

T.C.
YEDITEPE UNIVERSITY
INSTITUTE OF HEALTH SCIENCES
DEPARTMENT OF PHARMACEUTICAL TOXICOLOGY

**TOXICOLOGICAL EVALUATION OF SOME
ACTIVE AND INACTIVE MATERIALS
ACCORDING TO THE VENTILATION
CONDITIONS VARIATION DURING THE
MANUFACTURING IN NON STERILE
PHARMACEUTICAL INDUSTRY**

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APPROVAL

This thesis has been deemed by the jury in accordance with the relevant articles of Yeditepe University Graduate Education and Examinations Regulation and has been approved by Administrative Board of Institute with decision dated and numbered

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DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgment has been made in the text.



11.07.2023

Yasser BATSH

DEDICATION

Thanks with love to my parents, family, especially my spouse, Their belief in me has kept my spirits and motivation high during this process.

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ABSTRACT

Batsh Y. (2023). Toxicological Evaluation of Some Active and Inactive Materials According To The Ventilation Conditions Variation During The Manufacturing in Non Sterile Pharmaceutical Industry. Yeditepe University, Institute of Health Sciences, Department Of Pharmaceutical Toxicology, PhD Thesis, İstanbul.

The purpose of this thesis is to study the effect of air changes on particle number in pharmaceutical production facilities during production stage of non-sterile dosage forms. After those particle count measurements, a comparison of particle number with permitted daily exposure limit (PDE) and occupational exposure limit (OEL) was carried out.

In the international guidelines on Good Manufacturing Practices (GMP) like World Health Organization (WHO) (2018), heating, ventilation and air conditioning (H.V.A.C.) requirements for non sterile pharmaceutical dosage form production is strictly defined. Controlling and measuring of particles count in production area before starting the production is an important request, either in term of prevention of cross contamination, or in term of safety for workers. However in the same guidelines, there is no request for measuring and controlling the limit of the particles which should not be exceeded after starting the production in non sterile facilities. So that there is no real data about the concentrations of these particles count. The aim of this study to produce new data for some active and inactive materials which helps to increase the safety of the workers in pharmaceutical field. For this purpose, particle count measurement was done for different antihypertensive and anti diabetic active ingredients and excipients which have different physical properties during production. In parallel with particle counting, possibility of absorption of these active ingredients were calculated. The effect of the number of air changing on particle concentration during the fluctuation phase of production was studied as a worst case scenario. For this study metformin, gliburide, enalapril, hydrochlorthiazide, losartan, aerosil, and talc were selected. The number of air change of the production area and particle number has been measured by air velocity instrument Alnor model, and a particle counter - Lasair - II model. The number of air

change was adjusted between 7 and 12 times /hour and a comparison between each other was done. It was found that high particle concentration of the measured materials was detected in the environment of the manufacturing area especially at 7 times/hour of air changes. As a conclusion it is the first study to measure particle count during the operation phase of non sterile pharmaceutical dosage forms production area. It was concluded that this type of measurements should be included by international guidelines and an extra care needed for each case for the manufacturing conditions to protect workers.

Key words: Occupational Toxicity, Particles Count, Toxicity in Pharmaceutical Industry, Number of Air Changes.

ÖZET

Batsh Y. (2023). Steril Olmayan İlaç Üretim Esnasında Havalandırma Koşullarının Değişimine Göre Bazı Aktif ve Yardımcı Hammaddelerin Toksikolojik Değerlendirilmesi. Yeditepe Üniversitesi, Sağlık Bilimleri Enstitüsü, Farmasötik Toksikoloji Anabilim Dalı, Doktora Tezi, İstanbul.

Bu tezin amacı, farmasötik üretim tesislerinde steril olmayan dozaj formlarının üretim aşamasında hava değişimlerinin partikül sayısına etkisini incelemektir. Bu partikül sayım ölçümlerinden sonra, izin verilen günlük maruz kalma limiti (PDE) ve mesleki maruz kalma limiti (OEL) ile partikül sayısının karşılaştırılması yapılmıştır.

Dünya Sağlık Örgütü (WHO) (2018) gibi İyi Üretim Uygulamaları (GMP) hakkındaki uluslararası yönergelerde, steril olmayan farmasötik dozaj formu üretimi için ısıtma, havalandırma ve iklimlendirme (H.V.A.C.) gereksinimleri kesin olarak tanımlanmıştır. Üretime başlamadan önce üretim alanında partikül sayımının kontrol edilmesi ve ölçülmesi gerek çapraz kontaminasyonun önlenmesi gerekse çalışanların güvenliği açısından önemli bir taleptir. Ancak aynı kılavuzda steril olmayan tesislerde üretime başladıktan sonra aşılması gereken partikül sınırının ölçülmesi ve kontrol edilmesi yönünde bir gereksinim bulunmamaktadır. Öyle ki bu parçacıkların konsantrasyonları hakkında gerçek bir veri yoktur. Bu çalışmanın amacı, farmasötik alanında çalışanların güvenliğini artırmaya yardımcı olan bazı aktif ve inaktif maddeler için yeni veriler üretmektir. Bu amaçla üretim sırasında farklı fiziksel özelliklere sahip antihipertansif ve anti diyabetik etken maddeler ve yardımcı maddeler için partikül sayımı yapılmıştır. Partikül sayımına paralel olarak bu aktif bileşenlerin absorpsiyon olasılıkları hesaplanmıştır. Üretimin dalgalanma aşamasında değişen hava sayısının partikül konsantrasyonu üzerindeki etkisi en kötü durum senaryosu olarak incelenmiştir. Bu çalışma için metformin, gliburid, enalapril, hidrokloridiazid, losartan, aerosil ve talk seçilmiştir. Üretim alanındaki hava değişim sayısı ölçümü Alnor modeli hava hızı ölçüm cihazı ile partikül sayısı ölçümü ise Lasair - II modeli partikül sayım cihazı ile yapılmıştır. Hava değişim sayısı 7 ile 12 kez/saat arasında ayarlanarak birbirleri arasında karşılaştırma yapılmıştır. Üretim alanı ortamında özellikle 7 kez/saat hava

değişiminde ölçülen malzemelerin yüksek partikül konsantrasyonunun tespit edildiği bulunmuştur. Sonuç olarak bu çalışma, steril olmayan farmasötik dozaj formlarının üretimleri aşamasında partikül sayısını ölçen ilk çalışmadır. Bu tür ölçümlerin uluslararası kılavuzlarda yer alması ve çalışanları korumak için üretim koşullarında her durum için ekstra özen gösterilmesi gerektiği sonucuna varılmıştır.

Anahtar kelimeler: Mesleki Toksisite, Partikül Sayısı, İlaç fabrikasında toksisite, Hava Değişim Sayısı.

1. INTRODUCTION and PURPOSE

In the workplace, unlike in the general population, there exists a nominally healthy workforce. Employees who do not have the illness a particular drug is designed to treat have the potential to be exposed. Their exposure may be to an uncontrolled 'dose' and duration giving rise to the potential for adverse health effects and, due to the varied routes of exposure, health effects rarely seen in treatment can be observed. Inhalation Exposure may consider one of the most important ways for occupational hazards and is high related to the ventilation system applied in the production area.

In controlling exposure to therapeutic substances the pharmaceutical industry must control not only the toxic effects of substances, but also the pharmacological effects. These pharmacological effects, which may be either side effects or therapeutic effects, often occur at lower doses than toxic effects.

It is the therapeutic action of Active pharmaceutical ingredients APIs that often creates the primary occupational hazard. By design, desirable APIs have powerful positive effects on patient health with minimal adverse effects. However, effects that may be acceptable or even beneficial to a patient receiving treatment under medical supervision may be intolerable in workers subject to occupational exposure.

While epidemiological studies are few for morbidity and mortality in pharmaceutical workers, it is well documented that certain classes of pharmaceuticals can produce adverse health effects with both acute and chronic exposures. In a healthy worker, any effects of drugs, whether positive or negative, should be considered serious and should be prevented. [1]

Preventive action applied in pharmaceutical industry for work environment and especially against inhalation unwanted effects depended almost on the Clean rooms and clean air devices which routinely monitored in term of particle count and H.V.A.C. system activity in rest case for working and has the same parameters for all kinds of active and inactive materials if considered as generic non sterile products without taking in account a different particle size and count which may be produced during production according to the properties of materials and the fluctuation of HVAC system design.

So that more details should be taken in account through determining the criterias of preventive actions for workers in pharmaceutical production.

And in this research a study done for some of active and in active materials in term of particles count and number of air change in operation cases of work and the effect of hanging of these parameters in causing unwanted occupational effects that happen through inhalation route of workers .



2. LITERATURE SURVEY

2.1. Occupational Toxicology

Occupational toxicology is the application of the principles and methodology of toxicology to understanding and managing chemical and biological hazards encountered at work. The objective of the occupational toxicologist is to prevent adverse health effects in workers that arise from exposures in their work environment.

Because non occupational exposures can act as confounders or can increase the susceptibility of individual workers, occupational toxicologists must evaluate the entire spectrum of exposures experienced by the work force under consideration. Occupational toxicology is a discipline that draws on industrial hygiene, epidemiology, occupational medicine, and regulatory toxicology. The occupational toxicologist must have an intimate knowledge of the work environment and be able to recognize and prioritize exposure Hazards. Because the work environment can present exposures to complex mixtures, the occupational toxicologist must also recognize those that are particularly hazardous when occurring in combination. [2]

2.1. 1. Routes of Exposure of Hazardous Materials

Diseases arising in occupational environments involve exposure, primarily through inhalation, ingestion, or dermal absorption. In the vast majority of work environments, inhalation of toxicants is a primary concern. Inhalation exposures can occur with gases, vapors, liquid aerosols, particulate aerosols, fumes, and mixtures of these. [2]

2.1.2. Sources of Hazards in pharmaceutical Industry

- Handling and storage of huge quantity of hazardous chemicals.
- Transferring, loading and unloading of solvents and chemicals
- Human errors while handling hazardous chemicals.
- H.V.A.C. System design error. [3].

2.2. Anatomy of The Respiratory Tract

The respiratory tract is divided into the upper airways and lower airways:

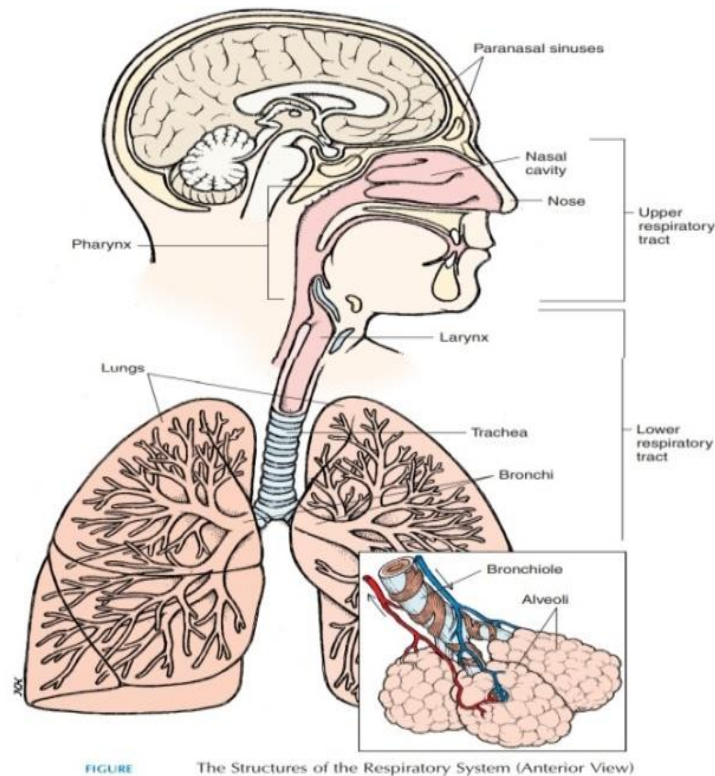


Figure 1. Anatomy of the respiratory tract

2.2.1 Upper Respiratory Tract

The upper respiratory tract can refer to the parts of the respiratory system lying above the sternal angle (outside of the thorax), above the vocal folds, or above the cricoid cartilage. The larynx is sometimes included in both the upper and lower airways. The larynx is also called the voice box and has the associated cartilage that produces sound. The tract consists of the nasal cavity and paranasal sinuses, the pharynx (nasopharynx, oropharynx and laryngopharynx) and sometimes includes the larynx. [4]

2.2.2. Lower Respiratory Tract

Parts of the lower respiratory tract are The lower respiratory tract or lower airway is derived from the developing foregut and consists of the trachea, bronchi (primary, secondary and tertiary), bronchioles (including terminal and respiratory), and lungs (including alveoli). It also sometimes includes the larynx. The lower respiratory tract is also called the respiratory tree or tracheobronchial tree, to describe the branching structure of airways supplying air to the lungs, and includes the trachea, bronchi and bronchioles. [4]

The human respiratory tree may consist on average of 23 generations, At each division point or generation, one airway branches into two or smaller airways. Proximal divisions (those closest to the top of the tree, such as the bronchi) mainly function to transmit air to the lower airways. Later divisions including the respiratory bronchiole, alveolar ducts, and alveoli, are specialized for gas exchange. [4]

The trachea is the largest tube in the respiratory tract and consists of tracheal rings of hyaline cartilage. It branches off into two bronchial tubes, a left and a right main bronchus, the bronchi branch off into smaller sections inside the lungs, called bronchioles. These bronchioles give rise to the air sacs in the lungs called the alveoli. [4]

The lungs are the largest organs in the lower respiratory tract; the lungs are suspended within the pleural cavity of the thorax. The pleurae are two thin membranes, one cell layer thick, which surround the lungs, The inner (visceral pleura) covers the lungs and the outer (parietal pleura) lines the inner surface of the chest wall, This membrane secretes a small amount of fluid, allowing the lungs to move freely within the pleural cavity while expanding and contracting during breathing. The lungs are divided into different lobes. The right lung is larger in size than the left, because of the heart's being situated to the left of the midline. The right lung has three lobes upper, middle, and lower (or superior, middle, and inferior), and the left lung has two upper and lower (or superior and inferior), plus a small tongue-shaped portion of the upper lobe known as the lingula. Each lobe is further divided up into segments

called bronchopulmonary segments. Each lung has a costal surface, which is adjacent to the ribcage; a diaphragmatic surface, which faces downward toward the diaphragm; and a mediastina surface, which faces toward the center of the chest, and lies against the heart, great vessels, and the carina where the two mainstem bronchi branch off from the base of the trachea. [5]

The alveoli are tiny air sacs in the lungs where gas exchange takes place; the mean number of alveoli in a human lung is 480 million. When the diaphragm contracts, a negative pressure is generated in the thorax and air rushes in to fill the cavity, when that happens, these sacs fill with air, making the lung expand. The alveoli are rich with capillaries, called alveolar capillaries. Here the red blood cells absorb oxygen from the air and then carry it back in the form of oxyhaemoglobin, to nourish the cells. The red blood cells also carry carbon dioxide (CO₂) away from the cells in the form of carbaminohemoglobin and release it into the alveoli through the alveolar capillaries. When the diaphragm relaxes, a positive pressure is generated in the thorax and air rushes out of the alveoli expelling the carbon dioxide. [5]

2.2.3. Microanatomy

The lungs are made up of thirteen different kinds of cells, eleven types of epithelial cell and two types of mesenchymal cell. The epithelial cells form the lining of the tracheal and bronchial tubes, while the mesenchymal cells line the lungs. There are glands and mucus produced by goblet cells in parts, as well as smooth muscle, elastin or cartilage. The epithelium from the nose to the bronchioles is covered in ciliated pseudostratified columnar epithelium, commonly called respiratory epithelium. The cilia beat in one direction, moving mucus towards the throat where it is swallowed. Moving down the bronchioles, the cells get more cuboidal in shape but are still ciliated. Glands are abundant in the upper respiratory tract, but there are fewer lower down and they are absent starting at the bronchioles. The same goes for goblet cells, although there are scattered ones in the first bronchioles. Cartilage is present until the small bronchi. In the trachea, they are C-shaped rings of hyaline cartilage, whereas in the bronchi the cartilage takes the form of interspersed plates. Smooth muscle starts

in the trachea, where it joins the C-shaped rings of cartilage. It continues down the bronchi and bronchioles, which it completely encircles. Instead of hard cartilage, the bronchi and bronchioles are composed of elastic tissue. [6]

The alveolar region has a very large surface area, about 50 times that of the skin. In addition, the alveoli consist of only a single layer of cells with very thin membranes that separate the inhaled air from the blood stream. [6]

2.3. Impact of Physical Factors on Absorption

2.3.1. Water Solubility

Oxygen, carbon dioxide, and other gases readily pass through this membrane. Gases and particles, which are water-soluble (and thus blood-soluble), are absorbed more efficiently from the lung alveoli compared to their absorption via the gastrointestinal tract or through the skin. Water-soluble gases and liquid aerosols can pass through the alveolar cell membrane by simple passive diffusion. Inhaled gases or vapors, which have poor solubility in the blood, have a limited capacity for absorption. The main reason for this is that the blood can become quickly saturated. Once saturated, blood will not be able to accept the gas and it will remain in the inhaled air and then get exhaled. One way that the amount of gas absorbed could increase is if the rate and depth of breathing were increased (this concept is known as ventilation limitation). More specifically, the amount of gas absorbed could be increased if the rate of blood supply to the lung were increased by flow limitation. In contrast, insoluble gases or vapours can be absorbed into the body by the lungs before getting exhaled (for example, nitrogen dioxide and carbon monoxide). This is quickly for relatively insoluble gases than for soluble gases. On the other hand the lipid soluble agent can rapidly distribute throughout the body, passing through the cell membranes of various organs or into fat depots. Lipid-soluble substances take a longer time to reach equilibrium than water soluble substances. Chloroform and ether are examples of lipid-soluble substances with high blood solubility. Some non-absorbed foreign material can cause damage reactions within the respiratory system. These reactions may take the form of chronic bronchitis, alveolar breakdown (emphysema), fibrotic lung disease, and even lung cancer. In some

cases, the toxic particles can kill the alveolar macrophages, which results in lowering the body's respiratory defence mechanism.[7]

2.3.2. Airborne Particles

The absorption of airborne particles is usually quite different from that of gases or vapours. The absorption of solid particles, regardless of solubility, depends upon particle size:

- Large particles ($>5\ \mu\text{m}$) are generally deposited in the nasopharyngeal (head airways region) region with little absorption.
- Particles $2\text{--}5\ \mu\text{m}$ can penetrate into the tracheobronchial region.
- Very small particles ($<1\ \mu\text{m}$) are able to penetrate deep into the alveolar sacs where they can deposit and be absorbed. Aerosol particle size is one of the most important variables in defining the dose deposited and the distribution of drug aerosol in the lung. Fine aerosols are distributed on peripheral airways but deposit less drug per unit surface area than larger particle aerosols which deposit more drug per unit surface area, but on the larger, more central airways [7].

Most therapeutic aerosols are almost always heterodisperse, consisting of a wide range of particle sizes and described by the log-normal distribution with the log of the particle diameters plotted against particle number, surface area or volume (mass) on a linear or probability scale and expressed as absolute values or cumulative percentage. Since delivered dose is very important when studying medical aerosols, particle number may be misleading, as smaller particles contain less drug than larger ones. Particle size is defined from this distribution by several parameters. Mass median diameter of an aerosol refers to the particle diameter that has 50% of the aerosol mass residing above and 50% of its mass below it. The median mass aerodynamic diameter MMAD relates the particle to the diameter of a sphere of unit density that has the same settling velocity as the particle of interest regardless of its shape or density. MMAD is read from the cumulative distribution curve at the 50% point. Geometric standard deviation (GSD) is a measure of the variability of the particle diameters within the aerosol and is calculated from the ratio of the particle diameter at the 84.1% point on the cumulative distribution

curve to the MMAD. For a log-normal distribution, the GSD is the same for the number, surface area or mass distributions. A GSD of 1 indicates a monodisperse aerosol, while a GSD of >1.2 indicates a heterodisperse aerosol. [8]

Particles can be deposited by inertial impaction, gravitational sedimentation or diffusion (Brownian motion) depending on their size. While deposition occurs throughout the airways, inertial impaction usually occurs in the first 10 generations of the lung, where air velocity is high and airflow is turbulent [3]. Most particles >10 μm are deposited in the oropharyngeal region with a large amount impacting on the larynx, particularly when the drug is inhaled from devices requiring a high inspiratory flow rate (DPIs) dry powder inhalers when the drug is dispensed from a device at a high forward velocity (MDIs) metered dose inhaler.[7]

The large particles are subsequently swallowed and contribute minimally, if at all, to the therapeutic response. One example is fluticasone propionate, with its poor oral absorption demonstrated by similar plasma levels when inhaled from an MDI or an MDI + spacer, hence the oral component of the inhaled dose provides no added therapeutic benefit. In contrast are drugs that can be absorbed orally including salbutamol and terbutaline, that when swallowed can produce a delayed therapeutic response. In the tracheobronchial region, inertial impaction also plays a significant role in the deposition of particles, particularly at bends and airway bifurcations.

Deposition by gravitational sedimentation predominates in the last five to six generations of airways (smaller bronchi and bronchioles), where air velocity is low

In the alveolar region, air velocity is negligible, and thus the contribution to deposition by inertial impaction is nil. Particles in this region have a longer residence time and are deposited by both sedimentation and diffusion. Particles not deposited during inhalation are exhaled. Deposition due to sedimentation affects particles down to 0.5 μm in diameter, whereas below 0.5 μm , the main mechanism for deposition is by diffusion. [8]

2.3.3. Ventilatory Parameters

Ventilation is the movement of air in and out of the respiratory tract and is a factor in determining the amount of an exposure atmosphere that is actually inhaled. Ventilatory parameters also affect the deposition of particles once inhaled.

The amount of air inspired (or expired) during a normal breath is the tidal volume (V_T); it averages 450–600 ml in resting healthy males and slightly less for females. The fraction of the V_T that does not reach the alveolated airways about 150–200 ml in resting males and 120–160 ml in females is termed the anatomic dead space volume. Ventilation is affected by numerous exogenous factors such as altitude, ambient temperature, and smoking, as well as endogenous factors such as body size. [6]

2.3.4. Physical Activity

Healthy humans at rest normally breathing through the nose, but when respiratory demand increases above a certain level there is a shift to oronasal (combined nose and mouth) breathing at which point ~40–60 percent of total airflow occurs through the nose. As respiratory demand increases further, the proportion of air entering the mouth increases, but even at high demand the oral pathway accounts for no more than 60 percent of the inhaled air. [6]

With mounting respiratory demand the maximum volume of air that can be inhaled per minute, or the maximum voluntary ventilation, may rise to more than 10 times the resting ventilatory level. As breathing frequency increases, expiratory time diminishes, but inspiratory time remains relatively constant. Furthermore, respiratory pauses, the gaps between expiration and inspiration which can occupy 25 percent of the breathing cycle in resting individuals, become shorter with increasing level of activity[6].

2.3.5. Growth and Aging

The volume of air in the lungs and the ventilatory capacity depend on body and lung size and, thus, increase with growth from childhood. Ventilatory function reaches a peak between the ages of 20 and 35 and then begins to decline. Although various models have been proposed to describe these changes, they differ in their assumptions

about the rate of functional decline. Furthermore, most of the reported data for age-related changes in lung function are derived from cross-sectional population studies and may not reflect the true aging process, especially since these studies may be measuring the heartiest survivors. The best way to avoid possible bias is to examine true aging patterns in longitudinal studies in which the same people are tested over a number of years. Such analyses are scarce, and those that do exist have measured only a few parameters.

Changes in lung function with aging are the result of deterioration of the lung tissue itself, a decrease in the strength of the respiratory muscles, and an increase in the stiffness of the thoracic cage. The time course varies from individual to individual and may be aggravated by chronic pollutant exposure. Some ventilatory indices are affected by age, whereas others are not. [6]

Aging is associated with regional inequalities in the distribution of ventilation and a decrease in the uniformity of perfusion. Nonuniform mixing of inspired air may result when sections of the lungs communicate poorly with others and, because of this, some alveolar regions may not be continuously ventilated during normal tidal breathing. Change with aging, the ability of the lungs to respond to increased activity is altered, and maximum voluntary ventilation declines by about 30 percent between ages 30 and 70.[6]

2.4. The Clearance Mechanisms of The Lung

Like all major points of contact with the external environment, the lung has evolved to prevent the invasion of unwanted airborne particles from entering the body. Airway geometry, humidity and clearance mechanisms contribute to this filtration process. The challenge in developing therapeutic aerosols is to produce an aerosol that eludes the lung's various lines of defence.[6]

2.4.1. Airway Geometry and Humidity

Progressive branching and narrowing of the airways encourage impaction of particles. The larger the particle size, the greater the velocity of incoming air, the greater the bend angle of bifurcations and the smaller the airway radius, the greater the

probability of deposition by impaction .The lung has a relative humidity of approximately 99.5%. The addition and removal of water can significantly affect the particle size of a hygroscopic aerosol and thus deposition. Drug particles are known to be hygroscopic and grow or shrink in size in high humidity, such as in the lung, as demonstrated in Figure 2. A hygroscopic aerosol that is delivered at relatively low temperature and humidity into one of high humidity and temperature would be expected to increase in size when inhaled into the lung. The rate of growth is a function of the initial diameter of the particle, with the potential for the diameter of fine particles $<1\text{ }\mu\text{m}$ to increase five-fold compared with two-to-three-fold for particles $>2\text{ }\mu\text{m}$.

The increase in particle size above the initial size should affect the amount of drug deposited and particularly, the distribution of the aerosolized drug within the lung. Ferron and colleagues have predicted that for initial sizes between $0.7\text{ }\mu\text{m}$ and $10\text{ }\mu\text{m}$, total deposition of hygroscopic aerosols increases by a factor of 2.[6]

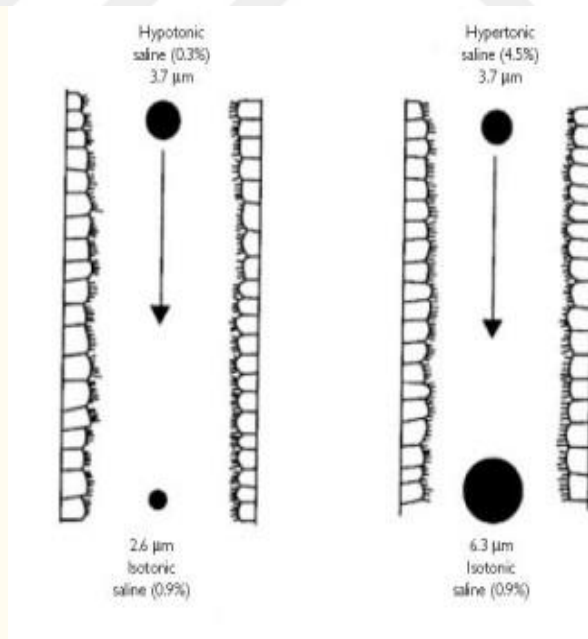


Figure 2.

Illustration of hygroscopic growth and shrinkage of hypertonic and hypotonic droplets of the same initial size ($3.7\text{ }\mu\text{m}$) in the humid environment of the respiratory tract.

2.4.2. Lung Clearance Mechanisms

Once deposited in the lungs, inhaled drugs are either cleared from the lungs, absorbed into the systemic circulation or degraded via drug metabolism. Drug particles deposited in the conducting airways are primarily removed through mucociliary clearance and, to a lesser extent, are absorbed through the airway epithelium into the blood or lymphatic system. Ciliated epithelium extends from the trachea to the terminal bronchioles. The airway epithelial goblet cells and submucosal glands secrete mucus forming a two-layer mucus blanket over the ciliated epithelium: a low-viscosity periciliary or sol layer covered by a high-viscosity gel layer. Insoluble particles are trapped in the gel layer and are moved toward the pharynx (and ultimately to the gastrointestinal tract) by the upward movement of mucus generated by the metachronous beating of cilia. In the normal lung, the rate of mucus movement varies with the airway region and is determined by the number of ciliated cells and their beat frequency. Movement is faster in the trachea than in the small airways and is affected by factors influencing ciliary functioning and the quantity and quality of mucus [8]

For normal mucociliary clearance to occur, airway epithelial cells must be intact, ciliary structure and activity normal, the depth and chemical composition of the sol layer optimal and the rheology of the mucus within the physiological range. Mucociliary clearance is impaired in lung diseases such as immotile cilia syndrome, bronchiectasis, CF and asthma. In immotile cilia syndrome and bronchiectasis, the ciliary function can be either impaired or non-existent. In CF, the ciliary structure and function are normal, but the copious amounts of thick, tenacious mucus present in the airways impair their ability to clear the mucus effectively. In these diseases, clearance of aerosolized drugs deposited in the conducting airways generally is decreased and secretions are cleared from the lung by cough. [8]

In addition to mucociliary clearance, soluble particles also can be removed by absorptive mechanisms in the conducting airways. Lipophilic molecules pass easily through the airway epithelium via passive transport. Hydrophilic molecules cross via extracellular pathways, such as tight junctions, or by active transport via endocytosis

and exocytosis. From the submucosal region, particles are absorbed into either the systemic circulation, bronchial circulation or lymphatic system. [8]

Drugs deposited in the alveolar region may be phagocytosed and cleared by alveolar macrophages or absorbed into the pulmonary circulation. Alveolar macrophages are the predominant phagocytic cell for the lung defence against inhaled microorganisms, particles and other toxic agents. There are approximately five to seven alveolar macrophages per alveolus in the lungs of healthy non-smokers. Macrophages phagocytose insoluble particles that are deposited in the alveolar region and are either cleared by the lymphatic system or moved into the ciliated airways along currents in alveolar fluid and then cleared via the mucociliary escalator. This process can take weeks to months to complete. Soluble drug particles deposited in the alveolar region can be absorbed into the systemic circulation, as discussed above. The pulmonary epithelium appears to be more resistant to soluble particle transport than the endothelium or the interstitium. The rate of protein absorption from the alveoli is size dependent. Effros and Mason demonstrated an inverse relationship between alveolar permeability and molecular weight. [8]

Very little is known about how the drug-metabolizing activities of the lung affect the concentration and therapeutic efficacy of inhaled drugs. All metabolizing enzymes found in the liver are found to a lesser extent in the lung (CYP450 enzymes are 5–20 times lower than in liver) distributed throughout the conducting airways and alveoli. Phase 1 cytochrome-450 (CYP450) enzymes, flavin-containing monooxygenases (FMO), monoamine oxidase (MAO), aldehyde dehydrogenase, NADPH-CYP450 reductase, for example, are all present in the lung [4].

The monooxygenase system metabolizes fatty acids, steroids and lipophilic xenobiotics. Esterase present in high concentrations in alveolar macrophages, and to a lesser degree in alveolar type I and II cells, hydrolyses beclomethasone dipropionate to its monopropionate and beclomethasone. The human lung, however, appears to be a poor site for sulphonation. Proteins and peptides are subject to hydrolysis by proteases, such as neutral endopeptidase and cathepsin H, present in the lung. The extent to which

various proteins and peptides are metabolized is unclear. Vasoactive intestinal polypeptide (VIP) is believed to be completely degraded during the passage across the pulmonary epithelium and into the bloodstream. Sources for the proteases are the alveolar macrophages and other inflammatory cells, such as neutrophils. Since these enzymes play a key role in the degradation of proteins and peptides, the effect of delivering aerosolized proteins and peptides to inflamed lungs where the level of proteases is higher, is unknown but may impair their efficacy. Co-administration with protease inhibitors, such as bacitracin and sodium glycocholate, have been shown to reduce the metabolism of proteins and thereby improve pulmonary absorption. However, for most proteins degradation in the alveoli is not a major clearance mechanism, with >95% of proteins, including insulin, being absorbed intact from the lung periphery [8].

2.4.3. Bronchial Circulation

The lung receives the entire cardiac output and thus is the best perfused organ in the body. However, only the alveolar region and respiratory bronchioles are supplied by the pulmonary circulation. Blood flow to the larger airways (trachea to terminal bronchioles) is via the systemic circulation and these airways receive approximately 1% of the cardiac output. The role of the bronchial circulation in distributing aerosolized drugs to regions distal from the original site of deposition or to nonventilated regions of the lung is unknown. The endobronchial circulation is recirculated to the peripheral airways and lung parenchyma via the bronchial veins and right atrium. Bronchial blood flow is augmented in diseases, such as bronchiectasis, from 1% to as much as 30% of cardiac output. In sheep, bronchial blood flow increased with antigen- and histamine-induced bronchoconstriction. Theoretically, inhaled drugs that are absorbed into the circulation from the tracheobronchial regions can be redistributed downstream and peripheral to airway obstructions, into otherwise poorly accessible areas of the lung which may aid in the drug's efficacy. Thus far, no experimental work in humans has been done to investigate the role of bronchial circulation in lung distribution of inhaled medications or its influence on their efficacy [9].

2.5. Some Of Respiratory Health Occupational Risks

2.5.1. Asthma

Is an inflammatory airways disease causing episodic, reversible airways obstruction “Work-related asthma” refers to both new-onset asthma caused by work and pre-existing asthma exacerbated by work. New-onset asthma associated with work can be occupational asthma caused by immunological sensitization to agents in the workplace; or irritant-induced asthma caused by irritant exposures in the workplace. Work-exacerbated asthma refers to exacerbation of pre-existing asthma by workplace exposures. Work-related asthma was recognized by Hippocrates (460 – 370 B.C.) and it was associated with occupations involving work with metals, textiles, and animals including fish. Today work-related asthma is commonly encountered in isocyanate production, in healthcare workers who use natural rubber latex gloves (although this is becoming less of a problem due to the substitution of other materials), and among office workers due to poor indoor environmental quality. It is estimated that between 15 and 30 percent of asthmatics have new-onset adult asthma or work-exacerbated asthma. Thus, over two million workers in the United States suffer from work-related asthma [10].

2.5.2. Chronic Obstructive pulmonary Disease (COPD)

Chronic obstructive pulmonary disease refers to chronic bronchitis, emphysema, and combined presentations of these two diseases. Chronic obstructive pulmonary disease is a leading cause of morbidity and mortality in the United States and world-wide. In 2003, 10.7 million United States adults were estimated to have COPD. However, close to 24 million adults were estimated to have impaired lung function, suggesting under-diagnosis of COPD.

Although often thought of as a disease caused by cigarette smoking, it is well-recognized that COPD is also caused by occupational exposures. It has been estimated that 15% of COPD is attributable to occupation. Coal mine dust and crystalline silica encountered in industries such as mining and construction are known risks. A study of participants in the Third National Health and Nutrition Examination Survey found

increased risk for COPD in a number of industries including rubber, plastics, and leather manufacturing; utilities; building services; textile manufacturing; and construction [2, 10].

2.5.3 Silica

Silica, also known as silicon dioxide, is the most common mineral on earth. Inhaling dusts that contain crystalline forms of silica can cause a fibrosing interstitial lung disease called silicosis. Silicosis usually takes at least 10 years after exposure to develop. However, it can develop more quickly, especially after large exposures. Quartz is a common type of crystalline silica and is a large component of many types of rock. Inhalation exposure can occur in many kinds of work, including tunnelling, quarrying, mining, construction, roadway repair and demolition, sandblasting or any type of work that breaks up quartz-containing rock. Cristobalite exposure can occur in foundry work as a result of heating amorphous silica. In addition to causing silicosis, crystalline silica exposure has been associated with pulmonary function impairment and COPD. Silica has also been classified by the International Agency for Research on Cancer (IARC) as a known human carcinogen. Crystalline silica is thought to damage lung tissue in large part as a result of oxidant mechanisms, with freshly fractured crystalline silica is being more potent than aged materials. Silica-induced diseases can be prevented by reducing exposures. It is encouraging that deaths from silicosis have fallen from more than 1060 in 1968 to less than 170 in 2005[2].

2.5.4. Asbestos

Asbestos is a commercial term that refers to six types of fibrous minerals, including one serpentine (chrysotile) and five amphiboles [crocidolite (riebeckite asbestos), amosite (cummingtonite-grunerite asbestos), anthophyllite asbestos, tremolite asbestos, and actinolite asbestos].

Asbestos mineral fibres are flame and heat resistant, pliable, strong, refractory to corrosive chemicals, and provide insulation. Because of its ability to insulate from heat and protect against fire, asbestos has been widely used as a building material. It has been especially important in the shipbuilding industry. It has also been woven into fabric to make fireproof, protective textile products, such as fireproof blankets and suits,

It has been used as a brake liner (e.g., in automobiles and railroad rolling stock) and for engine gaskets and has been used to make filters (e.g., in the chemical industry). Although known and used for its fire resistant properties as early as 3000 B.C., asbestos, use of the material grew markedly in the U.S. during the 20th century, peaking from the 1950s through the 1980s. Although its use in the US has largely disappeared, exposure continues to occur due to renovation or demolition of existing building stock or through exposure to asbestos-containing products such as brake linings or asbestos-containing cement products that continue to be imported. Inhalation of asbestos fibres can have many adverse health effects. Because these develop slowly, usually presenting a decade or more after exposure, asbestos-caused diseases remain an important problem. Asbestos fibres can cause asbestosis, a fibrosing interstitial lung disease. They can also penetrate through the lung tissue to the pleura, which are the tissues lining the lungs and chest cavity. In the pleura, they can cause conditions including pleural effusions (fluid collections), pleural plaques, and diffuse pleural fibrosis.

Asbestos fibres are also a cause of lung cancer. Risk increases multiplicatively if an exposed person also smokes. Another much-feared type of cancer caused by asbestos is mesothelioma, which arises in the pleura or peritoneum (lining of the abdominal cavity). Mesothelioma usually cannot be cured and thus has a high fatality rate. NIOSH has recently developed a “Roadmap” for research in the area of asbestos fibres and other elongated mineral particles [2].

2.5.5. Flock

Flock consists of short fibres that are cut from long filaments and glued to backing material such as cloth to provide a fuzzy, carpet-like surface texture. They are usually prepared from synthetic materials such as nylon, rayon, or polypropylene. The cutting process results in formation of airborne particles or fibres in the respirable range. Inhalation of flock dust has been associated with an interstitial lung disease called flock workers’ lung. NIOSH has made recommendations to companies that manufacture flock and make flock containing products that aim to reduce workers’

exposure to flock dust. These include: engineering controls and alternative methods to cleaning the workplace with compressed air, which re-aerosolizes settled dust. [12]

2.5.6. Severe Acute Respiratory Syndrome (SARS)

SARS was first reported in Asia in February 2003. The disease is a viral respiratory infection caused by SARS-associated coronavirus. It can cause fatal pneumonia associated with acute respiratory distress syndrome. Over a period of months, the illness spread to more than two dozen countries across the globe. According to the WHO, a total of 8,098 people worldwide became sick with SARS during the 2003 outbreak. Of these, 774 died. In the U.S., only eight people had laboratory evidence of SARS. Health workers contracted SARS from occupational exposure. Although there have been very few cases of occupationally acquired SARS in the U.S., there is potential for spread of the disease within the workplace, especially for healthcare workers and workers who experience daily contact with the general public [2].

2.5.7. Risk of Malignant Lung Diseases

A number of agents have been associated with work-related lung cancer. Examples include: asbestos, bischloromethyl ether (BCME), diesel exhaust, various metals (As, Be, Cd, Cr, Ni), polycyclic aromatic hydrocarbons (PAHs), second hand or environmental tobacco smoke (ETS) and silica. External icon Asbestos has already been discussed in the section on interstitial lung diseases. It causes lung cancer and mesothelioma, a type of cancer arising from the mesothelial lining of the chest cavity (pleura) and abdominal cavity (peritoneum) [10].

BCME external icon is used in the manufacture of other chemicals or it may be a by-product of chemical manufacture. Epidemiologic studies suggest that lung cancer (primarily small cell) is related to BCME exposure with a 15-20 year latency period and that risk increases with duration of exposure and cumulative exposure.

Diesel exhaust is the product of incomplete combustion of diesel fuel and is a complex mixture that contains soot, PAHs, nitro-PAHs, oxides of carbon, sulphur and nitrogen. It is both gaseous and particulate. Many components of diesel exhaust are known to be carcinogenic in laboratory animals. Since 1988, NIOSH has recommended

that whole diesel exhaust be regarded as a potential occupational carcinogen and that reductions in workplace exposure would reduce cancer risks. [5]

2.5.8 Metals (As, Be, Cd, Cr, Ni)

Each of the metals mentioned below has been classified as a Group I carcinogen by the International Agency for Research on Cancer.

Arsenic external icon has been associated with development of lung cancer in workers exposed to some pesticides and at copper smelters.

Cadmium external icon is a by-product of zinc-, copper- and lead-smelting, it is used in batteries, plastics, alloys and for electroplating – it is also encountered in welding. Cadmium exposure has been linked to lung and other cancers.

Chromium external icon exposure in welding, electroplating, steel manufacture and the production of pigments and dyes potentially affects 380,000 workers in the United States. These exposures have been linked to lung cancer. The current PEL, promulgated in 2006, of 5 µg (TWA) is still associated with a lung cancer risk of 45 per 1000 over a working lifetime.

Nickel external icon exposure can occur in nickel extraction/production, electroplating, welding, machining and grinding. Reported standard mortality rates for lung cancer and nasal cancer among workers in these industries have ranged from 110-550 and 870-26,670, respectively. [13]

2.5.9. Polycyclic Aromatic Hydrocarbons External Icon

Polycyclic aromatic hydrocarbons are formed in the incomplete combustion of vegetable materials and fossil fuels. They are essentially fused benzene ring structures that are chemically inert and require metabolic activation to exert their biological activity. They are metabolized by cytochrome P450 enzymes and epoxide hydrolases, which are polymorphic among the human population. Activated PAHs form highly reactive carbocations, which bind to biological molecules – like DNA. Covalent binding forms DNA adducts and can cause mutagenic events. Exposure to PAHs can occur in roofing, foundry work and in as a result of second-hand tobacco smoke exposure in indoor environments [14,15].

Environmental tobacco smoke (ETS) or second-hand tobacco smoke is composed of side-stream tobacco smoke and exhaled main-stream tobacco smoke. This complex mixture contains hundreds of noxious chemical and carcinogens, which include: nitrosamines, PAHs, aromatic amines, metals (As, Cd, Cr, Se, Pb among others), formaldehyde, pyridine, phenol, benzene. ETS has been shown to have both chronic and acute effects in some workers who do not otherwise smoke themselves. Although the use of tobacco products in workplaces has been largely banned, it continues in some places. Exposure of employees in hospitality industry workplaces such as restaurants, bars, and casinos remains an important concern. [14,15].

2.5.10. Nanoparticles

Nanoparticles in particular engineered nanoparticles, are an emerging occupational exposure. Their use in commercial products has grown markedly and is expected to continue to grow. A unique feature of nanoparticles is that they present a very large surface area to mass ratio. Thus, their toxic effects might be more related to surface area than mass. No epidemiological evidence from human studies has yet documented engineered nanomaterials as a human health hazard. However, early evidence from experimental toxicity studies provides cause for concern and continued vigilance. These studies suggest that engineered nanoparticles may, exhibit new or increased reactivity and increased toxic effects following inhalation exposure. Thus, workplace exposure to engineered nanoparticles is an important and emerging area of research [16].

2.6. Pharmaceutical Occupational Toxicity

2.6.1. APIs and Their Significance for Exposure

Whilst many airborne contaminants may be given consideration in the pharmaceutical research and manufacturing environment, the focus in this chapter will be on the hazards of the API themselves and the finished products.

In the workplace, unlike in the general population, there exists a nominally healthy workforce. Employees who do not have the illness a particular drug is designed to treat have the potential to be exposed. Their exposure may be to an uncontrolled 'dose' and duration giving rise to the potential for adverse health effects and, due to the varied routes of exposure, health effects rarely seen in treatment can be observed. Exposure inhalation may result in respiratory sensitization, effects rarely seen in treatment [10].

In controlling exposure to therapeutic substances the pharmaceutical industry must control not only the toxic effects of substances, but also the pharmacological effects. These pharmacological effects, which may be either side effects or therapeutic effects, often occur at lower doses than toxic effects [10].

It is the therapeutic action of APIs that often creates the primary occupational hazard. By design, desirable APIs have powerful positive effects on patient health with minimal adverse effects. However, effects that may be acceptable or even beneficial to a patient receiving treatment under medical supervision may be intolerable in workers subject to occupational exposure [10].

While epidemiological studies are few for morbidity and mortality in pharmaceutical workers, it is well documented that certain classes of pharmaceuticals can produce adverse health effects with both acute and chronic exposures. In a healthy worker, any effects of drugs, whether positive or negative, should be considered serious and should be prevented. For example:

- Side effects that are acceptable in treating serious illness such as cancer, suppressing the effectiveness of the immune system, are undesirable in healthy workers.

- Workers may have individual susceptibilities to a drug, e.g. there may be adverse effects on a worker who is pregnant, or a drug intended to reduce blood pressure may have an adverse effect on a worker whose blood pressure is already low.
- Workers who become sensitized to a drug may be unable to receive that drug subsequently to combat disease (e.g. penicillins) [10].

2.6.2. Excipients

Pharmaceutical excipients are substances other than the API which are included in the secondary manufacturing process and are contained in the finished pharmaceutical product dosage form. Tablet or capsule ingredients, for example, consist of Active Pharmaceutical Ingredient(s) (API) and excipients. In order to deliver an accurate amount of a drug for its intended clinical use in a convenient unit dosage form, excipients perform very important functions, specifically as:

Fillers, Diluents, Binders, Disintegrants, Lubricants, Glidants, Wetting, Surface Active Agents, Colors, Pigments, Plasticizer, Flavors, Sweeteners, Taste-Maskers.

Excipients, from an occupational hygiene perspective are commonly the least toxicological concern, whilst the API is the primary concern. However, the degree of dilution by excipients must be taken into account when interpreting gravimetric analysis results and in deciding on the control measures needed when handling materials.

Exposure assessment studies in manufacturing should be conducted with the highest drug loading to ensure worst-case exposure potential is taken into consideration with data interpretation and recommendations. This is especially important when assessing containment capabilities of facilities that will process highly potent compounds [10].

2.6.3. General Hazards of API

It should be noted that for all specific drugs and drug classes described below, potential health effects and health conditions aggravated are only provided here in abbreviated form. Within most product classes, there are subclasses categorized by API, chemical type or by mechanism that may have specific toxicological properties differing greatly from the information presented here. It is not possible to represent adequately all the effects for any group of pharmaceutical product.

For some of the newer drugs, the toxicological information is too limited to predict every potential health outcome as there has been less time to conduct toxicology studies and observe therapeutic side effects.

The information presented here is therefore limited to known effects for some of the most popular products chosen to represent the product class [10].

2.6.3.1. Central Nervous System

Intended clinical effects of many antidepressant treatments include regulation of patient mood by the reduction of extreme mood changes. This translates to a sedation of the central nervous system that can be seen even at moderate exposures along with tachycardia (high heart rate), tremors, irritability, and nausea. At higher levels of toxicity, reported effects were serotonin syndrome, hyperreflexia, and possible coma. Hypotension or hypertension has been observed as an acute health effect depending on the exposed antidepressant. Example:

Aripiprazole is an antipsychotic agent used for stabilizing patient's mood by moderating serotonin and dopamine receptors. In addition to depression, it is indicated for treatment of bipolar disorder, schizophrenia, and unintentional motor movements (psychomotor agitation) associated with autism and schizophrenia. Antidepressants primarily utilize interactions with signal receptors in the central nervous system. The most common health conditions potentially aggravated are existing psychological disorders, glaucoma, history of seizures, use. Antidepressants only directly affect the central nervous system but this can have indirect effects on many other organs, in particular the cardiovascular system, the liver, and the skeletal system [17].

2.6.3.2. Renal and Cardiovascular System

Anti-diabetic agents allow patients to manage symptoms of diabetes through regulation of insulin, glucose, or glucagon (and their associated metabolic pathways). Possible acute health effects from exposures to anti diabetic agents are hypoglycemia, pancreatitis, renal failure, rhabdomyolysis, and hypersensitivity reactions. Anti diabetic agents can aggravate conditions of the liver, kidney, dehydration, individuals who have recently had surgery. These agents target mostly the liver and pancreas, for example sitagliptin. It blocks the degradation of hormones that are important for insulin regulation. This product is indicated only for type 2 diabetes. The route of administration for clinical treatment with Januvia is through oral pill and can be with other medications for diabetes. [16].

Angiotensin II antagonists are used for the treatment of hypertension (high blood pressure) when patients cannot use angiotensin-converting-enzyme (ACE) inhibitors. Common acute health effects associated with angiotensin II antagonist exposures are associated with hypotension (the clinical effect) such as dizziness and headache. Some of these agents are also known to cause coughing, and in severe cases renal failure. Valsartan and hydrochlorothiazide combination is a kind combined product high blood pressure by promoting vasodilation and regulating hormones that control salt concentrations (18)

As with many other drug classes, angiotensin II antagonists can aggravate conditions involving the kidneys and liver. Additionally, there is a high risk of fetal toxicity to women who are pregnant. Some of these agents are known to cause inflammation to the skin and respiratory system as well [18].

Statins are a class of drugs for lowering the level of cholesterol in the blood by reducing the production of cholesterol by the liver, often used to offset the other source of cholesterol in the blood, which is dietary cholesterol. Statins block the enzyme in the liver that is responsible for making cholesterol called hydroxy-methylglutaryl-coenzyme A reductase (HMG-CoA reductase). Therefore, statins are referred to as HMG-CoA reductase inhibitors [19].

Statins most common adverse reactions include upper respiratory infection, headache, abdominal pain, constipation and nausea. Most notably, statins to a varying extent cause myopathy (muscle deterioration) manifested as muscle pain, tenderness or weakness. The target organs for statins are the liver, kidneys and muscles and present a multitude of drug interactions with various antibiotics and any other class of drug that interrupts the Cytochrome P450 liver enzyme.

Other pharmaceuticals are utilized for the control of blood cholesterol besides statins and include niacin and fibrates. They are often utilized when one component of cholesterol, such as triglycerides, is a factor and when the statins are contraindicated for the patient [19].

2.6.3.3. Gastrointestinal System

Anti-ulcers are typically indicated for treatment of gastro-esophageal acid reflux disease (GERD), ulcer disease, gastric ulcers, and erosive esophagitis. Example: Esomeprazole is an anti-ulcer agent. As with most of the current generation of anti-ulcers, esomeprazole operates by inhibiting proton pumps that secrete gastric acid in the digestive system. Common health effects from acute exposures include some of the most non-specific symptoms such as abdominal pain, diarrhea, dizziness, nausea, and headaches. Repeated exposures may aggravate osteoporosis-related conditions, as well as compromised renal and hepatic systems. Anti-ulcers clinically affect the digestive system but depending on exposure route, exposure levels and the specific agent may affect a variety of other organs [20].

2.6.3.4. Anti-Infective and Target Organs

HIV Antiviral products, like products for other viruses, focus on inhibiting the growth of the virus. HIV antiviral products are usually protease inhibitors or reverse transcriptase inhibitors, as above. Expected health outcomes for acute exposures to some protease inhibitor-class products included vomiting, abdominal pain, and headaches throughout a wide range of doses. For reverse transcriptase inhibitors, the most common reported effects from acute exposures were psychomotor agitation and psychiatric disturbances such as mania and agitation of mood. Whilst overdoses are

possible, no acutely toxic effects are document for some HIV antiviral products. Expected exposure outcomes from these products are limited to known side-effects associated with the therapeutic doses of the product such as: headache, dizziness, rash, nausea, vomiting, and hyperlipidemia.

Antiviral product mechanisms are directed towards the virus itself. At this time, there is no evidence of specific target organs for these effects other than the aforementioned effects that impact the nervous system. There is a significant list of contraindications and drug interactions that may cause additional health effects. Some examples of this are treatments for mental illness, hepatitis B or C, kidney problems, liver problems, or fat and cholesterol problems. Exposures to HIV antiviral products are also known to aggravate pre-existing impairment of the kidneys and the liver. [2, 10].

2.6.3.5. Respiratory Agents

Respiratory agents can range from potent corticosteroids (which are immunosuppressant's) to over-the-counter allergy medications and can therefore be placed in either the anti-infective or endocrine system categories. Common acute health effects can be headache, somnolence, fatigue, tachycardias, and dysrhythmias. Employees with impaired liver or renal function, as well as existing immune system suppression may have these conditions aggravated by exposure to respiratory agents. Respiratory agents all target the respiratory system, but in cases of acute exposures, the kidneys and liver may be affected as well. Chronic exposures have been shown in few cases to affect the cardiovascular system. Example: fluticasone/salmeterol combination is administered in multi-dose inhalers or dry powder inhalers. This combination is indicated for asthma, allergic rhinitis, and atopic dermatitis. This combination and other respiratory agents are mainly used to reduce or eliminate respiratory inflammation. In this combination, fluticasone is a glucocorticoid – a type of corticosteroid for which the adverse effects are described further below in the endocrine system category. [10].

2.6.3.6. Antibiotics

Are chemical substances capable of destroying micro-organisms such as bacteria and viruses that cause infection in animals and humans. The principal ones are:

erythromycin, the penicillin's, the tetracyclines, streptomycin, and clindamycin. The effects of occupational exposure to antibiotics can include:

- **Allergic reactions.** Itching and redness of the eyes, runny nose, skin rashes, asthma, and occasionally shock due to an allergic reaction (anaphylaxis).

- **Vitamin deficiency.** Workers with repeated exposure to antibiotics experience a change in the number and type of bacteria which are normally present in the intestines which break down and absorb vitamins in the intestines. [2,10]

- **Fungal infections.** Daily exposure to antibiotic dust can lead to fungal infections of the skin and nails. Additionally, women workers may develop vaginal yeast infections following exposure to antibiotics.

- **Toxic effects.** Exposure to certain antibiotics may lead to development of some of the toxic side effects that occur when that drug is given as medicine.

- **Other Effects.** Older female workers have been incorrectly told that flushing may be due to hormonal changes rather than the antibiotic drugs they are inadvertently taking in. Headaches and stuffy nose are other common complaints. Allergic heart disorders, bronchial asthma, poisoning, and allergic disorders of the liver have also been reported. Some experts are concerned that prolonged contact with antibiotics may cause cancer, although there have been no studies confirming this suspicion. Described below are common adverse reactions to some specific antibiotics:

- **Penicillins.** Because of the highly allergenic nature of the penicillins and their extensive use, many people have become allergic to them. The most serious reaction is shock. This type of acute reaction usually occurs minutes after exposure. Symptoms are tightness in the chest, asthmatic breathing, dizziness, swelling of the lips, tongue, or face, edema of the lungs, heart failure and in some cases, death. Other reactions are hives, "black hairy tongue," fungus infection, and rectal itch [2, 10, 17].

- **Tetracyclines.** Modification of the bacteria of the intestines and other organs has been reported following occupational exposure to tetracycline (as well as to streptomycin and penicillin). In workers exposed to tetracycline and to streptomycin, modification of the bacteria led to a drop in the body's vitamin content, especially of the

B vitamins. Another problem associated with occupational exposure to tetracycline is drug resistance. Workers may develop infections that are resistant to treatment with tetracycline. [10].

2.6.3.7. Immune System

Immunosuppressive products are used to prevent the immune system from rejecting its own cells or transplanted cells. Examples of immunosuppressive drugs include cytostatic drugs and glucocorticoids. Because of the nature of immunosuppressive products inhibiting the functions of the immune system the primary risk from occupational exposure to these products is the increase in risk of infections and infectious disease. Additional health effects from exposure to immunosuppressive drugs are highly variable to do the variety of products available in this category. When inflammatory functions of the immune system are inhibited the body can no longer prevent infection as effectively nor can it fight existing infections. Immunosuppressive drugs can potentially aggravate any worker with existing infections or infectious disease such as hepatitis or cancers. Example: Humira®, produced by AbbVie, is a Tumor Necrosis Factor (TNF) inhibitor that is indicated for Rheumatoid Arthritis, Plaque Psoriasis, Crohn's Disease, Ankylosing Spondylitis, Psoriatic Arthritis, and Juvenile Idiopathic Arthritis. It is in the class of antibody immunosuppressive drugs. [2.10]

Health effects from immunosuppressive drugs should be considered systemic an inhibited immune system can affect more than a single organ. The mechanism of immunosuppression can be specific as in the case of a specific organ transplant or systemic as in the case of antibody products treating joints throughout the body affected by rheumatoid arthritis [2].

2.6.3.8. Endocrine System

The sex hormones comprise the female sex hormones, the male sex hormones and antagonists and anabolic steroids. Exposure limits for sex hormones can be extremely low, in the low $\mu\text{g}/\text{m}^3$ or even ng/m^3 range.

Female sex hormones include the natural and synthetic estrogens and progestogens. Natural estrogens are used largely in Hormone Replacement Therapy

(HRT) and are often extracted from the urine of pregnant mammals. Synthetic estrogens are used in the treatment of breast and prostate cancer. [2,10]

Anabolic steroids (which were developed from the male sex hormones [androgens]) are used in the treatment of some aplastic anemias, but have been abused by some body builders and athletes. As well as the treatment of various conditions outlined above, the commonest use of the sex hormones is in the oral contraceptive pill. Contraceptive pills may contain estrogen only, progestogen (progesterone and testosterone analogues) only or both. Ethinyloestradiol is the commonest estrogen component in the pill. Esogestrel, levonorgestrel and norethisterone are the most commonly used progestogens. A series of reports throughout the 1970s and 1980s highlighted the problems caused by occupational exposure in both men and women. Studies confirmed the prevalence of problems at very low levels of exposure. The concern with the use of the sex hormones is the development of male traits in women and female traits in men and effects upon fertility. In women menstrual disorders, diminished fertility and increased frequency of spontaneous abortion have been observed. In men reduction in libido, breast growth and testicular pain have been reported. Progestogens may also inhibit spermatogenesis, resulting in decreased fertility. [10].

The effects of occupational exposure to hormones may be severe. For male workers, exposure to estrogens may give rise to breast development; for female workers, there may be menstrual disorders, abnormal overgrowth of the endometrium and excessive bleeding during menopause. Exposure of male workers to progestogen may bring about a lack of sexual drive and testicular pain. On the other hand, exposure of female workers to androgens is known to cause menstrual and ovarian function disorders, diminished fertility, increased frequency of spontaneous abortions, and symptoms of masculinity. The production of female contraceptive pills using diethylstilbestrol and the use in pharmaceuticals of other natural and synthetic steroid hormones has been associated with a number of adverse health effects. [6].

Corticosteroids are a drug class used to treat a variety of conditions ranging from generic inflammation, asthma, and allergic reactions, to more serious conditions such as hepatitis, Crohn's disease, and rheumatoid arthritis. They are produced as creams, lotions and foams for topical application, and as powders for use in inhalers. Many corticosteroids focus on regulating inflammation or an aspect of metabolism through their hormonal activity. Long-term overexposure to corticosteroids can affect many parts of the body:

- Adrenals.** Causes the adrenal glands to make less natural cortisol - a condition called adrenal suppression. This can be life threatening during times of stress, such as illness or surgery.

- Immune system.** Inhibits the normal activity of the immune system, causing increased risk of infection and prolonged wound healing.

- Muscles and bones.** Causes muscle shrinkage and thinning of bones (osteoporosis).

- Skin.** Thins and dries the skin, increases susceptibility to bruising and causes several kinds of skin rash. More frequent, severe infections and slow healing of wounds[2].

These effects have been observed as being reversible upon employee's removal from exposure. Occurring cases suggested that these occupational conditions can be caused by either local exposures or systemic exposures. The most common occupational effect is steroid withdrawal rash (SWR), an acne-like rash is usually accompanied by skin dryness, redness, flaking, and a burning sensation. SWR is triggered by an interruption in steroid overexposure occurs 2-10 days after the last exposure. Most commonly occurs on face, neck or hands typically last for two-three weeks does not usually cause long term effects. SWR is an indicator that over-exposure may have occurred and that current controls may not be effective. Skin is the most common route of overexposure. This can occur when touching a contaminated surface, such as doorknobs, tables, or the outside of a contaminated vial or metered dose inhaler, while not wearing gloves. It can also occur when you touch a contaminated glove to your neck

or face. Once on a surface, glucocorticoids tend to stay there. They are sticky, greasy chemicals which are not easily cleaned away with water-based cleaners. [2].

2.6.3.9. Hazardous Drugs

Drugs considered hazardous include those that exhibit one or more of the following six characteristics in humans or animals:

- Carcinogenicity.
- Teratogenicity or other developmental toxicity.
- Reproductive toxicity.
- Organ toxicity at low doses.
- Genotoxicity.
- Structure and toxicity profiles of new drugs that mimic existing drugs determined hazardous by the above criteria. [21].

Chemotherapy Agents. Traditional oncological (tumor treatment) agents work through mechanisms that kill cells, including both cancerous and normal cells alike. Such agents are called cytotoxic drugs. They have a greater toxic effect on rapidly dividing cells (such as malignant cells) and thus inhibit their growth and proliferation. Most cytotoxic are themselves carcinogens and/or mutagens and some may exhibit other forms of toxicity e.g. reproductive toxicity (teratogenicity or sterility). These drugs can exert biological effects even at very low levels of absorption. Also, as therapeutic dosages are high, the quantities handled by workers and therefore the level of potential exposure can be significant. Example: Methotrexate was developed and continues to be used for chemotherapy either alone or in combination with other agents. It is effective for the treatment of a number of cancers. The most common adverse effects from methotrexate treatment include ulcerative stomatitis, low white blood cell count and thus predisposition to infection, nausea, abdominal pain, fatigue, fever, dizziness, acute pneumonitis and rarely pulmonary fibrosis.

Monoclonal antibody products are an example of a therapy that is more targeted at cancerous cells. Potential occupational effects of monoclonal antibodies are severe projections of common adverse effects from therapeutic doses due to lack of

information. Commonly observed health effects associated with undergoing treatment include heart failure, anemia, nephrotoxicity, reactivation of hepatitis B, and dyspnea. Example: a monoclonal antibody Rituximab binds to specific sites on B lymphocytes that are commonly expressed in lymphomas (but not many other tissues). It is thought to induce cell lysis (rupture of membranes) of neoplasms and apoptosis (programmed cell death) in lymphoma cells. It is indicated for chronic lymphoid leukemia, non-Hodgkin's lymphoma, rheumatoid arthritis, and Wegener's granulomatosis. Observed health conditions that may be aggravated by exposure to the oncological drugs include a variety of respiratory and cardiac conditions, as well as viral infections. Oncological products are known to cause acute toxicity to the skin, liver, kidney, and the blood (if leukemia or blood conditions exist). Some oncological products were found to have reproductive and teratogenic health effects as well.

A relatively new class of drugs that are often oncolytic agents, referred to as antibody drug conjugates (ADC), are the combination of a potent oncolytic agent attached to a monoclonal antibody by a linker molecule. These targeted warheads move directly to the cancerous tissue via the antibody-antigen response in the body to create a targeted and precise impact on the tissue to be eradicated. As a result of their precision they often produce fewer adverse side effects in the patient during and after treatment. Their production is often smaller scale and in liquid-phase for most unit operations and therefore can potentially pose a reduced hazard to the pharmaceutical worker versus large-scale formulation with powders. [2,10,21].

2.6.4. Associated Material Hazards

Similar to industries such as health care and medical research, the pharmaceutical industry has a range of associated material hazards capable of producing acute and chronic health affects in workers. Respiratory sensitizations are common issues Examples of chemicals or other agents used in the industry that can produce sensitization are in the following paragraph [11].

2.6.4.1. Chemicals

Chemical hazards are toxic, corrosive, irritant, carcinogenic, flammable, and mutagenic. According to workplace hazardous materials information, chemical are classified according to physical properties as: [22].

Table 1: Classifications of chemicals according to the physical properties

Chemicals Class	Example
Class A	Compressed gases. Dissolved gases or liquefied gases
Class B	Flammable gases. Flammable and combustible liquids. Flammable solids Flammable aerosols. Reactive flammable material
Class C	Oxidizing materials.
Class D	Poisonous and infectious materials. E.g. Cyanides, tea salts, and Asbestos.
Class E	Corrosive materials. e.g.: Inorganic acids, bases as hydrogen fluoride.
Class F	Dangerous reactive materials. e.g.: Ethylene dioxide, organic azides, Na, Li, Ca.

And also it is classified according to the health hazards as:

- Skin Corrosion/Irritation.
- Serious Eye Damage/Eye Irritation.
- Respiratory or Skin Sensitization.
- Germ Cell Mutagenicity.
- Carcinogenicity.

- Reproductive Toxicology.
- Target Organ Systemic Toxicity - Repeated Exposure.
- Aspiration Toxicity [12, 22].

And as the exposure time of these materials classified as **Acute or chronic toxicity**.

Symptoms of these materials in general are resulting from acute exposures usually occur during or shortly after exposure to a sufficiently high concentration of a contaminant.

The number of exposures for a given contaminant, the symptoms of an acute exposure may be completely different from those resulting from chronic exposure. For either chronic or acute exposure, the toxic effect may be temporary and reversible or may be permanent (disability or death). Some chemicals may cause obvious symptoms such as burning, coughing, nausea, tearing eyes, or rashes. Other chemicals may cause general toxicity of the worker. An important exposure route of concern at a hazardous waste site is inhalation. The lungs are extremely vulnerable to chemical agents. Even substances that do not directly affect the lungs may pass through lung tissue into the bloodstream, where they are transported to other vulnerable areas of the body. Some toxic chemicals present in the atmosphere may not be detected by human senses, i.e., they may be colorless, odorless, and their toxic effects may not produce any immediate symptoms. Respiratory protection is, therefore, extremely important if 2-3 there is a possibility that the work-site atmosphere may contain such hazardous substances. [12].

2.6.4.2. Examples of Chemical Exposure in pharma Industry

2.6.4.2.A. Ethyl Alcohol:

Exposure Limits. TWA 1000 Ppm (1900 mg/m³).

Exposure Routes. inhalation, ingestion, skin and/or eye contact.

Symptoms. irritation eyes, skin, nose; headache, drowsiness, lassitude (weakness, exhaustion), narcosis; cough; liver damage; anemia; reproductive, teratogenic effects.

Target Organs. Eyes, skin, respiratory system, central nervous system. liver, blood, reproductive system.

Personal Protection/Sanitation:

Skin: Prevent skin contact.

Eyes: Prevent eye contact. Eye: Irrigate immediately.

Skin: Water flush promptly.

Breathing: Fresh air.

Swallow: Medical attention immediately.

2.6.4.2.B. Acetone:

Exposure Limits. TWA 250 ppm (590 mg/m³).

Exposure Routes. Inhalation, ingestion, skin and/or eye contact.

Symptoms. Irritation eyes, nose, throat; headache, dizziness, central nervous System depression; dermatitis.

Target Organs. Eyes, skin, respiratory system, central nervous system.

Personal Protection/Sanitation:

Skin: Prevent skin contact.

Eyes: Prevent eye contact.

Wash skin: When contaminated.

Remove: When wet (flammable).

Change: No recommendation.

First Aid:

Eye: Irrigate immediately.

Skin: Soap wash immediately.

Breathing: Respiratory support.

Swallow: Medical attention immediately.

2.6.4.2.C. Methylene Chloride:

Exposure Routes. Inhalation, skin absorption, ingestion, skin and/or eye contact.

Symptoms. Irritation eyes, skin; lassitude (weakness, exhaustion), drowsiness.

Dizziness; numb, tingle limbs; nausea; [potential occupational carcinogen]

Target Organs: Eyes, skin, cardiovascular system, central nervous system.

Cancer Site [In animals: lung, liver, salivary & mammary gland tumors]

Personal Protection/Sanitation:

Skin: Prevent skin contact.

Eyes: Prevent eye contact.

Wash skin: When contaminated.

Remove: When wet or contaminated.

Change: No recommendation.

Provide: Eyewash, Quick drench.

First Aid:

Eye: Irrigate immediately.

Skin: Soap wash promptly.

Breathing: Respiratory support.

Swallow: Medical attention immediately.

2.6.4.2.D. Isopropyl Alcohol:

Exposure Limits. TWA 400 ppm (980 mg/m³) ST 500 ppm (1225 mg/m³)

Exposure Routes. Inhalation, ingestion, skin and/or eye contact.

Symptoms. Irritation eyes, nose, throat; drowsiness, dizziness, headache; dry

Racking skin; In Animals: narcosis.

Target Organs. Eyes, skin, respiratory system.

Personal Protection/Sanitation:

Skin: Prevent skin contact.

Eyes: Prevent eye contact.

Wash skin: When contaminated.

Remove: When wet (flammable).

Change: No recommendation.

First Aid:

Eye: Irrigate immediately.

Skin: Water flush.

Breathing: Respiratory support.

Swallow: Medical attention immediately. [21].

2.7.Occupational Hazards Control and Elimination

In 1950, the World Health Organization (WHO) and the International Labor Organization (ILO) declared that the goals of occupational health should be to:

- Promote and maintain the highest degree of physical, mental and social well-being of workers.
- Prevent ill-health among workers caused by their working conditions.
- Protect workers from factors adverse to their health in their employment.
- Place and maintain workers in occupational environments adapted to their individual physiological and psychological conditions.

While more is known now about the occupational health but there still is much to learn about OHS hazards in the pharmaceutical sector.[2,22,23]

2.7.1. Occupational Exposure Limit (OEL)

TO control airborne exposure of different materials used in in the the work invironment in pharmaceutical industry there should be A control for the following values:

The ‘lead effect’ is often an endpoint related to the pharmacological action of the drug substance. The dose response for this ‘lead effect’ is then examined so that a ‘no observable adverse effect level’ (NOAEL) can be identified. This is then corrected for body weight (BW) and divided by the volume of air (V) breathed by a worker in 8 h (typically, 10 m³ is used as a default) and an appropriate uncertainty factor (UF) is selected. The **OEL** may also be adjusted to account for differences in bioavailability (α) between the inhaled route and the clinical route from which the NOAEL was derived, and for steady-state (S) plasma concentrations if they are higher due to accumulation following repeated exposure . Therefore the OEL can be described:

Employee’s average airborne exposure in any eight-hour work shift which shall not be exceeded.

$$OEL = NOAEL \text{ (mg / kg)} \times BW \text{ (kg)} / UF \times V \text{ (m}^3\text{)} \times S \times \alpha$$

Whereas the OELs for substances such as aspirin or paracetamol are in the mg/m^3 range, the majority of newer pharmaceuticals require exposure controls that reduce workplace exposure to levels $<100 \mu\text{g/m}^3$, with some in the sub- $\mu\text{g/m}^3$ range. These extremely low control limits not only present a significant challenge for bulk pharmaceutical manufacturing operations in controlling dust during solids handling, but also present significant challenges to the occupational hygienist when developing appropriately sensitive personal air sampling and analytical methods. Has to be maintained to such low levels,

For airborne exposures, there are another three types of limits in common use:

- **The Time-Weighted Average (TWA) Exposure Limit.** Which is the maximum average concentration of a chemical in air for a normal 8-hour working day and 40-hour week.
- **The Short-Term Exposure Limit (STEL).** The maximum average concentration to which workers can be exposed for a short period (usually 15 minutes).
- **The Ceiling Value.** The concentration that should not be exceeded at any time.

In addition, biological exposure indices (BEIs) represent the concentration of chemicals in the body that would correspond to inhalation exposure at a specific concentration in air. .[2,10,12]

2.7.2. Clean Rooms and Clean Air Monitoring in Pharmaceutical Industry

2.7.2.1. Cleanroom Classification

A cleanroom is a controlled environment where the concentration of airborne particles like dust, microbes and aerosol particles are controlled. Cleanrooms are maintained and utilized in a manner that minimizes the introduction, generation and retention of particles in the environment. All cleanrooms that meet the Good Manufacturing practices GMPs are classified according to the cleanliness level of the air inside them. Meeting requirements for cleanrooms is one of the most critical issues for laboratories adhering to GMP requirements. The International Organization for Standardization (ISO), a nongovernmental body charged with promoting worldwide standards to ensure safe, reliable and high-quality products, developed classifications

associated with the levels of cleanroom certification. ISO awards cleanroom designations based on a threshold of allowable particles within a specified area. According to GMPs requirements, cleanroom classifications should be carried out according to ISO 14644-1 (Table 2). This ISO classification impacts every cleanroom user in the GMP community.

Tablet 2: ISO 14644 classifications for clean rooms [24]

ISO Classification Number	Maximum allowable concentrations (particles/m ³) for particles equal to and greater than the considered sizes					
	≥0.1µm	≥0.2µm	≥0.3µm	≥0.5µm	≥1µm	≥5.0µm
ISO Class 1	10b	d	d	d	d	e
ISO Class 2	100	24b	10b	d	d	e
ISO Class 3	1,000	237	102	35b	d	e
ISO Class 4	10,000	2,370	1,020	352	83b	e
ISO Class 5	100,000	23,700	10,200	3,520	832	d,e,f
ISO Class 6	1,000,000	237,000	102,000	35,200	8,320	293
ISO Class 7	C	C	C	352,000	83,200	2,930
ISO Class 8	C	C	C	3,520,000	832,000	29,300
ISO Class 9g	C	C	C	35,200,000	8,320,000	293,000

a: All concentrations in the table are cumulative, e.g. for ISO Class 5, the 10,200 particles shown at 0.3µm include all particles equal to and greater than this size.

b :These concentrations will lead to air sample volumes for classification. Sequential sampling procedure may be applied.

c :Concentration limits are not applicable in this region of the table due to very high particle concentration.

d: Sampling and statistical limitations for particles in low concentrations make classification inappropriate.

e: Sample collection limitations for both particles in low concentrations and sizes greater than 1µm make classification at this particle size inappropriate, due to potential losses in the sampling system.

f: In order to specify at this particle size in association with ISO Class 5, the macro particle descriptor M may be adapted and used in conjunction with at least one other particle size.

g : This class is only applicable for the in-operation state.

Clean room classification according to EU GMP based on ISO14644-1 is developed mainly in the two states of the cleanroom, “at rest” and “in operation”.

- “At rest” state is the condition when the factory has completed the construction and installation of production equipment and is operating to meet the requirements, but the operator is not present. [23]
- “In operation” state is the condition when the machine is operating in a defined mode of operation and the presence of a specified number of employees.

By using a particle counter, each different level of cleanroom requires a maximum number of particles that are clearly specified below:

- For Grade A the airborne particle classification is ISO 4 dictated by the limit for particles $\geq 5.0\mu\text{m}$.
- For Grade B (at rest) the airborne particle classification is ISO 5 for both considered particle sizes.
- For Grade C (at rest & in operation) the airborne particle classification is ISO 7 and ISO 8 respectively.
- For Grade D (at rest) the airborne particle classification is ISO 8. [25]

The Guidelines Concepts about Clean Rooms. Because of the amounts of dust usually generated in a solid dosage facility some consideration to be taken in accounts:

- The clean area classifications would be rated for the “at-rest” condition. Room classification tests in the “operational” condition are normally carried out during the normal production process with equipment operating, and the normal number of personnel present in the room. Generally a room that is tested for an “operational” condition should be Able to be cleaned up to the “at-rest” clean area classification after

a short Clean-up time. The clean-up time should be determined through validation and is generally of the order of 20 minutes.

- The required degree of air cleanliness in most oral solid forms (OSD) manufacturing Facilities can normally be achieved without the use of high-efficiency particulate air (HEPA) filters, provided the air is not recirculated or in the case of a single-product facility.
- Many open product zones of OSD form facilities are capable of meeting ISO 14644-1 Class 8 or Grade D, “**at-rest**” condition, measured against particle sizes of 0.5 μ meter And 5 μ meter, but cleanliness may not necessarily be classified as such by manufacturers.
- A risk assessment should be carried out to determine the cleanroom conditions required and the extent of validation required. [25]

2.7.2.2. Basic Criteria of Clean Area

Areas for the manufacture of Pharmaceuticals, where pharmaceutical starting Materials and products, Primary packing materials and equipment are exposed to the environment, Should be defined as “clean areas”, “clean zones”, “controlled areas” or “Cleanrooms”. The achievement of a particular clean area Condition depends on a number of criteria that should be addressed at the Design and Qualification Stages. A suitable balance between the different criteria will be required in Order to create an efficient clean area.

Some of the basic criteria to be considered which affects room cleanliness should include:

- building finishes and structure.
- Air filtration.
- Air change rate or flushing rate.
- Room pressure.
- Location of air terminals and directional airflow.
- Temperature.
- Relative humidity.

- Material flow.
- Personnel flow.
- Equipment movement.
- Process being carried out (open or closed system).
- Outside air conditions.
- Occupancy.
- Type of product. [25]

Number of Air Changes. Air filtration and air change rates should be set to ensure that the defined clean area condition is attained. The air change rates should be determined by the manufacturer and designer, taking into account the various critical parameters using a risk based approach with due consideration of capital and running costs and Energy usage. Primarily the air change rate should be set to a level that will achieve the required clean area condition.

Air change rates are normally determined by the following Considerations (could normally vary between 6 and 20 air changes per hour:

- The product characteristics (e.g. odors, hygroscopicity, etc.)
- The quality and filtration of the supply air.
- Particulates generated by the manufacturing process.
- Particulates generated by the operators.
- Configuration of the room and air supply and extract locations.
- Sufficient air to achieve containment effect and to clean up the area.
- Sufficient air to cope with the room heat load.
- Sufficient air to balance extracts rates.
- Sufficient air to maintain the required room pressure. [25]

Filtration Criteria of Clean Area. The following Considerations should be taken in account:

- Airborne contaminants should be controlled through effective Ventilation and filtration.

- Airborne particulates and the degree of filtration should be considered critical parameters with reference to the level of product Protection required.
- The level of protection and air cleanliness for different areas should be determined according to the product being manufactured, the process being used and the product's susceptibility to degradation.
- Directional airflow within production or primary packing areas should assist in preventing contamination.
- Airflows should be planned in conjunction with operator locations, so as to minimize contamination of the Product by the operator and also to protect the operator from dust inhalation.
- Heating ventilation air conditioning system (HVAC) air distribution components should be designed, installed and located to prevent contaminants generated within the room from being spread.
- Unidirectional airflow (UDAF) should be used for weighing booths or sampling booths to provide operator and product protection and should also have a slight air in-flow from the room to enhance containment.

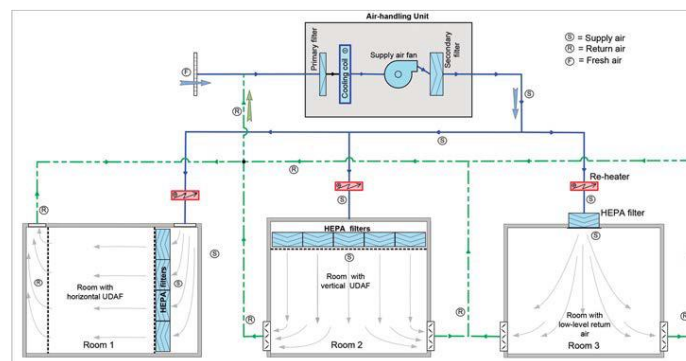


Figure 3. UDAF unidirectional airflow.

- Dust containment at the weigh booth should be demonstrated by smoke airflow Pattern tests or other appropriate tests. UDAF can also be used to provide Protection of other dusty processes.

- Sampling of materials such as starting materials, primary packaging Materials and products, should be carried out in the same environmental Conditions that is required for the further processing of the product.
- The position in which the operator stands relative to the source of dust liberation and airflow should be determined to ensure that the operator is not in the path of an airflow that could lead to contamination of the product. [25]



Figure 4. Operator protection in weighing room

- Where automated monitoring systems are used, these should be capable of indicating any out-of-specification condition without delay by means of an alarm or similar system. Sophisticated computer-based data monitoring systems may be installed, which can aide with planning of preventive maintenance and can also provide trend logging. (This type of system is commonly referred to as a building management system (BMS), building automation system (BAS) or system control and data acquisition (SCADA) system.). Failure of a supply air fan, return air fan, exhaust air fan or dust extract system fan can cause a system imbalance, resulting in a pressure cascade malfunction with a resultant airflow reversal. [25]
- In Recirculation system there should be no risk of contamination or cross-contamination (including by fumes and volatiles) due to recirculation of air. Depending on the airborne contaminants in the return-air system it may be acceptable to use recirculate air, provided that HEPA filters are installed in the supply air stream (or

return air stream) to remove contaminants and thus prevent cross-contamination. The HEPA filters for this application should have an EN 1822 classification of H13.

- HEPA filters may not be required where the air-handling system is serving a single product facility and there is evidence that cross contamination would not be possible.
- Air containing dust from highly toxic processes and/or solvents or flammable vapors should never be recirculated to the HVAC system.
- The required degree of filtration of the exhaust air depends on the Exhaust air contaminants and local environmental regulations.

HEPA filters in the exhaust system would normally only be required when handling hazardous materials. [25]

Filtered air Differential pressure Criteria of Clean Area. Manufacturing facilities should normally be maintained as the following:

- Positive Pressure relative to the outside, to limit the ingress of contaminants.
- Where Facilities are to be maintained at negative pressures relative to the ambient Pressure, special precautions should be taken.
- Refer to the WHO guideline for hazardous products, for further guidance on negative pressure facilities,

Highly potent products should be manufactured under a pressure Cascade regime that is negative relative to atmospheric pressure. [25].

2.7.3. Chemical Safety Guidelines

Before starting work with a chemical, a clean room environment should be used and many procedures have to be followed to protect workers where chemicals exist.

- a “chemical hazard pocket guide” should be consulted for necessary information about the chemical. It will give the type of reaction the chemical may produce, its inflammability, carcinogenicity, prevention, and treatment procedures, etc.
- No eating, drinking, or smoking where chemicals are used.
- Workers working in antibiotic-related products must be changed routinely so that an individual is not exposed to a certain antibiotic for a long period of time.

- Whenever a dust allergy or respiratory problem precipitates, the worker should immediately be removed from the workplace and put under proper health care.
- In case of inflammable gas or solvent leakage, the exhaust fans should be started, and all the source of fire should be extinguished.
- Provide on-going safety training.
- Never use any substance that is not properly labeled.
- Follow all chemical safety instructions, such as those listed in material safety data sheets or on chemical container labels, and precisely.
- Minimize your exposure to any chemical, regardless of its hazard rating, and avoid repeated exposure .
- Use personal protective equipment (PPE), as appropriate for that chemical.
- Use the buddy system when working with hazardous chemicals. Do not work in the
- Minimize your exposure to any chemical, regardless of its hazard rating, and avoid repeated exposure. [22]

2.7.4. General Management of Over Exposure To Chemicals or Hazardous Materials

- Prompt removal of the person from the exposure site is the first step.
- Air respirators and lifelines are a mandatory first aid.
- Resuscitation which means restoration of life of one who is apparently dead (collapsed or shocked). Further supportive care should be provided as with any other medical emergency.
- Decontamination a victim whose skin or clothing has been contaminated requires immediate removal of garments and shoes. Then, vigorous showering with soap and water, including attention to the fingernails and scalp is advised.
- Acute over exposure may result in a variety of signs and symptoms that require general supportive medical management regardless of the specific agent. Examples include the control of convulsive seizures bronchospasm.

- Specific management done after arriving to the specialized medical care center if needed. [11]

2. 8. Manufacturing Steps of Pharmaceutical Oral Solid Dosage Form

As tablets are the most common solid dosage form which manufactured in non-pharmaceutical industry and also it is considered the most production activity which can cause particles can be produced during work so we will explain the procedures followed in it in order to focus on the most critical steps.

The design and manufacture of pharmaceutical tablets is a complex multi-stage process whereby formulation scientists ensure that the correct amount of drug substance in the right form is delivered at the appropriate time, at the proper rate and in the desired location with its chemical integrity protected to that point. Most drug substances do not possess the required properties which give satisfactory flow from the hopper to the die cavity of tablet presses. As a result, they are subjected to pre-treatment either alone or in combination with suitable excipients to form free-flowing granules that lend themselves to tableting. [27]

Tablets are common manufactured by wet granulation, dry granulation or direct compression.

2.8.1. Primary Goals of Tablet Manufacturing process.

In general, the choice of formulation process employed during tablet manufacture is dependent upon such factors as:

- Compression properties of the Active Pharmaceutical Ingredient (API)/ drug substance.
- Physical and chemical stability of the API during the manufacturing process.
- Particle size of the formulation ingredients [26].

2.8.2. Personnel Requirements During Manufacture of pharmaceutical Tablets.

- Production pharmacists/ supervisors
- Manufacturing chemist
- Analytical chemist
- Quality assurance manager

- Machine operators
- Mechanics

In addition to the job-specific responsibilities of these personnel, all manufacturing employees must be versed and trained in Current Good Manufacturing Practices (CGMPs) and in the appropriate Standard Operating Procedures (SOPs) governing their area. [27]

2.8.3. Area Required for Manufacture of Tablets.

- Raw material warehouse.
- Dispensary.
- Mixing, Granulation, milling, sieving, and Drying Section.
- Tableting Section.
- Coating Section.
- Blistering section.
- Quality control section
- Packaging Section.

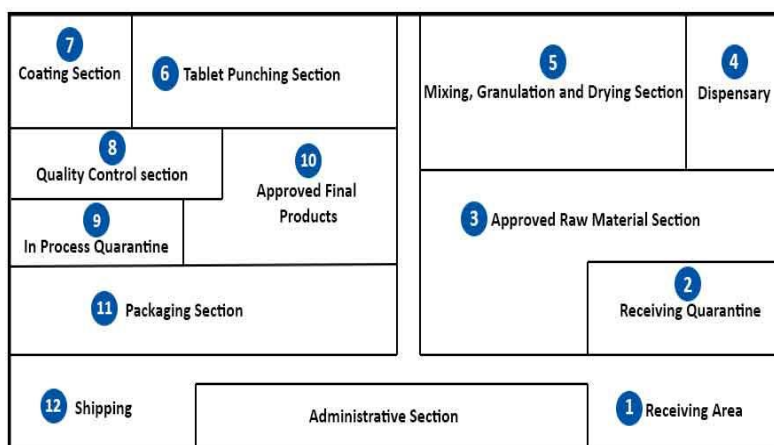


Figure 5. Area required for manufacture of tablets

2.8.4. Excipients Used in Tablet Formulation

Tablets in addition to the therapeutic agent(s) also contain excipients that are required to ensure satisfactory production process. These materials which are inert may be added to the drug substance to increase its bulk and give those desirable properties lacking in the drug substance alone. Depending on the intended use, tablet excipients may be subcategorized into:

- Those that help to impart satisfactory processing and compression properties to the formulation.
- Those that help to give additional desirable physical characteristics to the compressed tablet.

Many excipients used in tablet formulation are multi-functional that is, they may serve more than one function and thus can affect the properties of powders or tablets in various ways at varying concentrations. [28]

2.8.5. Some Multiple-Use Excipients in Tablet Formulation

All excipients used in tablet formulation may not be present in all tablet formula. Some such as lactose, stearates, microcrystalline cellulose are common to the vast majority of tablets. Excipients used in tablet formulations include binders or granulating fluid, diluents, disintegrates, lubricants, giants, colorants, sweeteners, flavourants, adsorbents, and surfactants. [27]

2.8.6. Techniques/ Methods Used in Tablet Formulation

Tablets are commonly manufactured by:

- Wet granulation.
- Dry granulation.
- Direct compression.

One important requirement is that the drug mixture flows freely from the hopper of the tableting machine into the dies to enable high-speed compression of the powder mix into tablets. [27]

2.8.6.1. Manufacture of Tablets by Wet Granulation Method

Wet granulation is a widely used method for the production of compressed tablet. It is essentially a process of size enlargement involving several steps and the use of an adhesive substance known as binder. The granules produced using this method of granulation has a greater probability of meeting all the physical requirements for tablet formation.

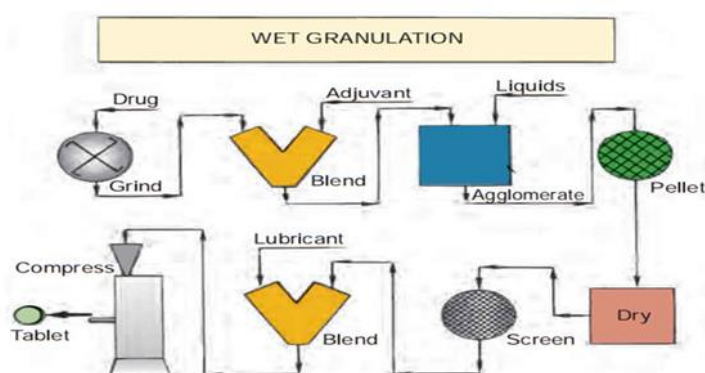


Figure 6. Flowchart of wet granulation process

A stepwise summary of the manufacturing steps used in the manufacture of tablets by the wet granulation method are listed below:

1. Weighing, milling and mixing of the APIs with powdered excipients (excluding the lubricant).
2. Preparation of binder solution.
3. Mixing of binder solution with powders to form a damp mass.
4. Screening the dampened powder into pellets or granules (wet screening) using 6- to 12-mesh screen.
5. Drying of moist granules.
6. Sizing the granulation by dry screening using 14- to 20-mesh screen.
7. Mixing of the dried granules with lubricant and disintegrates.
8. Compression of granules into tablets.

Tablets manufactured by wet granulation exhibit sufficient mechanical properties. [27]

2.8.6.2. Manufacture of Tablets by Dry Granulation Method

The formation of granules by compacting powder mixtures into large pieces or compacts which are subsequently broken down or sized into granules (often referred to as dry granulation, double compression or pre-compression) is a possible granulation method which, however, is not widely used in the manufacture of tablets. This method is used when tablet excipients have sufficient inherent binding properties. The procedure can also be used as a means to avoid exposure of drug substances to elevated temperatures (during drying) or moisture. Double compression method eliminates a number of steps but still includes weighing, mixing, slugging, dry screening, lubrication, and compression of granules into tablets. Compaction for the dry granulation process is generally achieved either by slugging or roller compaction. [27]

2.8.6.2. A Slugging. In this method, the powder mix is compressed into a soft large flat tablet (about 1 inch in diameter) using a tablet press that is capable of applying high stress. Following this, the slugs are broken by hand or milled using conventional milling equipment to produce granules of the required size. Lubricant is added in the usual manner, and the granules then compressed into tablets. Aspirin is a good example of where slugging is satisfactory. Other materials, such as aspirin combinations, acetaminophen, thiamine hydrochloride, ascorbic acid, magnesium hydroxide, and other antacid compounds, may be treated similarly. [27]

2.8.6.2. B. Roller Compaction. Results similar to those accomplished by the slugging process are also obtained with powder compactors. In roller compaction method, the formulation ingredients are mixed and are passed between high-pressure (oppositely) rotating rollers that compress the powder at 1 to 6 tons of pressure. The compacted material is then milled to a uniform granule size and compressed into tablets after the addition of a lubricant. The roller compaction method is often preferred to slugging. Excessive pressures that may be required to obtain cohesion of certain materials may result in a prolonged dissolution rate. [27].

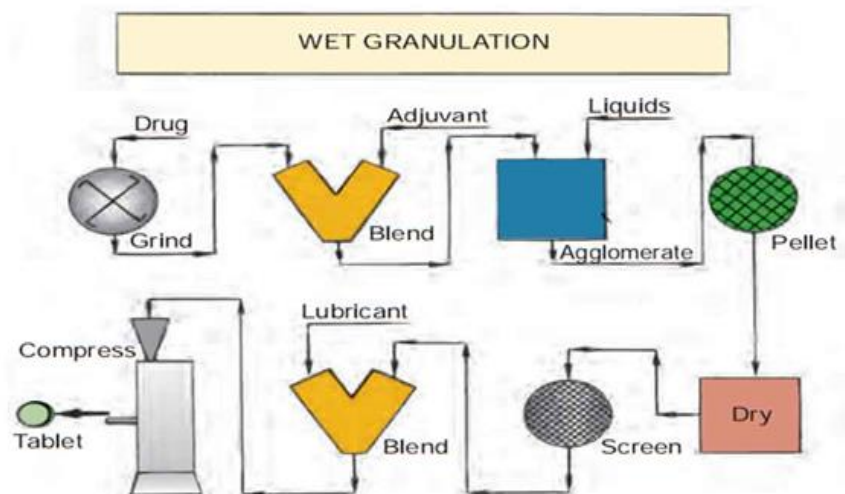


Figure7. Flowchart of dry granulation process

A stepwise summary of the manufacturing steps used in the manufacture of tablets by the dry granulation method are listed below.

1. Weighing and Milling of formulation ingredients (drug substance and excipients)
2. Mixing of milled powders.
3. Compression of mixed powders into slugs.
4. Milling and sieving of slugs.
5. Mixing with disintegrate and lubricant.
6. Compression into tablets. [27]

2.8.6.2.C Direct Compression:. As its name implies, direct compression involves direct compression of powdered materials into tablets without modifying the physical nature of the materials itself. The technology involved in this method assumes great importance in the tablet formulations, because it is often the cheapest means, particularly in the production of generics that the active substance permits.

Direct compression avoids many of the problems associated with wet and dry granulations. Its successful application in tablet formulation rests on two fundamental issues:

- The availability of suitable excipients.
- The availability of suitable machinery.

A stepwise summary of the manufacturing steps used in the manufacture of tablets by the dry granulation method are listed below.

1. Milling of therapeutic agent and excipients.
2. Mixing of milled powders, disintegrates and lubricants.

It is worth noting that tablets produced by direct compression are often softer than their counterparts that have been produced by wet granulation and therefore they may be difficult to film-coat. [27]

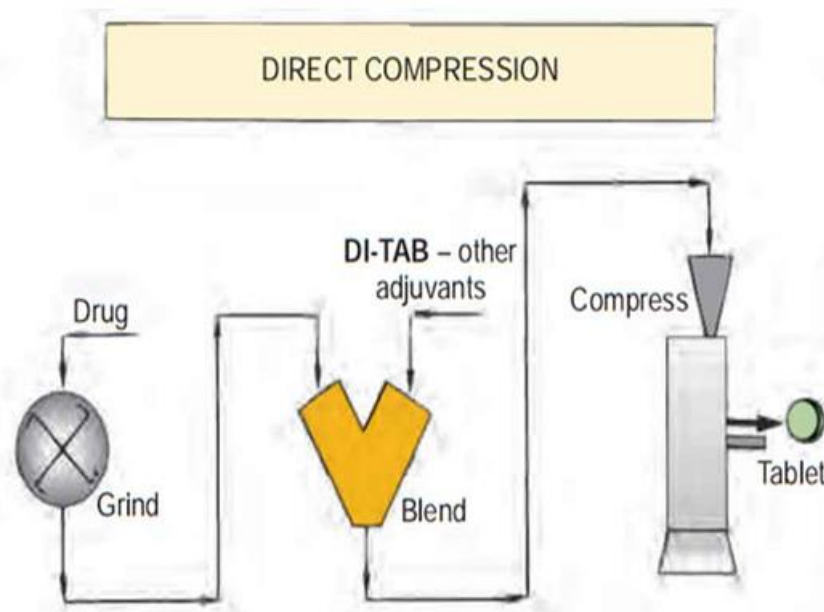


Figure 8. Flowchart of direct compression process

2. 9. The Pharmaceutical Materials Used In This Study

2.9.1. Metformin

Metformin is a biguanide drug used in conjunction with diet and exercise for glycaemic control in type 2 diabetes mellitus and used off-label for insulin resistance in polycystic ovary syndrome (PCOS). [29]

Currently, metformin is the first drug of choice for the management of type II diabetes and is prescribed to at least 120 million people worldwide.

Metformin is commonly described as an insulin sensitizer leading to a decrease in insulin resistance and a clinically significant reduction of plasma fasting insulin levels. Another well-known benefit of this drug is modest weight loss. Metformin is the drug of choice for obese type II diabetes patients.

Metformin was first approved in Canada in 1972 [8], followed by 1995 in the USA. This drug is available in regular and extended-release forms.[29]

2. 9.1.1. Absorption. Of Regular Tablet

The absolute bioavailability of a metformin 500 mg tablet administered in the fasting state is about 50%-60%. Single-dose clinical studies using oral doses of metformin 500 to 1500 mg and 850 to 2550 mg show that there is a lack of dose proportionality with an increase in metformin dose, attributed to decreased absorption rather than changes in elimination Label.

At usual clinical doses and dosing schedules of metformin, steady-state plasma concentrations of metformin are achieved within 24-48 hours and are normally measured at $<1 \mu\text{g/mL}$.[29]

2.9.1.2. Extended-Release Tablet Absorption

After a single oral dose of metformin extended-release, C_{max} is reached with a median value of 7 hours and a range of between 4 and 8 hours. Peak plasma levels are measured to be about 20% lower compared to the same dose of regular metformin, however, the extent of absorption of both forms (as measured by area under the curve - AUC), are similar . [29]

2.9.1.3. Effect of Food

Food reduces the absorption of metformin, as demonstrated by about a 40% lower mean peak plasma concentration (C_{max}), a 25% lower area under the plasma concentration versus time curve (AUC), and a 35-minute increase in time to peak plasma concentration (T_{max}) after ingestion of an 850 mg tablet of metformin taken with food, compared to the same dose administered during fast. Though the extent of metformin absorption (measured by the area under the curve - AUC) from the metformin extended-release tablet is increased by about 50% when given with food, no effect of food on C_{max} and T_{max} of metformin is observed. High and low-fat meals exert similar effects on the pharmacokinetics of extended-release metformin.[29]

2.9.1.4. Volume of Distribution

The apparent volume of distribution (V/F) of metformin after one oral dose of metformin 850 mg averaged at 654 ± 358

Protein binding of Metformin is negligibly bound to plasma proteins, in contrast to sulfonylureas, which are more than 90% protein bound [29]

2.9.1.5. Metabolism

Intravenous studies using a single dose of metformin in normal subjects show that metformin is excreted as unchanged drug in the urine and does not undergo hepatic metabolism (no metabolites have been identified in humans) or biliary excretion. [29]

2. 9.1.6. Route of Elimination

This drug is substantially excreted by the kidney. Renal clearance of metformin is about 3.5 times higher than creatinine clearance, which shows that renal tubular secretion is the major route of metformin elimination. After oral administration, about 90% of absorbed metformin is eliminated by the kidneys within the first 24 hours post-ingestion.

Half-life is Approximately 6.2 hours in the plasma and in the blood, the elimination half-life is approximately 17.6 hours, suggesting that the erythrocyte mass may be a compartment of distribution .[29]

2.9.1.7. Toxicity

Metformin (hydrochloride) toxicity data is as following:

Oral LD50 (rat): 1 g/kg; Intraperitoneal LD50 (rat): 500 mg/kg; Subcutaneous LD50 (rat): 300 mg/kg; Oral LD50 (mouse): 1450 mg/kg; Intraperitoneal LD50 (mouse): 420 mg/kg; Subcutaneous LD50 (mouse): 225 mg/kg.

A note on lactic acidosis. Metformin decreases liver uptake of lactate, thereby increasing lactate blood levels which may increase the risk of lactic acidosis. There have been reported post marketing cases of metformin-associated lactic acidosis, including some fatal cases. Such cases had a subtle onset and were accompanied by nonspecific symptoms including malaise, myalgias, abdominal pain, respiratory distress, or increased somnolence. In certain cases, hypotension and resistant Brady arrhythmias have occurred with severe lactic acidosis. Metformin-associated lactic acidosis was characterized by elevated blood lactate concentrations (>5 mmol/L), anion gap acidosis (without evidence of ketonuria or ketonemia), as well as an increased lactate: pyruvate ratio; metformin plasma levels were generally >5 mcg/mL[29].

Risk factors for metformin-associated lactic acidosis include renal impairment, concomitant use of certain drugs (e.g. carbonic anhydrase inhibitors such as Topiramate), age 65 years old or greater, having a radiological study with contrast, surgery and other procedures, hypoxic states (e.g., acute congestive heart failure), excessive alcohol intake, and hepatic impairment .[29]

A note on renal function. In patients with decreased renal function, the plasma and blood half-life of metformin is prolonged and the renal clearance is decreased. Metformin should be avoided in those with severely compromised renal function (creatinine clearance < 30 ml/min), acute/decompensated heart failure, severe liver disease and for 48 hours after the use of iodinated contrast dyes due to the risk of lactic acidosis. Lower doses should be used in the elderly and those with decreased renal function.

Metformin decreases fasting plasma glucose, postprandial blood glucose and glycosylated haemoglobin (HbA1c) levels, which are reflective of the last 8-10 weeks of glucose control. Metformin may also have a positive effect on lipid levels. [29]

A note on hypoglycaemia when used alone. Metformin does not cause hypoglycaemia, however, it may potentiate the hypoglycaemic effects of sulfonylureas and insulin when they are used together.[29]

2.9.1.8. Use in pregnancy

Available data from post-marketing studies have not indicated a clear association of metformin with major birth defects, miscarriage, or adverse maternal or fetal outcomes when metformin was ingested during pregnancy. Despite this, the abovementioned studies cannot definitively establish the absence of any metformin-associated risk due to methodological limitations, including small sample size and inconsistent study groups.[29]

2.9.1.9. Use in Nursing

A limited number of published studies indicate that metformin is present in human milk. There is insufficient information to confirm the effects of metformin on the nursing infant and no available data on the effects of metformin on the production of milk. The developmental and health benefits of breastfeeding should be considered as well as the mother's clinical need for metformin and any possible adverse effects on the nursing child.[29]

2.9.2. Losartan potassium

Losartan is an angiotensin receptor blocker used to treat hypertension and diabetic nephropathy, and is used to reduce the risk of stroke. [30]

2.9.2.1. Absorption

Losartan is approximately 33% orally bioavailable. Taking losartan with food decreases the C_{max} but does only results in a 10% decrease in the AUC of losartan and its active metabolite. 1,3,4 A . 50-80mg oral dose of losartan leads to a C_{max} of 200-250ng/mL. [30]

2.9.2.2. Volume of Distribution

The volume of distribution of losartan is $34.4 \pm 17.9L$ and $10.3 \pm 1.1L$ for the active metabolite E-3174. Protein binding Losartan is 98.6-98.8% protein bound and the active metabolite (E-3174) is 99.7% protein bound in serum. [30]

2. 9.2.3. Route of Elimination

A single oral dose of losartan leads to 4% recovery in the urine as unchanged losartan, 6% in the urine as the active metabolite.^{3,4} Oral radiolabelled losartan is 35% recovered in urine and 60% in feces. Intravenous radiolabelled losartan is 45% recovered in urine and 50% in feces.

Half-life the terminal elimination half-life of losartan is 1.5-2.5 hours while the active metabolite has a half-life of 6-9 hours. Clearance of **Losartan** has a total plasma clearance of 600mL/min and a renal clearance of 75mL/min.¹ E-3174, the active metabolite, has a total plasma clearance of 50mL/min and a renal clearance of 25mL/min.¹[30].

2.9.2.4. Toxicity

The oral TDLO in mice is 1000mg/kg and in rats is 2000mg/kg. In humans the TDLO for men is 10mg/kg/2W and for women is 1mg/kg/1D.

Symptoms of overdose are likely to include hypotension, tachycardia, or bradycardia due to vagal stimulation. Supportive treatment should be instituted for symptomatic hypotension. Haemodialysis will not remove losartan or its active metabolite due to their high rates of protein binding. [30]

2.9.3. Enalapril

Enalapril is a prodrug of an ACE inhibitor used to treat hypertension and congestive heart failure. Enalapril is a pharmacologically inactive prodrug that requires hepatic biotransformation to form enalaprilat, its active metabolite that works on the RAAS to inhibit ACE. Biotransformation is critical for the therapeutic actions of the drug, as enalapril itself is only a weak inhibitor of ACE. [31]

2.9.3.1. Absorption

Following oral administration, the peak plasma concentrations (C_{max}) of enalapril is achieved within 1 hour post dosing while the C_{max} of Enalaprilat occurs at three to four hours post dosing. The steady-state is achieved by the fourth daily dose and there is no accumulation with repeated dosing. However, accumulation of enalaprilat may occur in patients with creatinine clearance less than 30 mL/min. Food intake is reported to have a minimal effect on drug absorption. Following oral administration, about 60% of Enalapril was absorbed. Bioavailability of Enalapril averaged about 40% when intravenous Enalaprilat was used as a reference standard [30]

2.9.3.2. Volume of Distribution

The volume of distribution of Enalapril has not been established. Enalaprilat is shown to penetrate into most tissues, in particular the kidneys and vascular tissue, although penetration of the blood-brain barrier has not been demonstrated after administration at therapeutic doses. In dog studies, Enalapril and Enalaprilat cross the blood-brain barrier poorly. Minimal penetration occurs into breast milk but significant fetal transfer occurs. The drug crosses the placental barrier in rats and hamsters. It is reported that less than 50% of Enalaprilat is bound to human plasma proteins, based on limited data from binding studies of Enalaprilat in human plasma both by equilibrium dialysis and by ultrafiltration[31] .

2.9.3.3. Metabolism

About 60% of the absorbed dose is extensively hydrolysed to Enalaprilat via de-esterification mediated by hepatic esterases in humans. [31]

2.9.3.4. Route of Elimination

Enalapril is mainly eliminated through renal excretion, where approximately 94% of the total dose is excreted via urine or feces as either Enalaprilat or unchanged parent compound .About 61% and 33% of the total dose can be recovered in the urine and feces, respectively. In the urine, about 40% of the recovered dose is in the form of Enalaprilat. Half-life of Enalaprilat is 35-38 hours. The effective half-life following multiple doses is 11-14 hours. The prolonged terminal half-life is due to the binding of

Enalaprilat to ACE. Following oral administration in healthy male volunteers, the renal clearance was approximately 158 ± 47 mL/min.⁸ It is reported that Enalapril and Enalaprilat are undetectable in the plasma by 4 hours post-dosing. [31]

2.9.3.5. Toxicity

LD50 and Overdose Oral LD50 in rats is 2973 mg/kg. MSDS Lethality was observed with single oral doses of Enalapril above 1000 mg/kg in mice and greater than or equal to 1775 mg/kg in rats. Serum Enalaprilat levels 100- and 200-fold higher than usually seen after therapeutic doses have been reported after ingestion of 300 mg and 440 mg of Enalapril, respectively. While there is limited data about Enalapril overdose in humans, over dosage may result in marked hypotension and stupor based on the pharmacological properties of the drug. Most common adverse effects of Enalapril include cough, hypotension, stupor, headache, dizziness and fatigue. If hypotension is seen, usual treatment of intravenous infusion of normal saline solution is recommended. Enalaprilat may be removed from systemic circulation with the use of haemodialysis. It has been removed from neonatal circulation by peritoneal dialysis. [31]

Nonclinical toxicology. Maternal and fetal toxicity occurred in some rabbits treated with Enalapril at doses of 1 mg/kg/day or more. There was no fetotoxicity, expressed as a decrease in average fetal weight, or teratogenicity in rats treated with Enalapril at doses up to 200 mg/kg/day, which is about 333 times the maximum human dose. In mice and rats receiving Enalapril at doses ranging from 90 to 180 mg/kg/day, there was no evidence of a tumorigenic effect. Neither Enalapril nor its active metabolite were shown to be mutagenic or genotoxic in in vitro and in vivo studies. There were no adverse effects on reproductive performance of male and female rats treated with up to 90 mg/kg/day of Enalapril Use in special populations.

Caution is warranted in patients who are concurrently using another ACE inhibitors with Enalapril, as there have been incidences of agranulocytosis with the use of captopril, which is another ACE inhibitor. This adverse event may be particularly significant in patients with renal impairment or collagen vascular disease. As Enalapril and Enalaprilat were shown to be secreted in human milk in trace amounts, the use of

Enalaprilat in nursing women is not recommended. Significant fetal transfer occurs with Enalapril and Enalaprilat thus the use of the drug in pregnant women should be strongly avoided. Caution is advised when Enalapril is used in patients who are elderly or with renal impairment, as dosage adjustments may be appropriate. The antihypertensive effect of angiotensin converting enzyme inhibitors is generally lower in individuals of African descent, usually a low-renin hypertensive population. [31]

2.9.4. Hydrochlorothiazide

is a thiazide diuretic prevents the reabsorption of sodium and water from the distal convoluted tubule, allowing for the increased elimination of water in the urine., it is indicated alone or in combination for the management of edema associated with congestive heart failure, hepatic cirrhosis, nephrotic syndrome, acute glomerulonephritis, chronic renal failure, and corticosteroid and oestrogen therapy. Hydrochlorothiazide is also indicated alone or in combination for the management of hypertension. [32]

2.9.4.1. Absorption

An oral dose of hydrochlorothiazide is 65-75% bioavailable, with a Tmax of 1-5 hours, and a Cmax of 70-490ng/mL following doses of 12.5-100mg.^{10,11} When taken with a meal, bioavailability is 10% lower, Cmax is 20% lower, and Tmax increases from 1.6 to 2.9 hours[32].

2.9.4.2. Food Interactions

Avoid alcohol. Alcohol may potentiate orthostatic hypotension. Avoid multivalent ions. Take this medication 2 hours before or after the administration of antacids, calcium supplements, or iron supplements to avoid decreased absorption of hydrochlorothiazide. Avoid natural liquorice. Liquorice potentiates the hypokalaemia effect of hydrochlorothiazide. Increase consumption of potassium-rich foods. This medication may cause potassium depletion. Foods containing potassium include bananas and orange juice. Limit salt intake. Avoid excessive salt, unless recommended by a physician. Take with or without food. [32]

2.9.4.3. Volume of Distribution

The volume of distribution varies widely from one study to another with values of 0.83-4.19L/kg. Protein binding is 40-68% protein bound in plasma. [32].

2.9.4.4. Metabolism

Hydrochlorothiazide is not metabolized. [32]

2.9.4.5. Route of Elimination

Hydrochlorothiazide is eliminated in the urine as unchanged hydrochlorothiazide. The plasma half-life of hydrochlorothiazide is 5.6-14.8h. [32]

The renal clearance of hydrochlorothiazide in patients with normal renal function is 285mL/min. Patients with a creatinine clearance of 31-80mL/min have an average hydrochlorothiazide renal clearance of 75mL/min, and patients with a creatinine clearance of ≤ 30 mL/min have an average hydrochlorothiazide renal clearance of 17mL/min.[32]

2.9.4.6. Toxicity

The oral LD50 of hydrochlorothiazide is >10 g/kg in mice and rats. Patients experiencing an overdose may present with hypokalaemia, hyponatremia, and hypochloremia. Treat patients with symptomatic and supportive treatment including fluids and electrolytes. Vasopressors may be administered to treat hypotension and oxygen may be given for respiratory impairment. [32]

2. 9.5. Glyburide

Glyburide is a second generation sulfonylurea used to treat patients with diabetes mellitus type II. It is typically given to patients who cannot be managed with the standard first line therapy, metformin [33]

2.9.5.1. Absorption

Elderly patients taking glyburide reached a C_{max} of 211-315ng/mL with a T_{max} of 0.9-1.0h, while younger patients reached a C_{max} of 144-302ng/mL with a T_{max} of 1.3-3.0h. Patients taking glyburide have and AUC of 348ng*h/mL with absorption rate of about 40% [33]

Oral bioavailability is variable according to the oral dosage form [34]

2.9.5.2. Volume of Distribution

Elderly patients have a volume of distribution of 19.3-52.6L, while younger patients have a volume of distribution of 21.5-49.3L. Protein binding is 99.9% bound to protein in plasma with >98% accounted for by binding to serum albumin. [33]

2.9.5.3. Route of Elimination

Unlike other sulfonylureas, glyburide is 50% excreted in the urine and 50% in the feces. Glyburide is mainly excreted as the metabolite 4-trans-hydroxyglyburide. Elderly patients have a terminal elimination half-life of 4.0-13.4h, while younger patients have a terminal elimination half-life of 4.0-13.9h. . Elderly patients have a clearance of 2.70-3.55L/h, while younger patients have a clearance of 2.47-4.11L/h [33]

2.9.5.4. Toxicity

The oral LD50 in rats is >3200mg/kg, in mice is >1500mg/kg, in rabbits is >10,000mg/kg, and in guinea pigs is >1500mg/kg.

Patients experiencing an overdose may present with hypoglycaemia. Mild hypoglycaemia should be treated with oral glucose and adjustments to drug doses or meal schedules. Severe hypoglycaemia may present with coma, seizure, and neurological impairment. This should be treated immediately in hospital with intravenous glucose and monitoring for 24-48 hours. [33].

2.9.6. Talc

Chemically it is hydrous magnesium silicate.

It is used as lubricant in tablet formulation.

2.9.6.1. Physical State

Solid Powder, White, Greyish White Insoluble in water, Specific gravity 2.7- 2.8

2.9.6.2. Toxicity Symptoms

Skin Contact. May cause skin irritation. It may cause mild skin irritation. Do not get on broken skin. It will contaminate the wound and can cause a severe granulomatous reaction.

Eye Contact. May cause eye irritation.

Inhalation. May cause irritation of respiratory tract. Symptoms may include coughing, labored breathing, sneezing, cyanosis, and vomiting. It may produce permanent effects in the lungs.

Ingestion. Low hazard. May cause digestive (gastrointestinal) tract irritation.

Aspiration hazard. No information available.

Chronic Toxicity. Prolonged or repeated exposure by inhalation may cause Talc pneumoconiosis (Talcosis) which affects the lungs. [41]

2.9.7. Aerosil

It is chemically silicone dioxide. It is used as lubricante in tablet formulation.

2.9.7.1.Physical Properties

White Solid Powder insoluble in Water with Specific Gravity 2.2

2.9.7.2. Exposure Routes

Inhalation, ingestion, skin and/or eye contact.

2.9.7.3. Symptoms of Toxicity

Irritation eyes, skin, upper respiratory system; cough.[42]

3. MATERIALS AND METHOD

Materials

- Clean D classified production area in a generic pharmaceutical company
- Oral dosage form preparing machines which are as the following with the manufacturer name:

Dispensing Booth.	(Airpac)
Rapid Mixer Granulator Machine.	(Prism pharma machine)
Fluid bed Drier Machine.	(Prism pharma machine)
Oscillator Granulator machine.	(Prism pharma machine)
Multi Mill Machine.	(Prism pharma machine)
Vibro Sifter Machine.	(Prism pharma machine)
Tablet Press Machine.	(Cadmach pharma machine)
Coating Machine.	(Sejong machine)
Blistering Machine.	(Jornen machine)

The active and inactive materials which have been studied are chosen according to these criterias:

- validated products from the factory product list to confirm fixing all the parameters of the manufacturing steps.
- not one of over the counter O.T.C. products to be more critical products clinically than O.T.C .

As a result Anti Diabetic and Anti Hypertensive products have been chosed and here is the materials whith its batch size and strenght of the products:

- Metformin Anti Diabetic whith tablet strength 1000 mg / tab.
- Gliburide Anti Diabetic with tablet strength 5 mg /tab.
- Enalapril Anti Hypertension with tablet strength 10 mg /tab.
- Hydrochlorthiazide Anti Hypertension with tablet strength .25 mg /tab .
- Losartan Anti hypertension with tablet strength 50 mg/tab.

- Aerosil.
- Talc.

And These Instruments have been used For doing the measaurment :

- Air velocity instrument Alnor model.
- Lasair® - II model particle counter from particle measuring system.

Methods

During the manufacturing process of the mentioned materials as oral dosage forms in operation state I measured the number of air change of the production area by air velocity instrument Alnor model and adusted it at to cases one on 7 times air changes each hour and second is 12 times air change each hour.

Particle counter - Lasair® - II model used for particle measuring System Company. Germany to count the particles of the following materials during production in non-sterile facility Metformin, Glyburide, Losartan, Aerosil, Enalapril, Talc, and Hydrochlorothiazide.



Figure 9. Air velocity instrument



Figure 10. Particle counter instrument

4. RESULTS

During preparing the following products using wet granulation preparing method for metformin and gliburide, and direct compression for Enalapril, losartan and hydrochlorthiazide different batch sizes have been used according to the validated batches in the factory as the following:

- Metformin coated tablet (500 kg batch size):

1000 mg*30 tablet / 1500 mg tablet weight (total quantity of metformine is 333 kg).

- Gliburide uncoated tablets (500 kg batch size).

5 mg *30 tablet / 200 mg tablet weight (total quantity of gliburide is 12.49 kg).

- Enalapril coated tablet strength 10 mg /tab. (100 kg batch size).

10 mg * 30 tablet / 200 mg tablet weight (total quantity of Enalapril is 5 kg).

- Hydrochlorthiazide tablet strength 25 mg /tab (100 batch size kg).

25 mg*30tablet/ 400 mg tablet weight (total quantity of Hydrochlorthiazide is 6.25kg).

- Losartan. tablet strength 50 mg/tab. (100 batch size kg)

50 mg *30 tablet/ 400 mg tablet weight (total quantity of losartan is 12.5kg).

- Talc in the total formula is 3 kg.

- Aerosil in the total formula is 5 kg .

Then to see the limit of its particles in worke place environment during production process (at operation) and the effect of changing HVAC conditions at it in a clean D classified production area in a generic pharmaceutical company, the worst case which worker can face is applied by do the calculation directly above the preparing vessles in the praparing area and by intering the particle counter inside the laminar flow in the dispensing room as by mistake may the worker go inside the laminar flow during weight procedure and after that all the values calculated as a worst case if we considered that the same two worker go through all the steps of preparation.

Then the air vilocity and number of air change measured by air velocity instrument .

Then particle counter - Lasair® - II model. Is used to count the particles during production in non-sterile facility.

Then change in number of air change from 7 to 12 time /hour was applied and a comparison between each other is done.

Then the total particle counts of each Material at two values of number of air change have been calculated and a comparison with the permitted limits of OEL was done.

Then the method of calculations and the results was as the following:

According to ISO classification of class D which is used during the production of the mentioned products, a measuring for two particles size which is 0.5 and 5 micro meter μm was done. and if we considered according to the previous survey for the absorption of particles as these two values are the higher and lower values which include in between the absorbable particles size of the material through inhalation and as a result it is the risky limit of particle size for absorption . the weight of these absorbable particles was calculated according to the below method :

If we considered that the average density **D** of the pharmaceutical powders is about 2 (as it is varies for the same material according to the micronizing of the powder) and as the particles varies among many shapes and are so small, so we can consider the particle size value as a particle volume v value , then we can calculate the mass m of the 0.5 and 5 μ particles according to this formule :

$$\mathbf{D=m/v.}$$

SO: The mass of 0.5 μm particle is 1 μg :

The mass of 5 μm particle is 10 μg .

And then we calculate the total mass of the particles for each materials by multiply with the particle counts which have been measured by the particle counter, and here is an example of the calculation:

Measured values of For Gliburide:

The number of counted particles For size 0.5 μm in the case of 7 times air change is :
122087000 particles/ 1000 litre

so the mass of these particles in 1000 liter according to the previous explaining is:

$$122,087,000 \mu\text{m} \times 1 = 122,087,000 \mu\text{g} / 1000 = 122,087 \text{ mg}$$

And the number of counted particles for size 5 μm in the case of 7 times air change is :

4,596,718 particles/ 1000 litre

so the mass of these particles in 1000 liter according to the previous explaining is:

$$4596,718 \mu\text{m} \times 10 = 45967180 \mu\text{g} / 1000 = 45967.18 \text{ mg}.$$

This method is used for other materials which is included in this study.

If we considered that all the particles can be inhaled from two unmasked worker (either non well fixed mask or damaged mask) as the produced quantities need at least two persons because the line is not close system , then we have to take in account all this particles in consideration for each person in two cases one with 7 times of air change and the second is for 12 times of air chanhes and here is an example of the calculation which is done as the following:

Tablet 3: Values Of Measured Particles in case of 7 Times Air Changes condition

Material name	0.5 µm particle number in 1000 liter	Milligram of 0.5 µm particles in 1000 liter	5µm particle number in 1000 liter	Milligrams of 5 µm particles in 1000 liter
Metformin	388,534,040	388,534.04	247,215,960	2,472,159.0
Gliburide	122,087,000	122,087.0	4,596,718	45,967.1
Aerosil	35,311,360	353,113.60	1,428,660	14,286.0
Enalapril	132,943,320	132,943.32	2,272,080	22,720.8
Hydrochlorthiazide	85,649,900	85,649.9	3,037,360	3037.3
Losartan	98,853,360	98,853.6	3,978,060	39780.6
Talc	75,298,660	75,298.6	1,821,760	18217.6

Tablet 4: Values Of Measured Particles in case of 12 Times Air Changes condition

Material name	0.5 µm particle number in 1000 liter	Milligram of 0.5 µm particles in 1000 liter	5µm particle number in 1000 liter	Milligrams of 5 µm particles in 1000 liter
Metformin	9,287,360	928.73	124,240	1240.0
Gliburide	12,323,360	12,323.6	722,440	7224.0
Aerosil	9,023,200	9,023.2	247,480	2474.0
Enalapril	5,796,860	5,796.86	54,180	541.0
Hydrochlor thiazide	7,337,580	7,337.58	3,000	30.0
Losartan	9,269,920	9,269.9	60,420	604.0
Talc	26,751,820	26,751.8	12,800	128.0

MSDS [35,36,37,38,39,40,41,42] of the brand name company have been used to obtain the OEL occupational exposure limits and also OEL calculated from the PDE values which is calculated according to European medicine agency [43] (EMA) guidelines to compare the PDE permitted daily exposure for the same materials as the PDE is considered for oral route and this will reflect the importance of inhalation route in occupational toxicity of active materials and made a comparison between the measured and the theoritical values.

According to documents issued by European Medicine Agency (EMA), permitted daily exposure (PDE) values for drug molecules produced in shared facilities were calculated using following formula:

No observed adverse effect level (NOAEL) or lowest observed adverse effect level (LOAEL) for critical effects which are found from experimental studies multiply with average body weight and divided to a series of uncertainty factors.

$$\text{PDE} = \frac{\text{NOAEL (or LOAEL)} \times 50}{\text{F1} \times \text{F2} \times \text{F3} \times \text{F4} \times \text{F5}}$$

• **Modifying factors which will be used are as follows:**

- **F1:** A factor to account for extrapolation between species
 - **F1 = 5** for extrapolation from rats to humans
 - **F1 = 12** for extrapolation from mice to humans
 - **F1 = 2** for extrapolation from dogs to humans
 - **F1 = 2.5** for extrapolation from rabbits to humans
 - **F1 = 3** for extrapolation from monkeys to humans
 - **F1 = 10** for extrapolation from other animals to humans
- **F2:** A factor of 10 to account for variability between individuals
- **F3:** A variable factor to account for toxicity studies of short-term exposure
 - **F3 = 1** for studies that last at least one half lifetime (1 year for rodents or rabbits; 7 years for cats, dogs and monkeys).
 - **F3 = 1** for reproductive studies in which the whole period of organogenesis is covered.
 - **F3 = 2** for a 6-month study in rodents, or a 3.5-year study in non-rodents.
 - **F3 = 5** for a 3-month study in rodents, or a 2-year study in non-rodents.
 - **F3 = 10** for studies of a shorter duration.
 - In all cases, the higher factor has been used for study durations between the time points, e.g., a factor of 2 for a 9-month rodent study.
- **F4:** A factor that may be applied in cases of severe toxicity, e.g., non-genotoxic carcinogenicity, neurotoxicity or teratogenicity. In studies of reproductive toxicity, the following factors are used:
 - **F4 = 1** for fetal toxicity associated with maternal toxicity
 - **F4 = 5** for fetal toxicity without maternal toxicity
 - **F4 = 5** for a teratogenic effect with maternal toxicity
 - **F4 = 10** for a teratogenic effect without maternal toxicity

- **F5:** A variable factor that may be applied if the no-effect level was not established. When only an LOAEL is available, a factor of up to 10 could be used depending on the severity of the toxicity.
- **50:** Adult body weight is accepted as 50 kg.
- If the human data are used, restriction to use of F2 and F5 factors may be acceptable.

4.1. Permitted Daily Exposure (PDE) Determination For Enalapril Maleate

4.1.1. PDE Calculation Using Repeated Dose Toxicity Test Data

According to search for published document, repeated dose toxicity data are seen in Table 5.

Table 5: PDE values of Enalapril maleate calculated by using repeated dose toxicity test data.

Species	Dog [46] Oral, 1 year	Rat [47] Oral, 1 year
NOAEL (mg/kg/day)	15	10
F1	2	5
F2	10	10
F3	10	1
F4	1	1
F5	1	1
PDE*(mg/day)	3.75	10

* Body weight is 50 kg.

4.1.2. PDE Calculation for Critical Effects

4.1.2.A. Calculation of PDE for Carcinogenic Effect

No content of this product is defined as probable or confirmed carcinogens according to The International Agency for Research on Cancer (IARC). Calculated PDE values for carcinogenicity of Enalapril maleate are seen in Table 6.

Table 6: PDE values calculated by using carcinogenicity data for Enalapril maleate[48]

Species	Rat Oral, 2 years	Mice Oral, 2 years
NOAEL mg/kg/day	90	180
F1	5	12
F2	10	10
F3	1	1
F4	1	1
F5	1	1
PDE* (mg/day)	90	75

* Body weight is 50 kg.

4.1.2.B. PDE Calculation for Genotoxic Effect

Enalapril maleate was not mutagenic in the Ames mutagenicity test with or without metabolic activation. Enalapril was also negative in the following genotoxicity studies: reverse mutation assay with E. coli, Sister Chromatid Exchange with cultured mammalian cells, and the micronucleus test with mice, as well as in an in vivo cytogenic study using mouse bone marrow [49].

4.1.2.C. PDE Calculation for Reproductive/ Developmental Toxicity.

Calculated PDE values for reproductive and developmental toxic effect of Enalapril maleate are seen in Table 7. Enalapril maleate's FDA pregnancy category is D[45]. Enalapril maleate did not cause any congenital malformations.

Table 7: PDE values of Enalapril maleate for reproductive and developmental toxicity.
[49]

Species	Rat Oral, Development toxicity including whole organogenesis period	Rabbit Oral, Development toxicity including whole organogenesis period
NOAEL (mg/kg/day)	200	-
NOAEL (mg/kg/day)	-	1
F1	5	2.5
F2	10	10
F3	1	1
F4	1	1
F5	1	10
PDE* (mg/day)	200	0.2

* Body weight is 50 kg.

4.1.2.D. PDE Calculation for Hypersensitivity

There are no data regarding hypersensitivity reactions of Enalapril maleate for PDE calculation. Hypersensitivity reactions are not expected under normal use of Enalapril maleate.

4.1.3. Final PDE Calculation

According to the PDE calculation for critical toxic effects of Enalapril maleate final PDE values are seen in Table 8.

Table 8. Final PDE values for Enalapril maleate.

Administration route	PDE* (mg/day)
Oral	0.2

* Body weight is 50 kg.

4.2. Permitted Daily Exposure (PDE) Determination for Hydrochlorothiazide

4.2.1. PDE Calculation Using Repeated Dose Toxicity Test Data.

The PDE values calculated from repeated dose toxicity data in animals for hydrochlorothiazide are given in Table 9.

Table 9: PDE values calculated by using the repeated dose toxicity tests for hydrochlorothiazide.

Species	Dog [51] Oral, 9 months	Rat [52] Oral, 2 years	Mice [53] Oral, 2 years Male
NOAEL (mg/kg/day)	-	100	-
LOAEL (mg/kg/day)	50	-	600
F1	2	5	12
F2	10	10	10
F3	10	1	1
F4	10	10	10
F5	10	1	10
PDE* (mg/day)	0.125	10	2.5

* Body weight is 50 kg.

4.2.2. PDE Calculation for Critical Effects of Hydrochlorothiazide

4.2.2.A. Calculation of PDE for Carcinogenic Effect

Hydrochlorothiazide is defined as **possible carcinogen** (Group 2B) according to the International Agency for Research on Cancer (IARC) [53]. PDE values calculated from long-term carcinogenicity studies on animals with hydrochlorothiazide are listed in Table 10.

Table 10: PDE value calculated by carcinogenicity test data of hydrochlorothiazide

Species	Dog [51] Oral, 9 months	Rat [52] Oral, 2 years	Mice [52] Oral, 2 years Male
NOAEL (mg/kg/day)	-	100	-
LOAEL (mg/kg/day)	50	-	600
F1	2	5	12
F2	10	10	10
F3	10	1	1
F4	10	10	10
F5	10	1	10
PDE* (mg/day)	0.125	10	2.5

* Body weight is 50 kg.

4.2.2. B. PDE Calculation for Genotoxic Effect

Hydrochlorothiazide was not found to be mutagenic in the Ames microbial mutation test in vitro. However, positive test results were obtained in the in vitro CHO sister chromatid exchange (clastogenicity) and in the mouse lymphoma cell assays [52]. In vivo the mutagenic potential of hydrochlorothiazide was evaluated in a micronucleus test in Chinese hamsters, a spermatogonia assay and dominant lethal test in mice. All assays were negative. An in vivo sister chromatid exchange assay in Chinese hamsters after treatment with hydrochlorothiazide was also negative. No induction of sex linked recessive lethal mutations was seen in germ cells of male *Drosophila melanogaster* following feeding or injection of 10.000 mg/kg hydrochlorothiazide. It was concluded that hydrochlorothiazide has equivocal

mutagenic activity in vitro. The negative results of the in vivo tests indicate that hydrochlorothiazide and its metabolites do not represent a genotoxic hazard [51].

4.2.2.C. PDE Calculation for Reproductive/ Developmental Toxicity.

Studies conducted with hydrochlorothiazide on animals, pregnancy and / or embryonic / fetal development and / or delivery are not verifying risk probability in terms of the effects on postnatal development and classified in the **category B** during the entire pregnancy. Often, pregnant patients with heart disease or essential hypertension tend to reserve diuretic medications. According to the risk classification of FDA for drug use in pregnancy, thiazide group diuretics indicated as **category D** in **pregnant women with reduced uteroplacental perfusion**. In general, the majority of findings indicate that thiazide diuretics are not teratogenic in the first trimester, but most experts prefer to use diuretics in the second/third trimester[50]. PDE data on reproductive and developmental toxicity for hydrochlorothiazide are given in Table 11.

Table 11: PDE values of hydrochlorothiazide for reproductive and developmental toxicity.

Species	Mice [51] Developmental toxicity during whole organogenesis Oral, Female	Mice [51] Reproductive toxicity, Oral	Rat [52] Reproductive toxicity Oral	Mice [52] Developmental toxicity during whole organogenesis, Oral	Rat [52] Developmental toxicity during whole organogenesis Oral
NOAEL (mg/kg/day)	-	100	4	3000	1000
LOAEL (mg/kg/ day)	300	-	-	-	-
F1	12	12	5	12	5
F2	10	10	10	10	10
F3	1	1	1	1	1
F4	10	10	10	10	10
F5	10	1	1	1	1
PDE*(mg/day)	1.25	4.17	0.4	125	100

* Body weight is 50 kg.

4.2.2.D. PDE Calculation for Hypersensitivity

There is no available data for hypersensitivity reactions associated with hydrochlorothiazide use. An important risk is not expected. [53]

4.2.3. Final PDE Calculation

According to the PDE calculation for critical toxic effects of hydrochlorothiazide, final PDE values were shown in Table 12.

Table 12: Final PDE for hydrochlorothiazide

Administration route	PDE* (mg/day)
Oral	0.125

* Body weight is 50 kg.

4.3. Permitted Daily Exposure (PDE) Determination for Losartan

4.3.1. PDE Calculation Using Repeated Dose Toxicity Test Data.

Repeated dose toxicity tests for losartan could not be found.

4.3.2. PDE Calculation for Critical Effects of Losartan

4.3.2.A. Calculation of Pde for Carcinogenic Effect

Losartan is not defined as probable or confirmed carcinogens according to The International Agency for Research on Cancer (IARC)[57]. Long-term studies in animals to assess the carcinogenic potential of losartan have been showed in Table 13

Table 13: shows the PDE values calculated by using the carcinogenicity tests for losartan

Species	Mice [57] Oral, 92 weeks	Rat [57] Oral, 105 weeks
NOAEL (mg/kg/day)	200	270
LOAEL (mg/kg/day)	-	-
F1	12	5
F2	10	10
F3	1	1
F4	10	10
F5	1	1
PDE* (mg/day)	8.33	27

* Body weight is 50 kg.

4.3.2.B. PDE Calculation for Genotoxic Effect

Losartan potassium was negative in the microbial mutagenesis and V-79 mammalian cell mutagenesis assays and in the in vitro alkaline elution and in vitro and in vivo chromosomal aberration assays. In addition, the active metabolite showed no evidence of genotoxicity in the microbial mutagenesis, in vitro alkaline elution, and in vitro chromosomal aberration assays. [57]

4.3.2.C. PDE Calculation for Reproductive/Developmental Toxicity

PDE data related to reproductive and developmental toxicity for losartan are given in Table 2. Losartan is classified as **category C** in the first trimester; during second and third trimester it is classified in D category because of its effects on pregnancy and / or fetus / newborn, as a result of animal experiments and controlled cross studies conducted in pregnant women. It is known that contact with angiotensin II receptor antagonists during the second and third trimesters induces fetotoxicity (decreased renal function, oligohydramnios, skull ossification) and neonatal toxicity (renal failure, hypotension, hyperkalemia) in humans. [55]

Table 14: PDE values of losartan for reproductive and developmental toxicity

Species	Rat[57] Developmental toxicity Oral	Rat[57] Developmental toxicity during organogenesis Oral	Rat[57] Developmental toxicity during organogenesis Oral	Rat[57] Reproductive toxicity Oral
NOAEL (mg/kg/day)	-	-	-	150
LOAEL (mg/kg/day)	10	135	100	-
F1	5	5	5	5
F2	10	10	10	10
F3	10	1	1	1
F4	10	10	10	10
F5	10	10	10	1
PDE* (mg/day)	0.01	1.35	1	15

* Body weight is 50 kg.

4.3.2.D. PDE Calculation for Hypersensitivity

There is no available data for hypersensitivity reactions associated with losartan use.

An important risk is not expected. [55]

4.3.3. Final PDE Calculation

Critical toxic effects of losartan, final PDE values were shown in Table 15.

Table 15: Final PDE for losartan

Administration route	PDE* (mg/day)
Oral	0.01

* Body weight is 50 kg.

4.4. Permitted Daily Exposure (PDE) Determination for Metformin

4.4.1. PDE Calculation Using Repeated Dose Toxicity Data.

PDE calculation according to repeated dose toxicity and carcinogenicity tests for Metformin HCl was given at Table 16.

Table 16: PDE values by using the repeated dose toxicity and carcinogenicity tests for Metformin HCl

Species	Rat [59] Oral, 104 weeks	Mice [59] Oral, 91 weeks
NOAEL (mg/kg/day)	900	1500
LOAEL (mg/kg/day)	-	-
F1	5	12
F2	10	10
F3	1	1
F4	1	1
F5	1	1
PDE* (mg/day)	900	625

* Body weight is 50 kg.

4.4.2. PDE Calculation for Critical Effects of Metformin Hcl

4.4.2.A. Calculation of PDE for Carcinogenic Effect

Metformin HCl is not defined as probable or confirmed carcinogens according to The International Agency for Research on Cancer (IARC)[60] Long-term carcinogenicity study on animals with metformin HCl in literature were listed in Table16

4.4.2.B. PDE Calculation for Genotoxic Effect

There was no evidence of a mutagenic potential of metformin in the following in vitro tests: Ames test (*S. typhimurium*), gene mutation test (mouse lymphoma cells), or chromosomal aberrations test (human lymphocytes). Results in the in vivo mouse micronucleus test were also negative [59].

4.4.2.C. PDE Calculation for Reproductive/ Developmental Toxicity.

Metformin should only be used during pregnancy if it is clearly needed. The results of the narrow-scale studies show that metformin passes into human milk. There is a possibility that hypoglycemia or other side effects may occur in infants. However, it has not been reported in the human research that the baby has an adverse effect due to the plasma glucose level. In addition, it was reported that the use of 2550 mg/day

metformin for 6 months for women who did not affect the growth, motor or social development of the baby; The effect on periods of more than 6 months is unknown. Metformin has been classified as **category B** throughout pregnancy because drug has an unconfirmed risk potential, which is preferred over pregnancy and/or fetal/neonatal effects and benefit/risk ratio in controlled trials in animal trials and pregnant.¹ Studies with Metformin to evaluate reproductive and developmental toxicity were indicated in Table 17.

Table 17: PDE values of Metformin HCl for reproductive and developmental toxicity

Species	Rat[59] Reproductive Toxicity Oral	Rabbit[59] Developmental toxicity Oral	Rat[61] Developmental toxicity during organogenesis period , Oral
NOAEL (mg/kg/day)	600	600	-
LOAEL (mg/kg/day)	-	-	300
F1	5	2.5	5
F2	10	10	10
F3	1	10	1
F4	1	1	1
F5	1	1	10
PDE* (mg/day)	600	120	30

* Body weight is 50 kg.

4.4.2.D. PDE Calculation for Hypersensitivity

There is no available data for hypersensitivity reactions associated with Metformin HCl use. An important risk is not expected [62].

3. Final PDE Calculation

According to the PDE calculation for critical toxic effects of Metformin HCl, final PDE values were shown in Table 18 [60]

Table 18: Final PDE for Metformin HCl

Administration route	PDE* (mg/day)
Oral	30**

* Body weight is 50 kg. ** Since there is no Metformin HCl data, PDE data for metformin was accepted.

4.5. Permitted Daily Exposure (PDE) Determination for Gliburide

4.5.1. PDE Calculation Using Repeated Dose Toxicity Data.

The PDE values calculated from repeated dose toxicity data in animals for **Gliburide** are given in Table 19.

Table 19: PDE values calculated by using the repeated dose toxicity tests for Gliburide

Species	Rat [40] Oral, 18 Month
NOAEL (mg/kg/day)	-
LOAEL (mg/kg/day)	0.12
F1	5
F2	10
F3	1
F4	1
F5	10
PDE* (mg/day)	0.012

* Body weight is 50 kg.

4.5.2. PDE calculation for critical effects of Gliburide

4.5.2.A. Calculation of PDE for Carcinogenic Effect

It is not identified as probable, possible or confirmed human carcinogen by IARC[40]

Long-term studies in animals to assess the carcinogenic potential of glyburide have been showed in Table 20

Table 20: PDE values calculated by using the carcinogenicity tests for Gliburide

Species	Mice [40] Oral, 24 months	Rat [40] Oral, 18 month
NOAEL (mg/kg/day)	3000	300
LOAEL (mg/kg/day)	-	-
F1	12	5
F2	10	10
F3	1	1
F4	1	1
F5	1	1
PDE* (mg/day)	1250	300

* Body weight is 50 kg.

4.4.2.B. PDE Calculation for Genotoxic Effect

Bacterial Mutagenicity (Ames) Salmonella is Negative.

Glyburide Chromosome Aberration Hamster Lung Cells is Negative.

In Vivo Micronucleus Mouse Negative [40].

4.4.2.C. PDE Calculation for Reproductive/ Developmental Toxicity.

Calculated PDE values for reproductive and developmental toxic effect of Glyburide are seen in Table 21[40]. its US FDA pregnancy category is C [64]

Table 21: PDE values of Gliburide for reproductive and developmental toxicity

Species	Rat[40] Oral, Reproductive Toxicity	Rabbit[40] Oral, Developmental toxicity	Rat[40] Oral, Developmental toxicity during organogenesis period
NOAEL (mg/kg/day)	100	-	-
LOAEL (mg/kg/day)		100	100
F1	5	2.5	5
F2	10	10	10
F3	1	1	1
F4	1	1	1
F5	1	10	10
PDE* (mg/day)	100	20	10

* Body weight is 50 kg.

4.4.2.D. PDE Calculation for Hypersensitivity

There is no available data for hypersensitivity reactions associated with gliburide use.

3. Final PDE Calculation

According to the PDE calculation for critical toxic effects of Gliburide, final PDE values were shown in Table 22

Table 22: Final PDE for Giburide

Administration route	PDE* (mg/day)
Oral	0.012

* Body weight is 50 kg. *

OEL Calculation:

According to the European Medicine Agency document (EMA) [63], for calculation (OEL) value Depending On (PDE) values through this formula:

$$\text{OEL} = \frac{\text{PDE } (\mu\text{g/day})}{10 \text{ m}^3}$$

Oral bioavailability studies of the studied materials are as follows:

60% for Metformin as mentioned under subtitle 2.9.1.2.

33% for Losartan as mentioned under subtitle 2.9.2.2.

75% for Hydrochlorthiazide as mentioned under subtitle 2.9.4.2.

60% for Enalapril as mentioned under subtitle 2.9.3.2.

100 % for Gliburide as its bioavailability is variable depending on the oral dosage form as mentioned under subtitle 2.9.5.2. so that I considered it as worst case 100%.

The calculated (OEL) value for the studied materials after taking in account the oral bioavailability percentage of each material and after considering the inhalation route absorption as 100 % as worst case are as the following formula:

$$\text{OEL} = \text{Oral PDE} * \text{bioavailability} / 10\text{m}^3$$

And the result are in the Table 23.

Table 23: PDE and oel values for the studied materials

Material name	PDE Oral mg/ day according to EMA guidelines[43]	OEL/TWA (8 Hours) according to EMA guidelines[63]	OEL/TWA (8 Hours) according to brand product MSDS [35,36,37, 38,39,40,41,42]
Metformin	30	1.8 mg/m ³	5 mg/ m ³
Gliburide	0.012	0.0012 mg/m ³	0.001 mg/m ³
Aerosil	-	N.A	10 mg/m ³
Enalapril	0.2	0.012 mg/m ³	0.1mg/m ³
Losartan	0.01	0.00033 mg/m ³	0.01-0.1 mg/m ³
Hydrochlorthiazide	0.125	0.0094 mg/m ³	0.25 mg/m ³
Talc	-	N.A	2 mg/m ³

5. DISCUSSION and CONCLUSION

To study the limit of particles in work place environment during production process (at operation) and the effect of changing HVAC conditions at it in a clean D classified production area in a generic pharmaceutical company, the number of air change of the production area was measured. And the particles during production in non-sterile facility was counted.

Change in number of air change from 7 to 12 time /hour was done, and also a comparison between each other. The worst case scenario quantities were calculated and a comparison with the permitted limits of OEL which has been got from generic product MSDS and the PDE values which is calculated according to European medicine agency.

Regarding to the value we can see that an extremely concentration of the measured materials has been detected in the environment of the manufacturing, and this is because the measurements exactly done above the vessels used in the manufacturing area and without taking in the account the personal protective equipment.

And that means high extra care and more air change needed for the manufacturing area.

On the other hand there was a big difference in the values between materials as we noted that some of measured particles although have small concentration but because of different particles properties it can produce more quantities of particles like talc and glyburide. That means there should be an individual measuring for the particles for each formula at the developing stage to determine the exact manufacturing conditions before starting production for each.

Big decrease in the particles number happened after increasing the number of air change and that means it is highly appreciated step to determinate exact HVAC conditions for each product in the product batch to be controlled for each product differently.

And as a compare study between the OEL value which was calculated according to the EMA guidelines and the MSDS of the brand products companies some differences has been noted which didn't affect the study but it also pay the attention to

the fact that this values should be done as dedicated job by each factory to determine its own values according to its especial conditions which it follows in the production process.

As mentioned above that Preventive action applied in pharmaceutical industry for work environment and especially against inhalation unwanted effects depened almost on the Clean rooms and clean air devices which routinely monitored in term of particle count and H.V.A.C. system activity in rest case for working and has the same parameteres for all kinds of active and inactive materials if considered as generic non sterile products, without taking in account the different particle size and count which may be produced during production according to the properties of materials and the fluctuation of HVAC system design. So deep review needed in order to keep workeres in safe side, and also measurements of API particles count during production should be included by international guidelines.

Although cleanroom is a controlled environment where the concentration of airborne particles like dust, microbes and aerosol particles are controlled, and although Cleanrooms are maintained and utilized in a manner that minimizes the entering, generation and retention of particles in the environment, And although All cleanrooms that meet the GMPs and classified according to the cleanliness level of the air inside them. Calculating the values of occupational exposure limit for each material need more effort to protect the worker for worst cases which may happened.

The occupational toxicologist must have an intimate knowledge of the work environment and be able to recognize and prioritize exposure hazards. Because the work environment can present exposures to complex mixtures like properties and conditions of work, the occupational toxicologist must also recognize those that are particularly hazardous when occurring in combination

So that more details should be taken in account through determining the criterias of preventive actions for workers in pharmaceutical production.

And finally high care should be taken if the manufacturing is done in not close system as the quantities of exposure will be very high.

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