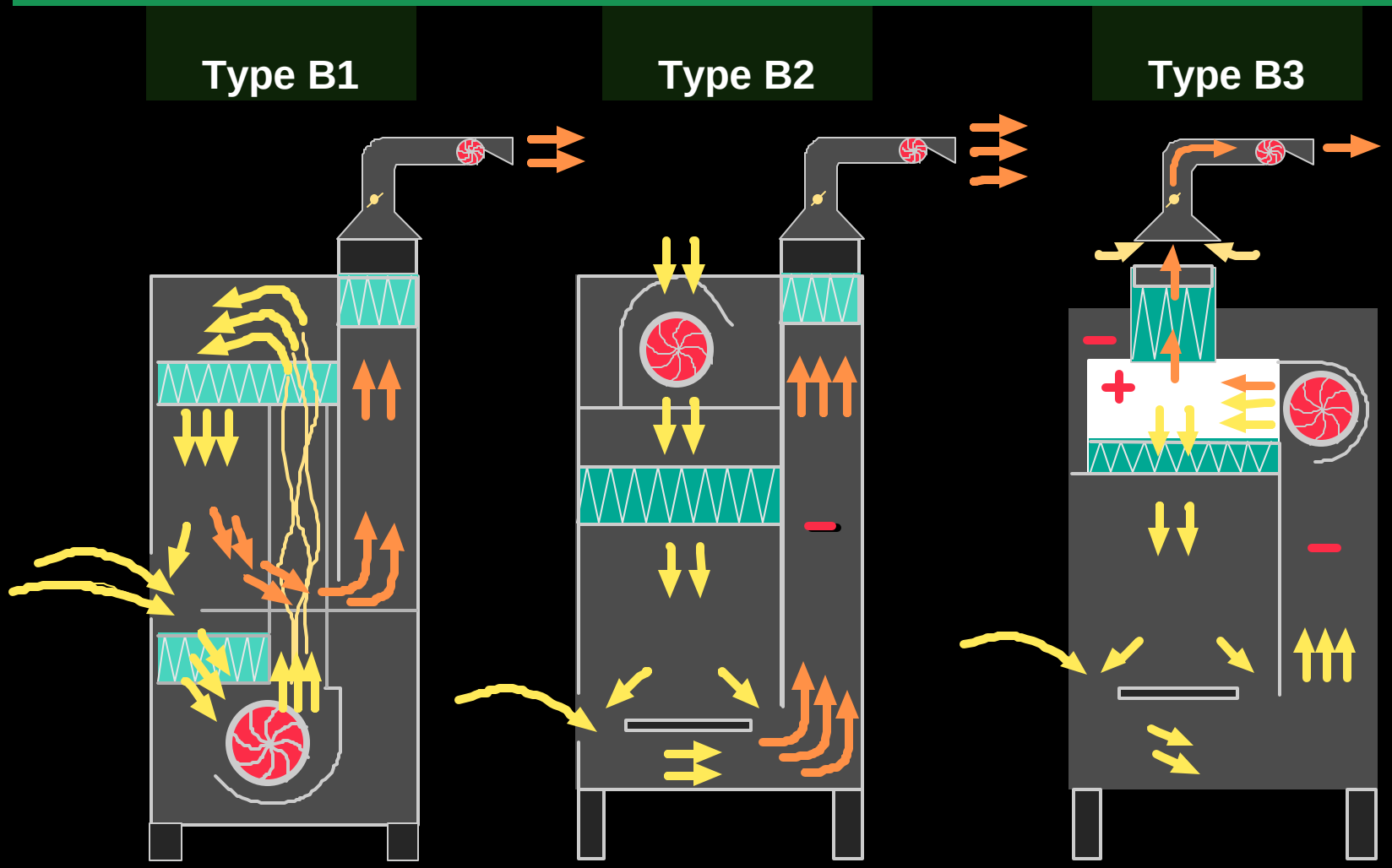
Class II Type B3 Airflow Schematic



Class II BSCs



Class II BSCs



Class II BSCs