# Chemical Disinfectants

In the laboratory setting, chemical disinfection is the most common method employed to decontaminate surfaces and disinfect waste liquids. In most laboratories, dilutions of household bleach is the preferred method but there are many alternatives that may be considered and could be more appropriate for some agents or situations. There are numerous commercially available products that have been approved by the Environmental Protection Agency (EPA). EPA Registered Sterilizers, Tuberculocides, and Antimicrobial Products Against Certain Human Public Health Bacteria and Viruses can be found at http://www.epa.gov/oppad001/chemregindex.htm. Most EPA-registered disinfectants have a 10-minute label claim. **However, OEHS Biosafety recommends a 15-20 minute contact time for disinfection/decontamination.**

**General Considerations**

Prior to using a chemical disinfectant always consult the manufacturer's instructions to determine the efficacy of the disinfectant against the biohazards in your lab and be sure to allow for sufficient contact time. In addition, consult the Safety Data Sheet for information regarding hazards, the appropriate protective equipment for handling the disinfectant and disposal of disinfected treated materials. Federal law requires all applicable label instructions on EPA-registered products to be followed (e.g., use-dilution, shelf life, storage, material compatibility, safe use, and disposal). Do not attempt to use a chemical disinfectant for a purpose it was not designed for.

When choosing a disinfectant consider the following:

- The microorganisms present
- The item to be disinfected or surface(s)
- Corrosivity or hazards associated with the chemicals in the disinfectant
- Ease of use

The OSHA Bloodborne Pathogen standard CFR 1910.1030 requires an EPA-registered disinfectant effective against HIV-1 and Hepatitis B virus. Therefore, diluted ethanol may not be used to disinfect materials and surface contaminated by human or non-human primate blood or other potentially infectious material (OPIM), as defined in the standard. However, alcohol-containing disinfectants, such as Cavicide are registered by the EPA as virucidal and tuberculocidal.

1. **Organism Sensitivity and Resistant Organisms**

   The innate characteristics of microorganisms often determine its sensitivity to chemical disinfection (**Table 1**). Some agents such as *Cryptosporidium*, *Clostridium difficile*, *Bacillus* spores and prions are very resistant to the usual disinfectants. OEHS Biosafety is available to assist you in determining the appropriate disinfectant and provides guidance on use of appropriate disinfection techniques and materials for researchers.

**Table 1. Sensitivity of Microorganisms to Chemical Disinfectants**

| | Type of Microbe | Examples |
|---|---|---|
| **More Resistant** | Bacterial or Fungal Spores | *Bacillus subtilis, Clostridium difficile/perfringens, Cryptococcus* |
| | Mycobacteria | *Mycobacterium tuberculosis, Mycobacterium bovis* |
| | Hydrophilic Viruses (non-enveloped) | Coxsackievirus, Rhinovirus, Adenovirus, Poliovirus |
| | Fungi | *Aspergillus., Candida sp.* |
| | Vegetative Bacteria | *Streptococcus pneumoniae, Staphylococcus aureus, E. coli, Pseudomonas spp., Klebsiella spp.* |
| **Less Resistant** | Lipophilic Viruses (lipid containing, enveloped) | Herpes Simplex virus, Cytomegalovirus, HIV (Lentiviruses) |

**2. Chemical Disinfectant Groups (Table 2)**

**a. Halogen-Based Biocides: (Chlorine Compounds and Iodophores)**

**i. Chlorine Compounds (e.g., Household Bleach)**

Chlorine compounds are good disinfectants on clean surfaces, but are quickly inactivated by organic matter, thus, reducing their biocidal activity. They have a broad spectrum of antimicrobial activity and are inexpensive and fast acting. Hypochlorites, the most widely used of the chlorine disinfectants, are available in liquid (e.g., Sodium hypochlorite), household bleach and solid (e.g., calcium hypochlorite, sodium dichloroisocyanurate) forms. Household bleach has an available chlorine content of 5.25%, or 52,500 ppm. For most purposes, **a 1:10 dilution of bleach** (approximately 0.5% or 5,000 ppm sodium hypochlorite) is recommended in the laboratory. Because of its oxidizing power, diluted bleach loses potency quickly and **should be made fresh** and used within the same day it is prepared. **Bleach should be diluted with cold water in order to prevent breakdown of the disinfectant.** The free available chlorine levels of hypochlorite solutions in both opened and closed polyethylene containers are reduced to 40% to 50% of the original concentration over a period of one month at room temperature.

Bleach should be stored between 50 and 70°F. Undiluted household bleach has a shelf life of six months to one year from the date of manufacture, after which it degrades at a rate of 20% each year until totally degraded to salt and water, and a **1:10 bleach solution has a shelf life of 24 hours**.

There are two potential occupational exposure hazards when using hypochlorite solutions. The first is the production of the carcinogen bis-chloromethyl ether when hypochlorite solutions come in contact with formaldehyde. The second is the rapid production of chlorine gas when hypochlorite solutions are mixed with an acid. Care must also be exercised in using chlorine–based disinfectants, which can corrode or damage metal, rubber, and other susceptible surfaces. Bleached articles should never be autoclaved without reducing the bleach with sodium thiosulfate or sodium bisulfate.

Chloramine T, which is prepared from sodium hypochlorite and p-toluenesulfonamide, is a more stable, odorless, less corrosive form of chlorine but has decreased biocidal activity in comparison to bleach.

**ii. Iodophors (e.g. Wescodyne)**

Iodophors are used both as antiseptics and disinfectants, typically at a concentration of 25-1600 ppm of titratable iodine: for Wescodyne the recommended final concentration is 75 to 150ppm. Wescodyne, Betadyne, Povidone-Iodine and other iodophors are commercially available Iodine-based disinfectants, which give good control when the manufacturer's instructions for formulation and application are followed. **Iodophors should be diluted in cold water in order to prevent breakdown of the disinfectant.**

An iodophor is a combination of iodine and a solubilizing agent or carrier; the resulting complex provides a sustained-release reservoir of iodine and releases small amounts of free iodine in aqueous solution. Antiseptic iodophors are not suitable for use as hard-surface disinfectants because they contain significantly less free iodine than do those formulated as disinfectants.

**b. Alcohols (ethanol and isopropanol)**

Alcohols work through the disruption of cellular membranes, solubilization of lipids, and denaturation of proteins by acting directly on S-H functional groups. Ethyl and isopropyl alcohols are the two most widely used alcohols for their biocidal activity. These alcohols are effective against lipid-containing viruses and a broad spectrum of bacterial species, but ineffective against spore-forming bacteria and many non-enveloped viruses. They evaporate rapidly, which makes extended contact times difficult to achieve unless the items are immersed.

The optimum bactericidal concentration for ethanol and isopropanol is in the range of 70% to 85% by volume. Their cidal activity drops sharply when diluted below 50% concentration. Absolute alcohol is also not very effective. They are used to clean sensitive equipment and are generally regarded as being non-corrosive.

Due to the evaporative nature of the solution, aqueous alcohol is not recommended as the primary disinfectant of spills, especially in areas with significant airflow, such as a Biosafety cabinet. For surface decontamination, a spray, wipe, spray approach is recommended to achieve the desired contact time.

EPA-registered alcohol containing disinfectants, such as Cavicide, are appropriate for surface decontamination.

**c. Aldehydes: (Formaldehyde, Paraformaldehyde, Glutaraldehyde)**

**i. Glutaraldehyde:**

Glutaraldehyde is a colorless liquid and has the sharp, pungent odor typical of all aldehydes, with an odor threshold of 0.04 parts per million (ppm). It is capable of sterilizing equipment, though to effect sterilization often requires many hours of exposure. Two percent solutions of glutaraldehyde exhibit very good activity against vegetative bacteria, spores and viruses. It is ten times more effective than formaldehyde and less toxic. However, it must be limited and controlled because of its toxic properties and hazards. It is important to avoid skin contact with glutaraldehyde as it has been documented to cause skin sensitization. Glutaraldehyde is also an inhalation hazard. The NIOSH ceiling threshold limit value is 0.2 ppm.

Cidex, a commercially prepared glutaraldehyde disinfectant is used routinely for cold surface sterilization of clinical instruments. Glutaraldehyde disinfectants should always be used in accordance with the manufacturer's directions.

**ii. Formaldehyde:**

Fomaldehyde and its polymerized solid paraformaldehyde have broad-spectrum biocidal activity and are both effective for surface and space decontamination. As a liquid (5% concentration), formaldehyde is an effective liquid decontaminant. Its biocidal action is through alkylation of carboxyl, hydroxyl and sulfhydryl groups on proteins and the ring nitrogen atoms of purine bases. Formaldehyde's drawbacks are reduction in efficacy at refrigeration temperature, its pungent, irritating odor, and several safety concerns. Formaldehyde is presently considered to be a carcinogen or a cancer-suspect agent according to several regulatory agencies. The OSHA 8-hour time-weighted exposure limit is 0.75 ppm.

**d. Quaternary Ammonium Compounds: (Zephirin, CDQ, A-3)**

Quaternary ammonium compounds are generally odorless, colorless, nonirritating, and deodorizing. They also have some detergent action, and they are good disinfectants. However, some quaternary ammonium compounds activity is reduced in the presence of some soaps or soap residues, detergents, acids and heavy organic matter loads. They are generally ineffective against viruses, spores and *Mycobacterium tuberculosis*. Basically these compounds are not suitable for any type of terminal disinfection. They are typically diluted to 0.1 to 2%.

The mode of action of these compounds is through inactivation of energy producing enzymes, denaturation of essential cell proteins, and disruption of the cell membrane. Many of these compounds are better used in water baths, incubators, and other applications where halide or phenolic residues are not desired.

e. **Phenolics: (O-phenophenoate-base Compounds)**

Phenolics are phenol (carbolic acid) derivatives and typically used at 1- 5% dilutions. These biocides act through membrane damage and are effective against enveloped viruses, rickettsiae, fungi and vegetative bacteria. They also retain more activity in the presence of organic material than other disinfectants. Cresols, hexachlorophene, alkyl- and chloro derivatives and diphenyls are more active than phenol itself. Available commercial products include Lysol, Pine-Sol, Amphyl, O-Syl, Tergisyl, Vesphene, and LpH se.

**Table 2. Summary and Comparison of Liquid Disinfectants**

| Class | Recommended Use | How They Work | Advantages | Disadvantages | Comments & Hazards | Examples |
|---|---|---|---|---|---|---|
| **Chlorine Compounds** | Spills of human body fluids<br><br>Good against:<br>Vegetative Bacteria<br>Fungi<br>Enveloped Viruses<br>Non-enveloped Viruses<br><br>Good at >1000ppm Sodium Hypochlorite:<br>Spores<br><br>Good with extended contact time:<br>Mycobacteria | Free available chlorine combines with contents within microorganism, reaction byproducts cause its death<br><br>Need 500 to 5000 ppm<br><br>Produce chemical combination with cell substances<br><br>Depends upon release of hypochlorous acid | Kills hardy viruses (e.g. hepatitis)<br><br>Kills a wide range of organisms<br><br>Inexpensive<br><br>Penetrates well<br><br>Relatively quick microbial kill<br><br>May be used on food prep surfaces | Corrodes metals, such as stainless, aluminum<br><br>Organics may reduce activity<br><br>Increase in alkalinity decreases bactericidal property<br><br>Unpleasant taste and odor<br><br>Tuberculocidal, with extended contact time | Follow spill procedure and dilution instructions<br><br>Make fresh solutions before use<br><br>Eye, skin and respiratory irritant<br><br>Corrosive<br><br>Toxic | Bleach solutions (sodium hypochlorite)<br><br>Clorox<br><br>Cyosan<br><br>Purex |
| **Iodophors (Iodine with carrier)** | Disinfecting some semicritical medical equipment<br><br>Very Good:<br>Fungi<br>Viruses<br>Bacteria<br>Some Spores<br><br>Good with extended contact time: | Free iodine enters microorganism and binds with cellular components<br><br>Carrier helps penetrate soil/fat<br><br>Probably by disorder of protein synthesis due to hindrance and/or blocking of hydrogen bonding | Kills broad range of organisms<br><br>Highly reactive<br><br>Low tissue toxicity<br><br>Kills immediately rather than by prolonged period of stasis<br><br>Not affected by hard water | May stain plastics or corrode metal<br><br>May stain skin/laundry<br><br>Stains most materials<br><br>Odor<br><br>Some organic and inorganic substances neutralize effect | Dilution critical<br><br>Follow directions!<br><br>Use only EPA registered hard surface iodophor disinfectants<br><br>Don't confuse skin antiseptic iodophors for disinfectants | Wescodyne<br><br>Bactergent<br><br>Hy-Sine<br><br>Ioprep<br><br>Providone (iodine/betadine) |

| Class | Recommended Use | How They Work | Advantages | Disadvantages | Comments & Hazards | Examples |
|---|---|---|---|---|---|---|
| | Mycobacteria | | May be used on food prep surfaces | Tuberculocidal, with extended contact time<br><br>Sporicidal: Some | Skin and eye irritant<br><br>Corrosive<br><br>Toxic | |
| **Alcohols** | Cleaning some instruments<br><br>Cleaning skin<br><br>Good Against:<br>Vegetative Bacteria<br>Enveloped Viruses | Changes protein structure of microorganism<br><br>Presence of water assists with killing action | Fairly inexpensive | < 50% or >90% Solution not very effective<br><br>Not active when organic matter present<br><br>Not active against certain types of viruses<br><br>Evaporates quickly<br><br>Contact time may not be sufficient for killing | Flammable<br><br>Eye Irritant<br><br>Toxic | 70% Ethanol<br><br>Cavicide |
| **Glutaraldehyde** | Good Against:<br><br>Vegetative Bacteria<br>Fungi<br>Mycobacteria<br>Viruses<br>Spores | Coagulates cellular proteins | Non-staining, relatively noncorrosive<br><br>Useable as a sterilant on plastics, rubber, lenses, stainless steel and other items that can't be autoclaved | Not stable in solution<br><br>Has to be in alkaline solution<br><br>Inactivated by organic material | Eye, skin and respiratory irritant<br><br>Sensitizer<br><br>Toxic | Cidex<br><br>Calgocide 14<br><br>Vespore |
| **Quaternary Ammonium compounds (QUATS)** | Ordinary housekeeping (e.g. floors, furniture, walls)<br><br>Good Against:<br><br>Vegetative Bacteria<br>Enveloped Viruses<br>Fungi | Affects proteins and cell membrane of microorganism<br><br>Releases nitrogen and phosphorous from cells | Contains a detergent to help loosen soil<br><br>Rapid action<br><br>Colorless, odorless<br><br>Non-toxic, less corrosive<br><br>Highly stable<br><br>May be used on food prep surfaces | Does not eliminate spores, TB bacteria, some viruses<br><br>Effectiveness influenced by hard water<br><br>Layer of soap interferes with action | Select from EPA list of hospital disinfectants<br><br>Skin and eye irritant<br><br>Toxic | Coverage 258<br><br>End-Bac<br><br>Hi Tor<br><br>Bacdown |
| **Phenolic Compounds** | Good Against:<br><br>Vegetative Bacteria<br>Fungi | Gross protoplasmic poison<br><br>Disrupts cell walls | Nonspecific concerning bactericidal and fungicidal action | Unpleasant odor<br><br>Some areas have disposal restrictions | Skin and eye irritant<br><br>Sensitizer<br><br>Corrosive | Hil-Phene<br><br>LpH se<br><br>Metar |

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| --- | --- | --- | --- | --- | --- | --- |
| | Enveloped Viruses<br>Some non-enveloped Viruses<br>Mycobacteria | Precipitates cell proteins<br><br>Low concentrations inactivate essential enzyme systems | When boiling water would cause rusting, the presence of phenolic substances produces an anti-rusting effect | Effectiveness reduced by alkaline pH, natural soap or organic material<br><br>Not Sporicidal | Toxic | Vesphene<br><br>Decon-Cycle |