⟨1072⟩ DISINFECTANTS AND ANTISEPTICS

INTRODUCTION

A sound cleaning and sanitization program is needed for controlled environments used in the manufacture of Pharmacopeial articles to prevent the microbial contamination of these articles. Sterile drug products may be contaminated via their pharmaceutical ingredients, process water, packaging components, manufacturing environment, processing equipment, and manufacturing operators. Current Good Manufacturing Practices (cGMPs) emphasize the size, design, construction, and location of buildings and construction materials, and the appropriate material flow to facilitate cleaning, maintenance, and proper operations for the manufacture of drug products. When disinfectants are used in a manufacturing environment, care should be taken to prevent the drug product from becoming contaminated with chemical disinfectants as a result of the inherent toxicity of the disinfectants. The requirements for aseptic processing include readily cleanable floors, walls, and ceilings that have smooth and nonporous surfaces; particulate, temperature, and humidity controls; and cleaning and disinfecting procedures to produce and maintain aseptic conditions. The cleaning and sanitization program should achieve specified cleanliness standards, control microbial contamination of products, and be designed to prevent the chemical contamination of pharmaceutical ingredients, product-contact surfaces and/or equipment, packaging materials, and ultimately the drug products. These principles also apply to nonsterile dosage forms where the microbial contamination is controlled by the selection of appropriate pharmaceutical ingredients, utilities, manufacturing environments, sound equipment cleaning procedures, products especially formulated to control water activity, inclusion of suitable preservatives, and product packaging design.

In addition to disinfectants, antiseptics are used to decontaminate human skin and exposed tissue and may be used by personnel prior to entering the manufacturing area. Chemical sterilants may be used to decontaminate surfaces in manufacturing and sterility testing areas. Furthermore, sterilants may be used for the sterilization of Pharmacopeial articles, and UV irradiation may be used as a surface sanitizer.

This general information chapter will discuss the selection of suitable chemical disinfectants and antiseptics; the demonstration of their bactericidal, fungicidal, and sporicidal efficacy; the application of disinfectants in the sterile pharmaceutical manufacturing area; and regulation and safety considerations. Biofilm formation and its relationship to disinfectants are outside the scope of this chapter. Additional information not covered in the chapter may be obtained from standard texts on disinfectants and antiseptics.[1]

DEFINITIONS

Antiseptic—An agent that inhibits or destroys microorganisms on living tissue including skin, oral cavities, and open wounds.

Chemical Disinfectant—A chemical agent used on inanimate surfaces and objects to destroy infectious fungi, viruses, and bacteria, but not necessarily their spores. Sporicidal and antiviral agents may be considered a special class of disinfectants. Disinfectants are often categorized as high-level, intermediate-level, and low-level by medically oriented groups based upon their efficacy against various microorganisms.

Cleaning Agent—An agent for the removal from facility and equipment surfaces of product residues that may inactivate sanitizing agents or harbor microorganisms.

Decontamination—The removal of microorganisms by disinfection or sterilization.

Disinfectant—A chemical or physical agent that destroys or removes vegetative forms of harmful microorganisms when applied to a surface.

Sanitizing Agent—An agent for reducing, on inanimate surfaces, the number of all forms of microbial life including fungi, viruses, and bacteria.

Sporicidal Agent—An agent that destroys bacterial and fungal spores when used in sufficient concentration for a specified contact time. It is expected to kill all vegetative microorganisms.

Sterilant—An agent that destroys all forms of microbial life including fungi, viruses, and all forms of bacteria and their spores. Sterilants are liquid or vapor-phase agents.

Microorganisms differ greatly in their resistance to disinfection agents. The order of resistance of clinically significant microorganisms to chemical disinfectants from most to least resistant is listed in *Table 1*.

Table 1. The Resistance of Some Clinically Important Microorganisms to Chemical Disinfectants (Listed in Order of Decreasing Resistance)

Type of Microorganisms	Examples
Bacterial spores	*Bacillus subtilis* and *Clostridium sporogenes*
Mycobacteria	*Mycobacterium tuberculosis*
Nonlipid-coated viruses	Poliovirus and rhinovirus
Fungal spores and vegetative molds and yeast	*Trichophyton, Cryptococcus,* and *Candida* spp.
Vegetative bacteria	*Pseudomonas aeruginosa, Staphylococcus aureus,* and *Salmonella* spp.
Lipid-coated viruses	Herpes simplex virus, hepatitis B virus, and human immunodeficiency virus

CLASSIFICATION OF DISINFECTANTS

Chemical disinfectants are classified by their chemical type. This includes aldehydes, alcohols, halogens, peroxides, quaternary ammonium compounds, and phenolic compounds (see *Table 2*).

Table 2. General Classification of Antiseptics, Disinfectants, and Sporicidal Agents

Chemical Entity	Classification	Example
Aldehydes	Sporicidal agent	2% Glutaraldehyde
Alcohols	General purpose disinfectant, antiseptic, antiviral agent	70% Isopropyl alcohol, 70% alcohol
Chlorine and sodium hypochlorite	Sporicidal agent	0.5% Sodium hypochlorite
Phenolics	General purpose disinfectant	500 µg per g Chlorocresol, 500 µg per g chloroxylenol
Ozone	Sporicidal agent	8% Gas by weight
Hydrogen peroxide	Vapor phase sterilant, liquid sporicidal agent, antiseptic	4 µg per g H_2O_2 vapor, 10%–25% solution, 3% solution

[1] Ascenzi, J.M., Ed. *Handbook of Disinfectants and Antiseptics,* 5th ed.; Marcel Dekker: New York, 1995; Block, S.S., Ed. *Disinfection, Sterilization, and Preservation,* 5th ed.; Lippincott Williams & Wilkins Publishers: Philadelphia, 2000. Russell, A.D.; Hugo, W.B.; Ayliffe, G.A.J., Eds. *Principles and Practices of Disinfection, Preservation and Sterilization,* 3rd ed.; Blackwell Science Inc.: London, 1999.

Table 2. General Classification of Antiseptics, Disinfectants, and Sporicidal Agents *(Continued)*

Chemical Entity	Classification	Example
Substituted diguanides	Antiseptic agent	0.5% Chlorhexidine gluconate
Peracetic acid	Liquid sterilant, vapor phase sterilant	0.2% Peracetic acid, 1 µg per g peracetic acid
Ethylene oxide	Vapor-phase sterilant	600 µg per g Ethylene oxide
Quaternary ammonium compounds	General purpose disinfectant, antiseptic	Concentration dependent on application, Benzalkonium chloride
β-Propiolactone	Sporicidal agent	100 µg per g β-Propiolactone

The effectiveness of a disinfectant depends on its intrinsic biocidal activity, the concentration of the disinfectant, the contact time, the nature of the surface disinfected, the hardness of water used to dilute the disinfectant, the amount of organic materials present on the surface, and the type and the number of microorganisms present. Under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), the Environmental Protection Agency (EPA) registers chemical disinfectants marketed in the United States and requires manufacturers to supply product information on the use dilution, type of microorganisms killed, and the necessary contact time. Certain liquid chemical sterilizers intended for use on critical or semicritical medical devices are defined and regulated by the U.S. Food and Drug Administration (FDA).

SELECTION OF AN ANTISEPTIC FOR HAND AND SURGICAL SITE DISINFECTION

Hands and surgical sites are disinfected in a hospital setting to reduce the resident flora and to remove transient flora (e.g., *Streptococcus pyogenes*) and methicillin-resistant *S. aureus* and *P. aeruginosa* that have been implicated in hospital-associated infection. Use of antiseptics to disinfect hands has been shown to be more effective than soap and water in reducing the counts of bacteria on the skin; repeated antiseptic use further reduces these counts. These principles may be applied to clean-room operators in the pharmaceutical industry.

Common antiseptics include 4% chlorhexidine, 10% povidone–iodine, 3% hexachlorophene, 70% isopropyl alcohol, and 0.5% chlorhexidine in 95% alcohol.

SELECTION OF A DISINFECTANT FOR USE IN A PHARMACEUTICAL MANUFACTURING ENVIRONMENT

When selecting a disinfectant for use in a pharmaceutical manufacturing area, the following points should be considered: the number and types of microorganisms to be controlled; the spectrum of activity of commercially available disinfectants; the claims as a sterilant; the disinfectant or sanitizer supported by the EPA registrations; the concentration, application method, and contact time of the disinfectant; the nature of the surface material being disinfected and its compatibility with the disinfectant; the amount of organic compounds on the surface that may inactivate a disinfectant; the possible need to maintain a residual bactericidal activity of the disinfectant on the surface; the corrosiveness of the disinfectant to equipment with repeated application; the safety considerations for operators applying the disinfectant; the compatibility of the disinfectant with cleaning agents and other disinfectants; the planned disinfectant rotation; and the steps that need to be taken to avoid the contamination of pharmaceutical products by a disinfectant.[2]

THEORETICAL DISCUSSION OF DISINFECTANT ACTIVITY

Plots of the log of the number of microorganisms per mL surviving in a disinfectant solution indicate that first-order kinetics can be applied as a gross approximation to the reduction in microbial count with respect to time. In practice, the plots show a more sigmoid curve with a slower initial reduction in numbers followed by an increasing rate with respect to time.

The rate constant, K, for the disinfection process can be calculated by the formula:

$$(1/t)(\log N_0/N)$$

in which t is the time, in minutes, for the microbial count to be reduced from N_0 to N; N_0 is the initial number of organisms, in cfu per mL; and N is the final number, in cfu per mL, of organisms.

As with a first-order chemical reaction, the same concentration of disinfectant reduces the number of organisms more rapidly at elevated temperatures. This can be expressed as a temperature, T, coefficient per 10° rise in temperature, Q_{10}, calculated by the formula:

$$\text{Time to decontamination at } T° / \text{Time to decontamination at } T$$

in which T is T° – 10.

Further evidence that a first-order reaction is an inadequate description of disinfection is that the Q_{10} values for chemical and enzyme reactions are 2 to 3, while the common disinfectants phenol and alcohol have a Q_{10} of 4 and 45, respectively.

Critical to the successful employment of disinfectants is an understanding of the effect of disinfectant concentration on microbial reduction. A plot of the log of the time to reduce the microbial population in a standard inoculum to zero against the log of the disinfectant concentration is a straight line with the slope of the line termed the concentration exponent, n. The relationship can be expressed as follows:

$$n = (\log \text{ of the kill time at concentration } C_2) - (\log \text{ of the kill time at concentration } C_1)/(\log C_1 - \log C_2)$$

in which C_1 and C_2 are the higher and lower disinfectant concentrations, respectively.

The wide differences in concentration exponents, n, have practical consequences in picking the use dilution of different disinfectants and in using dilution to neutralize a disinfectant in disinfectant-effectiveness testing and routine microbial monitoring of the manufacturing environment. For example, mercuric chloride has a concentration exponent of 1, so a 3-fold dilution will reduce the disinfectant activity by 3^1 (or by one-third), while phenol with a concentration exponent of 6 will have a 3^6 (or a 729-fold) reduction in disinfectant activity. Disinfectants with a larger concentration exponent or dilution coefficient rapidly lose activity when diluted. The concentration exponents for some disinfectants are listed in *Table 3*.

[2]Denny, V.F.; Marsik, F.J. Current Practices in the Use of Disinfectants within the Pharmaceutical Industry. *PDA J. of Pharmaceutical Sci. and Tech.*, **1997**, *51*, (6), 227–228.

Table 3. Concentration Exponents of Common Antiseptics, Disinfectants, and Sterilants

Disinfectant	Concentration Exponents
Hydrogen peroxide	0.5
Sodium hypochlorite	0.5
Mercuric chloride	1
Chlorhexidine	2
Formaldehyde	1
Alcohol	9
Phenol	6
Quaternary ammonium compounds	0.8 to 2.5
Aliphatic alcohols	6.0 to 12.7
Phenolic compounds	4 to 9.9

Another important consideration may be the pH of the disinfectant. Many disinfectants are more active in the ionized form, while others are more active in the nonionized form. The degree of ionization will depend on the pK_a of the agent and the pH of the disinfection environment. For example, phenol, with a pK_a of 10, will be more effective at a pH below 7 where it is nonionized.

MECHANISM OF DISINFECTANT ACTIVITY

Table 4 lists the sites and modes of action of some representative disinfectants.

Table 4. Mechanism of Disinfectant Activity Against Microbial Cells

Target	Disinfectant
Cell wall	Formaldehyde, hypochlorite, and glutaraldehyde
Cytoplasmic membrane, action on membrane potential	Anilides and hexachlorophene
Membrane enzymes, action on electron-transport chain	Hexachlorophene
Action on ATP	Chlorhexidine and ethylene oxide
Action on enzymes with –SH groups	Ethylene oxide, glutaraldehyde, hydrogen peroxide, hypochlorite, and iodine
Action on general membrane permeability	Alcohols, chlorhexidine, and quaternary ammonium compounds
Cell contents, general coagulation	Chlorhexidine, aldehydes, and quaternary ammonium compounds
Ribosomes	Hydrogen peroxide
Nucleic acids	Hypochlorites
Thiol groups	Ethylene oxide, glutaraldehyde, hydrogen peroxide, and hypochlorite
Amino groups	Ethylene oxide, glutaraldehyde, and hypochlorite
General oxidation	Hypochlorite

MICROBIAL RESISTANCE TO DISINFECTANTS

The development of microbial resistance to antibiotics is a well-described phenomenon. The development of microbial resistance to disinfectants is less likely to occur at significant levels, as disinfectants are more powerful biocidal agents than antibiotics. In addition, they are normally applied in high concentrations against low populations of microorganisms usually not growing actively, so the selective pressure for the development of resistance is less profound. However, the most frequently isolated microorganisms from an environmental monitoring program may be periodically subjected to use-dilution testing with the agents used in the disinfection program to confirm their susceptibility, as there are real differences among different species in resistance to the lethal effects of different sanitizers.

DISINFECTANT CHALLENGE TESTING

Under FIFRA, the EPA requires companies that register public health antimicrobial pesticide products including disinfectants, sanitization agents, sporicidal agents, and sterilants to ensure the safety and effectiveness of their products before they are sold or distributed. Companies registering these products must address the chemical composition of their product, include toxicology data to document that their product is safe if used as directed on the label, include efficacy data to document their claims of effectiveness against specific organisms and to support the directions for use provided in the labeling, and provide labeling that reflects the required elements for safe and effective use. While these directions provide valuable information, they may not be helpful in terms of the products' use as disinfectants in a manufacturing environment.

In the United States, the official disinfectant testing methods are published by AOAC International[3] and include the Phenol-Coefficient Test, Use-Dilution Method Test, Hard Surface Carrier Method, and Sporicidal Carrier Test. A scientific study submitted for EPA review in support of disinfectant registration must be conducted at a laboratory facility that follows the Good Laboratory Practices (GLP) regulations (21 CFR 58). To demonstrate the efficacy of a disinfectant within a pharmaceutical manufacturing environment, it may be deemed necessary to conduct the following tests: (1) use-dilution tests (screening disinfectants for their efficacy at various concentrations and contact times against a wide range of standard test organisms and environmental isolates); (2) surface challenge tests (using standard test microorganisms and microorganisms that are typical environmental isolates, applying disinfectants to surfaces at the selected use concentration with a specified contact time, and determining the log reduction of the challenge microorganisms); and (3) a statistical comparison of the frequency of isolation and numbers of microorganisms isolated prior to and after the implementation of a new disinfectant. This is considered necessary because critical process steps like disinfection of aseptic processing areas, as required by GMP regulations, need to be validated, and the EPA registration requirements do not address how disinfectants are used in the pharmaceutical, biotechnological, and medical device industries. For the surface challenge tests, the test organisms are enumerated using swabs, surface rinse, or contact plate methods. Neutralizers that inactivate the disinfectants should be included in either the diluent or microbiological media used for microbial enumeration or both. Information on disinfectant neutralization may be found in *Validation of Microbial Recovery from Pharmacopeial Articles* ⟨1227⟩.

The disinfectant efficacy test must have realistic acceptance criteria. In practice, sufficient organisms need to be inoculated on a 2-inch × 2-inch square of the surface being decontaminated, i.e., a coupon, to demonstrate at least a 2 (for bacterial spores) to 3 (for vegetative bacteria) log reduction during a predetermined contact time (i.e., 10 minutes over and above the recovery observed with a control disinfectant application). The efficacy of the neutralizers and their ability to recover inoculated microorganisms from the material should be demonstrated during the use-dilution or surface-challenge studies. Points to remember are that disinfectants are less effective against the higher numbers of microorganisms used in laboratory challenge tests than they are against the numbers that are found in clean rooms (see *Microbiological Evaluation of Clean Rooms and Other Controlled Environments* ⟨1116⟩); that inocula from the log growth phase that are typically employed in laboratory tests are more resistant, with the exception of spores formed during the static phase, than those from a static or dying cul-

[3] AOAC International Official Methods of Analysis, 15th, 16th, and 17th editions. Arlington, VA.

ture or stressed organisms in the environment; and that microorganisms may be physically removed during actual disinfectant application in the manufacturing area.

Although not all inclusive, typical challenge organisms that may be employed are listed in *Table 5.*

Table 5. Typical Challenge Organisms

AOAC Challenge Organisms	Typical Environmental Isolates
Bactericide: *E. coli,* ATCC 11229; *S. aureus,* ATCC 6538; *P. aeruginosa,* ATCC 15442	Bactericide: *M. luteus, S. epidermidis, Coynebacterium jeikeium, P. vesicularis*
Fungicide: *C. albicans,* ATCC 10231 or 2091; *Penicillium chrysogenum,* ATCC 11709; *A. brasiliensis*, ATCC 16404	Fungicide: *P. chrysogenum, A. brasiliensis*
Sporicide: *B. subtilis,* ATCC 19659	Sporicide: *B. sphaericus, B. thuringiensis*

Because a wide range of different materials of construction are used in clean rooms and other controlled areas, each material needs to be evaluated separately to validate the efficacy of a given disinfectant. *Table 6* contains a list of common materials used in clean room construction.

Table 6. Typical Surfaces to be Decontaminated by Disinfectants in a Pharmaceutical Manufacturing Area

Material	Application
Stainless steel 304L and 316L grades	Work surfaces, filling equipment, and tanks
Glass	Windows and vessels
Plastic, vinyl	Curtains
Plastic, polycarbonate	Insulation coating
Lexan® (plexiglass)	Shields
Epoxyl-coated gypsum	Walls and ceilings
Fiberglass-reinforced plastic	Wall paneling
Tyvek®	Equipment wraps
Terrazzo tiles	Floors

DISINFECTANTS IN A CLEANING AND SANITIZATION PROGRAM

The selection of suitable disinfectants and the verification of their effectiveness in surface challenge testing is critical in the development of a cleaning and sanitization program.

Issues associated with the successful implementation of such a program are the development of written procedures, staff training, decisions on disinfectant rotation, institution of application methods and contact times, environmental monitoring to demonstrate efficacy, and personnel safety.

The cGMP 21 CFR 211.67, *Equipment Cleaning and Maintenance,* details the requirements for written procedures for cleaning, maintenance, and sanitization of pharmaceutical manufacturing equipment. These procedures should address the assignment of responsibility, establishment of schedules, details of cleaning operations, protection of clean equipment prior to use, inspection for cleanliness immediately prior to use, and maintenance of cleaning and sanitization records.

Staff involved in disinfection require training in microbiology, industry practices for cleaning and sanitization, safe handling of concentrated disinfectants, the preparation and disposal of disinfectants, and appropriate application methods. It should be emphasized that the preparation of the correct dilutions is critical because many disinfectant failures can be attributed to use of disinfectant solutions that are too dilute. Typically disinfectants used in aseptic processing and filling areas are diluted with Sterile Purified Water, and are prepared aseptically. Alternately, the disinfectant may be diluted with Purified Water, and then sterile filtered to eliminate microorganisms that may potentially persist in a disinfectant. Diluted disinfectants must have an assigned expiration dating justified by effectiveness studies.

The rotation of an effective disinfectant with a sporicide is encouraged. It is prudent to augment the daily use of a bactericidal disinfectant with weekly (or monthly) use of a sporicidal agent. The daily application of sporicidal agents is not generally favored because of their tendency to corrode equipment and because of the potential safety issues with chronic operator exposure. Other disinfection rotation schemes may be supported on the basis of a review of the historical environmental monitoring data. Disinfectants applied on potential product contact surfaces are typically removed with 70% alcohol wipes. The removal of residual disinfectants should be monitored for effectiveness as a precaution against the possibility of product contamination.

The greatest safety concerns are in the handling of concentrated disinfectants and the mixing of incompatible disinfectants. For example, concentrated sodium hypochlorite solutions (at a concentration of more than 5%) are strong oxidants and will decompose on heating, on contact with acids, and under the influence of light, producing toxic and corrosive gases including chlorine. In contrast, dilute solutions (at a concentration of less than 0.5%) are not considered as hazardous. Under no circumstances should disinfectants of different concentrations be mixed. Material Safety Data Sheets for all the disinfectants used in a manufacturing area should be available to personnel handling these agents. Appropriate safety equipment such as face shields, safety glasses, gloves, and uniforms must be issued to personnel handling the disinfectant preparation, and personnel must be trained in the proper use of this equipment. Safety showers and eye wash stations must be situated in the work area where disinfectant solutions are prepared.

⟨1074⟩ EXCIPIENT BIOLOGICAL SAFETY EVALUATION GUIDELINES

INTRODUCTION

This informational chapter presents a scientifically-based approach for the safety assessment of new pharmaceutical excipients (i.e., those excipients that have not been previously used or permitted for use in a pharmaceutical preparation). The guidelines presented herein provide a protocol for developing an adequate database upon which to establish conditions for the safe use of a new excipient intended for use in products administered by various dosage routes. [NOTE—The final section of this chapter, *Definition of Terms*, lists some terms referred to in this chapter.]

An excipient may perform a variety of functionality roles in a pharmaceutical product; but, unlike pharmacologically active drug entities, the excipient displays either no pharmacological activity or very limited and directed activity. Because of these differences between excipients and active drug substances in terms of risk and benefit relationships and expected biological activities, the approaches for safety assessments of excipients and active drug substances will differ. Therefore, it is important to note that the guidelines presented in this informational chapter apply only to the safety assessment of excipients, not to the safety assessment of active drug substances.

These testing guidelines are informational in nature and are intended to be used by professionals having a knowl-