

does not have a chemical shower to decontaminate the suit, a decontamination station will be set up for this purpose. Suits maintained for emergency use will be inspected at least quarterly and respiratory equipment will be inspected monthly.

(g) *One-piece positive pressure suits.* A life-support system will be provided with alarms and emergency backup breathing tanks. The air provided will be HEPA-filtered meeting OSHA requirements found in the 29 CFR 1910.134, the requirements of Grade D breathing air as specified in the Compressed Gas Association pamphlet G-7.1 and ANSI Z86.1-1973. A HEPA-filter will be in-line between the disconnect on the suit and the breathing space in the suit. When these are used in other than an emergency situation, a chemical shower must be provided to decontaminate the surfaces of the suit as the worker leaves the containment area. Suits will be inspected before each use to check for indications of significant wear or leakage. The suits will be worn with impervious boots over the foot area of the suit and the outer gloves will be attached over the hand portion.

(h) *Respiratory protection equipment.* (1) Respirators and their use will be approved by the safety officer. The selection will be based on the conditions of the activities and the risks involved. In general, National Institute for Occupational Safety and Health (NIOSH) approved respirators that use aerosol filters for dusts and fumes having a Threshold Limit Value (TLV) of less than 0.05 mg/m³ have been found acceptable for use in microbiological laboratories. Alternatively, the Army M-17 or M-9 masks may be used. Air-supplied hoods are used in situations where greater respiratory protection is required without the need for body protection. One-piece suits are used when total body and respiratory protection are required.

(2) When respirators are used, a respirator protection program will be established that conforms to AR 11-34 and OSHA standards in the 29 CFR 1910.134. In general, a medical authority will designate who is to wear respirators, they will be fitted by individuals trained in their use and limitations, and wearers will be responsible

for the proper storage and regular inspection of their assigned respirators. Air-purifying respirators will not be worn in oxygen deficient environments.

(3) Reusable respirators that have been worn in a contaminated area will be decontaminated before reuse. At the end of each workday when a respirator has been worn in an area where it was required, the wearer will wipe it down with an appropriate liquid decontaminant. A damp cloth soaked in the decontaminant, with the excess liquid squeezed out, will be used for the wipe-down process, taking care to ensure that all crevices are reached. The respirator will be rinsed with clean, warm water. Visibly contaminated respirators will be decontaminated and discarded.

(4) Respirator programs will comply with AR 385-10 and AR 11-34.

(i) *Shoes.* All shoes specially issued for use in controlled access areas should be identified so that they can be segregated from other areas. Safety shoes or boots meeting OSHA requirements stated in the 29 CFR 1910.134 will be issued wherever heavy items or corrosive chemicals are handled. These will be sterilized appropriately after visible contamination. In certain situations (excluding BL-4 operations), it is desirable to wear disposable booties over street shoes, especially when product protection is required.

Subpart E—Decontamination and Disposal

§ 627.32 Introduction.

All material or equipment that is potentially contaminated with etiologic agents must be rendered nonhazardous before disposal. This chapter describes the acceptable physical and chemical decontamination methods and the general applicability of each. In general, all infectious materials and all contaminated equipment or apparatus will be sterilized before being washed and stored or discarded.

§ 627.33 Methods of decontamination.

(a) *Autoclave.* The use of wet heat is the most dependable procedure for destroying all forms of microbial life. An autoclave employs saturated steam under a pressure of approximately 15

pounds per square inch (psi) to achieve a chamber temperature of at least 121 ° C for a minimum of 15 minutes. The time is measured after the temperature of the material being sterilized reaches 121 ° C. Other combinations of temperature and pressure (some of which are dependent on the equipment used) can be used to accomplish sterilization provided that the efficacy of sterilization is validated as described below. The most critical factor in ensuring the reliability of this sterilization method, other than proper temperature, is preventing entrapped air that is not replaced by steam. Material to be autoclaved must come in contact with steam and heat and, as a result, it may be necessary to add water to a load of waste to aid in the formation and penetration of steam. Autoclaves use either a steam-activated exhaust valve that remains open during the replacement of air by live steam until the steam triggers the valve to close, or a pre-cycle vacuum to remove air prior to steam introduction.

(b) Sterilization will be verified using biological indicators (for example, *Bacillus stearothermophilus* spores) at locations throughout the autoclave, to include placement in the center of test loads, when the autoclave is first put into service, and after any maintenance or repairs. The primary means of verifying routine sterilization will be through using chemical indicators (for example, autoclave tape or labels) at locations throughout the autoclave. In addition each autoclave will be equipped with a permanent means to record time and the temperature of each operational event as a means of ensuring sterilization. The type of materials being handled must be reviewed and standard conditions for sterilization of each established. As a guide, the manufacturer's manual for the autoclaves will be consulted as a starting point in establishing these conditions. Treatment conditions to achieve sterility will vary in relation to the volume of material treated, the contamination level, the moisture content, and other factors that should be considered and which may cause the times to lengthen. In each case, the conditions will be established based on tests which verify that the conditions

selected are effective. In addition to being effective from viable agents, autoclaving effectively inactivates most protein toxins.

(c) Dry heat. Dry heat requires longer times or higher temperatures or both than does wet heat. If used, the specific sterilization times and temperatures must be determined for each type of material being sterilized. In general, sterilization by dry heat can be accomplished at 169-170 ° C for periods of 2 to 4 hours. Higher temperatures reduce the time requirements. The heat transfer properties and spatial relation or arrangement of materials in the load are critical in ensuring effective sterilization.

(d) Liquid disinfectants. Liquid disinfectants may be used in surface treatment, in dip tanks, and, at sufficient concentration, as sterilants of liquid waste for final disposal. If liquid disinfectants are used, they must have been shown to be effective against the organisms present. Important considerations include: temperature, time of contact, the negative logarithm of hydrogen ion concentration (pH), concentration and state of dispersion, penetrability, and reactivity of organic material at the site of application. Small variations in these factors may make large differences in the effectiveness of disinfection, so complete reliance should not be placed on liquid disinfectants when the end result must be sterility. If evidence of efficacy under the proposed procedures has not been reported previously, preliminary studies to verify the efficacy of liquid disinfectants must be conducted. Such studies may include attempts to recover and quantitate the agent in question from liquid or swab samples, or sealed patches, by animal inoculation, plaque assay, agar or broth cultivation, and similar methods, following controlled decontamination under the same experimental conditions envisioned for the proposed studies.

(1) *Alcohol*. Ethyl or isopropyl alcohol at the concentration of 70-85 percent by weight will denature proteins but is slow in its germicidal action. Alcohols are effective disinfectants for lipid-containing viruses. These alcohols exhibit no activity against bacterial spores.

(2) *Phenolic compounds.* These are effective disinfectants against vegetative bacteria, including *Mycobacterium tuberculosis*, fungi, and lipid-containing viruses. The phenolics are not effective against bacterial spores or non-lipid-containing viruses. The concentrations used will be in accordance with the manufacturer's recommendations.

(3) *Formaldehyde solutions.* Formaldehyde in solution at a concentration of 8 percent (formalin) is effective against vegetative bacteria, spores, and viruses. It loses considerable disinfectant activity below room temperature. Due to the toxic properties of formaldehyde, the use of formalin is restricted to surfaces or materials that are contained within appropriate engineering controls.

(4) *Quaternary ammonium compounds.* These cationic detergents are strongly surface-active. They lose effectiveness in the presence of proteins and are neutralized by anionic detergents, such as soap. At low concentrations, they are bacteriostatic, tuberculostatic, sporostatic, fungistatic, and algistatic. At medium concentration, they are bactericidal, fungicidal, algicidal, and virucidal against lipophilic viruses. They are not tuberculocidal, sporicidal, or virucidal against hydrophilic viruses, even at high concentrations. The manufacturer's recommended dilution will be used.

(5) *Chlorine.* Sodium hypochlorite is normally used as a base for chlorine disinfectants. Free available chlorine is the active ingredient and, at concentrations of at least 2,500 parts per million (ppm) (0.25 percent), is a disinfectant that is active against most microorganisms and bacterial spores. Chlorine solutions at 2.5 percent free available chlorine are effective against most toxins. Chlorine solutions lose strength if exposed to air, so fresh solutions must be prepared whenever the free chlorine content falls below desired minimums.

(6) *Iodine.* The characteristics of chlorine and iodine are similar. Iodophor compounds with 1,600 ppm free available iodine provide a relatively rapid inactivation of all microorganisms, including some bacterial spores. A commonly available iodophor is Wescodyne. The manufacturer of

Wescodyne recommends a range of dilution from 1 to 3 ounces per 5 gallons of water, giving a solution containing from 25 to 75 ppm of free iodine. At these concentrations, available iodine may be rapidly taken up by any extraneous protein present and will not be an effective sporocide. A solution providing 1,600 ppm iodine is recommended for hand washing or for use as a sporocide.

(7) *Mercurials.* Although the mercurials exhibit good activity against viruses, they are toxic and are not recommended for general use. They have poor activity against vegetative bacteria and are totally ineffective sporicides. The dilution recommendations stated by the manufacturer will be followed.

(e) Vapors and gases. Formaldehyde, ethylene oxide, peracetic acid, beta-propiolactone, methyl bromide, and glutaraldehyde have all been used successfully as space sterilants where they can be employed in closed systems and with controlled conditions of temperature and humidity. Of these, methyl bromide, beta-propiolactone, and glutaraldehyde are not recommended because of their toxic properties. Peracetic acid can readily decompose with explosive violence in a concentrated state and must be used only in a diluted state and with extreme care. Formaldehyde and ethylene oxide are both regulated by OSHA for their potential human carcinogenicity, but do have permissible exposure levels (unlike beta-propiolactone, for example) and can be used safely under controlled conditions.

(1) *Formaldehyde.* Formaldehyde gas is, in general, the chemical of choice for space disinfection. Biological safety cabinets and associated effluent air-handling systems and air filters, incubators, laboratory rooms, buildings, or other enclosed spaces can be disinfected with formaldehyde. The procedures found in appendix E of the National Sanitation Foundation Standard Number 49 will be followed for the disinfection of biological safety cabinets. Other enclosures or areas will be disinfected by following the same principles. To disinfect rooms, the generation of formaldehyde gas from heating powdered or flake paraformaldehyde is

the preferred method. When area decontamination is performed, use 0.3 grams of paraformaldehyde for each cubic foot of space to be treated. The room or area must be above 70° F, the relative humidity above 70 percent, and the exposure time at least 2 hours (overnight is preferred). After the required time for disinfection, the room must be cleared of the formaldehyde gas (a small room with nonporous surfaces and no materials or equipment in the room can be cleared of all detectable formaldehyde by aeration for one hour, while larger areas with equipment in them may take a full day). Before formaldehyde is used as a space disinfectant, the area to be treated must be surveyed to ensure that there are no open containers of any acidic solution containing chloride ion in order to prevent the possible formation of bis(chloromethyl)ether, a human carcinogen. Specific OSHA requirements for posting of rooms and equipment, personnel protection, and other requirements are found in 29 CFR 1910.1048.

(2) *Ethylene oxide (EtO)*. EtO sterilization will only be conducted in a sterilizer designed for that purpose and designed to maintain potential exposure levels below the current OSHA standard. EtO is effective against all microorganisms, including spores, molds, pathogenic fungi, and highly resistant thermophilic bacteria. All materials to be used in contact with human skin (for example, clothing, shoes, masks, adhesive tape) must be aerated for at least 24 hours after sterilization and prior to use. Concentrations of 500 to 1000 ppm are required for sterilization. Specific OSHA requirements for the use of ethylene oxide are found in 29 CFR 1910.1047.

(f) *UV Radiation*. UV radiation at a wave length of 253.7 nanometers is a practical method for inactivating airborne viruses, mycoplasma, bacteria, and fungi. The usefulness of UV radiation on exposed surfaces is limited by its low penetrating power. UV radiation shall only be relied upon to sterilize surfaces when conventional methods, such as autoclaving or the use of liquid disinfectants, would make the product unusable. An example is data sheets that must be brought out of a

biocontainment facility. The UV intensity must be at least 40 microwatts/cm³ on the surface to be treated. Single sheets of paper may be treated by exposing them to this radiation for a minimum of 15 minutes. A calibrated photoelectric UV intensity meter, capable of measuring UV radiation at a wave length of 253.7 nanometers, will be used whenever a new UV source is installed, and quarterly thereafter, to ensure the UV source is providing at least 40 microwatts/cm³ at the work surface. Bulbs should be cleaned routinely to remove any accumulated dust and prolong bulb performance and assure proper energy output. Protective eye wear and clothing may be necessary when working around UV radiation.

§ 627.34 Disposal.

Inactivation is the first step in the disposal of etiologic agents or materials that are potentially contaminated with them. All contaminated or potentially contaminated materials must be effectively disinfected or sterilized by an approved procedure discussed in § 627.33. After decontamination, reusable items, such as clothing or glassware, may be washed with other uncontaminated or decontaminated items.

(a) *Combustible items*. Combustible disposable items should be bagged and incinerated in an appropriate approved incinerator or otherwise disposed of in accordance with State and local regulations.

(b) *Noncombustible disposable items*. Items will be packaged as stated in § 626.34(e) and disposed of by a licensed waste hauler.

(c) *Equipment*. Equipment that cannot be autoclaved will be decontaminated by gaseous sterilization or with a suitable liquid disinfectant. Such equipment will be certified as decontaminated by the safety officer.

(d) *Waste*. Materials generated, such as solvents, acids, chemical carcinogens, radioactive isotopes, medical waste, or dead animals must be decontaminated, packaged, and then disposed of in accordance with EPA, NRC, local, State, and Federal regulations.