Sterilization (Part II)

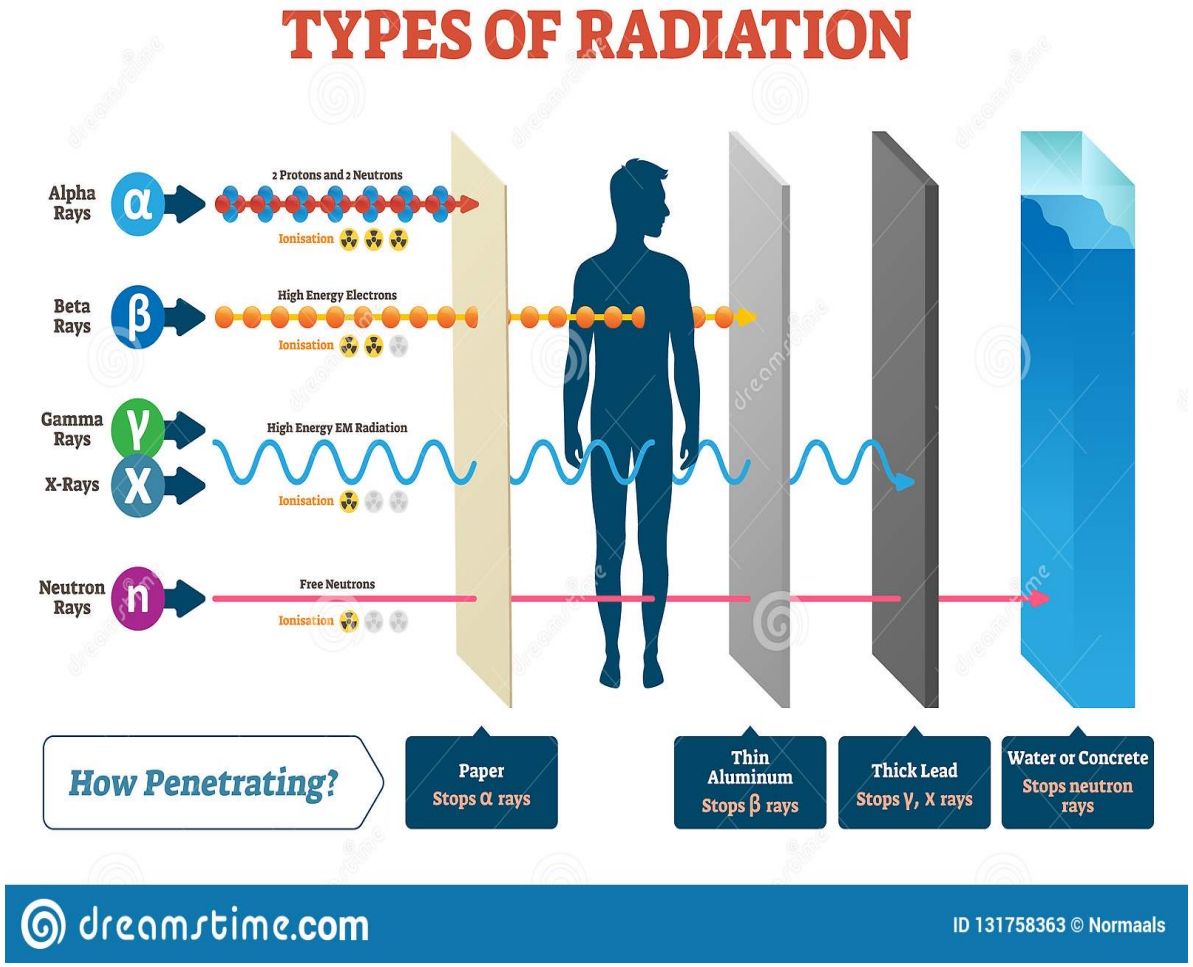
Radiation Sterilization
Ionization Radiation

- High energy radiations emitted from radioactive isotopes such as cobalt-60 (gamma rays), or produced by mechanical acceleration of electrons to very high velocities and energies (cathode rays, beta rays). Radiation is divided into two types:

I. Electromagnetic radiation such as gamma rays and x-rays.

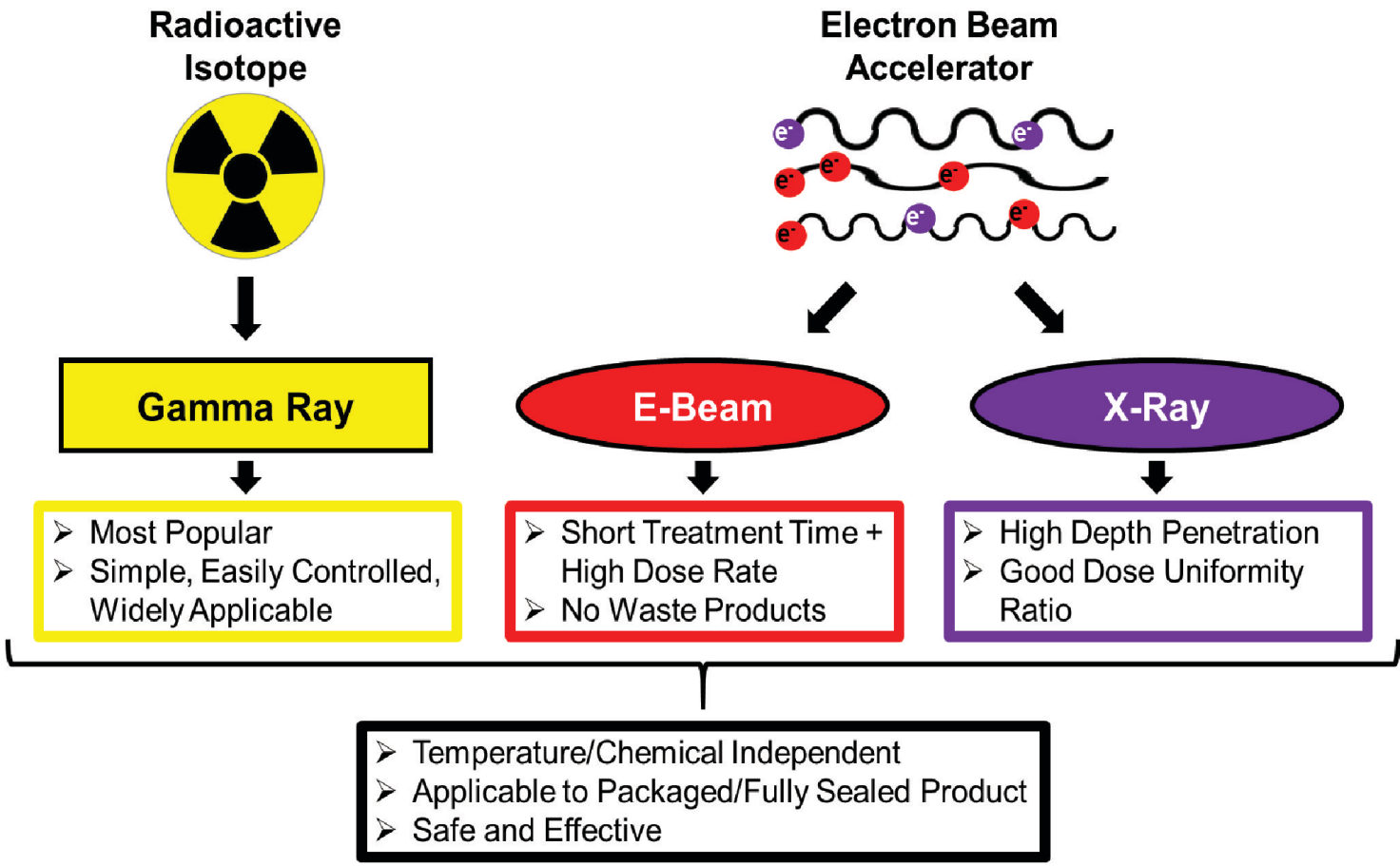
II. Particulate radiation such as alpha, beta, and neutron rays:

- Both alpha and beta do not have good penetration → cannot be used in sterilization.
- Neutrons: posses a safety hazard → not used



Ionization Radiation

- Of these, only **γ-rays (Gamma)** and **high-speed electron beams** are used for sterilization of pharmaceutical products, since other forms of radiation have not been shown to be effective as sterilant and/ or are not suitable.



Ionization Radiation
Electromagnetic Radiation

- **Gamma rays**
- **Advantages:**

a) Absolutely **reliable**, no mechanical breakdown

b) Providing a **higher** and more **uniform dose** rate output.

https://youtu.be/hblMTH09KJQ

- **Disadvantages:**
- Their source (**radioactive material**) is relatively expensive.
- **Emission cannot be shut off** as it can from the mechanical source of accelerated electrons.
- **Require special experience** which is not available in most pharmaceutical plants so they need to contract with another company which will **add cost** to their product

Gamma Radiation

- **Mechanism**:
- **Direct**: Ionizing radiation destroys microorganisms by **stopping reproduction** as a result of **lethal mutations**. The DNA is the principal target here.
- This happened by **transfer of energy from radiation beam** to molecules in their path.
- Or mutation can happen **indirectly** in which water molecules are transformed into highly-energized entities such as hydrogen and hydroxyl ions. → this will cause energy changes in nucleic acids and other molecules. And eliminating their availability for the metabolism of bacterial cell.
- Ionizing radiation **differ form ultraviolet rays** in their effects on matter primarily in that radiation **has higher energy** that produce ionization in the target molecules.

Units of Radioactivity

- The unit of absorbed radiation is gray (Gy).

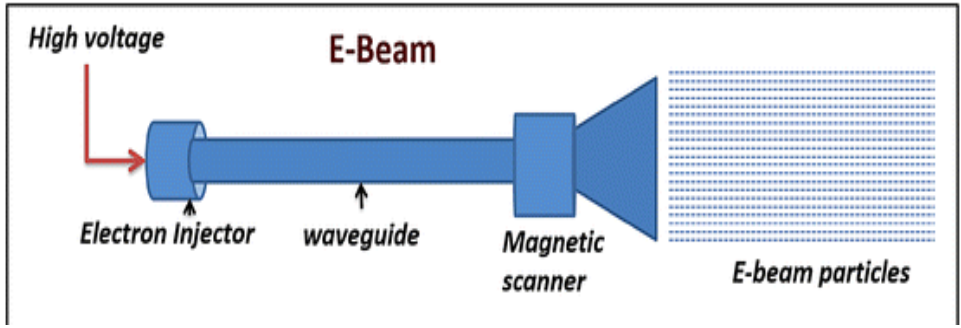
- 1 Gray = 100 rad (energy absorbed per gram of substance).
- The energy of radiation is measured in electron volts (eV) or millions of electron volts (MeV).
- The depth of penetration within a target of a given dose is **directly related** to the electron voltage of the source and **indirectly** related to the density of the material to be irradiated.
- This explain **why Gamma rays are reliable** method because it is easy to control the amount of radiation required with no extra damage to the material.
- **Note:** Bacterial spores and viruses are generally four to five times more resistant than vegetative bacteria and molds.

Ionization Radiation
Electron Accelerator

- Electron accelerators are of two general types:

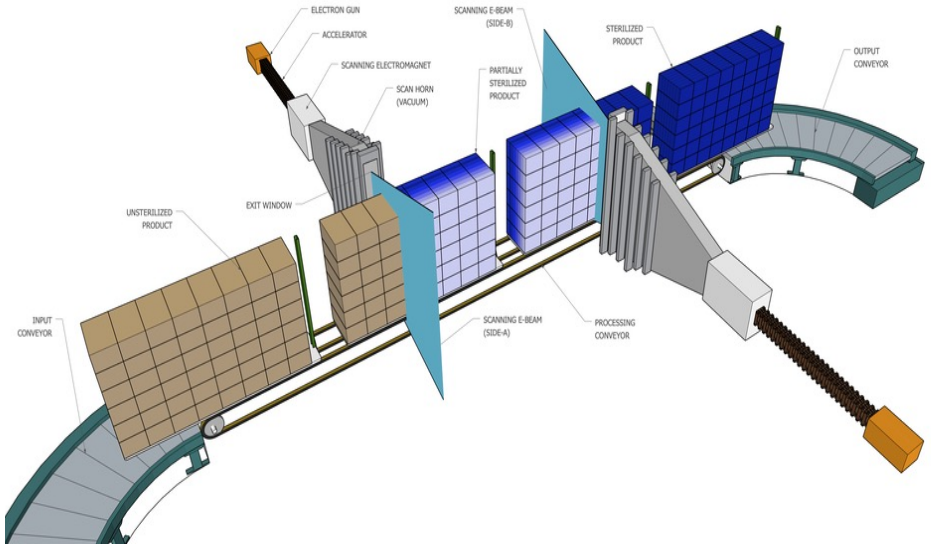
1. linear accelerators

- **Principle**: very high-frequency microwaves (radar) collect electrons from a cathode and accelerate electrons as they travel through the vacuum tube reaching the speed of light
- The electrons are emitted and directed to the target at an energy range of 3 to 15 million electron volts (MeV).
- However, the most efficient acceleration used is 10 MeV or higher.



2. The Van de Graaff accelerators

- Are capable of energy potentials up to 3 MeV.
- **Principle**: utilize the force exerted on a charged particle by a high voltage potential in an electric field as a means of direct particle acceleration.



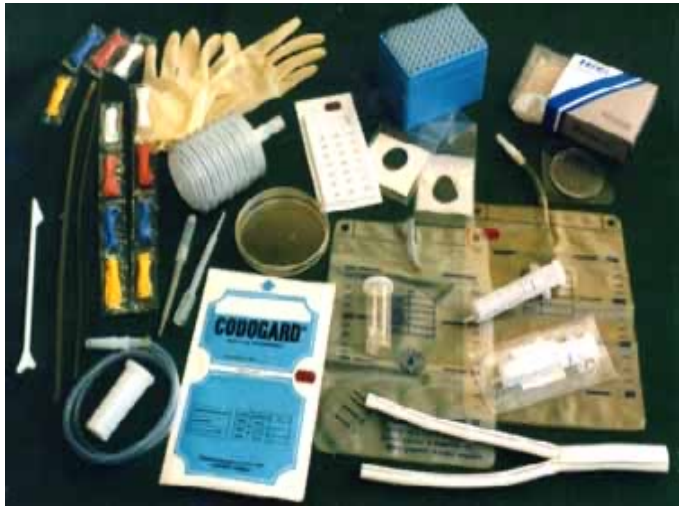
Ionization Radiation
Electron Accelerator

- **Summary**:
- Electron accelerator depends on **energy produced by accelerated electrons** to **produce ionization** inside the material to be sterilized.
- There is **no radioactivity** involved like gamma ray.
- **Very fast sterilization** compared to gamma ray since the material is irradiated in matter of seconds.
- However, it requires short to medium box and box should **be flipped** over to sterilize the other side **because** the energy of the beam diminish when pass through the box and not enough to sterilized the whole box.

https://youtu.be/Tv4KreXY_RU

Application for Sterilization

- Accelerated electrons or gamma rays used to sterilize selected products by a **continuous process**. (sterilization done in the **final** package)
- Most **other** product sterilization procedures performed in batches.
- **Application of radiation**:
- Sterilization of medical **plastic devices**.
- Number of vitamins, antibiotics, and hormones in the dry state.



- **Not Applied**: Liquid pharmaceuticals are difficult to sterilize →
- Because of the **potential effect of the radiations** on the vehicle system as well as the drug.

https://youtu.be/7pTsrBRtFSk

Process validation and Limitation of Radiation sterilization

- **Validation**:
- Assurance of adequate dose delivery determined by:
- Effect of the absorbed energy at the maximum depth of penetration, on **photographic film.**
- Biologic indicator *Bacillus pumilus* (Gram-positive, aerobic, spore-forming bacillus commonly found in soil which shows high resistance).

- **Limitation** (disadvantages of radiation):
- Risk to **operator** (that is why this method required a specialized facility).
- **Product damage** due to water radiolysis.
- Change in **potency**.
- **Discoloration** of come glasses and plastic (e.g. PVC) occur with radiation.
- **Poor penetration** and significant product heating at high dose of some radiation.

Filtration Sterilization

- **Filtration used for**:
- Main Advantage: Removal of particles (M.O.) from solutions and gases **without the application of heat**.
- **Ideally**:

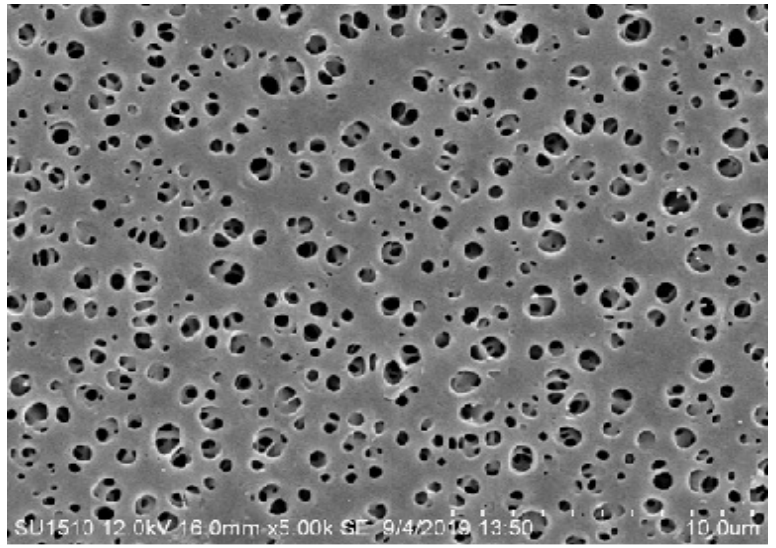
1. Filter should **not alter the solution or gas** in any way.
2. Must **not remove desired constituents** or imparting undesired components.

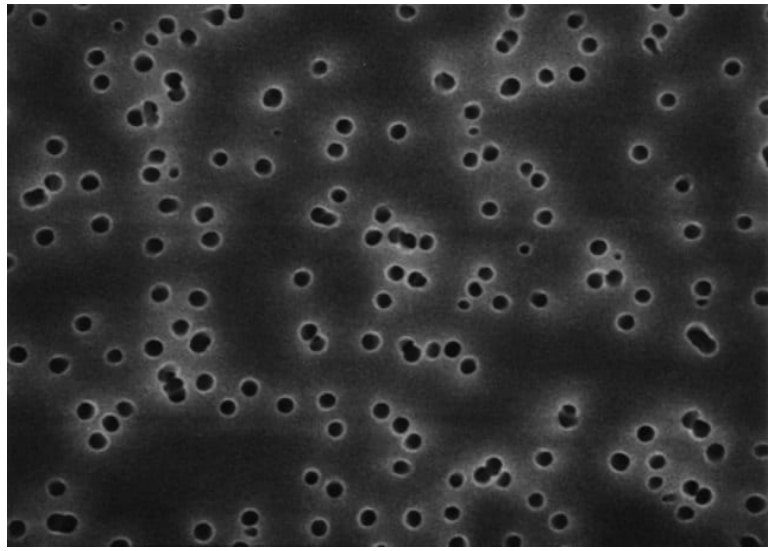
- These requirements essentially limits the types of filters currently employed to the **polymer type** .
- Composition of membrane filters is plastic polymers (such as cellulose, nylon, polycarbonate, and Teflon).
- All currently in use filters with parenteral solutions and gases are of the **membrane type** (tissue-thin material) removing particles **primarily by sieving.**

Filtration Sterilization

- **Limitation**:
- Because of the filter used has an **average** pore size of **0.22 μm**. →

1. This mean that there may be a pore size is larger than that → it is found there are pore of a size up to 0.5 μm but they are **very low** in number. → this may cause a problem in sterility →
   - **A solution for this problem is** :
   - Use a series of 0.2 μm filters.
   - Use a filter with a smaller pore size (such as 0.1μm) → but this would greatly reduce the flow rate.
2. **Filter blockage** → the **solution** is to use a pre filtration using a filter with a larger pore size such as 0.45μm may help.





Filtration Sterilization

- **Applications**:
- Sterilization by filtration is applied for pharmaceuticals which can not be sterilized by terminal processes, or to which agents like additives, heparin and vitamins are added post sterilization.
- It is used for pharmaceuticals that are **thermolabile**.

Chemical Processes of Sterilization
Gas Sterilization

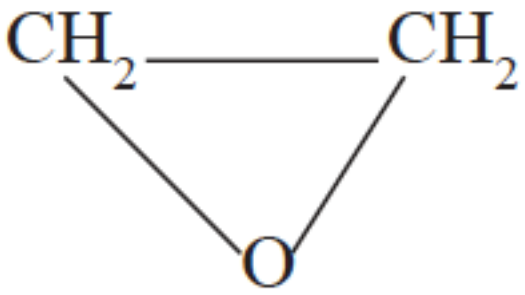
I. **Old gases** (formaldehyde and sulfur dioxide)
   - **Limitation**: highly reactive chemicals so difficult to remove from many materials after exposure.

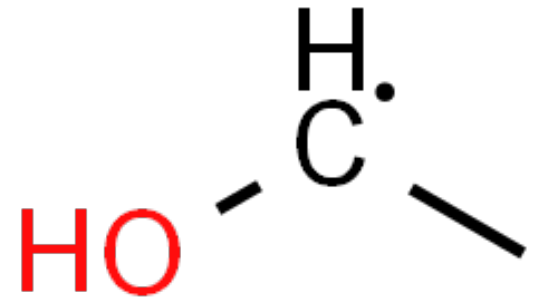
II. **New gases** (ethylene oxide and β-propiolactone)

- Have fewer disadvantages than the older agents so importance in sterilization. Ex: Sterilizing plastic materials.
- Gas sterilization is either **alkylating gases** such as ethylene oxide, β-propiolactone (BPL)
- Or **oxidizing gases** such as hydrogen peroxide, ozone.

Alkylating Gases

- **Ethylene oxide** is the most widely used and it will be discussed in this lecture.
- **Ethylene Oxide (EO):**
- It is cyclic ether ($[CH_2]_2O$) and it is a **gas at room temperature**.
- Highly flammable and when mixed with air → explosive → **solution of this safety problem**:
- Admixed with inert gases CO_2 or fluorinated H.C. (Freons) in certain proportions so rendered **nonflammable and safe to handle**.
- So in sterilization it is used as a mixture of 90% CO_2 and 10% EO.





Ethylene Oxide (EO)

- As a gas, **it penetrates plastic**, **paperboard**, and **powder**) dissipates from the materials simply by exposure to the air.

- **Mechanism**:
- Alkylating gases are believed to exert their lethal effect upon microorganisms by **alkylating essential metabolites**, affecting particularly the **reproductive process**.

hydroxyethyl radical

- Alkylating occur by replacing **active hydrogen** on sulfhydryl-amino, carboxyl-, or hydroxyl-groups with **a hydroxyethyl radical**.
- The **altered metabolite** will not be available for microorganism so it will die without reproducing.

Ethylene Oxide (EO)

- **Advantages:**
- Effective **on all microorganisms** and spores nearly equally.
- No microorganism of **genetically determined high resistance** has been found.
- It is **chemically inert towards** most solids materials.
- **Disadvantages:**
- **Toxicity problems** include burns and blistering, when comes into contact with the **skin**, whereas inhalation results in lachrymation, headache, dizziness and vomiting.
- Great care must be taken to ensure the removal of residual ethylene oxide from treated products (e.g. rubber gloves) to avoid the risk of skin reactions.
- Limited to dry powder because if the material was in liquid state → alkylation may occur in pharmaceutical product
- Requires a particular care for handling.

Sterilizing Process with EO

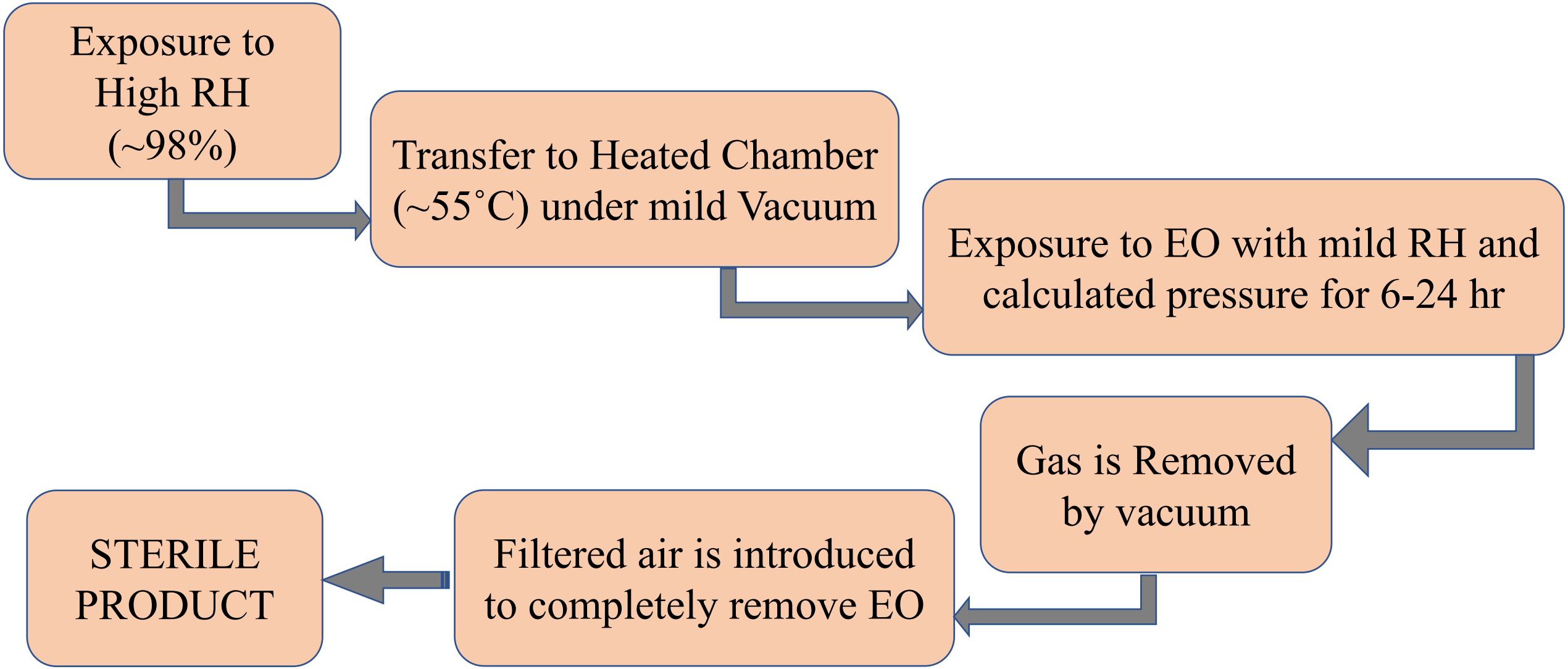
- Sterilization with EO is validated procedure using **a** pressure chamber under an elevated temperature of about 50-60 ˚C this is because **bactericidal** activity of EO proportional to:

1. **Partial pressure** of gas in the reaction chamber.
2. **Time** of exposure.
3. **Temperature** of treatment (best temperature is 50-60 ˚C).
   - A heated chamber is decreasing the time required for the sterilization process.
4. **Level** and type of contamination.
5. **Relative humidity** has a most pronounced effect.
   - Studies showed that microorganism must be hydrated if to be killed by EO within the usual cycle time.

Sterilizing Process with EO

1. The material is placed in a room or chamber and exposed to a **relative humidity** of up to 98% for a period of 60 min or longer.
   - This is required because of the moisture introduced with the gas will not be very effective to rehydrate the microorganism.
2. Then placed in chamber previously heated to 55˚C and an initial vacuum of 27 in. Hg is drawn.
3. EO is introduced with moisture to achieve a relative humidity of 50 to 60% to the pressure required to give the desired concentration of ethylene oxide which is maintained throughout the exposure period (6-24h)
   - **Note**: This period depends on degree of contamination, the penetrability of the material and the concentration of EO.
4. Gas is exhausted and a vacuum of 25 inches Hg is drawn.
5. Filtered air is then introduced into the chamber until atmospheric pressure is attained.

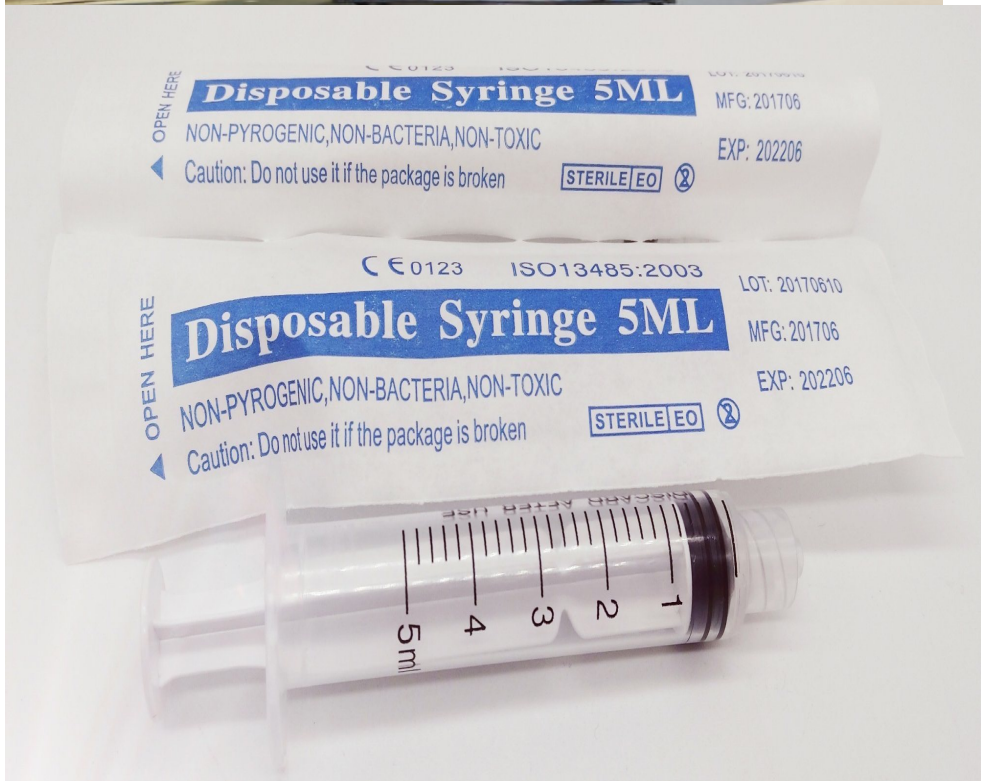
Sterilization Process Summary



Application of EO Sterilization

1. **Dry powders** of substances are unaffected.
2. **Plastic materials**, rubber goods, and delicate optical instruments.
3. **Stainless steel** equipment has a **longer useful** life when sterilized with ethylene oxide instead of steam.

- Due to penetration of EtO → it is possible to sterilize **parenteral administration sets, hypodermic needles, plastic syringes** and other material **in enclosed distribution packages** of paperboard or plastic.



Chemical Agents
Disinfectants, Antiseptic, Preservatives

- **Disinfectants**: are agents used to destroy microorganisms on inanimate objects.
- **Antiseptics**: are agents used to treat living tissues, as in wound irrigation, cleansing of burns or eye washes.
- **Preservative**: describes those antimicrobial agents used to protect medicines, pharmaceutical formulations, cosmetics, foods and general materials against microbial spoilage.
- In many instances concentration and time of contact are the critical factors.
- Chemical agents may **weaken the cell wall**, thereby allowing the extrusion of cell contents, distortion of cell shape, filament formation or complete lysis.

Surface Disinfection

- The use of chemical disinfectants to reduce microbial population so that asepsis can be maintained in a limited, controlled environment.
- Most disinfectants **do not destroy spores** during any reasonable contact period; therefore they do not sterilize the surface.
- The effectiveness of a disinfectant depends on:

1. Nature of the surface (rough vs smooth).
   - **Hard, smooth** surfaces are much easier to disinfect than rough porous ones
2. Nature and degree of contamination (load and type).
3. Microbicidal activity of the agent.

Disinfectants, Antiseptic, Preservatives

1. Phenolic:

- Various distillation fractions of **coal tar** yield phenolic compounds, including cresols, chloroxylenol and phenol itself
- All of which are toxic and caustic to skin and tissues.➔
- **Addition of chlorine** and methyl groups as in chlorocresol and chloroxylenol; This has the dual effect of 1) **eliminating** toxic and corrosive properties while at the same time 2)**enhancing and prolonging** antimicrobial activity.
- Thus, chlorocresol is used as a bactericide in injections and to preserve oil-in-water creams, whereas **<u>chloroxylenol</u>** is employed as a household and hospital antiseptic.

Disinfectants, Antiseptic, Preservatives

2. Alcohols:

- **Ethanol** has long been used, usually as 'surgical spirit' or rapid cleansing of preoperative areas of skin before injection.
- It is most effective at concentrations of 60–70%.
- It is rapidly lethal to bacterial vegetative cells and fungi but has **no activity** against bacterial endospores and **little** effect on viruses.

3. Organic Acids:

- The organic acids such as sorbic and benzoic and their esters, because of their low toxicity, are well established as preservatives or food products and medicines.

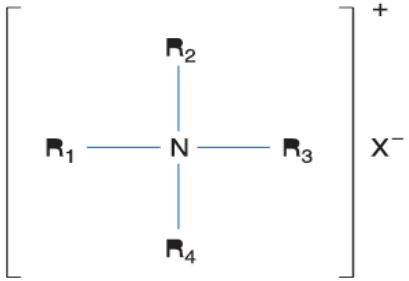
Disinfectants, Antiseptic, Preservatives

4. Aldehydes:

- **Formaldehyde** and glutaraldehyde are both powerful disinfectants, denaturing protein and destroying vegetative cells and spores.
- Formaldehyde is used in sterilization procedures both as a gas and as a solution in ethanol.
- Glutaraldehyde solutions are also used to sterilize surgical instruments.

5. Quaternary Ammonium Compounds

- Their surface active properties make them powerful cleansing agents, a useful adjunct to their common use as **skin antiseptics** and **preservatives** in contact lens cleansing and soaking solutions.
- Examples: chlorhexidine



renu® Advanced Formula multi-purpose solution has a unique triple disinfectant system. When used daily, **renu Advanced Formula** cleans and helps prevent the formation of deposits on lenses.

Contents: A sterile, isotonic solution that contains poloxamine, poloxamer 181, diglycine, sodium citrate, boric acid, sodium borate, edetate disodium and sodium chloride and preserved with a triple disinfectant system (polyaminopropyl biguanide 0.00005%, polyquaternium 0.00015% and alexidine 0.0002%).

Always consult your eye care professional prior to switching to any other multi-purpose solution or if you have questions about your eyes.