STERILIZATION AND DISINFECTION
STERILIZATION AND DISINFECTION

✓ Chemical and physical methods of disinfection and sterilization
✓ Principles and application of each method
✓ Common disinfectants and antiseptics used in healthcare settings
✓ Sterilization control
STERILIZATION AND DISINFECTION

Historical background

• The scientific use of disinfection and sterilization methods originated more than 100 years ago

• Ignatz Semmelweis (1816-1865) and Joseph Lister (1827-1912)
  – important pioneers for the promotion of infection control
STERILIZATION AND DISINFECTION

Historical background

• More than 100 years ago, Semmelweis demonstrated that routine handwashing can prevent the spread of disease
• He worked in a hospital in Vienna when maternity patients were dying at an alarming rate
• He recognized that medical students worked on cadavers during an anatomy class and afterwards they went to the maternity ward.
• Students did not wash their hands between touching the dead and the living!!!
• After administering the handwashing before examining the maternity patients the mortality rate decreased
STERILIZATION AND DISINFECTION

Historical background
STERILIZATION AND DISINFECTION

Historical background

• **Lister**, for the first time, used **carbolic acid** in operating theatres that significantly reduced mortality rates.

• Later when it was accepted that microorganisms were the causative agents of infections in 1867, **Lister** introduced British surgery to **hand washing** and the use of **phenol as antimicrobial agent** for surgical wound dressings.

• His principles were gradually and adopted in Britain and later in US.

• This was the beginning of **infection control**.
STERILIZATION AND DISINFECTION
Historical background

Sir Joseph Lister
STERILIZATION AND DISINFECTION

Historical background

Early Disinfection

Phenol being sprayed over an operation wound by 19th Century surgeons
PRINCIPLES OF STERILIZATION AND DISINFECTION

Sterilization

- destruction of **ALL** forms of life, including the bacterial spores, viruses, prions
- no degrees of sterilization: an **all-or-nothing process**
- physical or chemical methods
Disinfection

- a process that eliminates a defined scope of microrganisms, except most spores, viruses and prions
- the purpose - prevent transmission of certain microorganisms with objects, hands or skin and prevent spreading the infection
- physical or chemical methods
- most disinfectants are chemical agents applied to inanimate objects!
PRINCIPLES OF STERILIZATION AND DISINFECTION

Disinfection

- **decontamination** - removal of microorganisms contaminating an object
- **preservation** - preventing methods of microbe-caused spoilage of susceptible products (pharmaceuticals, foods)
- **sanitisation** - removal of microbes that pose a threat to the public health, food industry, water conditioning
  - **sanitizer** - an agent, usually a detergent, that reduces the numbers of bacteria to a safe level
PRINCIPLES OF STERILIZATION AND DISINFECTION

Disinfection

- **aseptic techniques**- prevent microbial contamination of materials or wounds
- **antisepsis**- disinfection of living tissues (e.g., in a wound), achieved through the use of **antiseptics**
- **antiseptics** are applied (do not kill spores) to reduce or eliminate the number of bacteria from the skin
Factors that influence the degree of killing

- Types of organisms
- Number of organisms
- Concentration of disinfecting agent
- Presence of organic material (e.g., serum, blood)
- Nature (composition) of surface to be disinfected
- Contact time
- Temperature
- pH
- Biofilms
- Compatibility of disinfectants and sterilants
Factors that influence the degree of killing

Types of organisms

✓ Organisms vary in their ability to withstand chemical and physical treatment, e.g.,
  - **Spores** – have coats rich in proteins, lipids and carbohydrates
  - **Mycobacteria** – cell walls are rich in lipids
  - **Biofilms** - microorganisms living together in communities
  - **Prions** – the most resistant known organisms to the action of heat, chemicals, and radiation !!!

**Prions** can withstand temp. exceeding 121 C for several hours while immersed in acid or basic solutions
Factors that influence the degree of killing

Different types of organisms and their resistance to killing agents
Factors that influence the degree of killing

Number of organisms

Microbial load - the total number of organisms which determine the exposure time of killing agent
- is composed of organisms with varying degrees of susceptibility to killing agents
- not all organisms die at the same time
- the death curve is logarithmic!!!
- higher numbers of organisms require longer exposure
Factors that influence the degree of killing

The effect of exposure time versus number of organisms
Factors that influence the degree of killing

Concentration of disinfecting agent

✓ a proper concentration of disinfecting agents ensure the activation of target organisms, e.g.,

- povidone-iodine should be diluted with water before use because there is not enough free iodine to kill microorganisms in concentrated solution
Factors that influence the degree of killing

Presence of organic material
(such as blood, mucus, pus)
✓ affects killing activity by inactivating the disinfecting agent, e.g,
- by coating the surface to be treated, prevents full contact between object and agent (Glutaraldehyde)
- easily inactivate bleach (Sodium hypochlorite)

For optimal killing activity, instruments and surfaces should be cleansed of excess organic material before disinfection !!!
Factors that influence the degree of killing

Nature (composition) of surface to be disinfected

- some medical instruments are manufactured of biomaterials that exclude the use of certain disinfection and sterilization methods because of possible damage, e.g.,

- endoscopic instruments cannot be sterilized by the heat in an autoclave
Factors that influence the degree of killing

Contact time

✓ the amount of time a disinfectant or sterilant is in contact with the object is critical! e.g.,
- **Betadine** (alcohol and iodine) must be in contact with object for at least 1 to 2 min. to kill microorg.
- the spores of bacteria and fungi need a much longer time

✓ determine whether it is disinfecting or sterilizing the object
Factors that influence the degree of killing

Temperature
✓ disinfectants are generally used at room temp. (20 C- 22 )
✓ their activity is increased by an increased temp. and decreased by a drop in temp.
Factors that influence the degree of killing

**pH**

✓ The pH of the material to be disinfected or sterilized can have an effect on the activity of disinfecting or sterilizing agent
Factors that influence the degree of killing

Biofilms
- communities of microorganisms
- can be on a surface of either inanimate or animate objects, e.g.,
  - catheters (critical place!), pipes that carry water and dionizing columns used to make processed water
- make disinfection more difficult
- the concentration of the disinfectant and the contact time need to be increased
Factors that influence the degree of killing

Compatibility of disinfectants

☑ a common mistake is to believe that two disinfectants are better than one!

☑ some of them may inactivate other, e.g.,

- the bleach and quaternary ammonium compound together negate the activity of both
STERILIZATION AND DISINFECTION

Medical materials are categorized into three device classifications:

- Critical materials
- Semicritical materials
- Noncritical materials
STERILIZATION AND DISINFECTION

- Critical materials
  - invade sterile tissues or enter the vascular system
  - most likely to produce infection if contaminated and therefore require sterilization
STERILIZATION AND DISINFECTION

- **Semicritical materials**
  - have contact with mucosal membranes
  - require high-level disinfection agents

- **Noncritical materials**
  - have contact with intact skin
  - require intermediate-level to low-level disinfection
STERILIZATION AND DISINFECTION

✓ **high-level disinfectants**
  - activity against bacterial spores

✓ **intermediate-level disinfectants**
  - tuberculocidal activity but not sporocidal

✓ **low-level disinfectants**
  - a wide range of activity against microorganisms but no sporocidal or tuberculocidal activity
<table>
<thead>
<tr>
<th>Device Classification</th>
<th>Disinfection Method</th>
<th>Spores</th>
<th>Mycobacteria</th>
<th>Nonlipid Viruses</th>
<th>Fungi</th>
<th>Bacteria</th>
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</thead>
<tbody>
<tr>
<td>Critical</td>
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<td>Low-level disinfection</td>
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<td>Ethyl, isopropyl</td>
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<td>-</td>
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<td></td>
<td>alcohol (70%-90%)</td>
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<tr>
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<td>Iodophors</td>
<td>-</td>
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<td>+</td>
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</tr>
</tbody>
</table>
STERILIZATION AND DISINFECTION

Physical methods

- Heat
  - moist heat
  - dry heat
  - pasteurisation
  - boiling
- Filtration
- Radiation
STERILIZATION AND DISINFECTION

Physical methods

Heat

The most common method used for elimination of microorganisms!
- reliably effects
- easy of use
- economic
STERILIZATION AND DISINFECTION

Physical methods

Heat

moist heat (heat under steam pressure)
- destroy ALL microorganisms (an exception being prions) and their spores
- steam under 1 atm of pressure, at 121 °C temp., 15 min. of exposure in autoclaves or
- 2 atm./ 134 °C/ 3 min.
- destroy the prions at higher temperatures, in longer times: 135 °C for at least 1 hour under 2 atm

Application: the sterilization method of choice for heat-stable objects
STERILIZATION AND DISINFECTION

Physical methods (autoclaves)
STERILIZATION AND DISINFECTION

Physical methods

➢ Heat

dry heat

- requires much longer exposure times and higher temperatures than moist heat
- 2 hours at 160°C in dry air ovens or
- 30 min. at 180°C

Application: sterilization for heat-stable substances that are not penetrated by moist heat, such as oils; for glasswear or surgical instruments
STERILIZATION AND DISINFECTION

Physical methods (dry heat oven)
STERILIZATION AND DISINFECTION

Physical methods

- **Heat**
  Boiling and pasteurisation achieve disinfection but not sterilisation (do not eliminates spores)!!!

**boiling** (decoctation)
- kills most microorganisms in **10 min. at 100°C**
- **tyndallisation** - an exposure of **100°C for 20 minutes** on 3 successive days; **sporicidal**
STERILIZATION AND DISINFECTION

Physical methods

**pasteurisation**
- do not kill spores
- **LTH** (low temperature holding) *batch method* - 63-65°C for 30 min.
- **UHT** (ultra-high temperature) - 135°C for 1-2 sec.
- **HTST** (high temperature short time) *flash method* - 72°C for 15 sec.

**Application:** in the food industry, eliminating food-born pathogens without affecting the taste (e.g. UHT milk)
# Sterilization and Disinfection

## Physical Methods (Heat)

<table>
<thead>
<tr>
<th>Method</th>
<th>Temperature (°C)</th>
<th>Time Required</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boiling water (steam)</td>
<td>100</td>
<td>15 minutes</td>
<td>Kills microbial vegetative forms; endospores survive</td>
</tr>
<tr>
<td>Autoclave (steam under pressure)</td>
<td>121.6</td>
<td>15 minutes at 15 psi</td>
<td>Sterilizes and kills endospores</td>
</tr>
<tr>
<td>Pasteurization</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Batch method</td>
<td>63</td>
<td>30 minutes</td>
<td>Disinfects and kills milk-borne pathogens and vegetable forms; endospores survive</td>
</tr>
<tr>
<td>Flash method</td>
<td>72</td>
<td>15 seconds</td>
<td>Same, but shorter time at higher temperature</td>
</tr>
<tr>
<td>Over (dry heat)</td>
<td>160-180</td>
<td>1.5-3 hours</td>
<td>Sterilizes; keeps materials dry</td>
</tr>
</tbody>
</table>

Adapted from VanDemark PJ, Batzing BL: *The microbes: an introduction to their nature and importance*, Redwood City, Calif, 1987, Benjamin-Cummings.
STERILIZATION AND DISINFECTION

Physical methods

Filtration

- of liquid
  - the membrane filters composed of plastic polymers or cellulose esters containing pores of certain size
  - liquid is pulled (vacuum) or pushed (pressure) through the filter matrix, organisms larger than the size of the pores are retained
  - pore size of:
    - 0.45 and 0.80 μm - most bacteria, yeasts and molds
    - 0.22 μm - for critical sterilizing, e.g. parenteral solutions
    - 0.01 μm - for retaining small viruses

Application: parenteral solutions (serum), vitamins, vaccines and antibiotic solutions
STERILIZATION AND DISINFECTION
Physical methods (filters)
STERILIZATION AND DISINFECTION

Physical methods

Filtration

- filters remove microorganisms larger than 0.3 µm
- the high efficiency participate air (HEPA) filters

Application: in laboratory hoods and in rooms of immunocompromised patients
STERILIZATION AND DISINFECTION

Physical methods (filters)

Groundwater Filtration

Filter Monitors
STERILIZATION AND DISINFECTION

Physical methods

Radiation
- used in two forms: ionizing and nonionizing

- ionizing radiation
  - gamma rays or electron beams
  - short wavelength and high energy

Application: for the medical industry: the sterilization of disposable supplies (syringes, bandages, catheters and gloves) and heat-sensitive pharmaceuticals
STERILIZATION AND DISINFECTION
Physical methods

Radiation
- **nonionizing**
  - in the form of **ultraviolet rays (UV)** (280-200 nm)
  - long wavelength and low energy
  - poor penetrability
  - the use is limited

**Application**: disinfect smooth surfaces with ultra violet lamps and to reduce airborne pathogens (surgical theaters, filling equipment)
STERILIZATION AND DISINFECTION

Just as physical methods are used mainly to achieve sterilization, chemical agents are used mainly as disinfectants.

- some chemical agents may be used to sterilize (chemosterilizers)
STERILIZATION AND DISINFECTION
Chemical agents

- Alcohols
- Aldehydes
- Halogens
- Phenols
- Surfactants
- Heavy metals
- Dyes
- Gases (ethylene oxide, oxidants)
STERILIZATION AND DISINFECTION

Chemical agents

- exert their killing effect by the following mechanisms:
  - reaction with components of the cytoplasmic membrane (sufactant compounds, alcohols)
  - denaturation of cellular proteins (alcohols, phenols, aldehydes, oxidants)
  - reaction with the thiol (-SH) groups of enzymes (heavy metals)
  - damage of RNA and DNA (aldehydes, oxidants, dyes)

- the agent can exert one or a combination of actions on microorganisms
STERILIZATION AND DISINFECTION

Chemical agents

Alcohols

- **ethanol 70%, isopropanol 70%, propanol 60%**
- inactivate microorganisms by denaturing proteins
- wide spectrum against *bacteria and fungi* but not **sporocidal**!
- **tuberculocidal and virucidal** for most viruses (15 min.)

- alcohols may be contaminated with spores – should be filtered through a 0.22 µm filter
- the most effective concentrations are between 60%- 90% (water is needed in chemical reactions)

**Application:** surgical and hygienic **disinfection** of the skin and hands
STERILIZATION AND DISINFECTION

Chemical agents

Aldehydes

- formaldehyde (HCHO) the most important
- is a water-soluble gas - formalin (35% solution of this gas in water) or glutaraldehyde (disinfectant and sterilizer!!!)
- denature proteins and nucleic acids
- irretate mucosa, skin contact may result inflamations or allergic eczemas
- broad-septrum: against bacteria, fungi, and viruses
- chemosterilizer in higher concentrations (sporicidal)

Application: - disinfection of surfaces and objects (plastic and rubber items)
  - the sterilizer of choice for heat-sensitive medical equipment
STERILIZATION AND DISINFECTION
Chemical agents

Halogens (chlorine, iodine, and their derivatives)

- chlorine
  - denatures proteins by oxidative effects after dissolution with water of chloride ions
  - used in the form of hypochlorite (e.g. liquid sodium hypochlorite - household bleach)
  - broad-spectrum activity, sporocidal required the long exposure time
  - corrosive

Application: disinfection of water and swimming pool, cleaning and washing products
STERILIZATION AND DISINFECTION

Chemical agents

Halogens

- **Iodine** (2 forms)
  - **tincture** (alcohol and iodine)
  - **iodophores** (iodine and surfactants)
  - denatures proteins by oxidative effects
  - **bactericidal, not sporocidal**
  - less irritant than pure iodine

**Application:** as aniseptics, disinfection of skin and small wounds
STERILIZATION AND DISINFECTION
Chemical agents

Phenols

- **Lister** was the first to use phenol (**carbolic acid**)
- today, chemically substituted with organic groups by halogens, alkyl, phenyl and benzyl groups
- denaturate proteins
- irritate the skin, corrosive
- **broad-spectrum**, but **not sporocidal, not virucidal**

**Application:** widely used, **disinfection** of hospital, institutional, and household environment (soaps)
STERILIZATION AND DISINFECTION

Chemical agents

Surfactants
- also known as surface-active agents, tensides, detergents
- anionic, cationic, amphoteric, and nonionic detergent compounds
- cationic and amphoteric types- the most effective!
- moderate bactericidal effect (good against Gram(+), not sporecidal, not tuberculocidal, not nonencapsulated viruses)
  BUT
- low toxicity level, lack of odor, good skin tolerance and cleaning effect

Application: disinfection of noncritical surfaces (bench tops, floors)
STERILIZATION AND DISINFECTION

Chemical agents

**Heavy metals**
- bind irreversibly to the sulfhydryl groups of proteins
- rather **bacteriostatic**

**Application:** - *rarly used* in clinical applications
  - **silver nitrate** (1% eyedrop solution) used in the prevention of eyes infections caused by *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in newborns
STERILIZATION AND DISINFECTION
Chemical agents

Dyes
- interact with bacterial nucleic acids
- acridine dyes, ethidium bromide, proflavine hemisulphate, triphenylmethane, brilliant green, crystal violet

Application: used topically as antiseptics to treat e.g. mild burns
STERILIZATION AND DISINFECTION

Chemical agents

Gases

- **Ethylene oxide**
  - the most commonly used for **sterilization**
  - alkylates of nucleic acids in the cell wall
  - at **low temp. 20-60 \degree C, 40-60\% humidity** in sterilizing chamber
  - highly reactive gas, flammable, toxic, a strong mucosal irritant
  - explosive in pure form!
  - active against **ALL** microorganisms and spores

**Application:** widely in hospitals for materials that cannot withstand steam sterilization
STERILIZATION AND DISINFECTION
Chemical agents

Gases

- Other Oxidants
  - ozone, hydrogen peroxide, potassium permanganate, and paracetic acid
  - split off oxygen, affects proteins and DNA acids

Application: as skin, mucosa, wound disinfectants
STERILIZATION AND DISINFECTION

Chemical agents

Plasma sterilisation

- plasma - ionized gas
- a collection of electrically charged particles and non-charged particles, "the fourth state of matter"
- sterilize at room temperature and pressure

Application: for sterilization
STERILIZATION CONTROL

- to ensure that potentially infectious agents are destroyed by adequate sterilisation regimes
- three levels:
  - **physical**: measuring device control (temp., time, pressure)
  - **chemical**:
    - substances that undergo a colour change or have melting points within the sterilizing range
      - Browne's tubes, Bowie Dick tape
      - give an immediate indication of a successful or non successful sterilization
STERILIZATION CONTROL

- **biological:**
  - *Bacillus stearothermophilus* spores \((10^4 - 10^6\) organisms)
    - survives steam heat at 121°C for 5 min. and is killed at 121°C in 13 min.
    - validate and determine the adequacy of steam or chemical vapor sterilisation
  - *Bacillus subtilis* spores
    - validate and determine the adequacy of ethylene oxide or dry heat sterilisation
Browne’s tubes are glass tubes that contain heat sensitive dyes. These change colour after sufficient time at the desired temperature.

Before heat exposure, the contents of the tube appear red.

As heating progresses, the colour changes to green.

Only when the tube is green sterilisation conditions can be considered adequate.
STERILIZATION CONTROL

Chemical indicators

*Bowie Dick tape* is applied to articles being autoclaved. Before heat exposure, the tape is uniformly buff in colour.

After adequate heating, the tape develops **dark brown** stripes.

The pack on the left has been properly sterilised; that on the right has not.
STERILIZATION CONTROL

Chemical indicators

Steam Sterilisation Indicators

Dry Heat Indicator Labels
STERILIZATION CONTROL

Chemical indicators

Raven Vacuum Test Pack
(a Bowie-Dick type test)
STERILIZATION CONTROL

Biological (the use of spores)
STERILIZATION AND DISINFECTION

Sterilization methods

✅ Physical methods
- Moist heat in autoclaves
- Dry-heat in ovens
- Gamma irradiation
- Filtration
- Plasma sterilization

✅ Chemical agents
- Ethylene oxide
- Glutaraldehyde (high concentration)

Disinfection methods

✅ Chemical agents
- Alcohols
- Aldehydes
- Halogens
- Phenols
- Surfactants
- Heavy metals
- Dyes
- Oxidants

✅ Physical methods
- Boiling and pasteurisation
- Ultraviolet radiation
## Table 4-1: Device Classification and Methods of Effective Disinfection

<table>
<thead>
<tr>
<th>Device Classification</th>
<th>Disinfection Method</th>
<th>Killing Action Against</th>
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<tbody>
<tr>
<td></td>
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<td>Spores</td>
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<tr>
<td>Critical</td>
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<td>Semicritical</td>
<td>High-level disinfection</td>
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<td>2% glutaraldehyde</td>
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<td>Chlorine dioxide</td>
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<td>Wet pasteurization</td>
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<td>Low-level disinfection</td>
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<tr>
<td></td>
<td>Sodium hypochlorite</td>
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<td>Quaternary ammonium compounds</td>
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<td>Ethyl, isopropyl alcohol (70%-90%)</td>
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<td>Phenolics</td>
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</tr>
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<td></td>
<td>Iodophors</td>
<td>−</td>
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</tbody>
</table>
PRACTICAL DISINFECTION

- Hygienic hand disinfection
- Surgical hand disinfection
- Disinfecting patient’s skin
- Disinfection of excretion
- Surface, instrument, laundry, final room, hospital disinfection
- Disinfection of drinking water and swimming-pool
PRACTICAL DISINFECTION

- **Hygienic hand disinfection**
  - disinfect hands contaminating with pathogenic organisms
  - Not necessary to kill spores
  - *Alcohols* are the agent of choice!

- **Surgical hand disinfection**
  - render a surgeon’s hands as free of organisms as possible
  - after washing the hands throughly,
  - *alcoholics preparation* or *alcohols combined with disinfectants* (e.g. quaternary ammonium comp.), *iodophores*
PRACTICAL DISINFECTION

- Disinfecting patient’s skin
  - in preparation for surgery and injections
  - alcohols, iodine compounds

- Disinfection of excretion
  - feces, sputum, urine, etc.
  - not necessary to kill spores
  - strong-smelling agents, like phenolic preparations
Surface, instrument, laundry, final room disinfection

- **surfaces** - aldehyde and phenol derivatives combined with surfactants
- **laundry** - heat, phenols, aldehyde, and chlorine derivatives, surfactants
- **final room disinfection** - atomization of formaldehyde, ultra-violet radiation
PRACTICAL DISINFECTION

- **Disinfection of drinking water and swimming-pool**
  - *chlorine* is the agent of choice!
  - easily dose, acts quickly, broad disinfectant range
  - the recommended concentration level of drinking water 0.1-0.3 mg/l
  - for swimming-pool water 0.5 mg/l