

AMBIENT FORMALDEHYDE LEVELS BEFORE, DURING, AND AFTER BIOLOGICAL SAFETY CABINET DECONTAMINATION

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ABSTRACT

The Biological Safety Cabinet (BSC) is the most commonly used piece of engineering control in laboratories working with infectious agents or recombinant DNA, offering personnel, product, and environmental protection. BSCs are routinely decontaminated with formaldehyde, an effective biocide (which is then neutralized with ammonium bicarbonate). During these procedures, laboratory personnel vacate the room, and the decontamination technicians wear respiratory protection. Nevertheless, because formaldehyde is an irritant, a sensitizer, and a suspect human carcinogen, it is important to determine the potential laboratory levels. Using an electrochemical direct-reading instrument, this study examined ambient formaldehyde levels in five research laboratories undergoing decontamination. In four of the processes, formaldehyde levels were below the limit of detection (0.4 ppm) the entire time. However, the fifth one had two concentration peaks, one at 1.5 ppm, when formaldehyde was released into the cabinet, the other at 37.8 ppm, when the cabinet was opened up after the procedure. During the first peak, the ambient levels remained above the OSHA PEL of 0.75 ppm for 10 minutes. During the second spike, the levels stayed above the STEL of 2.0 ppm for 90 minutes and did not fall below the PEL for another 170 minutes. The first peak probably resulted from the cabinet not being tightly sealed. The second peak may have been the result of incomplete neutralization and the size of the cabinet relative to the room. These results indicate that while the usual controls are effective, some additional controls, such as measuring out the amount of ammonium bicarbonate more carefully (the current procedure involves making "eyeball" estimates), may still be needed to counteract and/or prevent such occasions. Further investigation is needed to determine the frequency

of elevated formaldehyde levels during BSC decontamination procedures and the role that the ratio of cabinet-to-room size plays in determining these levels.

INTRODUCTION

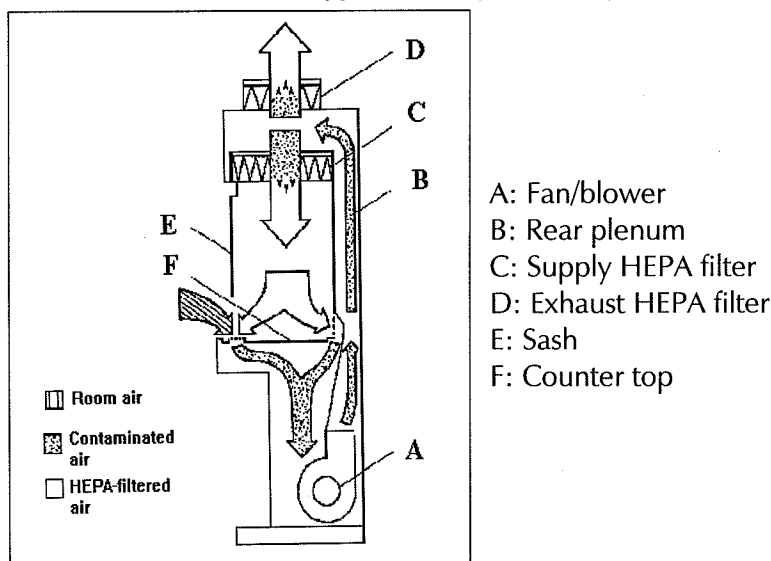
Biological Safety Cabinets

In biomedical research, biological safety cabinets (BSCs) are probably the most frequently used engineering control. The most common type of these cabinets, the Class II cabinet, is designed to have a face velocity of 75 to 100 fpm for personnel protection, High Efficiency Particulate Air (HEPA)-filtered downward laminar airflow for product protection, and HEPA-filtered exhaust air for environmental protection. Class II BSCs are further divided into two categories (Types A and B) based on construction, air flow velocities and patterns, and exhaust systems. Type A cabinets (See Figure 1), more commonly used than Type B cabinets, are suitable for microbiological research not involving flammable, volatile, or toxic chemicals since air is recirculated within the laboratory (after HEPA filtration) and because the fan could provide an ignition source. Type B cabinets, on the other hand, are hard-ducted to the exhaust system, and contain negative pressure plena. These features, plus an increased face velocity of 100 fpm, allow work to be done with toxic chemicals or radionuclides [11].

Formaldehyde Toxicology

Federal guidelines specify that primary containment equipment may not be opened until after sterilization by a validated procedure. Institutional biosafety committees also establish policies for the circumstances requiring sterilization (prior to moving or disposal, for example). Biosafety cabinets are routinely disinfected by fumigation with formaldehyde [8]. Formaldehyde is known to be effective in

FIGURE 1
Side View of a Class II, Type A Biological Safety Cabinet (11)



killing off microbes, but it is also a suspect carcinogen [1-4, 6, 9, 10, 12, 13] and an irritant as well. There have been several epidemiological studies on occupational formaldehyde exposure in industrial settings [2, 3, 9] and some on formaldehyde disinfection [5, 14], but, to the author's knowledge, none on the occupational exposure of those working in the laboratory during or after a formaldehyde decontamination of the BSC. Although researchers vacate the lab during the decontamination procedure, it is not known what the levels are upon their return and what the minimal time should be before re-entry. This study examined the levels of formaldehyde before, during, and after the decontamination by an outside vendor of several BSCs in biomedical research laboratories.

OSHA has set the Permissible Exposure Limit (PEL) for formaldehyde at 0.75 ppm and the STEL (Short-Term Exposure Limit) at 2.0 ppm. Because of its potential carcinogenicity, NIOSH has a REL (Recommended Exposure Limit) of 0.016 ppm for 10-hour exposures and a ceiling of 0.1 ppm for 15-minute exposures.

Decontamination Procedure

A total of five decontamination procedures of Class II Type A hoods were evaluated. The method used to decontaminate the hoods followed NSF Standard 49 [7]. First, the fan was shut off. Then two

frying pan, one with a pre-measured amount of paraformaldehyde (0.3 g/cu ft of hood volume), and the other with an equal volume of ammonium bicarbonate, were placed in the cabinet. Then all the openings of the hood were sealed up with thick plastic sheeting and duct tape. Next, the pan with the paraformaldehyde in it was turned on. The chemical depolymerized to formaldehyde, and the cabinet circulation was turned on briefly a few times so that the gas could permeate throughout the unit. After two hours, the ammonium bicarbonate pan was plugged in. This thermally decomposed to ammonia, which neutralized the formaldehyde by forming hexamethylene tetramine (six moles of formaldehyde reacting with four moles of ammonia, with one mole of water as a by-product). The ammonia was also allowed to flow throughout the unit by turning the circulation on briefly a few times. After a contact time of roughly an hour, the plastic sheeting was removed, and any remaining unneutralized formaldehyde gas was left for the laboratory's general room ventilation to remove; the sash was raised and air was allowed to diffuse out into the room with the BSC fan off. No researchers or other lab personnel were present in the laboratory during this procedure, and the room remained vacated for several hours. (The exact amount of time depended on what time of the day the decontamination was done and how soon the researchers

needed to re-enter their space.) The technicians wore full-face respirators with formaldehyde cartridges during the entire process.

One potential point of exposure was when the paraformaldehyde pan was first plugged in; if there were a leak in the plastic sheeting, formaldehyde could escape into the room. And if for some reason, not all the formaldehyde were neutralized by the ammonium bicarbonate before the plastic sheeting was removed, the formaldehyde level in the room could rise as well at this point, since the formaldehyde cannot be trapped by the HEPA filters.

METHODS

Using an Interscan 4160SP, an electrochemical system, with a formaldehyde sensor and an MIE PDL-10 Personal Data Logger, the air directly outside the biosafety cabinet (roughly one foot away) was measured and recorded for formaldehyde concentration. The data logger was set to take one-minute averages. The Interscan sensor had a range up to 20 ppm, with a limit of detection of 0.4 ppm and a sensitivity of ± 0.4 ppm.

The measurements were started a few minutes before the formaldehyde was released and ran until a few hours after the sheets had been removed. Based on the formaldehyde decay curve after the sheets had been removed, the time it would take for the level in the room to reach non-detectable

levels could be estimated. The decay equation is as follows:

$$\ln C = -Et + \ln C_0 \quad (1)$$

where C is the concentration at time t, E is the air exchange rate, and C_0 is the concentration after which the levels began to fall. Of course, if the levels are already non-detectable, estimation would be unnecessary.

Five processes on Class II Type A cabinets were monitored. Some decontaminations were done in large rooms (floor area roughly 20 ft by 40 ft) and others were in small rooms (floor area approximately 10 ft by 10 ft). Two sizes of cabinets were decontaminated: large cabinets (6 ft long) and small cabinets (4 ft long). Of the five processes, one was a large room with a large cabinet, two were small rooms with small cabinets, one was a large room two small cabinets, and one was a small room with a large cabinet.

RESULTS

The results are summarized in Table 1. The formaldehyde levels for four of the five decontamination procedures were non-detectable the entire time. One run, the one with the large BSC in the small room, however, had a small peak (1.5 ppm) at the time the formaldehyde was generated and a much larger one (37.8 ppm) when the sheets were removed three hours later. The first peak remained

TABLE 1
Summary of Results

Floor Dimensions (ft)*	Cabinet Length (ft)*	Measured Levels (ppm)
10 x 10	4	ND [§]
10 x 10	4	ND
10 x 10	6	1.5 when formaldehyde was released 37.8 when cabinet was opened up
20 x 40	6	ND
20 x 40	4 [†]	ND

* The ceiling heights of all the rooms were approximately the same, as were the cabinet heights and depths, so these dimensions have been omitted.

[§] The limit of detection was 0.4 ppm.

[†] Two cabinets, each 4 ft long, were monitored in this procedure.

above the OSHA PEL for 10 minutes, while the second spike stayed above the OSHA STEL for 90 minutes and did not fall below the PEL for another 170 minutes. The concentration of 37.8 ppm is not entirely reliable, because the instrument is only calibrated to go up to 20 ppm, but one can be sure that a high concentration did exist. The concentrations are plotted over time in Figure 2.

The measurements were terminated while the levels were roughly 0.60 ppm. Regressing Equation 1, it was estimated that the level would have fallen to the detection limit of 0.4 ppm 610 minutes after the run had begun. This corresponds to roughly 380 minutes after the plastic sheeting had been removed.

DISCUSSION

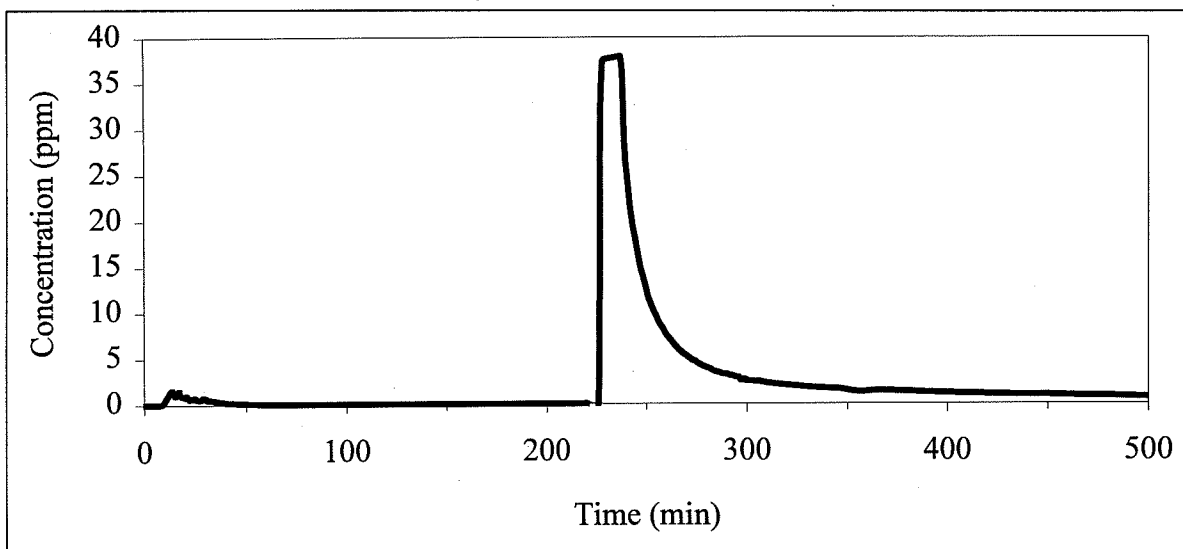
Although four of the runs had no detectable levels of formaldehyde, the fact that one had high levels that persisted for quite some time is a slight cause for concern. The current administrative control of having the researchers vacate the room is good, provided that the levels are low enough by the time they re-enter.

The reason that the one decontamination process led to such high levels in the room is probably due to the fact that not all the formaldehyde was neutralized. This could happen if the ammonia was not allowed to mix thoroughly inside the cabinet during the neutralization time, which could happen if the cabinet blowers were not turned on long and often enough.

Another explanation for the high formaldehyde levels is there simply was not enough ammonia in the first place. The vendors who conducted this procedure pre-weighed the paraformaldehyde before they left their company, but they only used an eyeball estimate for the amount of ammonium bicarbonate needed; they poured this salt straight out of a large bottle, so it is very possible that they did not pour enough.

However, it is important not to use too much ammonium bicarbonate either, because ammonia is an irritant, too. Ammonia has an OSHA PEL of 50 ppm. Like formaldehyde, this gas is not trapped by the HEPA filter and is exhausted back into the laboratory. This is an example of how a method to control one hazard can introduce another. Future studies are needed to determine if ammonia expo-

FIGURE 2
Formaldehyde Concentration Over Time



While four of the runs had no detectable levels of formaldehyde during the entire procedure, one run had a small peak (1.5 ppm) when the formaldehyde was released and a much larger one (37.8 ppm) three hours later when the plastic sheets were removed. The first peak stayed above the OSHA PEL for 10 minutes, while the second spike remained above the OSHA STEL for 90 minutes and did not fall below the PEL for another 170 minutes.

sure is a problem in these decontamination procedures. It is recommended that the respirator cartridges worn by the decontamination technicians provide protection for both formaldehyde and ammonia.

The vendors determined the amount of ammonia bicarbonate not possible to always get this amount so precisely with eyeball estimates of the volume of the two solids. Therefore, it is recommended that the ammonia bicarbonate be pre-weighed like the formaldehyde; this should avoid the problem of having too much or too little of this neutralizing agent.

This study did not consider the ventilation rates and cabinet volume relative to room volume. The fact that the one process that had high levels in this study was a small room with a large cabinet suggests that these factors may have played a part in determining the levels. The different combinations of cabinet and room sizes are all very common in biomedical research. Perhaps future studies can consider these parameters when addressing formaldehyde concentration.

Because the instrument used in this study could only detect down to 0.4 ppm, it could not be determined whether the runs with undetectable levels were above the NIOSH ceiling value 0.1 ppm. If one considers that formaldehyde is carcinogenic via a genotoxic route [4], then perhaps there is no safe threshold, and simply being below the OSHA PEL is not good enough. Further investigation using more sensitive equipment is suggested.

It is not possible from this study to conclude how often such high levels of formaldehyde are encountered. Additional research using a larger sample size may better ascertain this.

CONCLUSION

Even though four of the five decontamination processes monitored had no detectable levels of formaldehyde, the fact that one had very high levels suggests that more controls may be needed. It is suggested that the vendor pre-weigh the ammonium bicarbonate to ensure that the correct amount gets used each time. Respirators with ammonia cartridges should be used as well. Future studies may be needed to determine if there is an ammonia exposure issue and what roles the room ventilation and the cabinet-to-room-volume ratio plays. Studies

with larger sample sizes should also be used to determine how often such excursions occur, and studies using more sensitive equipment should be conducted as well.

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