Toxicology for the Health and Pharmaceutical Sciences

Edited by
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Antonio Peña-Fernández—This book is dedicated to my parents Ángela Fernández and Cástor Peña, for their unconditional support, and to my dear brothers and nephews. I love you so much.

Mark D. Evans—Dedicated to Jill for her understanding, love and support.

Marcus S. Cooke—Dedicated to Emily, Evie and Harrison, for their understanding, patience, willingness to travel, and their courage and strength wherever we arrive.

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Preface

There is an increasing need for a knowledge of toxicology to safeguard the use of chemicals in industry, public and private sectors. In particular, the health sector requires workers to have a basic knowledge of toxicology due to the large burden of disease and mortality caused by chemicals and drugs of abuse. Moreover, current anthropogenic activities and the development of new techniques for chemical creation, production, manufacturing and use have made necessary the development of different tools to evaluate the safety of these substances to protect human health. Therefore, the study of toxicology should be part of the education of all fully formed professionals in the health sciences.

Knowledge of toxicology is critical for the scientific evaluation of the risks that chemicals can pose to human health and to the environment. Such knowledge is also important to manage risk, respond to any accidental or deliberate release of chemical substances to the environment and to implement remediation strategies. Moreover, a modern healthcare sector requires professionals with an education in toxicology to tackle the negative public perception of the chemical and pharmaceutical industries for the environment and public health. Thus, the subject of toxicology is of critical importance to protect human health against chemicals and/or drugs that may be present in the environment or may pose a threat to the population in the aftermath of a chemical incident (i.e. the release of one or more chemicals to the environment). For these reasons, academics should develop and offer toxicological education that will play an essential part of the education of future health workers to face these chemical threats.

Comprising a series of chapters from leading toxicology, pharmacy and public health academics and experts across Europe, the United States and beyond, *Toxicology for the Health and Pharmaceutical Sciences* provides a concise yet comprehensive volume that can be used as a relevant textbook on toxicology for the clinical, healthcare, educational and professional sectors. This book covers the fundamentals and recent developments in toxicology, to respond to local and global chemical and pharmaceutical threats due to globalization and human activities. Thus, this volume has chapters specifically designed to support the understanding of the most current, toxicology-related subjects for any undergraduate/postgraduate health programmes, as well as aiding with the delivery of continuing professional development training on up-to-date topics in toxicology for current practicing health professionals wishing to improve their background knowledge in toxicology.

The textbook begins with 10 introductory chapters that provide basic and cutting-edge information on toxicology. Chapters 11–26 were written by researchers who are experts in their fields and further cover fundamental and applied topics, together with descriptions of novel tools in relevant toxicology specialties. The final section of this volume provides practical guidance in the form of two detailed case studies based on real-world/developed scenarios for characterising human risks to environmental pollutants and on how to use innovative guidance and tools to respond to chemical incidents, which will facilitate the user to acquire and practice these relevant skills highlighted in previous chapters.

This textbook is therefore a vital, comprehensive resource and reference for students, academics, researchers and employees in the pharmaceutical-, health- and environmental health-related subject areas. Arguably, this textbook is also vital reading and reference for policymakers and others that influence and decide regulations that have an impact on the environment and human health. Although there is a particular focus on Europe and the US, reflecting the current and emerging toxicological issues in those areas, this text has global relevance.

Acknowledgments

The editors would like to thank each of the contributors for their efforts, dedication and patience in the production of this first edition of *Toxicology for Health and Pharmaceutical Sciences*. Without their input, there would be no book.

About the Editors

Antonio Peña-Fernández is a senior lecturer in toxicology and medical sciences in the Faculty of Health and Life Sciences at De Montfort University, Leicester, UK. He has a BSc in chemistry from the CEU San Pablo University and an MSc in toxicology from the University of Seville. His PhD in toxicology is from the University of Alcalá, Spain (2011), where he has held an honorary professorship since 2019. His main research work focuses on human biomonitoring as a tool for the assessment of internal doses of contaminants, and the study of emerging chemical and biological threats in urban, industrial and rural environments for the characterisation of risks and the development of protocols to protect human health and decontaminate impacted environments. Many of his current projects are international collaborations with different universities and research institutions from England, Spain and Sierra Leone. Projects relate to environmental monitoring, exposure assessment, building capabilities and the development of protocols, training materials and tools for the protection of human health to different emerging chemical and biological contaminants. He became a European Registered Toxicologist (EUROTOX) in 2013 and is a Senior Fellow of the Higher Education Academy (SFHEA, 2016).

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Marcus S. Cooke is a professor in the Department of Cell Biology, Microbiology and Molecular Biology at the University of South Florida, Tampa, where he heads the Oxidative Stress Group. His background is in the environmental health sciences, in particular environmental toxicology. He studies oxidative stress and genomic instability: from basic mechanisms to translational application of validated biomarkers to study human health and disease; specifically, the formation and repair of damage to nuclear/mitochondrial DNA and nucleotide precursor pools, and the development and application of DNA adductomics; that is, the totality of DNA adducts in cellular DNA, and urine; and the genome-wide mapping of DNA damage. Translating such laboratory findings to the marketplace is a priority and is actively pursued. This has resulted in three US and one UK patents, license agreements, industry collaborations and an STTR award from NIEHS. As of March 2021, he has 119 peer-reviewed publications, which have attracted over 7500 citations, and he has an *h*-index of 40.

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1 Introduction to Toxicology

Mª Teresa Hernández, Mª del Carmen Berrocal, Domingo Ly-Pen and Mª Victorina Aguilar

CONTENTS

1.1 THE CONCEPT OF TOXICOLOGY AND AREAS OF ACTION

From the etymological point of view, the word "toxicology" is derived from two Greek words: *toxikon* (poison) and *logos* (treatise), which means "the science of poisons". According to Paracelsus (1493–1541), often called the Grandfather of Modern Toxicology, "all substances are poisons; there is none which is not poison. The right dose differentiates a poison from a remedy".

Orfila (1787–1853), the founder of the science of toxicology, in his famous *Treatise of Toxicology* (1813), defined a poison as "any substance, taken or applied in any way in small doses in a living organism, which destroys health or ends life entirely". According to this definition, substances would be classified as poisons and nonpoisons, the dose being crucial to establish their difference, and thus making the distinction between poisoning by overdose and poisoning itself.

With the advances of toxicology, this definition is now incomplete and the word "poison" is misleading. Since Paracelsus, it is well known that it is the dose that makes the poison; a chemical that is perfectly safe at one dose may be lethal at another. For example, at very high doses even table salt or drinking water can be harmful, especially if you consider the influence of other factors, such as stage of life, age, diet, diseases and concomitant exposures to various agents. Therefore, one of the most accepted definitions of toxicology today is "the study of the adverse effects of chemicals or physical agents on living organisms" (Gilbert 2012).

The change of paradigm in toxicology implies a transition from an *in vivo* science (in which the use of animals in experimental laboratory conditions were required to study parameters, such as the lethal dose for half of the population of animals) to a science in which the following are studied (even virtually): routes of exposure, mechanisms of action, events and key processes of the target molecule, cellular responses, and even the macroscopic and organographic effects on human health and the environment (Meek et al. 2014).

From a historical point of view, toxicology was consolidated as a scientific discipline, independent of medicine but related through forensic toxicology. For the development of this branch and of other more recent ones, a prerequisite was the development of increasingly sensitive and specific analysis techniques capable of analyzing toxic agents in biological samples, mainly blood and urine, of exposed subjects to confirm the presence of the toxin responsible (analytical toxicology). Analytical toxicology, with the advances in pharmacological toxicology and with the support of appropriate regulatory legislation, contributed to the current successes of forensic toxicology and clinical toxicology, which aims to diagnose and treat intoxications like any other disease that has a pathological character; that is, it can manifest in an acute and chronic way, before the death of the subject (Bello 2001).

New lifestyles, exposure to new substances and so forth have given rise to the development of different subdisciplines of toxicology. These subdisciplines are often closely related to each other, and their knowledge and application serves to protect public health.

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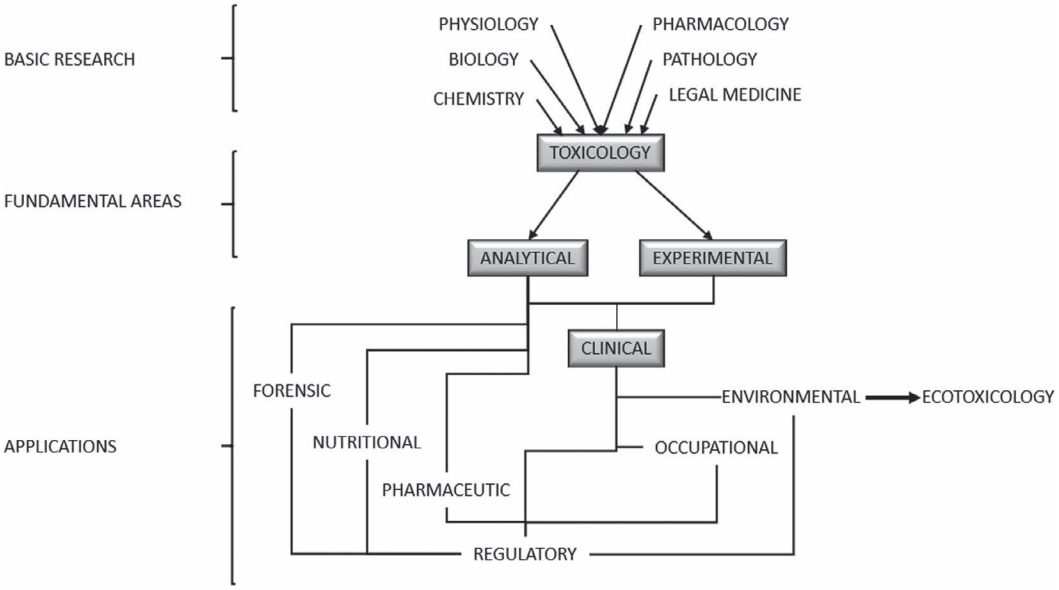

Figure 1.1 Subdisciplines of toxicology. The field of toxicology can divided into various subdisciplines, derived from the vertical and horizontal integration of toxicology with other sciences. Modern toxicology is a conglomerate of subdisciplines that cooperate to promote knowledge of physicochemical toxicity.

The development of the chemical industry has contributed to these advances in toxicology in the twentieth century, and interest has grown in increasing toxicological knowledge in relation to health in the workplace. The grouping of national toxicological societies by continental blocks has also been of crucial importance, represented by SOT in North America, EUROTOX in Europe, ASIATOX in Asia, ALATOX in Latin America and toxicological societies in Africa, such as those in South Africa and Cameroon. From the international cooperation of the different societies, the International Union of Toxicology (IUTOX) was created in 1980. It has played an important role in the applied knowledge of toxicology (Repetto and Repetto 2009).

The different subdisciplines of modern toxicology are outlined in Figure 1.1 and are established according to the way of addressing a toxic event in three areas that correspond to the type of work performed (Jaramillo et al. 2006):

1. General: the general basis of toxic actions. This includes mechanisms of action and ways of counteracting their effects.
2. Descriptive: the science of toxicity testing to provide information about safety evaluation and regulatory requirements. The different aspects and toxicological studies are grouped on toxic agents such as metal toxicology, toxicology of organic solvents, toxicology of pesticides and so forth.
3. Mechanistic: studies, identifies and attempts to understand the mechanisms by which toxic agents exert toxic effects on living beings, in order to produce safer substances and develop a rational treatment of intoxication.
4. Regulatory: integrates the information obtained from the mechanistic and descriptive areas to determine the level of risk to health and to the handling of exposure to chemical substances.

To further investigate the fundamental mechanisms and processes involved in toxic phenomena, it is necessary to integrate toxicological investigations with the knowledge of other basic sciences (Figure 1.1). This integration can be achieved by two methods (Bello 2001):

1. Vertical integration gives rise to the appearance of diverse subdisciplines: genetic, molecular toxicology, inmunotoxicology, neurotoxicology and so forth.

2. Horizontal integration is oriented towards practical applications with repercussions that affect the environment and human health. In this way, two areas appear:
   a. Retrospective toxicology: typical of the forensic branch oriented to the investigation of a toxin in corporal organs and its medicolegal aspects.
   b. Predictive toxicology: oriented towards the prediction of possible toxic effects in specific situations, including drug use, drug interactions and presence of contaminants or additives not allowed in food.

This horizontal integration of toxicology with other sciences gives rise to the appearance of different applied branches, among which we must highlight:

- Clinical toxicology, based on physiology, clinical chemistry and pharmacology, which studies the pathological changes caused by toxic agents, establishes treatments for intoxicated patients and analyzes new techniques to treat intoxications.
- Occupational or professional toxicology, based on occupational medicine and occupational hygiene. Occurrence of occupational diseases is related to toxic substances present in work environments. Therefore, toxicology investigates the harmful effects produced by substances for occupational use and determines safe exposure limits.
- Environmental toxicology, which relies on ecology and environmental chemistry to analyze the impact of pollutants present in the environment of living organisms. It is the subdiscipline responsible for evaluating the vast environmental impact produced by exposures to chemical products present in the environment, with special attention to living species other than humans in air, soil or water. A complex environment requires paying attention to the persistence of pollutants in soil, water and air, and knowing the capacity they have to join the food chain.

The joint work between ecologists and environmental toxicologists is increasing our knowledge about the impact of agrochemicals on native species. Monitoring changes of speciation, due to the effect of nearby mines or chemical plants, has led to development of the field of ecotoxicology. This is a branch of environmental toxicology, which studies the particular way(s) in which toxins impact the population dynamics of an ecosystem (Newman 2010).

- Food toxicology is a multidisciplinary approach, studying adverse effects of exposure by living organisms to chemical substances present in food. This area is supported by chemical analysis, food science and nutrition.

  It is important to know what products are safe to eat, and in what amounts; this discipline investigates the safety of the components that are added to food, deliberately or accidentally, as natural and synthetic additives or contaminants. Accidental contaminants are generally synthetic or natural environmental contaminants, such as polychlorinated biphenyls (PCBs) and methylmercury, which are found in fish; microbial toxins such as those produced by *Escherichia coli* in contaminated food; and fungal toxins, such as aflatoxins, which can contaminate grains. Recently, scientists have investigated and debated about the safety of genetically modified organisms (GMOs) as food products and the influence of new conservation technologies on food safety.
- Pharmacological toxicology studies the safety of pharmaceutical products. Toxicity testing helps ensure that pharmaceutical products are safe for humans. Advances in pharmacology and toxicological research help to ensure that the beneficial effects of therapeutic agents are not outweighed by undesired side effects.
- Forensic toxicology establishes the causes of death caused by toxins in humans and animals, their circumstances, and their medicolegal implications.

1.2 CONCEPT OF WHAT IS TOXIC

As with other fields of knowledge, toxicology has its own distinctive vocabulary: toxin, toxicant, poison and xenobiotic are often used interchangeably in the literature; however, there are subtle differences between them. The term "toxin" is best reserved for harmful substances made by living organisms (e.g. poisonous marine organisms, infectious pathogens or venomous spiders). The word "poison" is widely used for this purpose during everyday life, but it may convey a

misleading interpretation, because their action depends on the dose, the individual and environmental circumstances, as mentioned previously (Burcham 2014a).

According to toxicologists themselves, the word "toxin" should be used if the foreign material came from a biological source. This means a substance is only poisonous if produced by living cells or organisms.

A "toxicant", on the other hand, should only be used if the foreign material came from man-made sources; thus it is not produced biologically. The names of toxicants are especially informative when coupled with a prefix that designates the site of toxic action for a given substance; alcohol, for example, is a hepatotoxicant because it causes liver damage at high doses (Burcham 2014b).

The term "xenobiotic" describes chemicals found but not produced in organisms or the environment. This includes numerous substances such as food additives, contaminants, drugs, recreational drugs pesticides, herbicides and industrial reagents.

The term "endobiotic", in contrast, includes chemical compounds present in the body during normal physiological processes (androgens, neurotransmitters, glucocorticoids, bilirubin, etc.). According to this definition, any substance can damage an organism because all exogenous products (xenobiotics) as the own constituents of the organism (endobiotics) when they are in a certain amount, can produce toxic effects.

This does not mean that external or synthetic chemical substances can be more toxic than natural or endobiotic ones, but simply that xenobiotics attract more attention from modern toxicology because they are widely used in industry, they are produced on a large scale and they persist in the environment for a long time (Burcham 2017).

Classifying toxic substances into endogenous and exogenous substances is complicated, because some substances come from different sources. With the development of more sensitive analytical techniques in biological fluids or tissues, it is known that many chemical compounds of industrial origin can be formed at low levels in the body. For example, humans are exposed exogenously to acrolein (combustion of tobacco and fossil hydrocarbons) but also endogenously, as a result of different biochemical reactions in the body itself (e.g. lipid oxidation; Figure 1.2). For definitions of

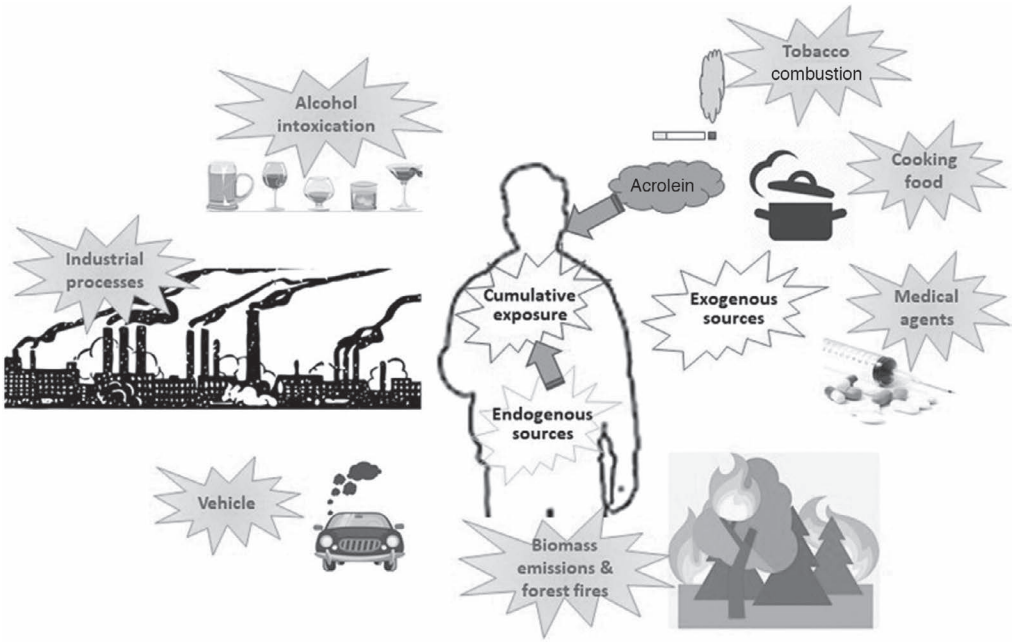

Figure 1.2 Complications of toxic substance origin based on source. Classification of toxic substances based upon their origin (endogenous or exogenous) is complicated because some substances come from different sources (e.g. acrolein). Humans are exposed to exogenous acrolein (e.g. tobacco, fossil fuels) but also to endogenous acrolein that forms via biochemical reactions within the body (e.g. lipid peroxidation).

Source: Figure drawn with inspiration from Burcham (2014a).

terms related to the study of toxicology, the reader is referred to the online resource provided by the *Encyclopaedia of Occupational Health and Safety* (Holmberg et al. 2015).

1.3 DETERMINING FACTORS OF TOXICITY

Toxicity is the activity specifically linked to the physicochemical properties of a substance due to its interaction with a receptor or receptors. This toxic activity is what determines that a substance is harmful to an organism under certain conditions. However, for each chemical agent there is a certain degree of toxicity. The range of doses necessary to produce damage in a living organism is very broad, as can be seen in Table 1.1. Toxic effect is assessed in terms of the median lethal dose (LD_{50}), which is the amount of a material, given all at once, which causes the death of 50% (one-half) of a group of test animals.

We should note that this concept is used in a relative manner, because several variables can influence the toxic activity of a substance. For a chemical compound to cause toxic effects, it is necessary for the substance or its metabolites (produced by biotransformation) to reach the appropriate place in the body and persist for a suitable amount of time at a concentration sufficient to produce a toxic manifestation. This toxic response, in turn, will depend on the chemical and physical characteristics of the substance, the size and route of exposure, the metabolism and the sensitivity of the system or individual.

1.3.1 Biological System

The biological system on which the toxic agent acts is of utmost importance, because the effect will vary markedly depending on the organism. This factor must be taken into account, because it is well known that there is a great variation in intra- and interspecies sensitivity towards toxic agents.

Therefore, we should consider that the toxic response of a substance, revealed in an animal toxicity test, does not imply that it will follow the same pattern when it is extrapolated to humans. Often the toxic effect is achieved at doses well above those of normal exposure for humans. In the history of toxicology, there are many examples of this interspecies variation. One of the most famous is the artificial sweetener saccharin, an additive widely used in foods and beverages. During the 1970s, reports of bladder tumours in rats were published after ingestion of high doses of saccharin. When investigating the mechanism related to the appearance of tumours, which only occurred in males and not in female rats, it was found that this bladder cancer was due to a specific protein in the rat called α-2u-globulin (Arnold 1983). This protein had little relevance in humans, and it was shown that the metabolism of saccharin was specific to rats. Numerous epidemiological studies provide no clear or consistent evidence to support the assertion that sodium saccharin increases the risk of bladder cancer in humans (Ellwein and Cohen 1990; NCI 2015). The US National Toxicology Program and the International Agency for Research on Cancer support the US Environmental Protection Agency's conclusion that saccharin is safe "at human levels of consumption" because, after assessing many saccharin-sweetened human foods, no association between saccharin and cancer could be established (Telišman 1998).

Table 1.1 Illustration of the Wide Order of Magnitude Observed for LD_{50} Values

Chemical	LD_{50} (mg/kg) rat
Ethanol	10,000
Sodium chloride	4000
Ferrous sulfate	1500
Morphine sulfate	900
Phenobarbital sodium	150
Nicotine	1
Botulinum toxin	0.00001

Note: The LD_{50} is one way to measure the short-term poisoning potential (acute toxicity) of a material. Toxicologists use many kinds of animals, but most often testing is done using rats and mice.

Source: Based on data in Klaassen (2005).

The different sensitivity within the same species is generally influenced, among others, by two parameters: age and sex.

Compared to adults, young children tend to be more susceptible to chemical toxicity because their inhalation volumes are relatively high compared to adults, and their gastrointestinal absorption rate is higher due to the increased permeability of the intestinal epithelium.

Children are highly susceptible to exposure to air pollutants. Minute ventilation is higher in children than in adults because children have higher basal metabolic rates and engage in more physical activity than do adults, and because children spend more time outdoors than do adults. On the basis of body weight, the volume of air passing through the airway of a child at rest is twice that of an adult under similar conditions (Künzli et al. 2010). In addition, because their enzymes for xenobiotic metabolism are immature, the excretion rate of toxic chemicals is relatively low, so the risk of toxicity may increase. In the early stages of their development, due to the immaturity of the central nervous system, children are especially susceptible to the neurotoxicity of various substances, such as lead and methylmercury, because these toxic compounds have direct (on intraneuronal regulatory mechanisms) and indirect (neurotoxic) effects. The neurological toxicity of methyl mercury at low doses in children especially affects memory (Freire et al. 2010; Oken et al. 2005; Weil et al. 2005), language and verbal skills (Lederman et al. 2008; Freire et al. 2010), and visual-motor function (Oken et al. 2008; Surkan et al. 2009). All these effects correlate with the loss of neurons from several areas of the brain (Korogi et al. 1998; Eto et al. 2010). In addition to cerebellar neurodegeneration, abnormal migration of neurons in the cerebellum and microtubule formation deficits were observed during fetal neural development (Choi et al. 1978; Castoldi et al. 2000).

Conversely, elderly people may be susceptible to adverse effects and toxicity due to age-related changes in body composition, organ-system function and consumption of a wide range of potent drugs. With advancing age, properties of functional systems that are involved in toxicokinetics process are altered (i.e. absorption metabolism, and excretion; Shibamoto and Bjeldanes 1996).

Relating to sex, there are susceptibility differences with respect to many toxic substances. These differences also occur in many mammalian species and are related to enzymatic activities, mechanisms of DNA repair and hormonal factors, and the presence of relatively greater fat deposits in women. In consequence, this produces a greater accumulation of some lipophilic toxins, such as organic solvents.

Sex differences in exposure, behavior, anatomy, physiology, biochemistry and genetics influence toxicokinetics and toxicodynamics from the molecular to whole animal level, accounting for male-female differences in responses to xenobiotics in humans and other animals. The Institute of Medicine (IOM; Wizemann and Pardue 2001) concluded that "sex matters" and exhorted: "Being male or female is an important fundamental variable that should be considered when designing and analyzing basic and clinical research". Aside from obvious differences related to sex-specific organs and reproductive events, xenobiotics can interact differently with the male and female sex hormones and their receptors. Some studies that observe male-female differences have determined that they are all due to the average body size differences (Schwartz 2003), without leading to mechanistic investigation. In other cases, statistical adjustment obscured differences. Laboratory research continued to focus on male animals or male cells, perhaps because of a mistaken belief that female development and physiology are intrinsically more variable than males (Itoh and Arnold 2015).

1.3.2 Routes of Exposure

The main ways in which toxic substances can enter the body are digestive (ingestion), pulmonary (inhalation), skin (topical, percutaneous or dermal) and other parenteral routes. In general, the most intense effect and the fastest response occurs when toxic substances are introduced directly into the bloodstream (intravenously). A decreasing order of efficacy would be inhalation, intraperitoneal, subcutaneous, intramuscular, intradermal, oral and dermal.

1.3.3 Toxicokinetic Processes

Four basic processes govern the concentrations toxicants achieve within vulnerable tissues: absorption, distribution, metabolism and excretion (Figure 1.3). These processes describe how a toxicant penetrates cell barriers to enter tissues (absorption), whether it is dispersed to particular organs and tissue compartments (distribution), how it undergoes chemical transformation within the liver (metabolism) and whether the parent compound or its metabolites are permanently eliminated in urine, faeces or both (excretion). The acronym ADME summarizes the four main processes involved in the toxicokinetic phase of xenobiotic action (Burcham 2014b).

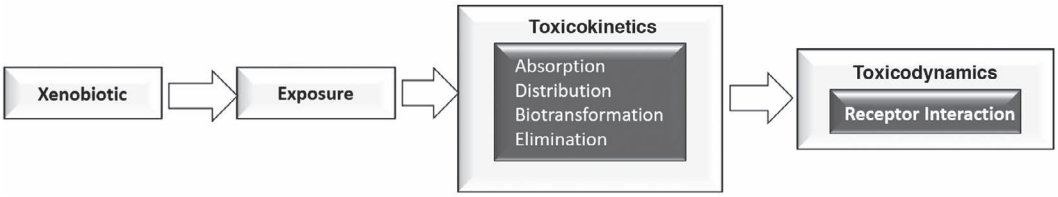

Figure 1.3 Processes affecting xenobiotic toxicity: exposure, toxicokinetics and toxicodynamics. Penetration of a xenobiotic from the environment to the sites of its toxic effect inside the organism can be divided into three phases. The exposure phase encompasses all processes occurring between various toxicants and/or the influence on them of environmental factors. The toxicokinetic phase encompasses absorption of xenobiotic into the organism and all processes which follow transport by body fluids; distribution and accumulation in tissues and organs; biotransformation (metabolism) to metabolites; and elimination (excretion) of toxicants and/or metabolites from the organism. The toxicodynamic phase refers to the interaction of toxicants with specific sites of action on or inside the cells (receptors), ultimately producing a toxic effect.

Some chemicals are readily absorbed and others are poorly absorbed. The rates and extent of absorption may vary greatly depending on the form of the chemical and the route of exposure. The behaviour of a xenobiotic during these processes is influenced by its basic physicochemical properties, including hydrophobicity, or more specifically the lipid/water partition coefficient, and the pH of the medium in which the xenobiotic is found (Ballantyne et al. 1993; Repetto 1995).

Absorption may occur through the alimentary tract, skin, lungs, eyes, mammary glands or uterus, and also from sites of injection. Toxic effects may be local, but the toxicant must be dissolved and absorbed to some extent to affect the cell. Solubility is the primary factor affecting absorption.

The skin, lungs, and alimentary canal are the main barriers that separate higher organisms from an environment containing a large number of chemicals. Toxicants must cross one or several of these incomplete barriers to exert deleterious effects. A chemical absorbed into the bloodstream through any of these three barriers is distributed throughout the body, including the site where it produces damage, which is the target organ or target tissue. A chemical may have one or several target organs; conversely, several chemicals may have the same target (Klaassen 2005). The distribution of toxicants and toxic metabolites throughout the body ultimately determines the sites where toxicity occurs.

Metabolism, also known as biotransformation, is a major factor in determining toxicity. The products of metabolism are known as metabolites, and there are two types of metabolism: detoxification and bioactivation. Detoxification is the process by which a xenobiotic is converted to a less toxic form. This is a natural defence mechanism of the organism. Generally, the detoxification process converts lipid soluble compounds to polar compounds. Except for the lungs, polar (hydrophilic) substances are more prevalent than lipid-soluble toxicants, which are excretable in bile and urine. Bioactivation is the process by which a xenobiotic may be converted to more reactive or toxic forms.

There are two phases of metabolism. Phase I includes oxidation, reduction and hydrolysis mechanisms. These reactions, catalyzed by hepatic enzymes, generally convert foreign compounds to derivatives for Phase II reactions. Products of Phase I, however, may be excreted as such, if polar solubility permits translocation. Phase II principally involves conjugation or synthesis reactions. Common conjugates include glucuronides, acetylation products, and combinations with glycine. Metabolism of xenobiotic agents seldom follows a single pathway. Usually, a fraction is excreted unchanged, and the rest is excreted or stored as metabolites (Klaassen 2005).

There are many pathways for the elimination of toxicants and/or metabolites: exhaled air via the lungs, urine via the kidneys, bile via the gastrointestinal tract, sweat via the skin, saliva via the mouth mucosa, milk via the mammary glands, and hair and nails via normal growth and cell turnover.

e) Toxicodynamic process, refers to the interaction between the molecules of the toxic substance and the cellular receptors through which the toxic effect is induced.

The toxic action of a chemical is a consequence of the physical/chemical interaction of the active form of that chemical with a molecular target within the living organism.

1.4 INTOXICATION AND TYPES

With the term "intoxication", reference is made to the damage exerted by a toxic agent on a living organism. Intoxications are classified based on different parameters (Burcham 2014b).

1.4.1 Duration of Chemical Exposure Needed to Produce Toxicity

Any intoxication usually offers a clinical picture whose toxic symptoms are usually related to the time needed to produce toxic effect. According to this criterion, they are classified as:

- Acute intoxications, characterized by the immediate appearance (usually less than 24 hours) of the pathological clinical picture. It usually occurs after exposure to a toxic agent at a high dose or after an accidental ingestion. Depending on the substance and the dose received, clinical symptoms may produce irritability, delirium, vomiting, diarrhea, seizures or even death.
- Subacute intoxication. Toxicities that manifest after repeated exposure to chemicals over several days or up to 1 month in duration are termed subacute intoxications. This broad category covers both repeated single dosing with a substance (e.g. an antibacterial drug taken every day to treat a persistent urinary tract infection) and prolonged exposures to chemicals present in our diet as food additives or contaminants of drinking water.
- Chronic intoxication. These are produced by repeated exposure to the toxic agent for a period of time, which can range from days to months or even years.

Some chronic effects caused by chemicals, such as cancer, have very long latency periods. The "latency period" is the time between the beginning of exposure and the appearance of disease caused by that exposure. The length of the latency period for chronic effects makes it difficult to establish the cause-and-effect relationship between the exposure and the illness.

The chronic effects can be varied and include neurological (damage to the nervous system), mutagenic (damage to genetic material that can be transmitted to future generations), carcinogenic (which can cause cancer), reproductive (damage to the female/male reproductive system) and teratogenic (damage to the embryo/fetus) effects.

1.4.2 Etiology of Toxic Agent

According to these criteria, intoxications can be classified into two types (Bello 2001):

- Voluntary: These intoxications are produced by an intentional action, among which are homicides, suicides, abortions, drugs, doping and aphrodisiacs.
- Accidental: These usually occur without any type of intentionality; examples are:

a. Environmental: Pollution in air, water or soil that often affects a large number of people. The causes are usually evacuation of industrial discharges, residues of pesticides in food, emissions of sulphur oxides and nitrogen to the environment by industry or use of fuels, and so forth.

b. Professional: These usually occur because of the slow but repeated action (for months or years) of a substance found in the work environment without adequate protection of ventilation and personal hygiene. Normally the legislation establishes the threshold limit value (TLV), which is the concentration of a substance whose daily exposure does not entail the development of adverse effects and thus regulates the permissible levels in the work environment (Miller et al. 1952).

c. Medicines: One of the most frequent causes of accidental poisonings due to confusion of one drug with another, overdose, drug-drug interactions or interactions between drugs and other substances, such as ethanol.

d. Food: Relatively frequent and produced by biotic or abiotic contamination of food (presence of toxic metals, pesticides, toxigenic microorganisms). In other cases it is due to certain components of a food that respond to an individual susceptibility (allergies, intolerances).

e. Domestic: These occur usually in children and the elderly, and those who inadvertently ingest medications, cleaning products, cosmetics and so forth.

Clinical manifestations can be classified as mild, moderate or severe in magnitude.

1.4.3 Characteristics of the Appearance or Location of the Toxic Effect

The intoxications can be classified as follows:

- Immediate versus delayed effects: Some substances show toxic effects in a few minutes, whereas others manifest themselves after a certain time or even after several years, as is the case with carcinogenic substances.
- Reversible versus irreversible effects: In the first case, the normal situation is restored when the toxin disappears, whereas the damage is irreparable in the second case.
- Local versus systemic effects:
  - a Local toxicity: Some toxic agents with extreme properties, such as irritants or vesicant gases, produce damage at the place of contact. Thus, very alkaline or acidic chemical compounds can cause damage directly if they contact body parts such as skin, the nasal cavity, or the eyes. Damage includes contact dermatitis, burns, blisters or irritation.
  - b Systemic toxicity (target organ toxicity): Most chemicals are absorbed and dispersed through the bloodstream and often cause damage to one or more organs. In some target organs, damage occurs due to a high accumulation of the toxin; an example is the damage to the lungs after the accidental ingestion of paraquat, a widely used herbicide (Burcham 2014b).

In addition to passive diffusion, membrane transporters have an important role in the transport of foreign substances, accumulating high amounts in target organs such as liver or kidneys. This is the case in heavy metals, such as cadmium or mercury, which can produce hepatotoxicity or nephrotoxicity.

In some cases, the specific toxicity of chemicals is localized in an organ because the tissue expresses high levels of enzymes that convert the compound into toxic metabolites that damage cells. This phenomenon is called toxicological bioactivation, and contributes to numerous pathologies induced by toxic substances; for example, German textile industry workers in the 1940s developed bladder cancer after producing the family of azo dyes, because toxic metabolites attacked DNA and proteins in exposed cells (Emiliani et al. 2014).

Other examples of toxicological bioactivation are the appearance of neurological diseases such as schizophrenia or neurological disorders such as autism related to oxidative stress. Oxidative stress is caused by an imbalance between the production of free radicals ROS (Reactive oxygen species are natural products inevitably generated along cellular metabolism. Due to their extreme reactivity, they can damage DNA, proteins and lipids) and the effectiveness of the antioxidant defences of the human body. A predominant physiological source of ROS is the mitochondria, where they are created naturally as by-products of energy generation. Mitochondrial dysfunction could lead to an overproduction of ROS and increase oxidative stress with changes in gene expression and even direct damage to DNA. If DNA repair is unsuccessful, it could lead to mutations in nuclear DNA and mitochondria, including promoting cell death (Telišman 1998). Several pathophysiological mechanisms have been implicated in these orders, including genetic predisposition, monoamine deficiency, circadian disruptions, hypercortisolemia and inflammation (Belmaker and Agam 2008). The involvement of oxidative stress mechanisms has also been suggested in some psychiatric illnesses, including depression, anxiety disorders, schizophrenia and autism spectrum disorders (Valko et al. 2007; Ng et al. 2008; Bouayed et al. 2009). Increased levels of ROS and RNS (Reactive nitrogen species are various nitric oxide–derived compounds, have been recognized as playing a crucial role in the physiologic regulation of many living cells, such as smooth muscle cells, cardiomyocytes, platelets, and nervous and juxtaglomerular cells) (Suzuki and Colasanti 2001; Dhir and Kulkarni 2011; Maes et al. 2011) and altered levels of the antioxidant glutathione (GSH) were reported in postmortem brain samples of depressed individuals (Gawryluk et al. 2011). Actually, oxidative stress mechanisms have been suggested as targets for novel antidepressants (Lee et al. 2013). This seems reasonable considering the reported occurrence of inflammation, oxidative and nitrosative stress as well as declining levels of plasma concentrations and activity of several key antioxidants in samples from depressed subjects (Maes et al. 2011). Perhaps psychologic stress disrupts the oxidant-antioxidant balance within the brain, causing impairment of antioxidant enzyme function. This leads to glutathione depletion and increases oxidative stress. Simultaneously occurring glutamate toxicity, calcium imbalance, and mitochondrial impairment intensify oxidative stress, causing biochemical distress in the brain. This disrupts neurocircuitry and weakens connections in the hippocampus, amygdala and cortex, ultimately causing behavioural and cognitive deficits. It seems reasonable to suggest that tight regulation of oxidative stress, either by enhancing the activity of enzymes of antioxidant defence or by directly quenching pro-oxidants, offers the potential to limit psychiatric symptoms (Salim 2017).

1.4.4 Dose Effect or Dose Response

"Dose" is usually defined as the amount (in units, such as mg/kg body weight) of a xenobiotic that enters an organism. According to current terminology, the adverse effect is the change in the morphology, physiology, growth, development or lifetime of an organism that results in a deterioration in the ability to compensate for an additional stress or an increase in susceptibility to harmful effects of other environmental influences. The decision of whether or not an effect is adverse is a matter that requires the judgment of the expert (Burcham 2014b). There are some toxic effects, such as death or cancer, that do not have degrees but are "all or nothing" effects.

The relationship between the response of the biological system and the amount of toxic substance administered takes such a consistent form that it is considered the most general and important concept of toxicology.

From a practical point of view, there are two types of relationships between dose and response (Burcham 2014b):

a. Individual or gradual effect dose relationship, the response of a single organism or individual at variable doses, is often called gradual because the effect measured increases according to the dose. This type of effect frequently appears in *in vitro* studies on isolated organs, tissues or cells that are very useful to study mechanisms of toxicity. It can also be observed in experimental animals but it is more difficult to interpret because the gradual effects are usually masked by the regulatory mechanisms of the organism. It should be noted that most toxic substances have several places of action and mechanisms of toxicity; each of them has its own dose-effect curve with its respective harmful effects.

b. Population or quantal dose-response relationship represents the distribution of responses to different doses in a population of organisms. Unlike the gradual dose-response relationship in a population is characterized because the individual responds or does not respond. Dose-response representations can provide important information. Experimentally it has been proven that the quantal dose-response relationship usually shows a normal distribution of frequency represented by a Gaussian, or bell-shaped, curve (Figure 1.4). In this curve, we can see how three groups of individuals can be described in relation to the action of the toxin: normal, hypersensitive or hyposensitive. This phenomenon of sensitivity to the effect of a certain toxic can occur among groups of individuals of the same population, races of the same species, species and so forth.

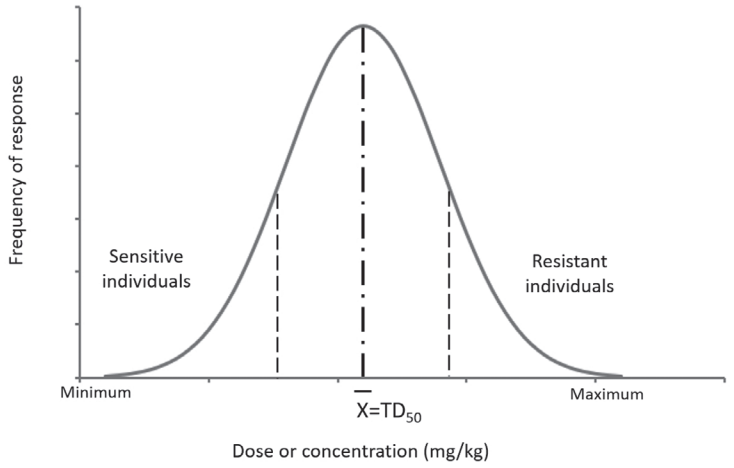

Figure 1.4 Quantal frequency dose-response relationship. This determines the dose required to produce toxic effects for each member of the population. Dose-response relationships typically proceed on the assumption that target populations are homogenous and comprise individuals who conform to bell-shaped Gaussian distributions. A median toxicant induces toxicity of comparable severity within most individuals in the population. However, the population also contains small numbers of individuals (sensitive individuals) who show toxicity at relatively low exposures (minimum dose) and other individuals (resistant individuals) who only exhibit toxicity at high exposure levels (maximum dose).

Abbreviation: TD_{50} = dose at which 50% of the exposed population experiences toxic effect.

Table 1.2 The Most Common Terms in Dose Response Curves

Term	Meaning
Dose (of a substance)	Total amount of a substance administered to, taken up, or absorbed by an organism, organ, or tissue. Often expressed as mg/kg/day.
Effective dose (ED)	Dose of a substance that causes a defined magnitude of response in a given system. Note: ED_{50} is the median dose that causes 50% of maximal response.
Dose effect	Relation between dose and the magnitude of a measured biological change.
LD_{50} (effective dose)	Dose that causes 50% lethality in an animal population.
Lethal dose (LD)	Amount of a substance or physical agent (e.g. radiation) that causes death when taken into the body.
Threshold	Dose or exposure concentration below which a defined effect will not occur.
Threshold dose (ThD 0.0)	The threshold dose (ThD 0.0) is measured as mg/kg/day.
Toxic dose (TD)	Amount of a substance that produces intoxication without lethal outcome.
Latent period	Delay between exposure to a harmful substance and the manifestations of a disease or other adverse effects. Period from disease initiation to disease detection.
NOAEL	No observed adverse effect level, or the highest dose that does not cause a toxic effect.
LOAEL	Lowest observed adverse effect level. The lowest observed effective dose on a dose-response curve, or the lowest dose that causes an effect. A safety factor is a formal, arbitrary number with which one divides the NOAEL or LOAEL derived from animal experiments to obtain a tentative permissible dose for humans. Safety factors range from 100 to 103.
Limit value (LV)	Limit concentration at or below which Member States of the European Community must set their environmental quality standard and emission standard for a particular substance according to Community Directives.
Potency (in toxicology)	Expression of relative toxicity of an agent as compared to a given or implied standard or reference.
Tolerance	Adaptive state characterized by diminished effects of a particular dose of a substance: the process leading to tolerance is called adaptation.

Source: IUPAC (2007).

A quantal dose-response curve supplies useful quantitative estimates (Table 1.2) that provide helpful insight into the toxicity of a given compound. For example, the dose eliciting the reported toxic response in 50% of the population can be easily determined (i.e. TD_{50}), as can the threshold dose at which toxicity is first observed, the lowest observed adverse effect level (LOAEL). A related concept is the no observed adverse effect level (NOAEL), which can be estimated from dose-response data of this kind (Figure 1.5).

Other parameters can also be determined, such as the threshold dose (ThD 0.0), which corresponds to the level of toxic substance below which no toxic effect is expected. The threshold is the dose below which no effect is detected or above which an effect is first observed. The threshold information is useful in extrapolating animal data to humans and calculating what may be considered a safe human dose for a given toxic substance. The threshold dose is measured as mg/kg/day. It is assumed that humans are as sensitive as the test animal used.

These parameters are insufficient, so it is necessary to establish safety margins, which are determined by the ratio between the safe dose, or threshold dose, and the lethal dose (LD). This margin of safety is very useful in environmental and food toxicology, which deals with parameters of high importance, such as NOAEL (the highest dose at which there was not an observed toxic or adverse effect), ADI (acceptable daily intake: maximum amount that an additive can be ingested in the diet

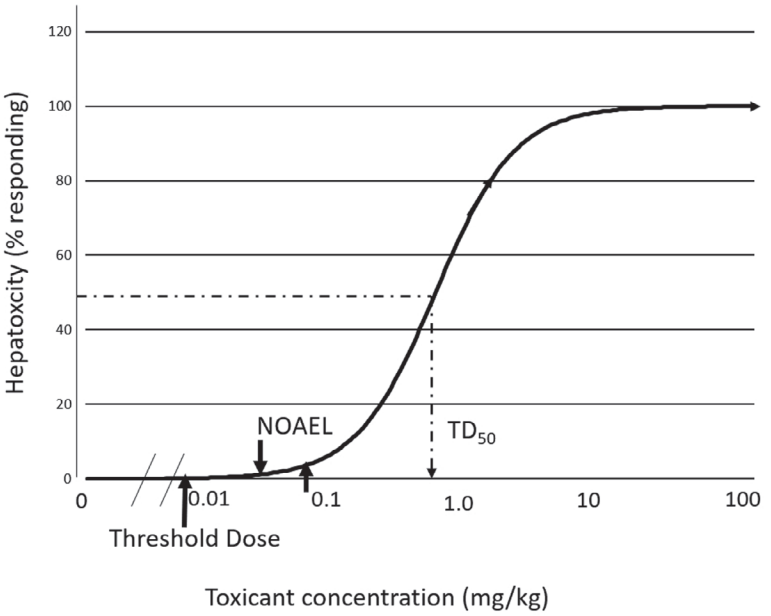

Figure 1.5 Semi-log dose response curves. The graph represents of the percentage of observed subjects with evidence of hepatotoxicity over the different dosages studied. Low doses are insufficient to generate a response, whereas high doses generate a maximal response. Toxic response in 50% of the population can be easily determined (TD_{50}), as can the threshold dose at which toxicity is first observed. Related concepts are the no observed adverse effect level (NOAEL), which can be estimated from dose-response data of this kind.

Source: Burcham (2014a).

throughout life, without adverse health effects) or VUL (limit threshold value that is the concentration of an agent to which a living organism can be exposed for 8 hours a day and 5 days a week without any damage to health).

When using a high number of doses with a high number of animals per dose, sigmoid dose-response curves can be obtained. The sigmoid curve has a relatively linear portion, and the slope of this region of the dose-response curve allows comparison between different toxins and determination of a toxin's potency and how effectively it induces a toxic effect. In Figure 1.6, the effects of three toxic compounds are compared, and we can observe that compound A shows a higher carcinogenic power than compounds B or C. This representation shows the LD_{50} or TD_{50}, which are defined as the single dose of a substance that is expected to cause death, or a certain toxic effect, in 50% of the animals subjected to the test, respectively (Burcham 2014a).

The shape of the dose-response curve has numerous and important consequences for the evaluation of toxicity. A conventional assumption of toxicology is that the dose-response relationship between a chemical and an adverse health effect will have a monotonic shape: that is, the slope of the curve does not change sign. In the case of chemicals that exhibit a U-shaped curve, effects are more prominent at low and high doses than they are at intermediate doses.

Nonmonotonic dose-response curves (NMDRCs) are mathematically defined as a change in the sign (positive/negative) of the slope of a dose-response relationship over the range of doses tested. Numerous studies have recognized the occurrence of NMDRCs in organisms' responses to nutrients, vitamins, pharmacological compounds, and other small molecules that interact with receptors including hormones (Vandenberg et al. 2012; Figure 1.7). That is, the magnitude of the harmful effects is great at low doses (or lack thereof) but decreases with increasing dose. When the dose reaches a point of nondeficiency, the harmful effects disappear, and the organism reaches a state of homeostasis. But if the dose increases to abnormally high values, an adverse response will

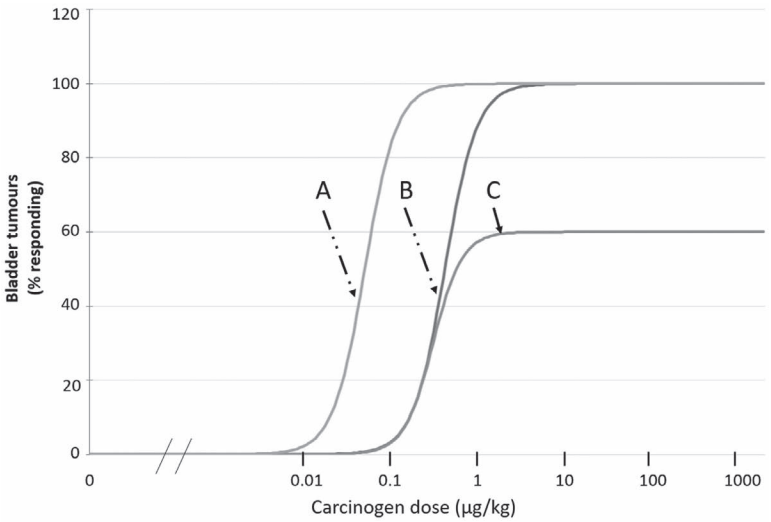

Figure 1.6 Comparison of carcinogenic potency and efficacy of three hypothetical toxicants (A, B and C) at inducing bladder cancer in a population of laboratory rats. Toxicant A has the highest potency because it increases tumour incidence most strongly at the lowest doses tested. The toxicants A and B both induce a maximal tumour response at high doses, indicating that they possess comparable efficacy. Toxicant C has comparable potency to toxicant B yet has lower efficacy.

Source: Burcham (2014a).

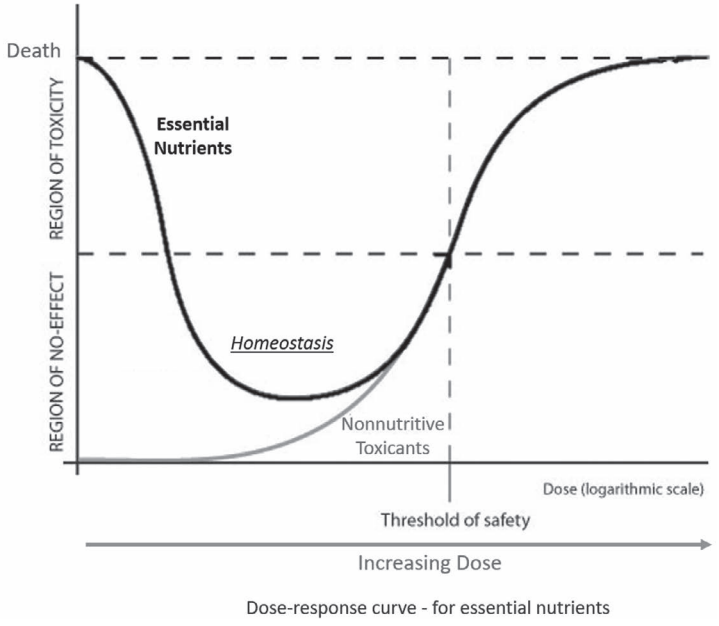

Figure 1.7 Dose-response relationships for essential vitamin or mineral nutrients and other chemical substances (black). The U-shaped hormetic response is shown with a region of homeostasis (the dose range with neither deficiency nor toxicity), which lies below the threshold for adverse response and is discontiguous with both the low-dose deficiency region (whose base is death) and the high-dose toxicity region (Hayes 2008). The grey line shows the dose-response relation.

appear, which will be different from the deficiency, and its magnitude will grow according with an increase of the dose. In the same way, some toxic substances may exert beneficial or stimulating effects at low doses, whereas at higher doses they produce toxic effects; this effect is called hormesis and the substances hormetins.

The term "hormesis" (Calabrese et al. 2007) has been most widely used in the toxicology field, where investigators use it to describe a biphasic dose response with a low-dose stimulation or beneficial effect and a high-dose inhibitory or toxic effect and those substances called hormetins. The response of the cell or organism to the low dose of some toxics substances is considered an adaptive compensatory process following an initial disruption in homeostasis. Thus, a short working definition of hormesis is: "a process in which exposure to a low dose of a chemical agent or environmental factor that is damaging at higher doses induces an adaptive beneficial effect on the cell or organism". The prevalence in the literature of hormetic dose responses to environmental toxins has been reviewed comprehensively (Calabrese and Blain 2005), as have the implications of toxin-mediated hormesis for understanding carcinogenesis and its prevention (Calabrese 2005).

These graphs also allow extrapolations, qualitative or quantitative estimations of the toxicity (extrapolations of the risk) obtained by transferring data from one species to another; or a series of dose-response data (generally in the high-dose range) to dose-response areas, for which there are no data and allows the health authorities to carry out the risk assessment for a specific toxin.

Extrapolations are theoretical qualitative or quantitative estimates of toxicity (risk extrapolations) that usually must be made to predict toxic responses outside the observation range. Mathematical modelling is used for extrapolations based upon an understanding of the behaviour of the chemical in the organism (toxicokinetic modelling) or the understanding of statistical probabilities that specific biological events will occur (biologically or mechanistically based models). Some national agencies have developed sophisticated extrapolation models as a formalized method to predict risks for regulatory purposes.

The procedures used to extrapolate from high to low doses are different for assessing carcinogenic effects and noncarcinogenic effects:

- Carcinogenic effects in general are not considered to have a threshold, and mathematical models are generally used to provide estimates of carcinogenic risk at very low dose levels.
- Noncarcinogenic effects (e.g. neurotoxicity) are considered to have dose thresholds below which the effect does not occur. The lowest dose with an effect in animal or human studies is divided by safety factors to provide a margin of safety. Based on this risk assessment, the health authorities will establish the necessary political decisions (risk management) to control the identified hazards and their communication to the population.

1.5 DOSE-RESPONSE RELATIONSHIPS TO MIXTURES OF CHEMICAL SUBSTANCES

Most of the toxicological databases of substances that produce adverse effects on health have been established using data from cell cultures, laboratory animals and even humans, but they are usually data related to exposure to a single toxin. These data can vary because, in daily life, multiple exposures usually take place simultaneously to many toxic substances that can potentially interact with each other. The latency period of the pathological response between exposure and disease is an additional complicating factor. This is the case of the appearance of tumours by accumulation of multiple genetic alterations for many years; the period of cancer manifestation can occur decades later.

The physical, chemical and biological agents can interact with each other in each phase of the toxicokinetic and/or toxicodynamic processes, with the result of three possible effects (Regl. (CE) Nº 1272/2008):

- Independent: Each agent produces a different effect due to a different mechanism of action.
- Synergistic: The combined effect is greater than that of each agent separately. Here we can distinguish two types: additive, when the combined effect is equal to the sum of the effects produced separately by each agent; and enhancer, when the combined effect is greater than the sum of the individual effects.
- Antagonist: The combined effect is less than the sum of the individual effects. There are four types of antagonism: functional, chemical, pharmacokinetic and receptor. Functional antagonism assumes that two substances are counteracted by opposite effects: chemical or inactivation. It involves the chemical reaction of two toxins that leads to a less toxic product.

Pharmacokinetic antagonism occurs when absorption, biotransformation, distribution or excretion is altered, and the result is a decrease in concentration or duration of a substance or both in the target organ. The receptor antagonism, also known as a blocker, supposes that two chemical substances share the same receptor and therefore achieve less effect together.

The lack of data from toxicological studies on chemical mixtures highlights one of the main challenges for regulatory authorities. In the European Union, the regulation of the classification, labelling and packaging of chemical substances and their mixtures (Baillie-Hamilton 2008) transferred the responsibility of performing *in vivo* tests of commercial mixtures to industry, to demonstrate the existence of a toxicological hazard. However, no regulatory provision has been adopted for noncommercial artificial mixtures that represent the real-life scenario of exposure.

In addition to genetic factors and cumulative exposure to various chemical substances present in the environment, water and food also influence our individual life habits decisively. Few life factors exert such a strong influence on toxic substances as the consumption of tobacco and alcohol or the performance of physical activity. The first two significantly lower the efficiency of the liver and kidneys in the elimination of toxins from the blood and produce numerous adverse health effects. Conversely, exercise can modify the toxicokinetics of toxic agents so that the amount of toxin that arrives at the target organs or blood can be modified, regardless of the protective effect it has on oxidative stress.

Other habits such as consuming barbecued meat and processed meat have received considerable attention due to likely exposure to cooking by-products that may alter the expression of xenobiotic-metabolizing genes within the gut wall and liver. Observational studies in recent years have associated heterocyclic aromatic amines and polycyclic aromatic hydrocarbons with colorectal cancer. Strong scientific evidence has demonstrated the relationship between cancer and nitrosamines (NA), heterocyclic amines (HCAs), and polycyclic aromatic hydrocarbons (PAHs), which are the major genotoxins derived from cooking and food processing. The mechanisms of the relationship between dietary toxic xenobiotics and cancer risk are not yet well understood, but it has been suggested that differences in dietary habits affect the colonic environment by increasing or decreasing the exposure to mutagens directly and indirectly through changes in the composition and activity of the gut microbiota. Several changes in the proportions of specific microbial groups have been proposed as risk factors for the development of neoplastic lesions and the enrichment of enterotoxigenic microbial strains in stool (Nogacka et al. 2019). Likewise, the consumption of fruits and vegetables in the diet seems to be able to influence the response to exposure to different toxic substances. An increasing number of studies suggest that these diets provide protection against cancer caused by DNA-damaging chemicals (Burcham 2014b). These effects can be mediated via epigenetics. Rising interest in the role of early epigenetic programming in the health of subsequent generations stems from the recognition that the cellular phenotype is influenced by factors other than changes in the underlying genetic sequence. The three major epigenetic determinants of prenatal gene expression are histone modification, DNA methylation and noncoding RNAs.

We must consider other effects. Since the beginning of the last decade, it was pointed out that environmental cumulative toxic chemical agents could contribute to the increase in the frequency of obesity in the population. Heindel (2003) analyzed the correlation between the increase of the frequency of overweight in the adult population and the increase in the production of industrial chemical substances; in 2002, they formulated a hypothesis of the causal relationship between both events. Recently, several studies have focused on the possible repercussion of the endocrine-disrupting effect of the energy regulation system (i.e. obesogens and their relationship with the increase in the prevalence of obesity) in practically all countries (Grün and Blumberg 2006; Gluckmen and Hanson 2004; Vallverdú 2005). Soon after, the term "obesogens" was coined, which defines xenobiotics that may be present in the environment and/or in foods and inappropriately regulate and promote lipid accumulation and adipogenesis (Heindel and Levin 2005). Among the xenobiotics considered as possible obesogenic compounds in humans, the most widely studied, have been diethylstilbesterol, bisphenol A, organic compounds derived from tin, genistein and phthalates. Interest focuses on the possibility that prenatal xenobiotic exposure disrupts normal fetal programming of energy homeostasis, conferring a lifelong predisposition towards weight gain. At present, there is evidence that the period of intrauterine development is the most vulnerable for the effect of obesogens, for the specific effect on the promotion of adipogenesis in adults (Gluckmen and Hanson 2004; Bern 1992; Giusti et al. 1995).

However, more studies are needed to demonstrate the causal relationship between the concentration of different obesogens in human biological fluids and the development of obesity.

1.6 MODERN TOXICOLOGY RESEARCH

Toxicology continues to advance rapidly, supported by the development of other disciplines and methodological tools. This is the case for the toxic response in tissues, based on the broader knowledge about toxicokinetics and toxicodynamics. From the large spectrum of toxic compounds, a greater number of studies have been carried out on those that have aroused greatest interest. The conducted studies include those with chronic, neurological, mutagenic, carcinogenic, teratogenic and reproductive toxic effects with high latency periods and presence in the environment, food and cosmetics. Of great interest to health professionals is the increasingly detailed study of the toxic effects of new drugs and the interactions among them, effects that sometimes have promoted their withdrawal from the available pharmacological stock. In 2016, a British team studied the causes for the market withdrawals of weight-loss drugs. Eighty percent of the market withdrawals were based on data from spontaneous reports involving cardiac disorders for eight drugs, psychiatric disorders for seven drugs, and abuse or dependence for 13 drugs. Fenfluramine was marketed for 24 years before its worldwide withdrawal in 1997 for heart valve disease. Benfluorex was marketed in France for 33 years before its withdrawal in 2009 for cardiotoxicity; yet the heart valve disorders resembled those caused by fenfluramine and the chemical structures of these two drugs are very similar (Onakpoya et al. 2016).

The advances in instrumentation, the development of increasingly sensitive, specific assays, and lower limits of detection, have allowed the progress of analytical toxicology. With these new tools, it is easier to obtain information to characterize toxicological mechanisms or describe the interactions between the toxic agents and the different biomolecules. Chiral organic pollutants are a major trend in environmental science research and include compounds with different physical chemical properties and applications such as pesticides, herbicides, pharmaceuticals, flame retardants, and synthetic polycyclic musk. In general, agrochemicals are commercialized as racemic mixtures. However, when a racemate reaches the environment, enantiomers of the compound can differ significantly in their environmental fate and their toxicological impacts; the evaluation of the enantiomeric fraction is of critical importance to assess the environmental risk of each enantiomer (Maia et al. 2017). Therefore, we need its analytical determination, and study of the toxic effects of the configurational isomers, in order to carry out a correct analysis of the environmental risk derived from its use (Ye et al. 2015). These advances in analytical toxicology give confidence that new products entering the market, will be safer at all levels of use, because *in silico, in vitro* and *in vivo* tests are increasingly reliable.

Until now, *in vivo* tests with experimental animals have been the basis of toxicity studies of chemical compounds for the evaluation of human risk, but the use of labour animals for evaluating toxic effects of chemicals raises ethical concerns. *In vitro* methods (which include technologies such as transcriptomics, proteomics and/or metabolomics), with organ-on-a-chip or toxigenomics and computational (or *in silico*) toxicology, are the applications that allow us to understand the complexity of the biological systems. These methods, which have been used as a complement to traditional *in vivo* studies, are now the paradigm of modern testing for the identification and characterization of chemical mechanisms of toxicity in humans (Raunio 2011; Saeidnia et al. 2015). In fact, the European Food Safety Authority (EFSA), which has as one of its priority objectives the evaluation of new methodologies and technologies that serve to improve the evaluation of risks in food and feed, launched a project in 2011 to know the current situation of technologies and the application of these technologies in the assessment of risk and its future prospects.

Another important problem is the great gap in the knowledge of environmental toxins. The industrial development of the last decades has resulted in the accumulation of substances that cause damage to the environment and health There are more than 1000 industrial chemicals which have been found to be neurotoxic in experimental studies. Although more than 200 are documented to produce neurotoxic effects in humans, only 11 of them have been shown to have a toxic effect on human neurodevelopment and lead to neurodevelopmental disorders. This does not mean, however, that exposure to any of the other chemicals is harmless to fetal development but simply that they have not been studied (Ornoy et al. 2015; Emiliani et al. 2014; Grün and Blumberg 2009). Some environmental pollution can modify the epigenome by altering the pattern of gene expression at susceptible life stages such as pregnancy, neonatal life, childhood or juvenile periods. These alterations can produce harmful effects on health in later adult life, or even worse, they could be transmitted to future generations (Burcham 2014b).

Other toxic environmental compounds are the chemical compounds that act as endocrine-disrupting chemicals (EDCs); as already mentioned, these are coupled to the hormonal receptors

and act as agonists, antagonists or hormonal modulators. Some EDCs frequently show non-monotonic dose-response relationships (Shanle and Xu 2011; Nadal et al. 2018). Although several molecular mechanisms have been proposed to explain NMDR relationships, they are largely undemonstrated (Nadal et al. 2018).

These environmental pollutants could be related to climate change (Europa Commission 2018). On the one hand, global warming can affect the movement and levels of chemicals, such as organochlorine pesticides in the environment, weakening the ability of animals and humans to tolerate these chemicals. On the other hand, as chemical exposure increases, sensitive populations of animals and humans may experience a reduced ability to handle extreme temperatures, severe storms, lack of food and other hazards of climate change.

The effect of toxic agents has been extended to include the study of nanomaterials, given the widespread presence in various facets of our lives: at work, pharmacological, food-related, domestic and so forth. Therefore, nanotoxicology has arisen, whose objective is to study the toxic effects of nanomaterials, of nanoparticles, compounds that can even change from a chemical to a biological nature due to their capacity to interact with biomolecules. The risk involved in their wide use has led the control agencies to balance the promotion of nanotechnology for its many beneficial effects on our way of life, with the assessment and regulation of their risks both at human and environmental level (Stone et al. 2017).

In summary, the awareness of the vulnerability of humans, and the environment, to the multiple substances to which we are exposed opens up new branches of toxicology as well as the need to study more techniques, especially at the molecular level, in order to establish prevention and diagnostic measures and treatment.

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2 Toxicokinetics

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CONTENTS

2.1 INTRODUCTION

When referring to toxicokinetics (TK), the reader will notice that the concepts and basis described herein are the same as those described in pharmacokinetics (PK). Indeed, both TK and PK characterize the absorption, distribution, metabolism and excretion (ADME) processes of xenobiotics, either drugs or toxins, from the time course of their concentrations (kinetics) as parent chemical(s) and/or metabolite(s) in biological media, tissues and excreta. Moreover, ADME studies identify factors influencing the different steps involved and try to represent it mathematically, to quantitatively estimate and predict temporal concentrations of chemicals in target tissues, where pharmacological or toxicological responses may be observed (El-Masri et al. 2015). However, it should be noted that PK and TK differ in terms of goals and technology as well as in the philosophical emphasis in the two approaches (Welling 1995). TK studies mainly refer to animals, either to determine toxicity mechanisms, appropriate species, study design and treatment regimen in subsequent nonclinical toxicity studies, or to assess the relevance of these findings to human safety. In veterinary medicine, TK will contribute to the avoidance of undesirable xenobiotic residues in animal tissues. It is also remarkable that TK focuses more on systemic *exposure*, and typically doses are higher relative to the therapeutic or effective dose (Van der Merwe and Buur 2018; Singh 2018; Rang and Hill 2013). Then, TK is defined as "the generation of pharmacokinetic data, either as an integral component in the conduct of non-clinical toxicity studies or specially designed supportive studies, to assess systemic exposure" (ICH 1995). However, its application towards risk assessment of industrial chemicals (fertilizers, pesticides, biocides, carcinogens, nanomaterials, etc.) and safety of nonpharmaceutical ingredients added to food, cosmetics and personal care products have brought a more clear definition: "the study of the time course of absorption, distribution, elimination (i.e., excretion and metabolism), and uptake of potentially harmful xenobiotics leading to a

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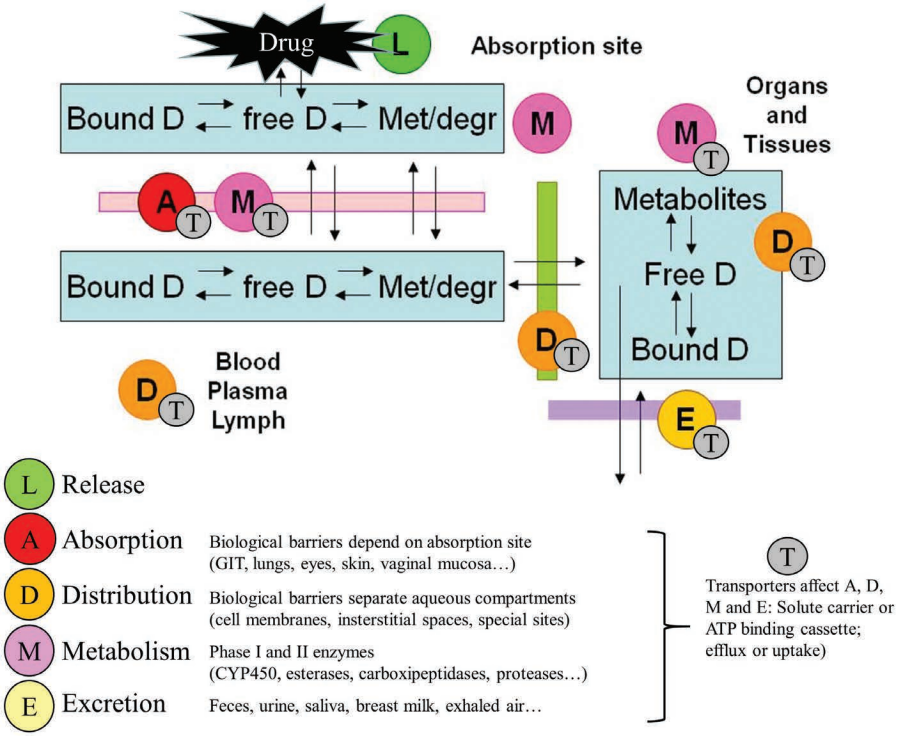

Figure 2.1 General overview of the LADME process.

toxic response" (Boroujerdi 2015). Then, the biological response, either toxicological or pharmacological, is a function of the ADME process associated with the time course evolution of the xenobiotic and/or its metabolite concentrations. It is usually assumed that the higher the dose, the higher the concentration and the longer the time the xenobiotic compound stays in blood, so that a higher body exposure and a more intense and long-lasting response will be attained. This principle was recognized by Paracelsus (1493–1541): "All substances are poisons; there is none which is not a poison. The right dose differentiates a poison from a remedy". Figure 2.1 shows the general scheme of the LADME process. L represents liberation or release of the toxicant from the matrix where it may be contained or the source where it is created.

2.2 DATA IN TOXICOKINETIC STUDIES

To obtain the data needed for TK studies, blood, plasma and urine are the most commonly used sample matrices. However, depending on the goal of the study, tissues, nails, hair or other samples might be relevant. The design of all TK experiments implies the selection of a test species and the response to be measured, an exposure period, the length of the observation period and the different dosing levels to be tested.

Data for TK studies can be obtained from occupational human epidemiology studies, clinical exposure studies, environmentally exposed epidemiologic studies, acute accidental poisonings, animal toxicity tests or other alternatives, such as the use of nonmammalian or nonavian species or *in vitro* systems. Because all have advantages and disadvantages, they should be selected according to the study objective (James et al. 2000). A proper selection and design of the approach best fitted to the study objectives should consider the factors affecting the ADME process (Table 2.1).

As shown in Table 2.1, these factors can be grouped into organism related factors or physicochemical specific factors. Some authors also consider intrinsic and extrinsic factors affecting susceptibility, referring to the differences in toxicity risks resulting from variation in the toxicological response (sensitivity) and exposure. Lifestyle, socioeconomic status, geographical factors and diet can affect exposure. Some ethnic groups eat fish or shellfish frequently or use ethnic medicines, both of which might increase ingestion of mercury. Certain population groups can be highly

Table 2.1 Factors Affecting the ADME Process in TK

Source	Factors	Facts	Example	
Organism	Route of exposure	Differences in epithelial barriers and physiological environment: Parenteral, GIT, skin, lungs, eyes, rectal, vaginal.	LD_{50} methadone in rats (mg/kg)	
			Oral	90
			SC	43
			IV	10
	Species (and strains)	The rate of production of reactive metabolites is different in humans, rabbits, rats, mice.	LD_{50} chloroform (mg/kg/day)	
			Human	602
			Rabbit	100
			Wistar rat	2180
			Sprague Dawley rat	908
			CD 1 mouse	250
			Swiss mouse	1100
	Sex	Metabolic differences, percentage fat of total BW.	Sensitivity to strychnine in rats: more toxic to females than to males when administered SC or IP due to higher rates of metabolism by males.	
	Genetics (ethnic group)	The largest contribution is metabolic differences. Extensive and poor metabolizers.	Acetylation of isoniazid; polymorphic CYP2C19, CYP2D6.	
	Age or life stage	Immature organ development, percentage fat, muscle or water of total BW. "Children are not little adults": chloramphenicol, cisapride.	Differential expression of enzymes during gestation: CYP3A, FMO and SULT1 and 2.	
	Diseases	GIT oedema in congestive heart failure, altered protein synthesis in renal or hepatic impairment.	Asthmatic individuals are more susceptible to air pollutants.	
Physicochemical	Chemical composition	Particle size, chirality, solubility, lipophilicity, pKa, product type, formulation.	LD_{50} (mg/kg) in rats	
			DDT	40
			morphine	265
			fentanyl	3.1
	Exposure conditions	Dose, single or repeated, time, substrate where toxic is included.	Morphine toxicity in rats	
			Single IV dose	LD_{50} 265 mg/kg
			Repeated dosing (35 mg/kg/day)	No toxic effects

Abbreviations: GIT = Gastrointestinal tract; SC = subcutaneous; IV = intravenous; BW = body weight; IP = intraperitoneal; SULT = sulfotransferase; FMO = flavin monooxygenase.

Source: Adapted from James et al. (2000) and Hines et al. (2010).

exposed to contaminants because of their geographic proximity to industrial plants, those near and/or using a polluted water body, or those living or working near roads with dense vehicular traffic. Smoking or drinking alcohol can modify metabolism as well. Indeed, this is another important source of variation because two chemicals playing simultaneously might exhibit additive,

synergistic, potentiated or antagonistic effects. These can be based on functional, chemical, dispositional or response mediated interactions. For example, ethanol increases gastritis associated with aspirin (functional), decreases the toxicity of ethylene glycol, thereby inhibiting the production of toxic metabolites (dispositional), the additive effects of ammonia and cyanide (chemical), or the induction of CYP1A2 in smokers, affecting antipyrine or theophylline metabolism (dispositional). A good example of several factors simultaneously affecting the toxicokinetics and metabolism of polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) has been described by Van den Berg et al. (1994). The absorption, body distribution and metabolism can vary greatly between species and also may depend on the congener (any individual of a specified genus) and dose. Besides, exposure to complex mixtures has the potential to alter the TK of each compound.

2.3 ROUTES OF EXPOSURE

As mentioned above, considering absorption is the first step after release, necessary for a xenobiotic to undergo disposition in the body; the different routes available to reach the systemic circulation will be described next. The administration routes have been classified into four categories (Boroujerdi 2015):

- Category 1 groups various routes that represent a biological barrier that may have different characteristics. The gastrointestinal tract (oral), the respiratory tract and parenteral or mucosal (rectal, vaginal nasal) administration are included here. Depending on the physicochemical and reactive properties of the compound, local and systemic effects can be obtained.
- In category 2, administration takes place directly in the bloodstream, circumventing all barriers (intravenous or intraarterial).
- Category 3 involves percutaneous absorption, given the particular multilayered structure of the skin.
- Category 4 includes all administration routes seeking targeted or local exposure (e.g. intracardiac, epidural, intrathecal, intraarticular, intracerebral, intravesical).

However, it should also be mentioned that in TK, very often the entry of xenobiotics into the body follows environmental exposure; skin (topical), lung (inhalation), eye (ophthalmic) and gastrointestinal tract (oral) are the major routes. Also, it is often challenging to determine the exact dose and the exposure pattern (frequency, duration and extent). Most accidental or autolytic acute poisonings occur after oral ingestion. Toxic reactions associated with gases happen through inhalation, and exposure to toxic industrial chemicals is often due to skin contact. The eyes represent a less frequent route, whereas toxicity after rectal and vaginal absorption is usually associated with illicit drug trafficking. Oral, parenteral and inhalation routes are predominantly linked to fatal poisoning (García et al. 2002).

2.3.1 Gastrointestinal Tract (GIT)

The oral route is preferred for drug product administration because of physiological reasons. It is also very important in TK because many toxicants can be ingested with food. Absorption in the gastrointestinal tract (GIT) is a complex process affected by several factors mainly classified as route related (physiology and dynamics of the GIT) and drug (product) related (physicochemical properties). Important route-related factors include pH, surface, secretory processes (e.g. bile, pancreatic juice), microflora, enzymes, transport proteins, gastric emptying and intestinal motility. Drug-related factors that can significantly affect oral absorption include polymorphism, solubility, chirality, particle size, porosity, partition coefficient, molecular weight, wettability, dosage form (excipients: pharmacologically inactive ingredients in the dosage form). Genetic differences or disease conditions might also affect the GIT.

The whole GIT is 20–25 feet long, and five absorption sites can be distinguished: buccal and sublingual mucosa, the stomach, small intestine, colon and rectum.

Buccal and sublingual absorption: The permeability of xenobiotics in the buccal and sublingual mucosa is ranked between that of the intestine and the skin. There are several epithelial layers with various degrees of differentiation, but the limiting step is permeability through the upper oral epithelium that shows different anatomical characteristics depending on absorption sites (Figure 2.2 and Table 2.2).

Therefore, sublingual absorption is faster than buccal and palatal (the roof of the mouth posterior to the ridge of bone behind the upper teeth). The absorption mechanism is either transcellular

passive diffusion or intercellular pathways, which means solubility and pKa are important determinants for absorption. Saliva volume and pH might affect ionization and accordingly modify absorption. Saliva is secreted at a highly variable rate depending on several factors such as stimulation of the taste buds (0.5–7 ml/min); it is hypotonic and has a pH close to neutral conditions (6.2 to 7.6) with buffering potential. Toxicants in the mouth can exert acute local reactions or systemic effects because of sublingual absorption or ingestion if they are lipophilic.

Gastric absorption: The gastric mucosa is a folded surface of 0.1 m^2, and according to the pH partition theory, weak acids at gastric pH (pH 1–3) will be in their unionized form and might be absorbed by passive diffusion. However, physiological gastric transit times are relatively short (half-life of 10–30 min), although various factors affect gastric emptying such as volume, viscosity, tonicity, drugs, food composition, pH, body position and temperature. The structure of the absorbing membrane in the stomach is heterogeneous, as shown in Figure 2.2. Then, regardless of the

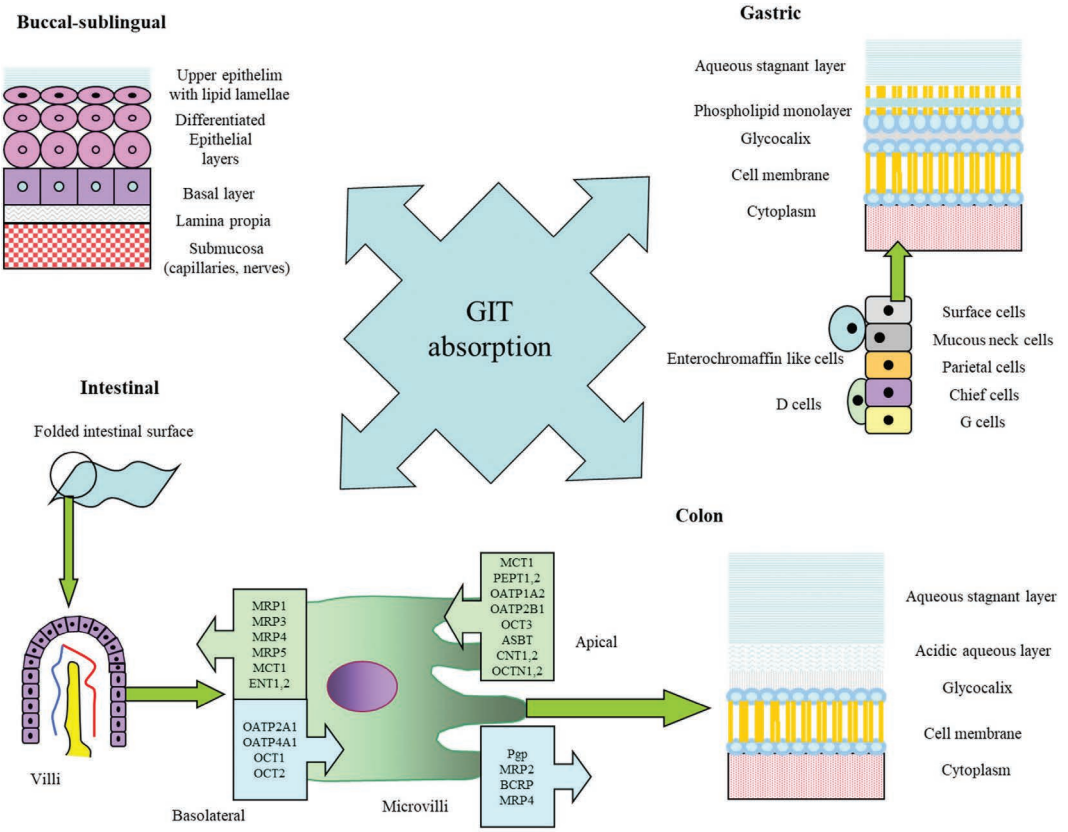

Figure 2.2 Absorption sites in the gastrointestinal tract.

Table 2.2 Differences in Absorption Site within the Oral Cavity

Absorption site	Thickness (μm)	Keratinized surface?	Lipids*
Cheeks and lips (buccal)	500–600	N	Polar
Under the tongue (sublingual)	100–200	N	Polar
Gums (gingival)	200	Y	Apolar
Buccal ceiling (palatal)	250	Y	Apolar

*Typical lipid composition.

Source: Adapted from Bermejo and Garrigues (2013).

potential absorption of acid chemicals in the stomach, the main absorption site in the GIT is the small intestine due to the huge surface area available.

Small bowel absorption: the small intestine is the biological site physiologically and anatomically adapted for absorption after ingestion, with a large surface area close to 200 m^2. The absorption process is conditioned by the absorbing membrane structure. Usually three steps are involved, passing from the lumen (1) through the aqueous luminal layer in the apical side, (2) through the lipid cell bilayer and (3) from the cytoplasm to the basolateral side and lymph or blood capillaries. The lipid membrane has the characteristic sandwich architecture (water–lipid bilayer–water). However, on top of the head groups and glycocalyx facing the luminal side there is a thin and acidic aqueous layer (20 µm, pH 5.3) covered by a thicker (300 µm) stagnant layer acting as a barrier to diffusion of hydrophobic molecules. The main mechanism responsible for absorption is diffusion, either transmembrane or through pores, but influx and efflux transporters are also expressed both in the luminal and basolateral side of enterocytes (Figure 2.2). The permeability of xenobiotics once in the cytoplasm is considered as a nonlimiting step because of the thin and porous structure of capillary and lacteal walls. Nevertheless, the presence of intracellular enzymes such as CYP450 or esterases may affect the bioavailability through a first-pass effect. The first-pass effect (also known as first-pass metabolism or presystemic metabolism) is a phenomenon of drug metabolism whereby the concentration of a drug is reduced before it reaches the systemic circulation. It is the fraction of lost drug during the process of absorption which is generally related to the liver and gut wall. All along the intestine, P450 enzymes and efflux transporters play a combined role to prevent the absorption of certain xenobiotics. However, a first-pass effect may occur in all administration routes depending on the xenobiotic compound. For example, Chang et al. (1994) demonstrated a significant metabolic first-pass effect for the pesticides carbaryl and parathion upon contact with porcine skin; both metabolized to naphthol and paraoxon and para-nitrophenol, respectively.

Absorption in the colon: As compared to the small intestinal site, the colonic mucosa is devoid of microvilli and the absorbing surface is reduced to 1/30. The number of transport proteins is also decreased, but in contrast, the longer residence time and a slightly higher pH of the acidic layer make the diffusion of basic compounds with pKa between 9 and 11 favoured. Besides, the presence of microflora with enzymes showing the ability to cleave specific bonds such as azoreductases, β-lyases, β-glucuronidases, nitroreductases and sulfatases may affect absorption and toxicity. There is clear evidence that air and food pollutants interact with GI microbiota. Liver metabolites of PAHs are deconjugated or transformed into CH_3S^-, nitro-PAHs and nitrotoluenes are reduced to amine metabolites, pesticides are dechlorinated or deconjugated, polychlorinated biphenyls (PCBs) are metabolized to $MeSO_2$ derivatives, metals are demethylated (Hg) or methylated (As, Bi), azo dyes are transformed into aromatic amines or melamine metabolized to cyanuric acid (Claus et al. 2016).

Rectal absorption: The absorption of toxicants in the rectum is mainly associated with illegal trafficking of drugs of abuse. The rectum is a richly perfused area divided into inferior, middle and upper segments according to venous drainage. The epithelium resembles that of the GIT but there is no serosa layer. The volume contained in the rectal ampoule is low (1–3 mL), viscous and holds a neutral pH (6–8) but without buffer capacity. The main absorption mechanism is transcellular passive diffusion and pKa becomes a determinant factor in rectal absorption, which is considered to be slow and erratic depending on the absorptive segment and the facts mentioned above.

2.3.2 Pulmonary Tract

The airways begin with a nasal mucosa of about 100–150 cm^2 and 20 mL connected to the trachea through the nasopharynx, oropharynx and larynx. The inner epithelium is folded into three turbinates (these are shell-shaped networks of bones coated with a thin mucosal layer, containing vessels and tissue located in the external wall of the nasal passageways) provoking a turbulent flow of inhaled air that enables it to intimately contact the mucosa. The nasal epithelium is made of ciliated and nonciliated cells, goblet mucus-secreting cells and basal cells. Mucociliary clearance acts as a physical barrier against the absorption of xenobiotics and prevents the inhalation of particles. The nasal fluid is a mixture of tears and local fluid, with the latter made of 96% water, 2% electrolytes and 2%–3% secretions containing proteins (e.g. albumin, α-macroglobulin, IgA, lactoferrin and aminopeptidases) or mucin (neutral and acidic glycoproteins). Nasal mucus is 2–5 µm thick, and it is cleared to the pharynx with a linear velocity of 5–6 mm/min, which means particle contact with the mucosa will last 20–30 minutes.

Nasal absorption can take place by passive diffusion, either through the membrane or pores, but a plethora of ABC transporters (P-gp, MRP and CFTR), solute carrier (SLC) transporters (POT, DAT, OAT, OATP, OCT, EAAT2/GLT1, and GLUT), amino acids and metal transport proteins have been identified as well (Anand et al. 2014). Also, phase I and II metabolic enzymes such as P450 (family 2 and 3), de-ethylases, hydroxylases, amino- and carboxypeptidases, esterases, glutathione or glucuronosyl transferases, dehydrogenases and oxidases are present with high interspecies variability and eventually higher activity than the liver (Chapter 3). Anilines and nitrosamines are metabolized in the nasal mucosa (Dhamankar 2013). Remarkably, certain disease conditions or toxic compounds can provoke local effects affecting the physiology of nasal mucosa, modifying mucociliary clearance and pH of mucus.

The pulmonary route is further divided into a conductive tract and a respiratory tract. Considering the anatomy of the airways, the upper conductive tract includes the trachea, bronchi and bronchioli up to generation number 16 (the upper airways branch into two or more smaller airways, and each division point or level is called a generation number). Then the respiratory tract goes deeper into the lungs to the alveolar region, corresponding to generation numbers 20–24 (Figure 2.3). The epithelial surface of the respiratory tract shares some features with the nasal epithelium, such as mucus secretion and mucociliary clearance to the pharynx. In contrast, the alveolar region shows a specialized epithelium for gas exchange and absorption with a huge surface (100 m^2). Type I alveolar cells are cells for O_2 and CO_2 exchange, whereas type II cells are type I precursors and produce the luminal surfactant coating containing phosphatidylcholine. The alveolar epithelium is not considered a restrictive barrier to absorption. Then, the lungs constitute an absorption site where local and systemic effects can be attained. Some toxicants can cause irritation and constriction of bronchi and bronchioli, altering mucociliary clearance and mucus production. In contrast, soluble and small particles can be absorbed in alveoli. From a toxicology

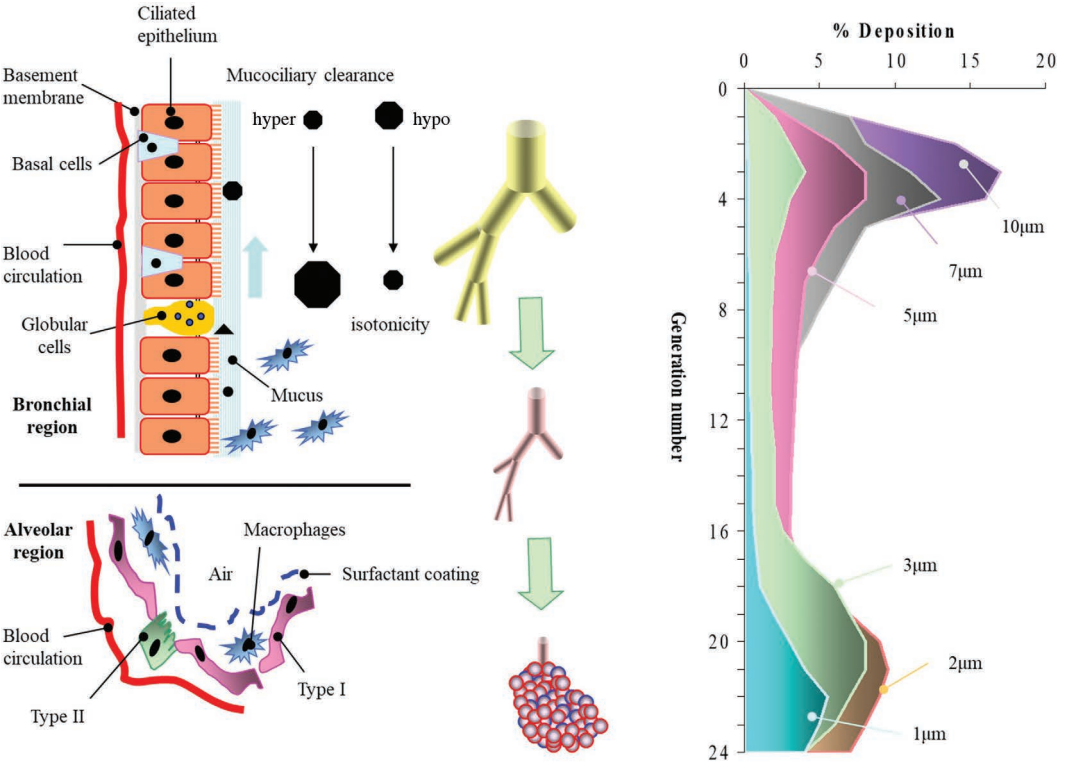

Figure 2.3 The inhalatory route and pattern for particle deposition according to aerodynamic diameter.

standpoint, the lungs can be exposed to gases or vapours and particles. Under normal breathing conditions, inflow of 10,000 L per day in the upper airways is fast, whereas in the deeper lungs, the narrower lumen and higher generation number favour gas exchange by diffusion and particle deposition. Particle disposition in the pulmonary tract depends on the particle aerodynamic diameter, relating particle size 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. According to their aerodynamic size, particles larger than 10 µm undergo inertial impact in the mouth and throat, and they are removed by nose-wiping, blowing, or by sneezing. Smaller sizes show diffusion and settling, causing 5–10 µm particles to be trapped in the upper conductive tract, which are removed by the mucociliary escalator. Those smaller than 1 µm are light and exhaled. Only 2–3 µm particles will reach and settle down in the alveolar region for further absorption (Figure 2.3). Some particulates (asbestos, fiberglass) that are phagocytized remain in the lungs, where they may have adverse effects and result in the development of respiratory disease.

Absorption in the lungs is related to solubility because only those chemical compounds that can get dissolved in the alveoli quickly will be able to penetrate the epithelium with the help of the surfactant coating. Gases and vapours of volatile compounds diffuse readily across the alveolar cell membrane. Although lipid solubility is important in determining the rate of absorption, the solubility of the toxic substance in the blood and its interaction with components of the blood is also crucial. The mechanisms for absorption are transcellular passive diffusion for lipophilic molecules and diffusion through pores (40–400 nm) for hydrophilic ones. Moreover, many protein transporters including organic cation transporters (e.g. OCT1, OCT2, OCT3, OCTN1), organic anion transporters (e.g. OATP2B1, OATP2B1, OATP3A1, OATP4C1), ABC transporters (e.g. MRP1, MRP3) and peptide transporters (e.g. PEPT2) are expressed. Also, a broad spectrum of P450 (CYP1A1, CYP3A4, CYP3A5, CYP1B1, CYP2B6, CYP2D6, CYP2E1 and CYP2J2), non-P450 (peptidases, flavin mono-oxygenases, esterases and cyclo-oxygenases) or phase II enzymes (UDP glucuronosyl transferases, glutathione S-transferases and sulfotransferases) have been identified.

2.3.3 Ocular Route

The eye is a highly protected organ. In humans, general toxic effects on the eyes are usually considered as mainly external due to the direct ocular exposure. Usually, upon contact with chemicals, the eyes show an acute local irritation reaction, with increased secretion of tear fluid outweighing the tear film volume stably associated with the corneal epithelium (7–30 µL), thus eliminating the chemical from the surface. However, some compounds can permeate the corneal epithelium to further diffuse through the stroma and endothelium into the aqueous humour. Then the trabecular meshwork, iris and ciliary body can be reached. The clearance of chemicals from these structures depends on molecular size. Also, chemicals can be absorbed from the conjunctiva and sclera to the iris and/or ciliary body without entering the aqueous humour due to the larger pore sizes than those found in the cornea. Very low local exposure of the anterior eye chamber is achieved after chemical contact with the eye surface (1%–5%). Factors such as short retention time on the eye surface (less than 2 min), low corneal permeability and systemic absorption from lacrimal fluid (tear fluid differs, in that it consists not only of fluid from the lacrimal gland but also contains components from ocular surface epithelial cells, stromal immune cells and meibomian gland acinar cells) in the palpebral and bulbar conjunctiva explain this fact. Small molecules permeate across the cornea and conjunctiva by passive diffusion (trans-cellularly and/or paracellularly) depending on physicochemical properties, such as logD, hydrogen bonding and polar surface area. Although transporters such as P-glycoprotein (P-gp) and multidrug-associated proteins (MRP-1 and MRP-4) have been identified in the cornea, their influence on ocular exposure remains to be determined (Subrizi et al. 2019). Ocular toxicity is usually associated with nonadverted exposure to environmental or occupational chemicals or accidental splashing. Jaga and Dharmani (2006) reviewed the ocular toxicity from pesticide exposure. They concluded that ocular toxicity resulted from inhalation, ingestion, dermal contact and ocular exposure. Effects of pesticides have been observed in the anterior chamber (conjunctiva, cornea, lens) but also retina and the optic nerve, suggesting permeation to the posterior chamber and/or systemic toxicity after absorption in the lungs, GIT or skin. Pesticide exposure has also been associated with retinopathy, Saku disease, optic-autonomic peripheral neuropathy and abnormal ocular movements. Ocular toxicity has been

reported after systemic administration of certain drugs such as antineoplastic agents, ethambutol or hydroxychloroquine.

2.3.4 Dermal Route

The skin is a relatively large organ (1.73 m^2 surface in adults) with a main protective role. It is a multilayered and nonpermeable barrier, where the outermost layer of the epidermis, stratum corneum, is considered as the rate-limiting step in skin absorption. It consists of a cell layer packed with keratin and devoid of blood vessels. The cell walls of the keratinized cells are double in thickness due to the presence of the keratin. Blood vessels are usually about 100 μm below the skin surface.

Drug permeability through the stratum corneum can occur via paracellular or transcellular pathways, but the first (involving the lamellar intercellular space) is the main one. The intercellular route is through the lipids of the stratum corneum and the transcellular route is through the corneocytes. In both cases, the drug must diffuse into the intercellular lipid matrix, which is recognized as the major determinant of drug absorption by the skin. Mainly, small lipophilic molecules can pass through the stratum corneum in small amounts. No active transport mechanisms functioning within the epidermis have been reported so far, but in 2014, Fujiwara et al. described the expression of human solute carrier family transporters in the skin.

Also, a minor absorption pathway is associated with sweat glands, sebaceous glands and hair follicles. Because these structures represent only a very small percentage of the skin's total surface area (0.1%), they are not ordinarily viewed as important contributors to dermal absorption. However, this route enables the permeation of charged molecules and large polar compounds (e.g. peptide-based drugs; Morais et al. 2016).

Once a substance penetrates the stratum corneum, it enters the viable epidermis, the dermis and subcutaneous tissue. These contain a porous, nonselective aqueous diffusion medium where toxicants can readily penetrate by simple diffusion into the circulatory system via the large numbers of venous and lymphatic capillaries in the dermis.

Skin absorption is influenced by several factors. The thickness of the stratum corneum shows regional variability. In the palms and soles, it is very thick (400–600 μm) but is much thinner in the arms, back, legs and abdomen (8–15 μm). The stratum corneum of the neck behind the ear, scrotum and the axillary and inguinal regions is the thinnest. Assuming diffusion is the absorption mechanism, skin permeability inversely relates to the thickness of the epidermis. Then any skin injuries, abrasion, scratching or cuts—either mechanically or chemically induced—will make it easier for toxicants to penetrate this layer. Certain chemicals such as methyl and ethyl alcohol, hexane, and acetone are lipid soluble and can degrade the lipid barrier of the cell membrane. Skin burns and dermatitis are the most prevalent conditions.

The skin's basal level of hydration (7% by weight) favours the absorption of polar substances 10-fold, and additional hydration increases penetration by 3–5 times.

Skin penetration can vary by species: monkey, pig, and guinea pig skin permeability is similar to that of humans; that of the rat and rabbit are generally more permeable; and the skin of the cat is generally less permeable. For safety reasons, the rat and rabbit have been used for preclinical dermal toxicity tests after shaving.

Some toxicants can gain entry into the body after skin contamination. Examples include poisoning of agricultural workers by organophosphate pesticides; death after skin contact with the neurological warfare agent sarin; and systemic toxicity from several industrial solvents such as carbon tetrachloride (hepatotoxic) and hexane (nerve damage). Dermal exposure to semivolatile organic compounds (SVOCs) has often been underestimated but it can occur even to a larger extent than the amount taken in via inhalation. (SVOCs include polybrominated flame retardants, endocrine-disrupting chemicals [EDCs], polycyclic aromatic hydrocarbons, phthalates, plasticizers, pesticides, and antimicrobials and are encountered indoors in cleaning products, detergents, aerosol cans, brushes and sponges.) Also, dermal exposure to particles and dust, the role of clothing and bedding as transport vectors, and the relevance of a potential absorption through hair follicles are all areas of research interest.

As happens in other routes of exposure (lungs, rectal, buccal, sublingual), chemicals entering the blood through the skin escape the hepatic first-pass effect and do not encounter the same detoxification pathways found when ingested and processed by the stomach, intestines and liver, making them potentially more toxic.

2.3.5 Intravaginal Route

The vaginal mucosa is a highly perfused region, usually acidic due to the presence of *Lactobacillus acidophilus* which buffers the pH between 3.8 and 4.2. Regional but also systemic toxicological effects can be expected after vaginal absorption. It takes place mainly by transcellular and paracellular passive diffusion. Although the protein expression of P-gp, BCRP and MRP-2 in endocervical and vaginal tissue of premenopausal women has been confirmed, no uptake transporters have been reported for this site (Grammen et al. 2014; Nicol et al. 2014). It should also be pointed out that the vaginal epithelium possesses protease enzymatic activity. Proteases are likely to be the prominent barrier for the absorption of intact peptide and protein molecules into the systemic circulation (Ashok et al. 2012).

Mucosal irritation induced by some chemicals, vaginal changes during the menstrual cycle, and menopausal changes modify the regional vascularity and thickness of the mucosa, which leads to significant differences in the permeability of the barrier, with inter- and intraindividual absorption variability. The vaginal absorption of therapeutic agents is influenced by their lipophilicity, degree of ionization, chemical structure and molecular weight, and interaction with vaginal barrier and secretion. The TK of a vaginally absorbed compound depends to some extent on the type of product used. For example, the absorption of a compound surrounded by a plastic film depends on the release rate. In general, the absorption through mucosal barriers of drugs and their related pharmacokinetic analysis are the same as other extravascular routes of administration.

2.4 MECHANISMS OF PASSAGE THROUGH BIOLOGICAL BARRIERS

The passage of a compound through physiological barriers can take place by different mechanisms. The GIT is the only site where all these absorption mechanisms are present and in some instances some play simultaneously: (1) passive diffusion, either transcellular or paracellular; (2) carrier-mediated transcellular diffusion or facilitated diffusion, or passive-mediated transport; (3) transcellular diffusion subject to P-gp efflux; (4) active transport; (5) pinocytosis and receptor-mediated endocytosis; (6) solvent drag, osmosis and two-pore theory; and (7) ion-pair absorption. The most important are passive diffusion, facilitated diffusion and active transport.

2.4.1 Passive Diffusion

Passive diffusion is the main mechanism for the transfer of xenobiotics through biological barriers. It can take place through the cell membrane (transcellular) or using pores between the cells (paracellular). Drug molecules on one side of the membrane start crossing because a concentration gradient acts as the driving force. According to Fick's first law, flux (J_{sm}) by passive diffusion can be written as

$$J_{sm}\ (\text{mass length}^{-2}\ \text{time}^{-1}) = \text{P}\ (\text{length time}^{-1})\ \Delta\text{C}\ (\text{mass length}^{-3}) \tag{2.1}$$

where P is the permeability constant and ΔC is the concentration gradient across the membrane corresponding to dissolved solutes. Therefore, to have a nonlimited mass transfer rate, the concentration gradient should always be in favour of the absorption. This usually happens in most biological barriers because blood flow withdraws absorbed drug molecules from the basolateral side. *In vitro* experiments designed to estimate mass transfer rates refer to this situation as a sink condition, and it means that solute concentrations passing through should never exceed 10%–30% of the solute solubility in the receiving chamber after drug passage. Equation 2.1 shows a clear dependency of drug diffusion on concentration following first-order kinetics. P depends on the diffusion coefficient (D), the partition coefficient (P_c) and the length of the diffusion pathway (x) as shown in Equation 2.2:

$$P\ (\text{length time}^{-1}) = D\ (\text{length}^2\ \text{time}^{-1})\ P_c\ (\text{unitless})\ /x\ (\text{length}) \tag{2.2}$$

Partition coefficient: P_c is an index of lipid solubility. It is very useful to classify chemical compounds in a rank order, because it has been shown that their values for nonionized forms of several series of representative chemicals and drugs can be correlated with their transfer rates through biological membrane systems—from intestinal lumen into blood, from plasma into brain and cerebrospinal fluid, and from lung into blood. In general, the higher the lipid solubility, the higher the P_c and the easier the passage through the membrane. In many instances, P_c is referred to as log P_c to make values more understandable. P_c can be determined experimentally by the shake flask method using hexane, heptane or n-octanol, or by chromatography. The classical shake flask method is based on the ratio of the equilibrium concentrations at 20–25°C (tolerance ±1°C) of a dissolved substance in a two-phase system consisting of two largely immiscible solvents. Different volumes ratio of n-octanol to water are used, and after shaking both phases are separated by centrifugation and the concentrations in each one determined by appropriate analytical techniques. It

can also be predicted using software packages such as ADMET Predictor's PCB, ACD/LogP from the individual contribution of functional groups or molecular structure.

When molecules contain ionizable functional groups like monoprotic weakly acidic or basic xenobiotics or polyprotic ampholytes, their true partition coefficient depends on the pH, and thus, the distribution coefficient D_c is estimated based on the presence of both ionized and unionized forms in the lipid and aqueous phases.

Diffusion coefficient: D is also a determinant feature for passive diffusion. As shown in the Stokes-Einstein equation (Equation 2.3) it is inversely proportional to molecular size:

$$D = k_B T/(N_A 6\eta r)\ (\text{length}^2\ \text{time}^{-1}) \tag{2.3}$$

where K and T are the Boltzmann constant (1.38×10^{-23} units J/K) and absolute temperature, respectively; N_A is Avogadro's number; η is viscosity; and r is hydrodynamic radius or molecular size, assuming a spherical shape. In this expression, the energy per molecule is kBT/N_A; the friction per molecule is $6\pi\eta r$ and is equivalent to a force divided by velocity: (g cm s^{-2})/(cm s^{-1}). Larger molecules show slower diffusion because of frictional resistance and in some cases steric hindrance. In contrast, very small molecules pass through membranes faster than expected based on their P_c. Smaller molecules are usually associated with lower P_c values than their larger homologs, and they may be able to move through membrane pores. Pores are present in all membranes, but their size varies with the nature and function of the membrane. For example, GIT cell membranes will not allow passage of water-soluble molecules larger than about 0.4 nm in diameter, whereas renal or hepatic blood capillary walls are permeable to molecules up to about 100 nm in diameter.

Diffusion through pores follows Equation 2.4:

$$J_{sp} = D_p \frac{A_p}{A_m} \frac{\Delta C}{x} \tag{2.4}$$

where Dp is the diffusion coefficient through aqueous pores and A_p/A_m represents the fractional area of pores in the whole membrane surface. In small pores, the effective pore area A_p is reduced because of entrance effects, friction between pore wall and solute, and solute-solute and solute-solvent interactions.

When transcellular and paracellular diffusion occur simultaneously, total flux is the sum of the two processes (Equation 2.5):

$$J = J_{sm} + J_{sp} = \frac{P_c D_m}{x}\left(1 - \frac{A_p}{A_m}\right)\Delta C + D_p \frac{A_p}{A_m}\frac{\Delta C}{x} \tag{2.5}$$

Ionization: When chemicals are ionized at physiological pH, only the nonionized forms will cross the membrane by transcellular diffusion, because small electrolytes will be able to diffuse through pores regardless of their ionization state and ionized larger molecules will show very low diffusion and partition coefficients to pass through the membrane (Figure 2.4).

The Henderson-Hasselbalch equation (Equation 2.6) predicts the fraction of the drug that remains unionized at a given pH depending on pKa.

$$\text{for acids}\ \ pK_a - pH = \log\frac{non-ion}{ion} \tag{2.6}$$

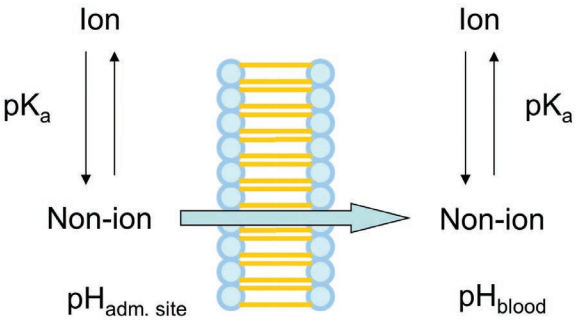

Figure 2.4 The pH-partition theory.

$$\text{for bases } pK_a - pH = \log \frac{ion}{non-ion}$$

Thus, acidic compounds (benzoic acid pKa = 4) will be more ionized at pH values above pKa (1% nonionized at pH 5 and 90% nonionized at pH 3), whereas bases will show an opposite behavior (aniline pKa = 5; 1% nonionized at pH 3 and 90% nonionized at pH 6). Most toxicants move across the cell membrane by diffusion. Organic substances such as nitrous oxide, ethylene and divinyl ether diffuse across the cell membrane of the alveoli easily because they do not have an electrical charge and are lipid soluble.

2.4.2 Facilitated Diffusion

Facilitated diffusion, also called passive mediated transport or carrier-mediated transcellular diffusion, is a mechanism to enable permeation through biological membranes using carrier proteins or ion channels in the membrane that facilitate transport. In contrast to active transport, it is a passive diffusion through a passageway without requiring energy or conformational change of the protein. Therefore, molecules do not cross the barrier against the concentration gradient. Lipid insoluble compounds or those larger than pores allowing for paracellular diffusion may use this mechanism. In facilitated diffusion, substances are transported at a much faster rate than expected based on the molecular size and polarity of the molecule. As opposed to passive diffusion, mass flux (J) is not just driven by the concentration gradient but rather by the amount of the protein expressed on the membrane, the diffusion coefficient and the affinity between the carrier and the substrate. Thus, saturation and/or competitive phenomena may occur, and mass transfer kinetics may deviate from linearity at high concentrations, influencing TK behavior. Compounds such as sugars, amino acids, steroids or vitamin B12 have been shown to use facilitated transport.

2.4.3 Transcellular Diffusion Subject to P-Glycoprotein Efflux

Nowadays, many proteins expressed on membranes have been identified, and their roles in the disposition of chemicals have been characterized. They are usually considered as phase III enzymes (phase III transporters affect drug disposition, including drug metabolism) because they affect the passage of many chemical compounds through biological barriers. These are both expressed in the apical and basolateral side of cells so that they can help in permeability across the membrane or work against it. These proteins are grouped into adenosine triphosphate (ATP)-binding cassette and soluble proteins. One of the most important proteins affecting the absorption of xenobiotics is P-gp, a 170 KDa protein embedded in the cell membrane of enterocytes, hepatocytes, renal tubular cells and endothelial brain cells. The net effect of the P-gp efflux on the kinetics of xenobiotics passage through biological barriers has been well established *in vitro* by using *in vitro* Caco-2 cells culture systems.

2.4.4 Active Transport

Active transport shares some features with facilitated transport, because a carrier protein is needed to help the xenobiotic to pass through a biological barrier. Then, saturation and competitive processes might occur as well. However, active transport also demands energy input and conformational changes of the carrier. It maintains transport against a concentration gradient, and the main energy source comes from ATP hydrolysis. These two steps of energy production or protein interaction can be affected by toxicants like fluorides or dinitrophenols.

Active transport can be referred to as primary transport when ions permeate the membrane because of protein interaction and energy expenditure. Examples are Ca^{2+}, Na^{+} or H^{+} pumps. Toxic substances such as cadmium, lead or strontium will also bind to the calcium-binding protein (CaBP) because their physicochemical characteristics are similar to calcium. However, in secondary transport, primary transport generates ion gradients that make other ions pass through either in the same or the opposite direction. This is also called cotransport. An example is the intestinal absorption of Na^{+} and glucose. Usually, when xenobiotics interact with proteins, the model that best explains how they pass through membranes is the well-known Michaelis-Menten kinetics equation (Equation 2.7):

$$T = T_{max}\, C/K_m + C \tag{2.7}$$

where T_{max} is the maximum transport rate, K_m the xenobiotic concentration when half of the maximum transport rate is achieved and C the xenobiotic concentration.

Active transport is an essential mechanism for nutrient supply and to detoxify the organism. Accordingly, transport proteins are expressed in body sites such as the liver, kidneys, intestine, placenta or brain. More than 49 ABC transporter subtypes have been reported in humans, and they are divided into seven subfamilies: ABCA, ABCB, ABCC, ABCD, ABCE, ABCF and ABCG1. In contrast, more than 384 unique protein sequences have been identified, classified into 52 distinct solute carrier families (SLC1 to SLC52; Fujiwara et al. 2014).

Chedik et al. (2018, 2019) have shown that some pesticides, such as organochlorine, pyrethroid and organophosphorus pesticides, interact with various uptake and efflux drug transporters, including the efflux pump P-gp and the uptake organic cation transporters (OCTs). Table 2.3 is a classification of the different transport proteins.

2.4.5 Pinocytosis and Receptor-Mediated Endocytosis

These mechanisms refer to the surrounding of particles or large molecules by the cell membrane to form vesicles within the cell cytoplasm which are further processed. The size and properties of engulfed material make a difference: if large entities are included in vesicles, the process is called phagocytosis; smaller molecules associated with the extracellular fluid are taken up by pinocytosis. Both are nonselective but require energy for vesicle formation and processing to phagosomes

Table 2.3 List of Transporter Proteins

Organ	Efflux	Uptake
Brain	Apical: P-gp, BCRP, MRP4, MRP5, OAT3, OATP-A, MRP1, MRP3, MCT1	Apical: OATP1A2, OATP2B1
Liver	MRP1, MRP2, MRP3–6, P-gp (MDR1), BCRP, MDR3, OAT7, BSEP, MATE1, OSTα-β	OCT1, OATP-C, OATP-B, OATP8, NTCP, OAT2, OAT7, OSTα-β
Intestine	Apical: BCRP, P-gp, MRP2 Basolateral: OCT1, OSTα-β	Apical: OATP, PEPT1, ASBT, MCT1 Basolateral: MRP3, OSTα-β
Kidney	Apical: OAT4, P-gp, MATE 1 and 2, MRP2 and 4, OCTN1 and 2 Basolateral: OCT1, OCT2, OAT1, OAT2, OAT3, MRP1	Apical: OAT4, URAT1, PEPT1 and 2, OCTN1 and 2
Eyes	MRP1–7, MDR1, LRP in the corneal epithelium MRP1–7, MDR1, LRP and BCRP in the conjunctival epithelium MRP1–2, MRP6–7, MDR1 and LRP in the iris-ciliary body MRP1–3, MRP6–7, MDR1 and LRP in the retina MRP1–3, MRP6–7, MDR1 and LRP in human corneal epithelial cell line (HCEC) MRP7, MDR1, LRP, and BCRP in the human retinal pigment epithelial cell line (ARPE-19)	PEPT1 and 2, OCT1 to 3
Placental membrane	Apical: P-gp, BCRP, MRP2 Basolateral: OCT3, OAT4, OATP2B1	Apical: NET, SERT, OATP4A1, OCTN2 and 1 Basolateral: MRP5 and 1

Transporters (some of the following acronyms are followed by numbers/letters indicating polymorphism, families and subfamilies of transporter) listed in order of occurrence: P-gp = P-glycoprotein (also known as MDR1); MRP = multidrug-resistant proteins; BCRP = breast cancer resistant protein; OAT = organic anion transporter; OATP = organic anion transporter polypeptide; MCT = monocarboxylate transporter; OCT = organic cation transporter; NTCP = Na^+-taurocholate cotransporting polypeptide; OST = organic solute and steroid transporter; BSEP = bile salt–exporting pump; MATE = multidrug and toxin exclusion protein; PEPT = peptide transporter; ASBT = apical sodium dependent bile acid transporter; URAT = uric acid transporter; OCTN = organic cation transporter, novel; LRP = lung resistance protein; NET = norepinephrine transporter; SERT = sodium-dependent serotonin transporter.

Transporter families: ABC transporters (ATP-binding cassette); family members include P-gp, MRP; SLC (solute carrier) family members include OCT, OAT, URAT, MCT.

Source: Adapted from Li et al. (2012), Chen et al. (2013) and the International Transporter Consortium et al. (2010).

and lysosomes. Proteins, polypeptides or particles (e.g. nanoparticles, liposomes, microemulsions) may permeate membranes by pinocytosis. Also, there is a highly selective transport, when endocytosis takes places after the molecular entity binds to a specific receptor or ligand on the cell surface. Phagocytosis happens in the alveoli of the lung and the reticuloendothelial system of the liver and spleen. Clathrin-mediated endocytosis, clathrin-independent, caveolar endocytosis, macropinocytosis or mixed pathways have been identified. The receptors commonly involved are transferrin receptor (TfR), low-density lipoprotein receptor (LDLR), epidermal growth factor receptor (EGFR), G-protein coupled receptor, integrins (αvβ3, αvβ5, α5β1, α6β4, α4β1 and αvβ6), folate receptor (FR), CD44, ICAM, CD55, CD59, CD90, the heavy chain of amino acid transporters CD98; CD147; or the glucose transporter Glut1 (Xu et al. 2013).

2.4.6 Solvent Drag and Osmosis

The passage of large molecules like proteins through membranes can be contributed by another mechanism related to the presence of pores that can be crossed by solvents as a function of hydrostatic and osmotic pressures. Then, solvent permeability may show a significant dragging effect for large molecules. However, this effect is not so important for small molecules because they permeate membranes faster by other mechanisms.

2.4.7 Ion-Pair Formation

Some chemicals can cross biological barriers to a significant extent and at a faster rate even when ionized at the pH of the absorption site. This fact cannot be explained by the pH-partition theory mentioned above for passive diffusion. However, it has been hypothesized that this ionized chemical species might form a neutral complex with a counter-ion in the absorption site with new physicochemical properties better suited for membrane penetration. It is accepted that the ion-pair formation increases the lipophilicity of hydrophilic ionized compounds and enhances their partition coefficient. The formation of the complex will be the rate-limiting step in the absorption process, and once absorbed its breakdown will release the charged molecule in the basolateral side of the membrane. Some counter-ion compounds for positively charged molecules are alkyl carboxylate, cholate, n-alkyl sulphates, n-alkyl carbonates and trichloroacetate. This hypothesis has been used to promote the absorption of peptides, prodrugs and antiviral compounds.

2.5 DISPOSITION OF XENOBIOTIC COMPOUNDS

Disposition is a term describing the combined processes of distribution, biotransformation and elimination (excretion).

2.5.1 Intravascular Distribution

After absorption or intravenous administration through the capillary walls into the blood, xenobiotics undergo first intravascular and then extravascular distribution to other organs and tissues (Figure 2.1). Within the blood compartment, binding to plasma proteins such as albumin, glycoproteins transferrin, globulin, and lipoproteins oppose distribution to other body sites. A dynamic equilibrium exists between the bound and unbound forms of a toxicant in plasma. The equilibrium will be determined by factors such as concentrations or affinity constant and only unbound toxicant passes through the endothelial cells of the capillaries into the extravascular space. Some examples of protein binding include metals such as mercury (95%), ochratoxin (80%), chlorinated phenoxy acid herbicides (Hagelberg et al. 1989; Roberts et al. 2011), or perfluorinated carboxylic acids (PFCAs; 98%) (Ohmori et al. 2003). El-Moneim and Afify (2010) described the protein binding of xenobiotics (especially pesticides) from a general standpoint, including potential displacement and competition between chemically related structures for the same binding sites. Sometimes binding involves different plasma elements, such as aluminium binding to small molecule plasma binders (citrate, fluoride, phosphate, bicarbonate, and low molecular weight proteins), albumin, transferrin and other larger molecular weight proteins (Wilhelm et al. 1990). Also, protein binding has been suggested as a potential biomarker of exposure (i.e. albumin binding of organophosphorus pesticides is considered a complement to the widely used measure of acetylcholinesterase (AChE) inhibition; Tarhoni et al. 2008).

2.5.2 Extravascular Distribution

After extravasation the toxicants may be stored in the target tissue, possibly resulting in an adverse response on other tissue types, preventing exposure of target organs which may not be readily affected. Uptake of xenobiotics may occur either by passive diffusion or by special transport processes. Organs or tissues differ in the amount of a chemical that they receive or to which they are exposed. This is primarily due to blood flow or permeability through a specific tissue barrier. Organs with larger perfusion rates, such as the liver and the kidneys, can potentially accumulate more of a given toxicant. These organs together receive almost 50% of cardiac output and are the main elimination pathways for xenobiotic compounds through metabolism and/or excretion. However, some poorly perfused tissues can be the primary storage sites for many toxicants because of affinity. For example, adipose tissue, which has a meagre blood supply, concentrates fat-soluble toxicants whereas others form complexes with minerals commonly found in bone. Once deposited in these storage tissues, toxicants may remain for long periods due to their solubility in the tissue and the relatively low blood flow. Examples of storage depots for specific chemicals in mammalian organisms are bones (lead, strontium, fluoride), kidneys (cadmium), transferrin (iron), fat (polychlorinated pesticides such as DDT), nerve tissue (lead) and skin or hair (arsenic; Savković-Stevanović 2011). A potential irreversible interaction between the xenobiotic and the tissue might occur (Pumford et al. 1997).

Distribution of toxicants to extravascular sites means mass transfer from and to different aqueous compartments: vascular, interstitial and intracellular. Human plasma accounts for about 5%–7% of the total body weight in comparison to interstitial tissue fluids (15%–20% of body weight) and intracellular fluids (35%). The key parameter characterizing the magnitude of the distribution process is the apparent volume of distribution. It is the total volume (in litres) of body fluids in which a toxicant is distributed. It is a virtual value because very often it exceeds the total volume of the aqueous compartments mentioned above (60% of body weight). However, it is a relatively constant value for given xenobiotics and species and is very useful for upscaling in interspecies comparisons.

During distribution, there are key sites with specific structural barriers that restrict the entrance of toxicants. The primary barriers are those of the brain, placenta and testes.

In the blood-brain barrier, specialized cells called astrocytes have many small branches, which form a barrier between the capillary endothelium and the neurons of the brain. Membrane astrocyte lipids, very tight junctions between adjacent endothelial cells and efflux transport proteins expressed on their surface, limit the passage of molecules. Nevertheless, the blood-brain barrier shows variable permeability with health status and disease state, mainly to very small lipophilic compounds. Thus, the rate at which toxicants cross into brain tissue is slowed down while allowing essential nutrients, including oxygen, to pass through. The placental barrier has also a protective role and effectively slows down the diffusion of most toxicants from the mother into the foetus. This barrier consists of several cell layers combining lipids, efflux transport proteins, and enzymes to limit the diffusion of water-soluble toxicants. In contrast, nutrients, gases and waste from the developing foetus can pass through the placental barrier.

Metabolism is one of the options to eliminate xenobiotics from the body, but it will not be discussed in this section because it is the focus of Chapter 3 in this book.

Finally, the excretion of xenobiotics can take place in different organs and biological fluids as parent or biotransformed chemical compounds. The most common are urine, faeces, saliva, breast milk, bile, sweat or exhaled air. Usually, biotransformed polar (hydrophilic) substances are more likely excreted from the body. Excretion shares with other ADME steps the passage of chemicals through biological barriers, and the same chemical and physical principles are also applicable.

2.5.3 Renal Excretion

The kidneys are the primary route of excretion, and there are three primary regions involved: the glomerulus, proximal convoluted tubule and distal convoluted tubule. There are also three processes: filtration, secretion and reabsorption.

Filtration takes place in the glomerulus, and an average value of 125 mL/min (10% of the blood flow) filters through the glomerulus into the nephron tubule. This results from the large pores (40 angstroms) in the glomerular capillaries and the hydrostatic pressure of the blood. Small molecules, both lipid-soluble and polar substances, will pass through the glomerulus into the tubule filtrate. The physiological volume of urine excreted daily is around 1.5 L, thus, about 99% of the aqueous filtrate is reabsorbed downstream in the nephron tubule.

Molecules with molecular weights greater than 60,000 (which include large protein molecules and blood cells) cannot pass through the capillary pores of healthy glomeruli. Therefore, binding to plasma proteins will influence urinary excretion. Polar substances usually do not bind with the plasma proteins and thus can be filtered out of the blood into the tubule filtrate. In contrast, substances extensively bound to plasma proteins remain in the blood.

Secretion occurs in the proximal convoluted tubule, affecting potassium ions, hydrogen ions and some xenobiotics. Secretion occurs by active transport mechanisms that are capable of differentiating among compounds based on polarity. Two systems exist: one that transports weak acids (such as many conjugated drugs and penicillins) and the other that transports basic substances (such as histamine and choline).

Reabsorption also happens in the proximal convoluted tubule of the nephron. The renal tubules reuptake nearly all the water, glucose, potassium and amino acids lost during glomerular filtration. Reabsorption occurs primarily by passive transfer, thereby urine pH has a great influence. If the urine is alkaline, weak acids (such as glucuronide and sulfate conjugates) are more ionized and excretion is increased. Because the urinary pH varies in humans (due to diet or drugs), the urinary excretion rates of weak electrolytes also vary. Examples of drugs are phenobarbital (acidic) and amphetamine (basic). Treatment of barbiturate poisoning (such as an overdose of phenobarbital) may include changing the pH of the urine to facilitate excretion. Then, molecular size and polarity are the main determinants of renal excretion. In some cases, large molecules (including some that are protein bound) may be secreted (by passive transfer) to enter the urine. Lipid-soluble toxicants can be reabsorbed, which lengthens their half-life in the body and potential for toxicity. Nephrotoxicity caused by some metals (Cd, As, Pb, Hg or U) and chemicals (e.g. melamine, herbal Chinese drugs, vancomycin) diminish the ability to excrete toxicants, thus making those individuals more susceptible upon exposure (Vervaet et al. 2017).

2.5.4 Excretion into Faeces

Excretion of toxicants in the faeces is contributed by biliary and/or intestinal excretion. The biliary route generally involves active secretion rather than passive diffusion. Specific transport systems exist for organic bases, organic acids, and neutral substances. Some heavy metals (As, Pb and Hg) and large, ionized molecules such as conjugates greater than 300 daltons are excreted in the bile. Bile excretion makes intestinal reabsorption possible, but not for water-soluble xenobiotics. However, enzymes in the intestinal flora can cleave glucuronide and sulfate conjugates, allowing the parent compound to undergo enterohepatic recycling.

Enterohepatic recycling prolongs the half-life of the xenobiotic or its phase I metabolites, and in some cases the metabolite is more toxic than the excreted conjugate. Continuous enterohepatic recycling can occur and lead to very long half-lives of some substances. Dimethylmercury is secreted in bile, and chelators or resins taken orally can bind this compound to prevent recycling. Changes in the production and flow of bile into the liver affect the efficiency of biliary excretion. Bile flow is a determinant factor of bile excretion, and some drugs can affect it. Phenobarbital increases bile flow rate, thereby enhancing the excretion of methylmercury.

Intestinal excretion is not a major route of elimination, but several substances, especially those that are poorly ionized in plasma (such as weak bases), may passively diffuse from blood capillaries through the intestinal submucosa and into the intestinal lumen. This process is only relevant for xenobiotics with large circulation times due to slow biotransformation, or slow urinary or biliary excretion. The presence of lipids in the intestinal tract favours intestinal excretion of lipophilic substances. Mineral oil (liquid paraffin, derived from petroleum) is sometimes added to the diet to help eliminate toxic substances, which are known to be excreted directly into the intestinal tract.

2.5.5 Pulmonary Excretion

The lungs are an important route of excretion for volatile xenobiotics (and metabolites) in the blood. Gases are excreted by passive diffusion according to a concentration gradient. The lower the gas solubility, the faster the excretion by alveoli. Vapor pressure of the volatile toxicant also has a significant influence on the amount excreted. Well-known examples of pulmonary excretion are the measurement of alcohol, metals (Pb, Cd, Al), solvents or toxic elements from cigarette smoking in exhaled air or exhaled breath condensates. In some cases, these have been suggested as biomarkers of disease or exposure to noxious pneumotoxic substances (Corradi and Muti 2005).

2.5.6 Excretion into Breast Milk

Alternative but minor routes of excretion are mother's milk, sweat, saliva, tears, and semen.