Chemical Disinfectants, Antiseptics & Preservatives

Hugo and Russell's Pharmaceutical Microbiology, 8th Edition

Chapter 19 – Page 312

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Introduction

- **Biocides**: chemical agents that have the ability to **destroy or inhibit** the growth of m.o.

  (Bactericide, sporicide, fungicide, virucide)
- **Uses**: medical, healthcare, livestock, environment, coatings, plastics, pharmaceuticals, food & beverage manufacturing and cosmetics
- Due to **increase in concerns** about bacterial & other pathogenic threats & the **increase in awareness** about hygiene at home or work, the biocide market in US increased to about $2 billions/year
- Biocides include **disinfectants, antiseptics and preservatives**

Disinfection

- Disinfection is the **destruction or removing** of microorganisms, including potentially pathogenic ones, from the surfaces of **inanimate objects**.
- According to British Standards Institution (BSI), disinfection **doesn't not necessarily mean the killing of all microorganisms** but **reducing them to a level acceptable for a defined purpose**, e.g. a level which is not harmful to health nor to the quality of goods.
- Disinfectants should have **bactericidal action** to destroy the bacterial population in order to make the environment free from the ability to cause infection

Disinfection

- Chemical disinfectants exert 3 different levels of disinfection:
  - **High level disinfection**: destruction of **all m.o.** but **not necessarily all bacterial spores**. Some have good **sporicidal** activity and hence they are called **'liquid chemical sterilants'** to indicate that they completely kill all microorganisms as in sterilization
  - **Intermediate level disinfection**: destruction of **all vegetative bacteria** (including *Mycobacterium tuberculosis*), **most viruses** & **most fungi**. They have little or **no sporicidal** activity
  - **Low level disinfection**: can destroy **most vegetative** bacteria (**excluding *Mycobacterium tuberculosis***), **some fungi** and **some viruses**

Antisepsis

- Antisepsis is the **destruction** or **inhibition** of microorganisms on **living tissues**; having the effect of preventing or limiting the harmful effects of infection
- Antiseptics means ‘against sepsis’ (where sepsis generally means infection), they **can be bacteriostatic** agent as they prevent sepsis in the body if the **body defenses** are normal
- The chemicals used have adequate antimicrobial activity & are applied to **skin and mucous membranes**, therefore they must be **non-toxic and non-irritant** for these tissues

Antisepsis

- **Antiseptics** usually have **lower concentrations** than agents used for **disinfection**
- Antiseptics are mostly used to reduce the microbial population on the **skin prior to surgery** or on the **hands** to help prevent spread of infection by this route.
- **Sanitizer**: in the public health context, refers to an agent that **reduces the number** of bacterial contaminants **to a safe level**.

Preservation

- Preservation is the addition of antimicrobial compounds to certain **products** (e.g. pharmaceutical preparations) **to prevent microbial spoilage** of the product and to **minimize the risk of causing infection** to the consumer during the use of the product
- Preservatives must be able to **limit proliferation** of m.o. that may be introduced unavoidably during **manufacture and use** of non-sterile products such as oral and topical medications
- Preservatives must be **non-toxic** in relation to the route of administration of the preparation, therefore, they are used in **concentrations lower** than that for **disinfection or antisepsis**

Factors Affecting Choice Of Biocide

1. Properties of the chemical agent
2. Intended application
3. Environmental factors
4. Toxicity of the agent
5. Microbiological challenge

Factors Affecting Choice Of Biocide

- Properties of the chemical agent
  - The process of killing or inhibiting m.o. by biocide is a chemical reaction which is influenced by **reactivity of the agent**, **concentration** of the agent, **pH**, **temp** & **formulation** (affect the antimicrobial activity).
  - Also, tissue toxicity influences the use of the agent (to be used as antiseptic or preservative)

Factors Affecting Choice Of Biocide

- Intended application
  - i.e. whether the agent will be used for **preservation, antisepsis or disinfection**
  - Certain considerations should be taken into account, such as:
    - **Preservatives** might be antagonized by **ingredients** in the formula
    - Whether the **antiseptic** will be used in close contact with **broken skin**
    - **Disinfectant** should be **not corrosive to instrument**, doesn't affect **clarity or integrity of lenses**, **not absorbed by rubber** (phenolics) or fabric (QACs)

Factors Affecting Choice Of Biocide

- Environmental factors
  - **Organic matter** affect antimicrobial activity either by **adsorption** or **chemical inactivation**, thus reducing the conc. of active agent in solution or by acting as a **barrier** to the penetration of the biocide.
  - Blood, body fluids, pus, milk, food residues, even in small amounts, reduce the effectiveness of antimicrobial agents to varying degrees and **some are seriously affected**. That's why it is important to **clean instruments** before disinfection.
  - **Ions in water** (hard water) can affect the activity of some disinfectants (check the label, hard or soft water)
  - Some organisms are specific to certain environments e.g. *Legionella* in cooling towers and non-potable water supply systems, *Listeria* in the dairy and food industry and HBV in blood-contaminated articles.

Factors Affecting Choice Of Biocide

- Toxicity of the agent
  - Increasing concern for health & safety is reflected in **COSHH (Control of Substances Hazardous to Health) regulations** which specify the **precautions required** in handling **toxic agents** e.g. gluteraldehyde, phenolics and formaldehyde.
  - Many disinfectants including gluteraldehyde may affect the **eyes, skin** (contact dermatitis) & induce **respiratory distress**. Face protection & impermeable gloves should be worn when using these agents
  - **Systemic toxicity** of **preservatives**!
  - **Toxic volatile substances**, should be kept in covered containers to reduce the level of exposure to **irritant vapour** and they should be used with an extractor facility.

Factors Affecting Choice Of Biocide

- Microbiological challenge
  - The **type** and **size** (**bioburden**) of microbial contamination to be dealt with.
  - If the **bioburden is high**, **long exposure** time and/or **higher concentration** will be needed
  - Different m.o. vary in their sensitivity to the action of a certain antimicrobial. Also different antimicrobials have different capacity & activity

    Therefore the user should have some awareness of what level of antimicrobial action is needed according to certain situation (biofilms, nosocomial infections, etc..)

Factors Affecting Choice Of Biocide

- Microbiological challenge (cont'd)
  - Vegetative bacteria:
    - At in-use conc, disinfectants should be capable of killing most vegetative bacteria (broad spectrum) within a reasonable contact period including problematic m.o. (*Listeria*, MRSA, *Campylobacter*, *Legionella* and VRE).
    - **G-ve bacilli** are **more resistant** than G +ve & responsible for most nosocomial infections, where the most resistant is ***P. aeruginosa***.

Factors Affecting Choice Of Biocide

- Microbiological challenge (cont'd)
  - Mycobacteria:
    - ➢Mycobacteria are **resistant to many biocides**.
    - ➢Resistance is either **intrinsic** (mainly due to reduced cellular permeability) or **acquired** (due to mutation or acquisition of plasmids)
    - ➢The greatest risk comes from **undiagnosed patients**
    - ➢Equipment used for respiratory investigation of a patient or a carrier will be contaminated & must be disinfected with a proper agent

Factors Affecting Choice Of Biocide

- Microbiological challenge (cont'd)
  - Bacterial spores:
    - ➢Bacterial spores are the **most resistant** of all microbial forms to chemical treatment. (prions?? not m.o)
    - ➢Spore-forming genera: ***Bacillus* & *Clostridium*** (e.g. *C. difficile*)
    - ➢The majority of antimicrobial agents have no useful sporicidal action.
      - ✓Exceptions: **aldehydes, halogens and peroxygen compounds**
      - ✓Such chemicals are sometimes used as alternatives to physical methods of sterilization (e.g. heat sterilization).

Factors Affecting Choice Of Biocide

- Microbiological challenge (cont'd)
  - Fungi:
    - ➢The **vegetative** fungal form is often as **sensitive as vegetative bacteria** to antimicrobial agents.
    - ➢**Fungal spores** are more resistant but **less than bacterial spores**
    - ➢The ability to kill pathogenic fungi, such as *Candida albicans* (yease), *Trichophyton* (filamentous fungi) & the spores of common spoilage-causing *Aspergillus niger* is an advantage of the disinfectant

Factors Affecting Choice Of Biocide

- Microbiological challenge (cont'd)
  - Viruses:
    - ➢Susceptibility of viruses to antimicrobial agents depends on whether the virus possesses a lipid envelope.
    - ➢**Non-enveloped viruses** are frequently **more resistant** to disinfectants
      - ✓These viruses are responsible for many **nosocomial infections**, e.g. **rotaviruses**, **picornaviruses** and **adenoviruses**
      - ✓**Hepatitis A** virus (a picornavirus) is considered one of the most resistant viruses to disinfectants
    - ➢Much concern for the hazards of **HIV & HBV**, even though they are **enveloped** viruses. Disinfectants must be able to treat rapidly and reliably accidental spills of blood, body fluids or secretions from patients infected with these viruses

Factors Affecting Choice Of Biocide

- Microbiological challenge (cont'd)
  - Viruses:
    - It is recommended to use **high level disinfectants** for decontamination of **HIV or HBV contaminated reusable equipment**. Although HIV is inactivated by most chemical disinfectants at in-use conc.
    - **Intermediate level disinfectants** are satisfactory for **patient care areas**.
    - Flooding with liquid biocide is only needed if large spills of concentrated infectious agents occur
    - **Influnzaviruses** (including H1N1) is **susceptible to most disinfectant** products. Chlorine at 1-3ppm in swimming pools is enough to disinfect avian flu virus (H5N1).

Factors Affecting Choice Of Biocide

- Microbiological challenge (cont'd)
  - Protozoa:
    - ***Acanthamoeba* spp**. can cause *Acanthamoeba* **keratitis** with corneal scarring and loss of vision in soft contact lens wearers. The cysts, in particular, present a problem of lens disinfection.
    - Only **hydrogen peroxide**-based disinfection is completely reliable and consistent in producing an acanthomoebicidal effect.
    - **Polyhexamethylene biguanide** & **polyaminopropyl biguanides** are also good acanthamoebicides.
    - The chlorine-generating systems in use are generally inadequate for such m.o.

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Factors Affecting Choice Of Biocide

- Microbiological challenge (cont'd)
  - Prions:
    - Small proteinaceous infectious particles associated with causing **mad cow disease**; bovine spongiform encephalopathy (BSE) in cattle and Creutzfeldt-Jakob disease (CJD) in humans.
    - They are **not m.o.** as they don't have plasma membrane or nucleic acids
    - Risk of infectivity is highest in **brain, spinal cord & eye tissues**.
    - Prions are **resistant to most disinfectants**. Immersion in **1N sodium hydroxide** or **high conc. of sodium hypochlorite** (20000 ppm) **for 1 hour** is advised by WHO followed by cleaning & sterilization (e.g. autoclaving)
    - Some prefer to **destroy surgical instruments** where procedures involve brain, spinal cord or eye in patients with confirmed or suspected CJD.

Biocides in Use

- Acids and esters
  - In pharmaceutical context, these biocides are usually **organic acids**
  - Such biocides are weak acids so they can dissociate as follows:

    $HA \rightleftarrows H^+ + A^-$
  - The **undissociated form** (HA) is usually the active antimicrobial agent, and hence a balance between their pKa and the formulation pH should be considered.

Biocides in Use

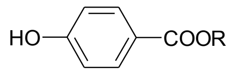
- Acids and esters
  - **Benzoic acid:**
    - Used as such, or in combination with other agents, for the **preservation** of pharmaceuticals
    - Although often added to the formulation as **sodium salt**, its **unionized acid is the active form**
    - Its **pKa is 4.2** (i.e. it is 50% ionized at pH 4.2) so it can only be used in formulations with a **maximum pH of 5.0**
    - Used for **oral preparations** at conc 0.05-0.1%
    - Also used with salicylic acid to treat **superficial fungal infections**
    - Resistance of m.o. to this agent may develop and sometimes metabolism of the acid occurs resulting in complete loss of activity

Biocides in Use

- Acids and esters
  - **Sorbic acid:**
    - A widely used **preservative** (as the acid or its **potassium salt**)
    - **pKa is 4.8** so, similar to benzoic acid, its activity decreases with increasing pH
    - Usually used to preserve pharmaceutical **gums, syrups and mucilages** with **acidic pH**
  - **Sulphur dioxide, sulphites and metabisulphites:**
    - **Sulphur dioxide** (SO_2) is an extensively used **preservative** in **food & beverage industry**
    - Sodium salts of **sulphites** (Na_2SO_3) and **metabisulphites** ($Na_2S_2O_5$) used in **pharmaceuticals** for their dual role: acting as **preservatives and antioxidants**

Biocides in Use

- Acids and esters
  - **Esters of p-hydroxybenzoic acid (parabens):**



R= methyl, ethyl, propyl or butyl

- Prepared to **overcome the pH-dependence** on activity of the acids
- These are less readily ionized, having **pKa** values in the range of **8.0-8.5**
- They have good **preservative** activity even at pH **7-8**, but **optimum** activity at acidic conditions
- They are **extensively used in pharmaceuticals** especially in combinations for **two-phase preparations** like creams, emulsions & lotions (i.e. methyl paraben (at 0.25%) protects aqueous phase while propyl or butyl paraben (at 0.02%) protects oil phase)
- They are mainly active **against fungi**, but less so against bacteria (*Pseudomonas* can even utilize them as C-source)

Biocides in Use

- Alcohols
  - Alcohols as **disinfectants & antiseptics**:
    - **Aliphatic** alcohols (ethanol & isopropanol) are bactericidal against vegetative bacteria and mycobacteria, but does not have sporicidal activity
    - Activity drops sharply at conc below 50%
    - General properties:
      - ✓ Poor penetration of organic material (so used in **clean conditions**)
      - ✓ Have **cleansing action**
      - ✓ **Volatile** (contact time?)
      - ✓ Reduce skin flora (prepare skin for injection or procedure)
      - ✓ Presence of **water is essential** for activity so 100% conc is relatively not effective.

Biocides in Use

- Alcohols
  - Alcohols as disinfectants & antiseptics: (cont'd)
    - ➢**Ethanol:**
      - ✓ Conc of **60-95 %** are bactericidal
      - ✓ Used at 70% for decontaminating skin & clean instruments
      - ✓ **Higher conc** (e.g. 90%) is **virucidal** (including HIV) & **fungicidal**.
      - ✓ Used as solvent & preservative in pharmaceuticals (e.g. gels) & cosmetic products.
    - ➢**Isopropyl alcohol:**
      - ✓ **less virucidal** (especially against naked viruses) but **more bactericidal** activity than EtOH.
      - ✓ It is 2X **more toxic**.
      - ✓ Used at conc 60-70% as **alternative for EtOH** for preoperative skin treatment and a preservative in cosmetics

Biocides in Use

- Alcohols
  - Alcohols as preservatives:

    Aralkyl alcohols & **highly substituted** aliphatic alcohols are used as preservatives

    - ➢**Phenylethanol** (0.25-0.5%):
      - ✓ **More active against g-ve bacteria** (e.g. *Pseudomonas aeruginosa*) than other bacteria
      - ✓ Used in **combination** with other compounds
    - ➢**Phenoxyethanol** (1%):
      - ✓ **More active against g-ve bacteria** (e.g. *Pseudomonas aeruginosa*) than other bacteria
      - ✓ Usually **combined** with parabens to broaden its activity
    - ➢**Chlorbutol** (Chlorobutanol, trichlorobutanol) (0.5%):
      - ✓ Used as a **preservative** in **injections & eye drops**.
      - ✓ **Unstable**; decomposes during autoclaving at acidic pH whereas in alkaline solutions it decomposes at room temperature

Biocides in Use

- Alcohols
  - Alcohols as preservatives:
    - ➢**Bronopol** (0.01-0.1%):
      - ✓**Broad spectrum** antibacterial including *Pseudomonas* sp.
      - ✓At alkaline pH especially if high temp →**light will decompose** its solution to yellow brown compound.
      - ✓Degradation compounds include **formaldehyde & nitrite ions**.
      - ✓Nitrite can react with **2° or 3° amines** forming **nitrosamines** (carcinogenic)
    - ➢**Benzyl alcohol** (2%):
      - ✓Has antibacterial & weak **anesthetic** properties.
      - ✓Used as preservative

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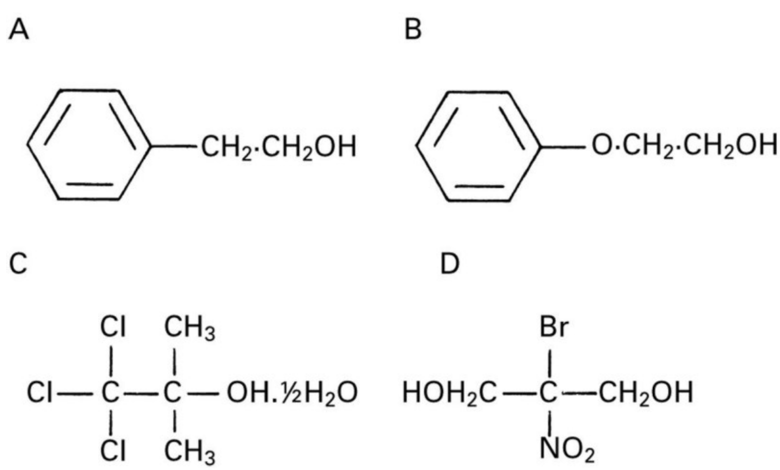

Figure 19.2 Structural formulae of alcohols used in preserving and disinfection: (A) 2-phenylethanol; (B) 2-phenoxyethanol, (C) chlorbutol (trichloro-tert-butanol); (D) bronopol (2-bromo-2-nitropropan-1,3-diol).

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Biocides in Use

- Aldehydes

  High level disinfectants, they also have sporicidal activity (**liquid chemical sterilants**)

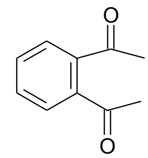
  - **Gluteraldehyde**: ($CHO\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CHO$)
    - ➢ It has 2 aldehyde groups (highly active)
    - ➢ It has broad spectrum of activity.
    - ➢ Rapid rate of killing to vegetative bacteria (i.e. few min), while **spores** usually need **longer exposure times** (i.e. few hours)
    - ➢ The monomer is in equilibrium with **polymer** form which is affected by **temp & pH**.

Biocides in Use

- Aldehydes
  - **Gluteraldehyde**: (cont'd)
    - ➢ At **pH 8, highest biocidal activity** but **low stability** (due to polymerization). **Acidic soln** are **stable** but **less active**. Increasing temp breaks the polymers of acidic soln & increase active free form
    - ➢ In practice acidic soln (2%) is used for storage, before use it is **activated** by addition of **alkaline soln** to increase pH to optimum (stable for ~2wks after activation).
    - ➢ Mainly used for cold liquid chemical **sterilization** of medical and surgical materials that cannot be sterilized by other methods (e.g. **endoscopes**)
    - ➢ Contact times varies according to m.o. Generally 20-90min (long time if HIV or *Mycobacteria* is suspected).

Biocides in Use

- Aldehydes
  - **Ortho-phthalaldehyde (OPA)**:



➢Also belongs to high level disinfectants

➢Compared to gluteraldehyde, OPA has **better mycobactericidal activity**

➢Also **less irritant** to eyes or nasal passages, **doesn't need activation** and stable at pH range 3-9.

➢Used mainly for **endoscope disinfection/sterilization**

Biocides in Use

- Aldehydes
  - **Formaldehyde (HCHO):**

➢Used in either **gaseous** or **liquid** (solution) state

➢Supplied as **liquid formalin** (34-38% aqueous soln),or as solid polymer **paraformaldehyde**

Heating or mixing with **potassium permanganate** & water liberates the free form from the solid polymer

➢Uses:

✓ **Vapour phase** is used for decontamination of **safety cabinets, isolators and rooms**

✓ Low temperature steam formaldehyde (**LTSF**) **sterilization** of heat-sensitive items

✓ Formalin in 1:10 dilution (~**4% formaldehyde**) is used to **disinfect** surfaces

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Biocides in Use

- Aldehydes
  - **Formaldehyde: (cont'd)**
    - ➢Limitations:
      - ✓Its solutions are **highly irritant** & cannot be used for skin
      - ✓Vapours are highly **toxic** (carcinogenic)
      - ✓Not very active at **temp** below 20 °C & needs relative **humidity** of 70%
      - ✓It has **poor penetration** ability & it tends to polymerize on surfaces which limits its use as disinfectant for pharmaceutical purposes

Biocides in Use

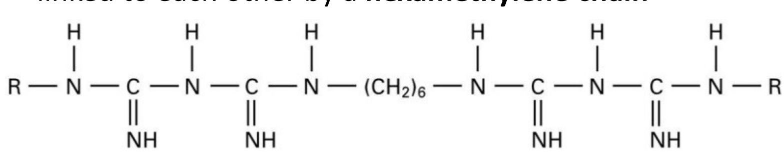
- Aldehydes
  - **Formaldehyde releasing agents:**
    - ➢Also called masked-formaldehyde compounds
    - ➢Developed to **reduce irritation** of formaldehyde while maintaining activity
    - ➢**Noxythiolin**:
      - ✓Supplied as a **dry powder** and upon aqueous **reconstitution** it slowly releases formaldehyde.
      - ✓Antimicrobial **activity** is due to **both** the noxythiolin molecule and the released formaldehyde
      - ✓Used **topically** and in accessible **body cavities** as an **irrigation solution** and in the treatment of **peritonitis**

Biocides in Use

- Aldehydes
  - **Formaldehyde releasing agents: (cont'd)**
    - ➢**Polynoxylin**:
      - ✓Similar to noxythiolin but usually used in **gel and lozenge** formulations for **skin** and **mouth** treatment
    - ➢**Taurolidine**:
      - ✓A condensate of two molecules of the **amino acid taurine** and three molecules of formaldehyde.
      - ✓It is **more stable** than noxythiolin in solution and has similar uses.
      - ✓It has even **greater activity** than formaldehyde

Biocides in Use

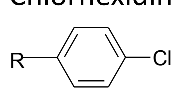
- Biguanides
  - Of special interest are the **bisbiguanides** which are more active than biguanides as antimicrobials
  - Bisbiguanides are composed of two biguanide groups linked to each other by a **hexamethylene chain**

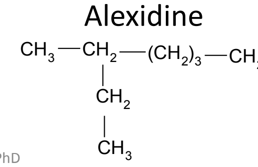


Chlorhexidine

Alexidine

R=





Biocides in Use

- Biguanides
  - **Chlorhexidine**:
    - Bisbiguanide with two **p-chlorophenyl** end groups
    - As a base it has poor water solubility while its **salts** (HCl, gluconate & acetate) have good solubility.
    - At **pH 7-8** the molecule exists as **di-cation** & has **greatest activity**.
    - **Anions** like carbonate, bicarbonate, borate, citrate, phosphate & soaps **reduce its activity** due to formation of **insoluble salts**.
    - Similarly **hard water** reduces its solubility (use deionized or distilled water for dilution).
    - Also **organic matter** like pus & blood **reduces its activity**

Biocides in Use

- Biguanides
  - **Chlorhexidine**: (cont'd)
    - It is used mainly as an **antiseptic**, generally **well tolerated** & non-toxic to skin & mucous membrane.
    - It is important **preoperative antiseptic** and frequently used in **mouthwash** products
    - Antimicrobial activity:
      - ✓ Effective against **G+ve** bacteria, but **higher conc needed for G-ve** bacteria.
      - ✓ **Not effective** against **spores or Mycobacteria**.
      - ✓ Contamination of aqueous chlorhexidine soln with ***Pseudomonas aeruginosa*** can be prevented by addition of small amounts of **ethanol or isopropanol**.
      - ✓ It has **limited antifungal activity** which restricts its use as preservative

Biocides in Use

- Biguanides
  - **Alexidine**:
    - Bisbiguanide with two **ethylhexyl** end groups
    - Used as an **oral antiseptic** and **antiplaque** agent
  - **Polyhexamethylene biguanide (PHMB):**
    - A **polymeric biguanide** that is more active than other biguanides
    - PHMB is repeating biguanide groups linked by hexamethylene chains.
    - Has a **broad spectrum** of activity against G+ve and G-ve bacteria
    - It has **low toxicity**.
    - PHMB is employed as an antimicrobial agent in various **ophthalmic products** since it also has good activity against ***Acanthamoeba***

Biocides in Use

- Halogens
  - **Chlorine compounds:**

  A. Liquid & gaseous chlorine:
  - In practical context, **liquid & gaseous chlorine** means **Cl_2**, while the word '**chlorine**' means mixture of **OCl^-**, HOCl, Cl_2 & other active chlorine compounds in **aq soln**.
  - Conc. or **potency** of chlorine is expressed in **ppm** or **% of available chlorine**
  - Liquid Chlorine is usually supplied as amber liquid prepared by compressing & cooling gaseous Cl_2

Biocides in Use

- Halogens
  - **Chlorine compounds: (cont'd)**

B. Hypochlorites (bleach):

- They exhibit a **rapid activity** against a **wide spectrum** of bacteria, fungi and viruses.
- **High conc** of chlorine is effective in eradicating acid-fast bacilli and bacterial spores and hence considered among **high-level disinfectants**
- It is **compatible with most anionic and cationic surface-active agents** and are relatively **inexpensive** to use.
- They are **corrosive**, suffer **inactivation by organic matter** and can become **unstable**
- Hypochlorites are available as **powders or liquids**, most frequently as Na or K hypochlorite salts which, in solution, exist **in equilibrium with hypochlorous acid**:

$$NaOCl + H_2O \rightleftarrows HOCl + NaOH$$

Biocides in Use

- Halogens
  - **Chlorine compounds: (cont'd)**

B. Hypochlorites (bleach): (cont'd)

- Undissociated hypochlorous acid (which releases Cl_2) is a strong oxidizing agent and has a **potent antimicrobial activity** much higher (~100x) than that of the hypochlorite form
- The **pH** affects the equilibrium between hypochlorous acid and hypochlorite anion and hence its antimicrobial activity:

$$HOCl \rightleftarrows H^+ + OCl^-$$

- **Lowering pH** of hypochlorite solutions **increases** the antimicrobial activity to an **optimum at about pH 5** but this **decreases the stability** of the solutions
- This problem may be alleviated by addition of NaOH to maintain a **high pH during storage** for stability. Upon dilution to use-strength, absence of buffer allows the **pH to be lowered sufficiently for activity**. It is preferable to prepare use-dilutions of hypochlorite on a daily basis

Biocides in Use

- Halogens
  - **Chlorine compounds: (cont'd)**

C. Organic chlorine compounds (chloramines):

- These are the **N-chloro derivatives** of, for example, sulfonamides such as **chloramine T, dichloramine T & halazone**; which are commonly used for **disinfecting drinking water**
- A second gp N-chloro derivatives of other heterocyclic compounds include Na or K salts of **dichloroisocyanuric acid** (NaDCC) are available in **tablet or granule** form and, if protected from moisture, are very stable on storage.
- Activity of chloramines under acidic conditions is similar to hypochlorites

D. Chloroform ($CHCl_3$):

- A **volatile compound** that has a **narrow spectrum** of activity, used in the past as a **preservative**. But due to its **evaporation**, reduction in its conc frequently occurs leading to microbial growth

Biocides in Use

- Halogens
  - **Iodine (I_2):**

- Has a **wide spectrum** of antimicrobial activity including G-ve & G+ve bacteria, mycobacteria, fungi and viruses and even bacterial spores (on extended exposure)
- The active agent is **molecular iodine** (I_2) but it is only **slightly soluble** in water, therefore iodide ions (I^-) are added for its **solubilization**

  e.g. aqueous iodine solution (**Lugol's solution**), BP: 5% iodine in 10% potassium iodide solution. **Iodine tincture**, BP: iodine (2.5%) in **ethanol** (90%) and potassium iodide (2.5%)

- The antimicrobial activity of iodine is **less dependent** than chlorine on **temperature and pH**, though alkaline pH should be avoided. I_2 is also less susceptible to **inactivation by organic matter**
- But when used as antiseptic on skin causes **staining** of skin and fabrics coupled with possible **sensitization** of skin and mucous membranes

Biocides in Use

- Halogens
  - **Iodophors (iodine carriers):**
    - Developed to **eliminate adverse effects** of Iodine.
    - In these compounds, iodine is **complexed** with other compounds to **slowly release** it
    - 4 compound groups are used as complexing agents or carriers for iodine:
      1. **Poloxamer iodophors** (i.e. with propylene oxide and **ethylene oxide** polymer)
      2. **Cationic** (quaternary ammonium) surfactants iodophors
      3. **Non-ionic** (ethoxylated) surfactant iodophors
      4. Polyvinylpyrrolidone iodophors (PVP-I or **povidone-iodine**)
    - In surfactant preparations, the iodine is present in the form of micellar aggregates which disperse upon dilution, especially below the critical micelle concentration (CMC) of the surfactant, to liberate free iodine.

Biocides in Use

- Halogens
  - **Iodophors (iodine carriers):**
    - **Povidone-iodine** (9-12%) produced by a **complexation** rxn between iodine & povidone resulting in **PVP-I_2 complex** which is water soluble
      - ✓ Dilution of PVP-I weakens the linkage between iodine & PVP carrier resulting in increase in molecular iodine in soln that exerts the antimicrobial activity.
    - **Cadexomer-I_2** (also called **Iodosorb**) is an iodophor similar to povidone-iodine. It is a water-soluble modified starch polymer (cross linked D-glucan carboxy methyl ether) containing 0.9% iodine
      - ✓ Used for its **absorbent** (absorbs **exudates** & particulate matter from wounds) and **antiseptic properties** in the management of **leg ulcers** and **pressure sores** where it is applied in form of microbeads. Also used as gels & pastes for healing wounds (was found to accelerate healing process of wounds).
    - The amount of free iodine the solution can generate is termed the 'available iodine'. Iodophor acts as a **reservoir** for active iodine releasing it slowly (sustained release) thus, avoiding the harmful side-effects of high iodine conc.
    - Consequently, when used for antisepsis, iodophors should be allowed to remain on the skin for **sufficient time** to obtain full advantage of the sustained-release iodine.

Biocides in Use

- Heavy metals
  - Silver and mercury compounds have been used in the past as antiseptics but they are replaced by others due to their toxicity
  - **Silver**:
    - ➢Is recently used by incorporating it in urethral catheters to prevent catheter related infections (i.e. **antimicrobial biomaterials**)
    - ➢Can be employed as **nanoparticles** or **silver salts** (e.g. halides, oxide, nitrate etc)
    - ➢Can also be used in some **topical and cosmetic products**

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Biocides in Use

- Heavy metals
  - **Mercurials**:
    - ➢Among the **organo-mercurials** that are still in use (to a small extent) are **thiomersal** (merthiolate) and **phenylmercuric compounds** (nitrate PMN or acetate PMA).
    - ➢They are generally **bacteriostatic** agents
    - ➢Disadvantages: **toxicity**, **hypersensitivity**, local irritation & **absorbed** by **rubber** closure & **plastic** containers significantly
    - ➢**Thiomersal**: used as **preservatives** in **eye drops & contact lens solutions**. It was also used in **vaccines** but **stopped** due to possible **neurotoxicity**.
    - ➢**Phenylmercuric salts** (PMN or PMA) (0.002%): for preservation of **eye drops**,

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Biocides in Use

- Hydrogen peroxide & peroxygen compounds

  Are **high level disinfectants** due to production of **highly reactive hydroxyl radical**. Their decomposition products are non-toxic & biodegradable

  – **Hydrogen peroxide:**
    - ➢ High level disinfectant
    - ➢ Used as **antiseptic** for **open wounds & ulcers** as it also oxidizes organic debris
    - ➢ Also used for **disinfection** of soft **contact lenses** as it is active against ***Acanthamoeba*** which causes keratitis in contact lens wearers
    - ➢ 3-6% solution can be used as **general disinfectant**
    - ➢ At **high concentrations** (up to 35%) and increased temperature hydrogen peroxide is **sporicidal**.
    - ➢ **Vapour-phase** hydrogen peroxide is used for decontamination of **laboratory equipment** and **enclosed spaces**

Biocides in Use

- Hydrogen peroxide & peroxygen compounds

  – **Peracetic acid (CH_3COOOH):**
    - ➢ A **more potent** biocide than hydrogen peroxide, with excellent rapid biocidal activity against bacteria, including mycobacteria, fungi, viruses and spores
    - ➢ It can be used in both the **liquid and vapour phases** and is active even in the presence of organic matter.
    - ➢ At concentrations of 0.2-0.35% it is used for the **sterilization** of medical equipment (e.g. **endoscopes**).
    - ➢ **Combination** of **hydrogen peroxide & peracetic acid** is synergistic & is marketed as **cold sterilant for dialysis machine**
    - ➢ Disadvantages: **corrosive** to some metals, **highly irritant** and must be used in an enclosed system.

Biocides in Use

- Phenols
  - Phenols have been widely used as **disinfectants** and **preservatives**
  - As disinfectants, they have good antimicrobial activity and rapid bactericidal effect but generally are **not sporicidal**. They are **more active at acidic pH**.
  - The main **disadvantages** of phenols are their **caustic effect** on skin and tissues and their **systemic toxicity**.
  - Their activity is **markedly diminished by dilution** and is also **reduced by organic matter**
  - The more **highly substituted phenols** are **less toxic** and can be used as **preservatives and antiseptics**; however, some are also less active than the simple phenolics, especially against Gram-ve organisms

Biocides in Use

- Phenols
  - **Phenol (carbolic acid):**
    - One of the **oldest** used biocides but no longer plays any significant role as an antibacterial agent, except in some multi-dose vaccines
    - In the past it was used as standard for comparison with other disinfectants (i.e. **phenol coefficient tests**)
  - **Tar acids:**
    - Phenols obtained by distillation of **coal, oil shale** or petroleum. These compounds can be separated by **fractional distillation** according to their **boiling point** range into phenol, cresols, xylenols and high boiling point tar acids

Biocides in Use

- Phenols
  - **Tar acids: (cont'd)**
    - As the boiling point increases the properties of the products change as follows:

Phenol	Boiling point increases
Cresols	Bactericidal **activity increases**
Xylenols	Inactivation by organic matter increases
High b.p. tar acids	Water **solubility decreases**
	Tissue toxicity decreases

    - Phenols from the higher boiling point fractions have greater antimicrobial activity but must be formulated so as to overcome their poor solubility.
    - A range of **solubilized and emulsified** phenolic disinfectants are available including the clear soluble fluids, black fluids and white fluids

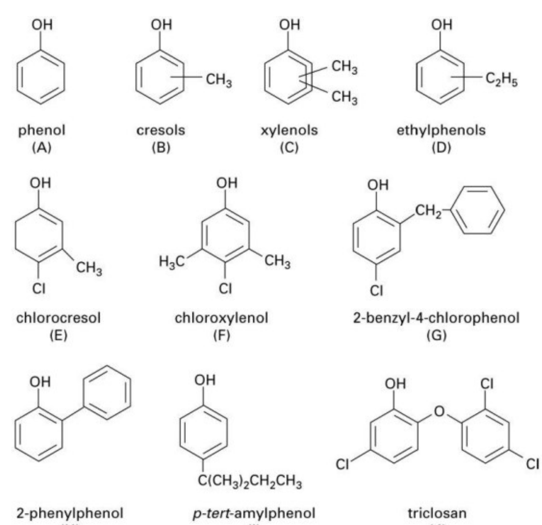


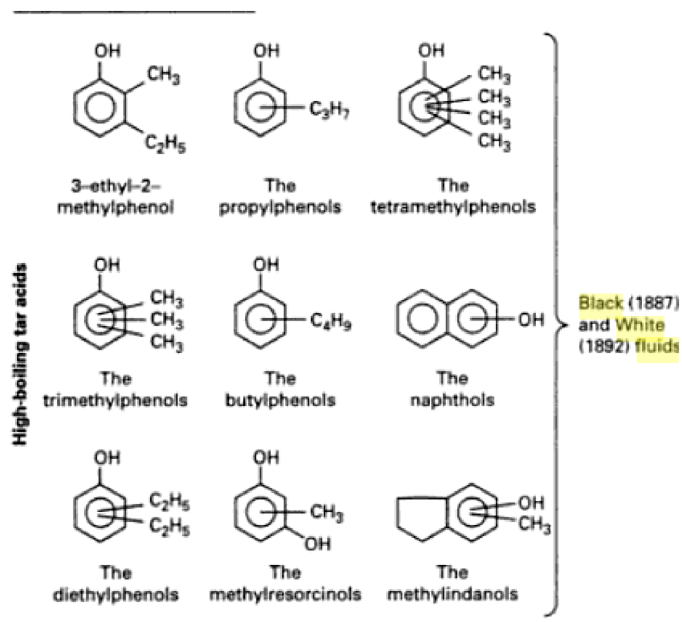
Figure 19.7 Structures of some common phenols possessing antimicrobial activity.

Biocides in Use

- Phenols
  - **Clear soluble fluids**:
    - Clear soluble fluids are produced from **cresols or xylenols**
    - A preparation known as **Lysol** (**cresol & soap** solution) is a soap-solubilized formulation of cresol. It has a wide use as **general purpose disinfectant**
    - A higher boiling point fractions consisting of **xylenols** and ethylphenols are also used for **general purpose disinfection**
    - **Advantages**: **More active** than phenol, **less corrosive** product which still retains activity in the presence of organic matter. They are solubilized with a soap to give a clear soluble fluid. It possesses rapid fungicidal & bactericidal activity, (including *Mycobacteria*)

Biocides in Use

- Phenols
  - **Black fluids and white fluids**:
    - Prepared by **emulsifying** or **solubilizing** high boiling point tar acids.
    - **Black fluids** are homogenous **solutions** which form an **emulsion on dilution** with water.
    - **White fluids** are finely dispersed **stable emulsions** of tar acids, which on dilution with water produce more stable emulsions than that of black fluids.
    - Both types have good bactericidal activity.
    - But such preparations are **irritant and corrosive to the skin** and are **strong smelling**; however, they are relatively **inexpensive** and are useful for household and **general disinfection purposes**.
    - They must be used in adequate concentrations as activity is **reduced by organic matter** and is markedly **affected by dilution**.



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Biocides in Use

- Phenols
  - **Chlorinated synthetic phenols**:
    - ➢Two of the most widely used chlorinated derivatives are
      - ✓ **Chlorocresol** which is mostly used as a **preservative** (0.1%)
      - ✓ **Chloroxylenol** (Dettol) which can be used for **skin antisepsis**, although less than formerly.
    - ➢**Chloroxylenol** is sparingly soluble in water and must be **solubilized**, e.g. in a suitable soap solution in conjunction with terpineol or pine oil.
    - ➢Its antimicrobial capacity is **weak** and is reduced by the presence of **organic matter**.

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Biocides in Use

- Phenols
  - **Bisphenols**:
    - ➢Bisphenols are composed of two phenolic groups connected by various linkages.
    - ➢They include hydroxy halogenated derivatives, such as **triclosan** & **hexachlorophane**.
    - ➢Among these, **triclosan** the **most widely used**. It is **bacteriostatic** at use-concentrations and have little antipseudomonal activity.
    - ➢Triclosan has application in medicated soaps, lotions & solutions, tooth pastes and **household products** (fabrics & plastics).
    - ➢Concerns of bacterial **resistance**!

Biocides in Use

- Surface active agents
  - Surfactants are classified as anionic, cationic, non-ionic or ampholytic according to the ionization of the hydrophilic group in the molecule. They have a range of **detergent and disinfectant** activity.
  - The **anionic and non-ionic** surfactants, have **strong detergent** properties but exhibit little or **no antimicrobial activity**. But they can, render certain bacterial species more sensitive to some antimicrobial agents, by altering the permeability of the outer envelope.
  - **Ampholytic** or amphoteric surfactants can ionize to give anionic, cationic and zwiterionic forms. Consequently, they possess the **detergent** properties of the anionic surface-active agents and the **antimicrobial activity** of the cationic agents. They are used quite extensively in Europe for pre-surgical **hand scrubbing**, medical instrument and floor **disinfection** in hospitals.
  - The **cationic** compounds play the **most important role as antimicrobials**.

Biocides in Use

- Surface active agents
  - **Cationic surface-active agents**:
    - ➢All the antimicrobial cationic agents fall within the group known as the **quaternary ammonium compounds** QACs, quats or onium ions.
    - ➢QACs are organically substituted ammonium compounds where the R substituents are alkyl or heterocyclic groups to give compounds like **cetyltrimethyl ammonium bromide (cetrimide), cetylpyridinium chloride and benzalkonium chloride**
    - ➢For good antimicrobial activity at least one R should have a chain of **C8 to C18**.
    - ➢QACs are most effective against microorganisms at **neutral or slightly alkaline pH** and become inactive below pH 3.5

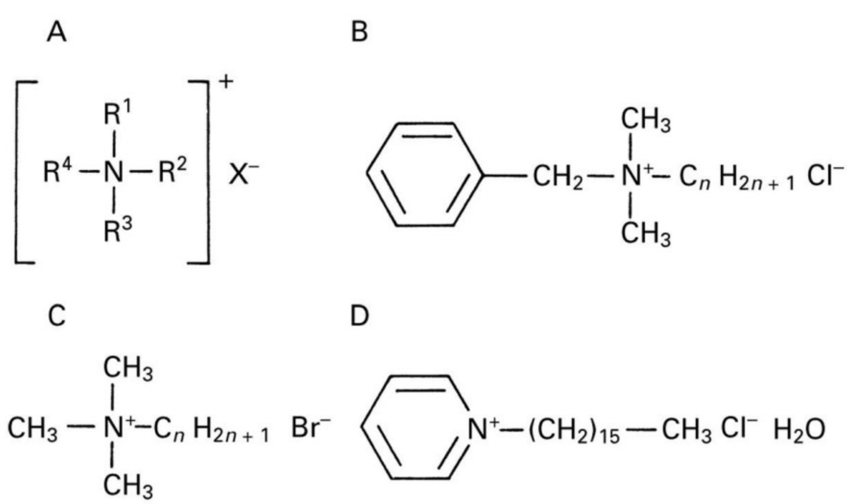

Figure 19.8 Quaternary ammonium compounds (QACs): (A) general structure of QACs; (B) benzalkonium chloride (n = 8–18); (C) cetrimide (n = 12–14 or 16); (D) cetylpyridinium chloride.

Biocides in Use

- Surface active agents
  - **Cationic surface-active agents: (cont'd)**
    - ➢**Incompatibility**: some **anions** greatly reduce the activity of these cationic agents. Also incompatible with non-ionic surfactants, due to the formation of micelles. The presence of **organic matter** such as serum, faeces and milk will also seriously affect activity.
    - ➢QACs have relatively narrow spectrum of activity, they exhibit greatest activity against **G+ve** bacteria with lethal effect at low conc. G-ve **bacteria are more resistant** requiring higher conc especially if *P. aeruginosa* is present.
    - ➢QACs have a **limited antifungal** (fungistatic) activity and they have **no sporicidal** activity
    - ➢Advantages: generally **well tolerated** and non-toxic when applied to skin and mucous membranes

Biocides in Use

- Surface active agents
  - **Cationic surface-active agents: (cont'd)**
    - ➢They are used in **treatment of wounds** and abrasions and they are used as **preservatives** in certain preparations.
    - ➢The detergent properties of the QACs are also useful, especially in hospitals, for general environmental sanitation.
    - ➢**Benzalkonium chloride and cetrimide** are employed extensively as **antiseptics** in surgery, urology and gynaecology as aqueous and alcoholic **solutions** and as **creams**.
    - ➢Many times they are used in **combination with biguanides** (e.g. cetrimide and chlorhexidine in **Savlon®**)
    - ➢**Alkyl dimethylbenzyl** ammonium chloride and **alkyl dimethylethylbenzyl** ammonium chloride are used for disinfection where **HIV & HBV** are present

Biocides in Use

- Other antimicrobials:
  - **Diamidines:**
    - ➢**Propamidine** in the form of **eye-drops** (0.1%) for **amoebic eye infections** and **dibromopropamidine** for topical treatment of minor infections
  - **Dyes:**
    - ➢**Crystal violet, brilliant green and malachite green** are triphenyl-methane dyes used to stain bacteria, they have **bacteriostatic & fungistatic** activity but their use is restricted due to **carcinogenicity** (rarely used nowadays)
    - ➢**Acridine dyes** (acriflavine & aminacrine) only have slow **bacteriostatic** activity, can be used for **skin antisepsis** & treatment of infected **wounds or burns**.

Biocides in Use

- Other antimicrobials:
  - **Quinoline derivatives**:
    - ➢Active against **G+ve**, less active against G-ve bacteria. **Dequalinium chloride** used as a **lozenge** for the treatment of **oropharyngeal infections**.
  - **Ozone (O_3):**
    - ➢**Highly active oxidizing agent**, used for **disinfection of water** (drinking, sewage, bottled water & swimming pools)& food (surface of eggs).
    - ➢Have some applications as **sterilizing agent**.
    - ➢Used as **gas & liquid**.
    - ➢Has **low stability**.

Biocides in Use

- Other antimicrobials:
  - **Isothiazolinones**:
    - Such as methylisothiazolinone (MIT) chloromethylisothiazolinone (CMIT)
    - Broad spectrum; active against G+ve bacteria, G-ve bacteria & fungi.
    - Used as preservatives in cosmetics and personal care products (e.g. shampoos).

Antimicrobial Combinations and Systems

- To overcome the limitations of antimicrobials (incompatibility, irritancy, narrow spectrum, resistance etc.) formulations containing combinations of agents are frequently used, examples:
  - Ethanol + chlorhexidine + iodine (gives more active preparation).
  - Chlorhexidine + cetrimide (Savlon®)
  - Gluteraldehyde + QACs or phenols to obtain same effect with less irritant conc of gluteraldehyde
  - Hydrogen peroxide + peracetic acid (synergistic activity)
  - Combination of disinfectant & **quorum sensing inhibitors** to inhibit or eliminate **biofilms**.
  - Incorporation of antimicrobials into materials that will form **antimicrobially active surfaces** or implants. These **"bioactive" surfaces** can be formed by incorporating silver salts & alloys, biguanides & triclosan. They reduce infection arising from microbial adherence & **biofilm formation**.

Disinfection Policy

- The aim of such policy is to control the use of chemicals for disinfection and antisepsis and give guidelines on their use.
- The control of microorganisms is of prime importance in hospital and industrial environments. Usually there is a committee (**Infection Control Committee**) who is responsible to set & implement the policy (i.e. what disinfectants to use, conc, where to use them (floors, ceiling, walls, air, instruments), contact time)
- In hospitals, **categories of risk to patients** may be assigned to **equipment** that come in contact with them dictating the level of decontamination needed.
  - **High risk items**: have **close contact** with **broken skin or mucous membrane** or are those introduced into a **sterile area of the body**. These should be **sterile**. e.g. surgical equipment, gloves, catheters, syringes and needles.
  - **Intermediate-risk items**: are in **close contact** with **intact skin or mucous membranes** and **disinfection** will normally be applied. e.g. endoscopes, respiratory and anaesthetic equipment, wash bowls and bed-pans.
  - **Low-risk items or areas**: which are **not in close contact with the patient**. e.g. walls, floors, ceiling, etc.