



## **A framework for describing diseases caused by toxic agents**

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Multiple Sclerosis is a neurological disorder of unknown etiology that affects approximately 400,000 Americans and over 2,500,000 people worldwide. It is believed that genetic and environmental factors act together in concert to trigger the development of this disease.

The goal of the Boston Cure Project is to systematically determine the causes of MS as the most direct route to developing a cure for this disease. We have divided all known disease causing agents into five categories – genetics, pathogens, toxic agents, nutrition and trauma. Toxic agents (also called xenobiotics) are a potential cause of MS, and several have been investigated for an effect on MS risk, including mercury, organic solvents, pesticides, and exposure to X-rays (diagnostic or occupational). However, none of these has yet been definitively ruled in or out as an MS risk factor. Phase 1 of our effort to determine whether and how toxic agents help to trigger MS is to organize the characteristics of toxic agents and how they cause their adverse effects into a logical framework, which is presented in this document. In subsequent phases we will assess to what extent MS fits these characteristics according to existing evidence, and based on that assessment, facilitate additional research needed to determine the role of toxic agents in triggering or causing MS.

This Phase I document defines categories that characterize the physical and chemical structure of toxic agents, the means of exposure to them, their movement through the body, and their action at the molecular level. Finally, there is a section on the factors that characterize the expression of the toxic effects in the population. The set of classes that describe a toxic agent and its effects include:

- Composition
- Origin
- Entrance
- Pharmacokinetics
- Pharmacodynamics
- Epidemiology

### **Composition of toxic agent**

It is important to know the composition of an agent that causes disease because its chemical structure determines its disposition and thus reactivity in the human body. In

fact, the toxic effect of an agent of unknown toxicity can often be interpreted by relating it to another with similar molecular structure and known toxicity, a method called molecular structure analysis.

However, it is important to remember that since the composition of a toxic agent only helps infer its potency by predicting its disposition in the body, it is not the only criterion for potency. Two other factors are also important – both the essentiality of the molecular function that the toxic agent disrupts and the functionality of the organ it damages. (See “Mechanisms of Action” and “Systemic Toxicity”). The most potent toxic agents are so because of one or more of these three reasons.

Toxic agents can be divided first into radiation and toxic substances on the basis of physical nature, that is, radiation is energy and substance is matter. Then, in terms of toxic effects, radiation only causes either direct traumatic effects or damage to DNA, while substances can have a variety of toxic endpoints.

### **Radiation**

Radiation is the propagation of energy as characteristic waves or through the emission of particles such as electrons, neutrons, alpha and beta particles. Radiation of energy as waves produced by the oscillation of electric and magnetic fields is called electromagnetic and includes examples such as gamma rays, x-rays, ultraviolet rays, microwaves, etc.

Radiation is responsible for causing either direct necrosis (death of cells) through trauma (which is addressed under the trauma section of the Cure Map), or DNA damage. If DNA damage occurs in somatic cells of the exposed individual, it may lead to dysfunctional proteins or cancer, whereas if the damage is in germ cells, it may cause reduced fertility and genetic defects in the following generations such as congenital abnormalities and decreased birth weight.

### **Substances**

Toxic substances include both elements and compounds. Traditionally, toxic agents have been divided into organic (carbon containing) and inorganic (non-carbon containing) on the basis of their origin. Organic substances are typically derived from biological systems, while inorganic substances often come from minerals and ores. However, the effects of a toxic agent that derive from chemical structure typically have less to do with their origin and whether they contain carbon than with other inherent differences that affect their ability to be transported around the body and cause a toxic effect.

Characteristics of substances that influence their disposition (absorption, distribution and storage) and therefore toxic effect are lipophilicity and size:

- **Lipophilicity** - a criteria used to differentiate substances by polarity and ability to mix with other solvents. Lipophilicity strongly influences a substance’s ability to diffuse across barriers such as cell membranes.
  - Hydrophobic (lipophilic) - non-polar or non-ionic, any substance that repels water. These substances are lipid soluble. Examples include alkanes, oils, fats, hormones, organochlorides and some amino acids.
  - Hydrophilic - polar or ionic, any substance that has affinity for water. These substances are only soluble in water, not in lipids. Examples include heavy

metals, salts, water and some amino acids.

Because of the distinct character of all cell membranes, there is a difference in the facility and rate of movement of hydrophilic and hydrophobic molecules at all stages of disposition involving transport across membranes, including absorption and distribution. The cell membrane is a phospholipid bilayer (a phospholipid has a hydrophilic 'head' composed of a negatively charged phosphate group and a hydrophobic 'tail' consisting of two highly hydrophobic fatty acid chains) in which the hydrophobic tails face in towards each other, with the heads forming the outer surface of the membranes. There is a direct relation between lipophilicity and permeability through the cell membrane. Only hydrophobic substances can pass through the hydrophobic interior of the cell membrane by simple diffusion down a concentration gradient. Hydrophilic molecules, which repel the hydrophobic interior, must be moved by facilitated diffusion or active transport through the proteins lodged in the cell membrane. Since hydrophobic substances can more easily pass through cell membranes to different organs, they are expected to have a wider range of effects in an exposed individual. For instance, PCBs, which are hydrophobic, can cause direct skin effects as well as cancer of the liver and biliary tract, and genetic birth effects.

The lipophilicity of weak acids and bases depends on the form they are in – in the non-ionized form, they are hydrophobic, and in the ionized form they are hydrophilic. These substances can exist in both forms in solution – the amount of each depends on the pKa of the substance and pH of the solution. Since the structure of cell membranes is designed to facilitate the transfer of non-ionized, lipophilic substances, the pH of the environment in some regions of the body (such as in the lumen of the GI tract and renal tubules) can influence transfer of the ionizable toxic agent by increasing or decreasing the amount of its non-ionized form. See 'Absorption- Gastrointestinal Tract' for more discussion.

The only exception to the generalization that lipophilic substances diffuse through membranes more easily is in the kidney. Since the purpose of this organ is to filter liquid for excretion, its membranes are more permeable to hydrophilic substances. Taking mercury as an example, the lipid-soluble form, called methylmercury can more easily penetrate the blood-brain barrier and act as a neurotoxicant, but the hydrophilic metallic form can only penetrate into the kidney and act as a nephrotoxicant.

Lipophilicity can also influence the toxicity of an agent through its ability to be stored in various places in the body. Hydrophobic molecules, which are lipid soluble, are stored in adipose tissue, the fat storage depot of the body. On the other hand, some (hydrophilic) ions, such as fluoride and strontium, can either displace OH<sup>-</sup> or substitute for Ca<sup>2+</sup> and be stored in the bone, leading to skeletal fluorosis and osteosarcoma respectively. Plasma proteins, also a storage site, bind toxic agents according to lipophilicity. For instance, metal binding proteins such as transferrin and ceruloplasmin bind the hydrophilic metal ions iron and copper respectively, while alpha- and beta- lipoproteins are important in transporting lipid soluble molecules such as steroids.

- **Size** - Like lipophilicity, size is an important determinant of a substance's ability to be absorbed and distributed in the body, especially for hydrophilic molecules.

(Hydrophobic molecules can usually dissolve into the hydrophobic interior of the phospholipid bilayer regardless of size, and pass through by simple diffusion.)

Smaller hydrophilic molecules, such as ions and small polar molecules, especially water, are transported across the membrane by facilitated diffusion through channels called aqueous pores in carrier proteins, a process that does not require energy. Most aqueous pores are about 4Å in size and allow chemicals of molecular weight (MW) 100-200 to pass through. Exceptions are membranes of capillaries and kidney glomeruli which have relatively large pores (about 40 Å) that allow molecules up to a MW of about 50,000 (molecules slightly smaller than albumin, which has a MW of 60,000) to pass through. Larger hydrophilic molecules mentioned above cannot easily fit through the channels, and so require energy to be transported by the processes of active transport called endocytosis and exocytosis. In these processes, with the help of energy, the cell membrane invaginates and forms a vesicle around the molecules, which gets transported in or out of the cell respectively.

As an example of a comparison between two hydrophilic toxic agents of different sizes, arsenic, which is small (MW 350.5), can distribute itself readily around the body and may cause respiratory, neurological, and gastrointestinal problems as well as skin cancer. On the other hand, botulinum toxin, which is large (MW 150,000), has its distribution and thus effects restricted to the peripheral nervous system (not the central nervous system, because it cannot pass through the blood brain barrier).

## Origin of toxic agent

This category distinguishes between the various sources from which a toxic agent can originate. Note that not all sources agree with these definitions, and some might use them interchangeably, but the usage can be interpreted from the context. A toxic agent can be classified under more than one of the following subclasses, if applicable. For example, arsenic, which is naturally found in the underground water of some regions, and therefore is an 'earth natural', can also be found in steel foundries, making it a 'toxicant'.

### Toxins

Toxins are specific substances released by biological systems to act as toxic agents either as a defense mechanism, or for the capture and digestion of food, as in the case of predators.

Toxins themselves can be grouped according to the biological system producing them:

- **Infectious agents** - In this category, only toxins that are released by the infectious agent outside the body are discussed here, as opposed to toxins released in the body after infection occurs (see our Phase I Cure Map document on infectious agents for discussion of infections involving toxin release).
  - Mycotoxins - secondary metabolic products of a type of fungus called toxic mold. An example is aflatoxin, which is produced by two types of mold, *Aspergillus flavus* and *Aspergillus parasiticus*. Humans are exposed to

aflatoxin through contaminated food. The toxin is a carcinogen—it may be biotransformed in the liver to aflatoxin 8,9 epoxide, which can damage DNA in the liver.

- **Bacterial** - consists of endotoxins and exotoxins. Endotoxins are a component of the bacterial cell wall released during growth and after its death. Since exposure to these require infection, they are discussed in our Phase I Cure Map document on infectious agents. Exotoxins are released as part of a normal metabolic process. They are proteins and often quite toxic. An example of an exotoxin is botulinum, which is a toxin found in canned foods that causes a neurological disease called botulism.
- **Animal venoms** - Venoms are toxins produced in secretory glands and delivered during a biting or stinging act. They contain a variety of compounds—both high and low molecular weight proteins, alkaloids, amines, lipids, steroids, histamine, etc. The main purpose is to immobilize the prey or predator. Therefore, most venoms are targeted towards acetylcholine receptors causing neuromuscular blockage. Some animal toxins can bind to sodium, potassium, calcium or chloride channel proteins, producing the same neurological effect. For example, black widow spiders release alpha-latrotoxin, a large molecular weight protein, which exerts its toxic effects by increasing the concentration of Ca<sup>2+</sup> and causing exocytosis of neurotransmitters from nerve terminals.

Venoms can also contain enzymes that break down specific components of tissue such as protein, nucleic acids or phospholipids. For example, crotalase, a snake venom enzyme that is isolated from a North American rattlesnake, acts on fibrinogen and prevents the formation of the polymer that allows blood clotting, thus leading to internal bleeding.

- **Plant toxins** - Plant toxins have a wide variety of active components. The most common outcome of ingestion of a toxic plant is gastrointestinal disturbance. For example, many nuts, such as buffalo bean, which contain quinolizidine alkaloids, and tung nuts cause abdominal pain, vomiting and diarrhea.

In addition, plant toxins also have a wide variety of other effects, including allergies, local and systemic effects. For example, ricin from castor beans is one of the most potent toxic agents, causing inhibition of protein synthesis and cell death. The active ingredient in Philodendron scandens, a common houseplant, is resorcinol, a member of the phenols family, which causes contact dermatitis as an allergic reaction. Herbaceous plants called foxgloves contain glycosides such as oleandrin and nerium which cause nausea, vomiting and cardiac arrhythmias. Other chemicals can also cause vasoconstriction, hepatitis, stomach cancer, etc. Many of the common drugs of abuse, such as nicotine, morphine and cocaine are all plant toxins.

### **Endogenous**

Endogenous toxic agents are often produced when a harmless or less harmful substance is biotransformed into a more toxic one in the body, a process called bioactivation (see 'Biotransformation' section under Pharmacokinetics). An example is aflatoxin, which becomes bioactivated in the liver to aflatoxin 8,9 epoxide, and acts as a liver carcinogen.

Endogenous toxic substances can also be produced through the chemical reaction in the body of other exogenous substances. For example, intestinal microflora and the acidic environment of the stomach facilitate the chemical reaction between nitrites, found as an additive in meat and smoked fish, and secondary amines, found in some fish, vegetables and fruit juices, to produce nitrosamines. These compounds have carcinogenic effects in the stomach and also cause T-dependent humoral immunosuppression.

A specific example of endogenous toxic agents are reactive oxygen species (ROS). ROS are radicals naturally formed as a result of some metabolic reactions, but they can also be produced by toxic agents such as cigarette smoke, alcohol and ionizing radiation. When a toxic source causes over-accumulation of these radicals, photoreceptor phospholipids donate electrons to stabilize them, but become oxidized and damaged in the process.

### **Toxicants**

A toxicant is extracted from, is produced by or is a by-product of anthropogenic (human-made) activities. Some examples of chemicals extracted to be used in man-made products are nicotine used in cigarettes, and various chemicals used in alcohol, drugs, medication, dietary supplements, cosmetics, insecticides and pesticides.

Some toxicants are themselves produced or are unwanted by-products of industry, mining and automobiles which can cause harm after exposure. Examples are carbon monoxide, sulfur dioxide and nitrogen oxides produced through combustion of chemicals in cars, lead mining, etc. Subsequently, these gases combine with rain to form a liquid of more acidic pH. This acid rain causes respiratory problems such as dry coughs, asthma, headaches, eye, nose, throat irritation and allergic reactions. Also, the photochemical action of UV radiation on nitrogen dioxide produces ozone in the troposphere (between 0 to 10 miles above the Earth's surface), that is harmful.

Some toxicants are radioactive. Anthropogenic sources of radiation include chemotherapy, x-ray exams, etc.

### **Earth naturals**

This class includes any substance obtained from a natural source that does not fall into any of the other three categories – all naturally occurring elements and compounds (such as minerals and metals found in rocks and drinking water), food, products of natural non-biological processes (e.g. combustion through a forest fire), and natural sources of radiation, such as UV from the sun.

## **Vector**

This category addresses the pathways of exposure to toxic agents. In studying transmission vectors for toxic agents it is obvious that these agents have numerous methods of reaching humans. Some of the most common examples include:

### **Food and drink**

These can be the carriers of essential nutrients, or components that become toxic at higher doses (excess manganese intake from food or dietary supplements which causes manganism; certain food additives and preservatives such as nitrates, antioxidants and

synthetic sweeteners in excess can impart toxicity), or may contain toxic agents by circumstance (for instance, botulinum toxin, which causes botulism, grows in canned foods; arsenic found in the underground water of some regions can cause poisoning; mercury can bioaccumulate in the fish of regions rich in the mineral, or contamination, which can cause overt toxicity, or at lower doses, may facilitate cognitive disorders and congenital birth defects).

### **Medication**

Medications may contain chemicals that cause adverse reactions in organs not specifically targeted. This is an important vector to consider since many medications can exude harmful side effects. For example, hydralazine, a medication used to lower blood pressure, can also cause inflammation of the skin, lungs and nervous system. In addition, non-prescription drugs may cause damage when taken in doses exceeding those recommended, for example the analgesic Advil increases the risk of stomach ulcers.

### **Substance abuse**

Excessive consumption or use of substances such as alcoholic beverages, drugs and tobacco can cause well-established toxic effects such as sclerosis of the liver, seizures and cancer respectively.

### **Domestic uses**

These include the use of household cleaners, insecticides, pesticides, air fresheners and cosmetics. Some important examples of these vectors are exposure to gases such as carbon monoxide and polycyclic aromatic hydrocarbons released from cooking and heating, as well as from the vapors and aerosols of cleaning products and pesticides, and through skin absorption from cosmetics.

### **Occupational exposure**

This includes the occupational use of insecticides and pesticides on crops and livestock and exposure to toxic chemicals which are reactants, products and by-products of industrial processes and mining (e.g. cryolite, which is used in the electrolytic production of aluminum, causes skeletal fluorosis; workers in lead mines are prone to lead poisoning).

### **Environmental exposure**

The combustion of fuel in automobiles releases into the air various harmful gases and particulate matter, such as carbon monoxide, sulfur dioxide, nitrogen oxides and hydrocarbons. Industrial processes can also release hazardous by-products into the air and water, such as acids, solvents, resins, gases (ammonia, chlorine, hydrogen sulfide) and metals (copper, lead, zinc).

### **Animal bites**

This includes bites of animals that secrete a venom or poison, and also transmission of flukes and other parasites e.g. spiders, snakes, etc.

## **From nature**

Many hazards exist in the natural world such as UV radiation from sunlight, radon gas emitted from radioactive radium found in rock and soil, toxins from contact with plants, metals and minerals from rocks, particulate matter from dust storms in many parts of the world, etc.

## **Entrance**

The extent of damage and type of health risk caused by a toxic agent is also dependent on the pathways of exposure and specifically, the route of entry. However, it is difficult to generalize any site of entry as being because of more importance in imparting toxicity since, as previously discussed, toxicity is also dependent on the innate chemical structure of the toxic agent. For example, snake venom is significantly less toxic when administered orally than intravenously because, as a toxin, it is degraded by digestive enzymes in the gastrointestinal tract. In contrast, a high percentage of dichloro-diphenyl-trichloroethane (DDT) powder will be absorbed if ingested orally, however very little will penetrate the skin. For this reason, a given toxic agent is often assigned several recommended exposure limits, based on the route of exposure.

The purpose of this category is to address the types of toxic agents that most commonly and effectively enter into the body through specific sites. Some of these sites of entry are:

### **Skin**

This may be the most common site of exposure for toxic agents. In terms of environment, animal bites and exposure to sunlight, plants and mineral-containing rocks all occur through the skin. Through occupational exposure, workers' skin may come in contact with the reactants, products and solvents used in industrial processes and mining (e.g. carbon tetrachloride, hexanes), as well as insecticides and pesticides, especially organophosphates and halogenated pesticides (which plays a role in birth defects and environmental health). Many cosmetic and pharmaceutical products are applied directly to the skin. Chemical warfare agents such as the deadly neurotoxin, sarin, can also enter the body through the skin.

Though exposure through skin is the most common route of exposure, the quantity of absorbed toxin tends to be low since the skin is least permissive in allowing injury by most toxic agents; the stratum corneum of the epidermis serves as a resilient barrier against entry of unwanted substances. Exceptions occur in the aforementioned cases and also under the circumstances that would expectedly allow toxic agents to penetrate this barrier more easily, such as pre-existing skin disease and the presence of certain chemicals. Toxic agents can also enter through wounds in the skin.

### **Lungs (inhalation)**

This is an important route of exposure of certain toxic agents because the membranes of the alveoli are very thin. The most common chemical exposure through this pathway, however, is exposure to cigarette smoke. Some pharmaceuticals, such as nasal aerosol inhalers, and abused drugs also utilize this route.

### **GI tract (ingestion)**

This route is important for environmental exposure to food and drinking fluid contaminants. The primary exposure route for insecticides and pesticides sprayed on crops is through ingestion. It is important to note that intake of pesticides does not only occur through low concentrations contained in drinking water and through residual quantities on food, but also through intake of fruits which have metabolized and absorbed the pesticides internally. In addition, it is the primary route of exposure for many pharmaceuticals and drugs.

### **Eyes**

Intentional introduction of agents through this route can occur during treatment of ocular conditions, such as through eye drops. However, exposure through eyes of toxic irritants can also occur unintentionally in the environment and workplace. Effects can be allergic reactions in the cornea, or sometimes, considerable absorption can occur, resulting in systemic toxicity.

### **Suppositories**

This route is used for medications that may not be adequately absorbed after oral administration or intended for local therapy. The locations for suppositories are the rectum and vagina.

### **Intravenous**

This route is primarily used for pharmaceuticals and drugs and is generally effective in causing toxicity because the toxin is absorbed directly into the bloodstream.

### **Implants**

Pharmaceuticals such as hormones (contained in time release capsules) may be implanted to permit slow release over time. Many medical devices are implanted where minimal or no absorption is desired, e.g. artificial lenses or tendons. Toxicity can arise, however, when chemicals in the implant (for example, silicon breast implants) have a harmful side effect.

### **Radiation**

The entrance of radiation into the body is an idiosyncrasy to the aforementioned modes of entry. Radiation can enter the body without any direct contact. It is capable of penetrating through us; it can, but often does not accumulate. It may still be detrimental upon exposure, however.

## **Pharmacokinetics – disposition of toxic agent**

Pharmacokinetics refers to metabolism of toxic agents over a period of time, including the processes of absorption, distribution, biotransformation, storage and excretion. All of these processes are collectively known as the disposition of the toxic agent. In studying these processes for specific toxic agents, we can observe the mechanisms responsible for observed toxic effects that may ultimately lead to a disease.

The following subsections describe the general pharmacokinetics of toxic agents through the body, and the effects that they may have at certain stages of the disposition:

## Local toxicity

Local toxicity refers to the effects that occur at the site of entry. Generally these effects must occur prior to absorption which can happen if the toxic agent is a caustic or corrosive agent, an irritant chemical, or when the chemical is present at high concentrations (acute effect) at the site of entry. For example, when inhaled, chlorine gas reacts directly with the lung tissue and causes damage and swelling. However, under some circumstances, local effects can occur subsequent to absorption; the effect is still considered local, however, provided it occurs at the site of entry (for example, localized nerve damage/injury).

Some examples of local toxicity are:

- **Alopecia** - hair loss from areas where it is normally present. This is caused by caustic or corrosive agents that immediately kill the hair follicles, resulting in hair loss. (Note that Alopecia can also be systemic- see 'Dermal' under 'Organ directed damage'.)
- **Allergy** - inflammation at the site of entry which may result from recruitment of lymphocytes to a localized area in an attempt to repair damage that the toxic agent may cause; the results may be mucositis or dermatitis. Occupational dermatosis is a condition promoted by either irritating or allergenic chemical exposure in the workplace.
- **Local nerve damage** - occurs as a result of the toxic agent tearing or causing compression of nervous tissue and producing effects only at the site of entrance. An example of this is paresthesia (an abnormal sensation such as burning, tingling, and/or numbness at site of entry); this is caused by acute contact with DDT.
- **Corrosion** - destruction of a tissue at the site of exposure on direct contact.
- **Ulcer** - a defect or cavity produced by the sloughing of inflammatory necrotic tissue, e.g. skin, lung and stomach ulcers.
- **Neoplasia** - tumors which can be produced at the site of entry in the case of industrial toxicants and both natural and anthropogenic sources of radiation. For example, asbestos fibers and beryllium can lead to lung cancer. Excessive UV radiation can cause cancer of the skin called melanoma.
- **Deposits** - especially important in the case of the lungs. Deposition of aerosols and particulate matter too large to be absorbed in the blood ( $> 2\mu\text{M}$ ) reduce the lung's permeability for essential gas exchange of oxygen and carbon dioxide. For example, coal dust and asbestos fibers may lead to black lung and asbestosis respectively.

## Absorption

The process by which toxic agents cross the cell membranes at the site of entry and enter the bloodstream is called absorption. When a toxic agent is absorbed, it passes through the endothelial cell lining of the absorbing organ into the interstitial fluid of that organ. (Later, passage of the toxic agent across the cell membrane into target cells also takes place, but this is considered under 'Distribution'.) As mentioned in the 'Substances' section of 'Composition of toxic agents', the chemical structure of the toxins determines the methods of transport that can occur across these cell membranes, i.e. passive transport, active transport and facilitated diffusion. One characteristic of absorption not yet mentioned is that down a concentration gradient, passive transport can occur; against a concentration gradient, energy must be expended to allow for active transport.

Each site of entry influences the toxicity of certain chemicals because of their effective absorption at that site. The main sites of absorption are:

- **GI tract** - Toxic agents are poorly absorbed within the mouth and esophagus, due to the short time that a substance resides within these portions of the GI tract. The stomach, which has high acidity (pH 1-3), is a site of significant absorption of weak organic acids (e.g. benzoic acid), which exist in a non-ionized, lipid-soluble form. In contrast, weak bases exist primarily in the ionized form, and thus, are poorly absorbed.

The greatest absorption of chemicals, as with nutrients, occurs in the small intestine. The intestine has a large surface area consisting of outward projections of the thin mucosa into the lumen of the intestine called villi, which facilitate diffusion. Since the pH is near neutral (pH 5-8), both weak bases and weak acids tend to exist in the unionized form, and as such, can be readily absorbed via passive diffusion. Small, lipid-soluble molecules such as DDT and PCBs effectively enter the body from the intestine by passive diffusion as well. Strong acids, strong bases, large molecules and metals (e.g. lead, thallium) undergo active transport.

- **Lungs** - The main absorption in lungs occurs in the pulmonary region, which consists of very small airways called bronchioles and the alveolar sacs of the lung. The alveoli consist of only a single layer of cells with very thin membranes that separate the inhaled air from the bloodstream. Therefore, the ability of a toxic agent to be absorbed highly depends on its physical form, i.e. whether it is gas, particulate matter or aerosol.

The rate of absorption of gases is dependent on their solubility in the blood. Highly blood-soluble gases such as chloroform can enter the bloodstream by passive diffusion. Another example, carbon monoxide, is the most frequent cause of death from poisoning. Gases with a low blood-solubility, such as ethylene, saturate the blood too quickly to allow passive diffusion to continue.

Absorption of aerosols and particulate matter is dependent on particle or colloid size. Any particles smaller than 1  $\mu\text{M}$  can readily pass through the nasopharyngeal and tracheobronchial regions, for absorption through the alveoli.

- **Skin** - The epidermis is the only layer of the skin that regulates the penetration of toxic agents. The epidermis consists of 7 layers; the most important layer is the outermost stratum corneum, which is high in keratin, making its cells more impenetrable and chemically resistant to toxic agents than other layers of the epidermis. Those toxic agents that are able to move across the stratum corneum do so by passive diffusion (there are no known active transport mechanisms functioning within the epidermis). Polar and non-polar toxicants diffuse through the stratum corneum via different mechanisms. Water-soluble compounds diffuse through the outer surface of the hydrated keratinized layer. Lipid soluble compounds (e.g. carbon tetrachloride, hexanes, organophosphate pesticides, sarin) can dissolve in and diffuse through the lipid material between the keratin filaments, thus allowing a greater quantity to be absorbed. If a toxic agent passes through the stratum corneum, it is subsequently absorbed into the blood vessels

approximately 100  $\mu\text{M}$  beneath the skin surface.

### **Distribution and systemic toxicity**

**Distribution** is the process by which an absorbed toxic agent moves away from the site of entrance to other areas of the body by blood or lymph. Transport can occur via blood or lymph. Since blood moves more rapidly than lymph, absorbed chemicals are distributed primarily via blood, with only minor distribution by lymph. The majority of tissues have a constant supply of blood, so all organs and tissues of the body are potentially exposed to the absorbed chemical.

Agents that enter the body through the GI tract must follow a specific distribution pathway; this can lower the extent of distribution. A toxic agent that is absorbed through the GI tract flows into the vascular system of that organ, where it is then carried via the blood directly to the liver; this occurs by the portal system, previous to flow through the heart and subsequently the lungs. Thus before distribution occurs throughout the rest of the body, the toxic agent undergoes immediate biotransformation by the liver and elimination by the lungs. For example, first-pass biotransformation of the drug propranolol (cardiac depressant) is approximately 70% when administered orally, thus making the blood level only about 30% of that of a comparable intravenous dose. No other site of exposure has this first-pass screening effect.

The distribution of a toxic agent is significantly affected by its binding capability to plasma proteins such as albumin. Since only dissolved, unbound compounds are able to pass through capillary membranes, the more tightly the toxin is bound to plasma proteins, the lower the chance of distribution throughout the body. In toxicology, a quantity called “apparent volume of distribution” ( $V_d$ ) is defined as the dose of a toxin (mg)/ plasma concentration of the agent (mg/L). A high  $V_d$  indicates a low plasma concentration and thus, greater distribution to tissues; a low  $V_d$  implies the opposite behavior. Thus, this measurement gives a general idea of distribution. It is problematic in that this concept can be oversimplified when considering toxic agent metabolism. Additional factors in determining distribution are dependent on the other phases of the toxic agent’s disposition; primarily, consideration must be given to binding to proteins and/or dissolution in storage sites of the body, as well as rates of biotransformation and excretion.

The amount of a toxic agent that an organ or tissue receives is dependent on the volume of blood flowing through the specific tissue, as well as the presence of special barriers to slow toxic agent entry. Generally, tissues receiving increased blood supply, such as the liver, kidney, heart and brain, are exposed to higher concentrations and more variable toxic agents. Structural barriers that restrict toxic agents include the blood-brain barrier, placenta and testes. These barriers are not completely impenetrable, but slow down the rate of entry into these organs.

Ultimately, the extent of distribution of a toxic agent to various organs depends on its permeability in the target tissue, and tissue affinity. Permeability is important because the toxic agent must penetrate the cellular (endothelial cell layer) membrane of the target tissue before it can exert toxic effects. Thus, lipophilic toxic agents have higher penetration for this reason. Tissue affinity addresses whether the toxic agent have comparable structures to essential substances that enter a specific tissue. For instance, carbon monoxide replaces oxygen on the hemoglobin molecule because it has a much

higher affinity for it. Adipose tissue, which is the storage depot for fat, attracts lipophilic toxic agents such as DDT and PCBs.

**Systemic toxicity** occurs subsequent to absorption and distribution, affecting one or more organs in addition to those at the site of exposure. The essentiality of the target organ is an important factor in determining the apparent potency of the toxin. Damage includes systemic effects (e.g. dysfunction and dysregulation of proteins, cell death, carcinogenesis, etc.) that occur with particularly significant toxicity at specific organs of the body.

- **Blood** - Because this tissue is highly proliferative, a toxic agent can spread to a large number of cells in a short period of time. Thus, blood is highly susceptible to intoxication. The observed effects tend to occur from toxins acting directly on cells in circulating blood and bone marrow. Examples are hypoxia due to carbon monoxide binding of hemoglobin, which prevents transport of oxygen, and decrease in circulating leukocytes due to chloramphenicol damage to bone marrow cells.
- **Cardiovascular** – Damage to this system results from toxic agents acting directly on cells in the heart to effectively disturb cardiac function – for example, chronic consumption of alcohol can lead to alcoholic cardiomyopathy (similar to congestive heart failure).
- **Dermal** - Systemic effects are produced by internal distribution to the skin. One effect is phototoxicity in which chemicals such as sulfonamides and some dyes more easily absorb UV light to elevate the incidence of cell death. Another is chloracne, caused by chlorine containing toxic agents. Another important effect is alopecia (hair loss)—many chemotherapy drugs and radiation can be distributed to the skin, where they destroy hair cells in the anagen (growing) phase.
- **Endocrine** – This includes damage to any of the endocrine glands (pituitary gland, adrenal cortex, adrenal medulla, thyroid gland, parathyroid gland and testis), agonistic/antagonistic binding to the receptors that the hormones normally bind to, or simply inhibition through reaction with the hormone(s). For example, aluminum can inhibit the secretion of the parathyroid hormone. Resorcinol and lithium can induce hypothyroidism through metabolic inactivation, and inhibition of the thyroid hormone. DES binds to estrogen receptors, thus preventing the action of the female hormone, and causing teratogenic effects.
- **Hepato-** This type of toxicity includes that to the liver, bile duct, and gall bladder. The liver is highly exposed, and thus susceptible to toxic agents as a result of the significant blood supply and its principle role in biotransformation. Some effects of toxicity are steatosis (lipid accumulation in hepatocytes), which can be caused by carbon tetrachloride or ethanol; fatty liver (abnormal accumulation of fat in hepatocytes) can be caused by high doses of acetaminophen; hepatocyte death can be caused by dimethylformamide or copper; canalicular cholestasis (impaired bile production) can be caused by manganese, or the drug phalloidin.
- **Immuno-** This includes toxicity of the immune system that is not an allergy at the locality of exposure (considered as a local effect). It can exist in several different

forms: hypersensitivity in terms of autoimmunity (not allergy), immunodeficiency, and uncontrolled proliferation (leukemia and lymphoma). Examples are systemic lupus erythematosus in workers exposed to hydrazine, immunosuppression by cocaine, and leukemia caused by benzene respectively.

- **Nephro-** The kidneys are a common site of toxicity because during the filtration process of urinary excretion (see section under 'Excretion'), the nephron functions to concentrate toxic agents for elimination. All organ-directed damage is to specific regions of the kidney. For example, structural injury to the glomerulus is caused by the drug doxorubicin. Lead in the ionic form can enter the proximal tubule cells and damage mitochondria, thus altering the normal absorptive function of the kidney.
- **Neuro-** This category includes toxic agent damage to cells of the central nervous system (i.e. the brain and spinal cord) and the peripheral nervous system. The primary types of neurotoxicity are neuronopathies (neuronal injury), axonopathies (axonal injury), demyelination and interference with neurotransmission. Many of the potent neurotoxins, such as botulinum, sarin and cobra venom, are so because they inhibit the essential function of the neurotransmitter acetylcholine. Some of the heavy metals are also potential neurotoxins- for instance, mercury in high doses can induce demyelination.
- **Ocular-** Chemicals in the circulatory system can distribute to the eye and cause corneal opacity, cataracts, retinal and optic nerve damage. For example, corticosteroids can cause cataracts and methanol can damage the optic nerve.
- **Reproductive (teratogenesis)-** This involves toxic agent damage to both the male and female reproductive systems and their germ cells (sperm and ova), which occurs via DNA damage and other subcellular injury. For example, in males, the fungicide DBCP causes oligospermia (low sperm count), and lead accumulation in the testes is associated with testicular degeneration and inhibition of spermatogenesis. In females, narcotics inhibit secretion of the hormone gonadotropin, thus suppressing ovulation.

Such damage leads to dysfunction of reproductive organs or reduced fertility. These in turn result in reduced productivity due to fetal or embryonic mortality, or the birth of offspring with physical, mental or developmental defects. This group of reproductive and developmental defects is collectively called teratogenesis. Developmental problems in the fetus can also occur when toxic agents are able to pass through the placental barrier. As examples of teratogenic effects, tobacco smoke can cause spontaneous abortions, perinatal deaths and lower birth weight. DES, a xenoestrogen, causes malformations and sterility in male and female reproductive organs of offspring. Thalidomide, a drug previously used to treat morning sickness, nausea and insomnia, caused various malformations in the offspring, such as amelia (absence of limbs), phocomelia (severe shortening of limbs), and defects of the muscles of the eye and face.

### **Modification**

This section discusses both the reactions that occur to alter the reactivity of a toxic agent (biotransformation) and the interactions that occur between different toxic agents that

humans are exposed to (interaction). Both of these modifications can occur at any step of the disposition:

- **Biotransformation** - This is the process by which some substances are changed from one chemical into others (called metabolites) through a chemical reaction in the body. Enzymes are essential in the process of biotransformation. These biotransforming enzymes exist primarily in the liver (in small vesicles called microsomes of the endoplasmic reticulum), but also in the kidneys and lungs.

Biotransformation pathways that occur via enzymes generally go through two stages, Phase I and Phase II, which are meant to occur in sequence and produce metabolites of lower toxicity, i.e. detoxification. In Phase I reactions, the chemical is modified by adding a functional group such as -OH, -NH<sub>2</sub>, -SH or -COOH, forming reactive metabolites. The three main Phase I reactions are oxidation, reduction and hydrolysis. A group of enzymes called cytochrome P450 (found mainly in the liver, but in other tissues as well) is very important as a result of its high versatility in performing Phase I oxidation reactions. The purpose of the Phase I reaction is to allow the toxic agent molecule to fit into the Phase II biotransforming enzyme, which conjugates it with another molecule normally present in the body. The most important Phase II reactions are glucuronide conjugation, sulfate conjugation and acetylation. The products formed at the end of Phase II are larger than the original molecules and generally polar in nature, thus decreasing their absorption ability through cell membranes (see previous 'Substances' and 'Distribution' sections) and increasing their elimination capacity (see 'Excretion' section below). That is why this biotransformation pathway is mostly important for detoxifying lipophilic toxic agents, which are easily absorbed but difficult to excrete.

Unfortunately some factors may cause the production of metabolites of higher toxicity, a process termed bioactivation. Generally, bioactivation is an unintended pathway, and only occurs upon administration of a high dose of the toxic agent or other circumstances. These factors cause biotransformation to cease after Phase I, producing a reactive metabolite, or causing the original toxic agent to undergo a different biosynthetic pathway. The Phase I enzyme systems most involved in bioactivation are those that catalyze oxidation reactions, especially cytochrome P450. In general, reactive metabolites are electrophiles (molecules containing negative centers) such as epoxides, quinones and unstable conjugates. These in turn can react with cellular nucleophiles (molecules with positive centers) such as glutathione, proteins and nucleic acids. For example, when excess acetaminophen (Tylenol) is taken, the normal level of biotransforming enzymes may not be sufficient to convert all acetaminophen, and thus, the excess is sent to a cytochrome P450 (Phase I) enzyme, which produces a reactive metabolite that is toxic to the liver.

Most substances that we term carcinogenic are actually in a pro-carcinogenic form that require bioactivation before inflicting damage. An example is the oxidation of vinyl chloride to vinyl chloride epoxide, which cannot undergo Phase II reactions. Instead, it undergoes covalent binding to DNA and RNA, inducing cancer of the liver.

Intestinal microflora also act in the processes of biotransforming toxic agents,

both detoxifying and bioactivating toxic agents. An example of their role in bioactivation is the conversion of secondary amines to carcinogenic nitrosamines.

- **Interaction** - In many cases, when toxic agents are administered simultaneously, the presence of one chemical may affect the response to another chemical. The various alterations that interactions can cause include modifications in pharmacokinetics, reactions at binding sites and receptors, physiological changes or just reactions between the chemicals.

The four basic types of interactions are:

- Additivity - The sum of responses produced by the interaction of two or more toxic agents is the sum of individual responses, e.g., chlorinated insecticides and halogenated solvents both produce liver toxicity. The hepatotoxicity of an insecticide formulation containing both is equivalent to the sum of the hepatotoxicity of each.
- Synergism - Exposure to one toxic agent causes a dramatic increase in the effect of the other toxic agents. In some cases this can be explained by the similarity of their signal transduction pathways. As the receptor of a toxic agent is activated, it initiates a cascade of messenger molecules to the effector. These messengers can, in turn, increase the affinity of the initial receptor for the toxic agent. Thus, if two or more toxic agents have some messenger molecules in common, they can increase receptor binding frequency. As an example, the combination of exposure to cigarette smoke, asbestos and radon results in a significantly greater risk for lung cancer than the sum of the risks of each.
- Potentiation - One toxic agent does not have a toxic effect on a certain organ or system, but when added to another toxic agent, makes the latter more toxic. Normally, warfarin, a widely used anticoagulant in cardiac disease, is bound to plasma albumin so that only 2% of the warfarin is active. Drugs which compete for binding sites on albumin increase the level of free warfarin to 4% causing fatal hemorrhage. In another example, piperonyl butoxide, which is non-toxic on its own, is often included in pesticide formulations because it increases the toxicity of the active pesticide ingredient by inhibiting its detoxification in the target's body.
- Antagonism - Exposure to one toxic agent results in the reduction of effect of the other one. Antagonism can cause any of the various alterations produced by interaction between chemicals (see introduction to 'Interaction'), but the most common one is that of one chemical causing the induction of detoxifying enzymes that lower the toxicity of another one. This concept is the basis for many antidotes. For instance, the administration of phenobarbital (a barbiturate) induces drug-metabolizing enzymes that can detoxify the carcinogens benzopyrene and aflatoxin. As an example of non-enzyme related antagonism, mercury toxicity can be reduced by chelating (binding) the mercury ions with dimercaprol.

### **Storage**

The compartment where a substance is in high concentration, because the body intends to hold onto it for future use, is its storage site. Because of structure and binding capacity, toxic agents may also be stored in various areas of the body. A toxic agent may or may not exert a toxic effect at its storage site. The toxic agents at these sites

always try to maintain equilibrium with the free fraction of the agent in the plasma. Deposition at the storage sites tends to be reversible – as a substance is biotransformed or excreted, there is less in the blood, so more is released from the storage site.

If there is elevated blood flow to a tissue, then it may receive and store more of a toxic agent. However, if a certain toxic agent has a greater affinity for a specific tissue, the substance will accumulate there, even if there are lower levels of blood flow to that tissue. For example, adipose tissue will easily attract lipophilic toxic agents though it has a small blood supply.

The major storage sites for toxic agents in the body are:

- **Plasma protein** - Toxic agents bind to plasma proteins while being distributed in the blood. Depending on how strongly the proteins can bind them, they may act as storage sites. Plasma proteins can bind hydrophobic, hydrophilic and neutral molecules, based on the type of protein. For example, the metal-binding proteins transferrin and ceruloplasmin bind to iron and copper respectively; metallothionein binds cadmium in the kidney and liver. The alpha- and beta-lipoproteins transport important lipid-soluble compounds such as vitamins and cholesterol, as well as lipophilic toxic agents. The most abundant protein in plasma is albumin. The forces in action for albumin are both hydrophilic (such as hydrogen bonding and Van der Waals forces) and hydrophobic, thus making it capable of binding a large number of different compounds such as metal ions (Ca<sup>2+</sup>, Zn<sup>2+</sup>), acid dyes, barbiturates and fatty acids.
- **Adipose tissue** - Fat is found in many areas of the body, but primarily in subcutaneous tissue. Lipophilic toxic agents can easily penetrate and dissolve into body fat. Examples include chlordane, DDT and polychlorinated biphenyls (PCBs). Storage in fat is advantageous in that it lowers the concentration of the toxic agent at the target organ. Thus between two people who receive the same dose of a toxic agent, the one with a greater percentage of body fat will be less intoxicated.

Lipids are in a continual exchange with blood, so the toxic agent may be mobilized into the blood for further distribution and elimination, or redeposited in other adipose tissue cells. For example, rapid mobilization of fat, such as during starvation, can release the toxic agent (e.g. organochlorine insecticides) into the blood and target organ.

- **Bone** - Bone is composed of proteins and the mineral salt hydroxyapatite. During the normal processes that form bone, calcium and hydroxyl ions are incorporated into the hydroxyapatite-calcium matrix. Several chemicals, primarily elements, follow the same kinetics as calcium and hydroxyl ions and thus can be substituted for them in the bone matrix. For instance, F<sup>-</sup> may readily displace OH<sup>-</sup>, and lead or strontium may substitute for calcium in the hydroxyapatite lattice matrix. The chronic effects of fluoride deposition include skeletal fluorosis; strontium deposition can induce osteosarcoma and other neoplasms.

The deposition of toxic agents in the bone is not irreversible. Through osteoclastic activity in which bone tissue is absorbed and replaced, toxic agents can be released from the bone and into the circulatory system.

- **Liver and kidney** - These are important storage sites because both organs have a high blood flow, and contain proteins that can bind tightly to a variety of toxic agents. For example, the protein ligandin transports organic anions (such as nitrates and sulfates), azo dye carcinogens (containing the N=N linkage) and corticosteroids (synthetic hormones) from the plasma to the liver. Another metal-binding protein, metallothionein, binds to cadmium and zinc in both the liver and kidney.

### **Excretion**

Elimination from the body is very important in determining the potential toxicity of a chemical, since rapid elimination of a toxic agent or its metabolites from the body generally infers less ability to concentrate in and instill damage to critical cells.

Transport of toxic agents to the elimination site generally occurs via the circulatory system, and thus is dependent on the water solubility of the toxic agent. Water-soluble chemicals can more easily leave the storage or damage site, dissolve into the aqueous component of blood, be transported to the elimination site and diffuse into excretory organs. More lipid-soluble toxic agents require lipid-binding plasma proteins for their transport, and then enter the excretory organ via endocytosis.

Toxic agents and their metabolites can be excreted from the body by several routes. Of these, the most important are the kidneys, GI tract and respiratory systems. Other modes of excretion that are less common but important for specific toxic agents are the cerebrospinal fluid (CSF), sweat, saliva, tears, vomit, milk, mucus, pus, etc.

- **Urine** - Elimination of substances by the kidneys via urine is the primary route of excretion of toxic agents. In the first process of urinary excretion, filtration, toxic agents dissolved in blood diffuse into the glomerulus of the nephron. Thus, this mode of excretion depends on two characteristics of the toxic agent—size and water solubility. Considering size, any substance with a molecular weight less than approximately 65,000 Da can pass through the capillary pores of the glomeruli. As for water solubility, lipid-soluble substances, which are transported to the kidney by plasma proteins, cannot diffuse through the capillary pores. These substances must undergo biotransformation in the liver, or elsewhere, before filtration in the glomerulus can occur.

The last process of urine excretion, reabsorption, depends on the polarity of the toxic agent. Ionized substances remain in the urine and leave the body; lipid-soluble toxicants can be reabsorbed, and re-enter the blood circulation, which lengthens their half-life (time required for half of the chemical to be lost from the plasma) and increases their potential for toxicity. This shows that at the two stages of the renal excretion processes, filtration and reabsorption, the elimination of lipid-soluble toxic agents is unfavorable.

Because many functions of the kidney are incompletely developed at birth, some toxic agents are eliminated more slowly in newborns than in adults, and thus, may be more toxic.

- **Feces** - Elimination of toxicants in the feces occurs through two processes, excretion in bile, which then enters the intestine, and direct excretion from the GI

tract. The biliary route is an important mechanism for fecal excretion of toxic agents and their metabolites, while direct intestinal excretion is a pathway of only minor importance. This biliary route generally involves active secretion in which specific transport systems exist for certain substances, e.g. organic bases, organic acids, and neutral substances. Some heavy metals are excreted in the bile, e.g. arsenic, lead, and mercury. However, the most likely substances to be excreted in the bile are comparatively large, ionized molecules, such as conjugates of MW > 300 Da.

A substance can be eliminated from the body via feces, or can be reabsorbed. In the latter case, the reabsorbed material is returned to the liver, a process called enterohepatic circulation. Since the majority of the substances excreted in the bile are water soluble, they are not likely to be reabsorbed (see 'Substance-Lipophilicity'). However, enzymes in the intestinal flora can biotransform these substances, making them sufficiently lipophilic for reabsorption. Thus enterohepatic circulation may prolong the life of toxic agents in the body. For this reason, drugs may be administered to bind to specific toxic agents and prevent reabsorption, e.g. polythiol resin binds to dimethylmercury secreted in the bile.

- **Exhalation** - The lungs are an important route of excretion for toxic agents and their metabolites that exist in the gaseous phase in blood at body temperature. Gases are excreted by passive diffusion from the blood into the alveoli, following a concentration gradient. Gases with a low solubility in blood (e.g. ethylene) are more rapidly eliminated than those with a high solubility (e.g. chloroform), a pattern that is approximately inverse of their absorption rate. Volatile liquids dissolved in the blood, for example diethyl ether, are also excreted via exhalation. The amount of a liquid excreted by the lungs is proportional to its vapor pressure.

Exhalation is an exception to most other routes of excretion in that it can be very efficient in eliminating lipid soluble substances that are in the form of gases or volatile liquids (because the capillary and alveolar membranes are in close proximity and are very thin).

- **Others** - Of all the other routes of elimination, milk is probably the most important. This is because the elimination of a toxic agent through milk represents a new mode of exposure of the agent to others, i.e. through food for babies and dairy products from cows or goats. The primary toxic agents that can be present in milk are basic substances, because the pH of milk is acidic at 6.5, and lipid-soluble substances, because they can readily diffuse into the mammary glands along with necessary fats. Substances that chemically resemble calcium, such as lead, can also be excreted in milk along with calcium.

Other modes of elimination may be important in particular cases. Vomit can expel toxic agents that have not yet been absorbed. If the toxic agent has been ingested but not absorbed, a substance called an emetic is administered to stimulate vomiting and thus, immediate expulsion. Cerebrospinal fluid (CSF) can remove toxic agents from the central nervous system through the arachnoid villi (local elimination). Under conditions of great sweat production, some metals, including cadmium, copper, iron, lead, nickel, and zinc, may be eliminated.

Expulsion of toxic agents through hair and other keratinous fibers, such as fingernails, is a critical pathway in heavy metal removal.

Finally, the excretion of toxic agents in tears, hair, skin and any other routes not mentioned is quantitatively of minor importance.

## Pharmacodynamics

The term pharmacodynamics refers to the effects that toxins have on the body, that is, their mechanisms of action through biochemical and physiological changes at the molecular level. Therefore, this section addresses all characteristics of toxic agents that influence or determine their effect on the body, including mechanisms of injury, dosage response patterns, and duration and frequency of exposure.

### Mechanisms of injury

There are a variety of mechanisms of injury through which toxic agents can induce local and systemic effects at the molecular level. The essentiality of the molecular function that a toxic agent disrupts is one of the factors that determines its potency. Toxic agents generally induce damage by some disruption or modification of a normal biochemical process or cellular structure (except for allergies, caused by a hypersensitive immune reaction). Non-genetic and genetic disruption are considered separately since the toxic endpoints of each are different:

- **Disruption involving essential, non-genetic components** can be caused by any of the following actions:
  - Replacement of an essential substance in the body by an inadequate substitute - For example, in CO poisoning, oxygen is replaced by CO (which has about 210 times the affinity) on the hemoglobin molecule, preventing the normal transport of oxygen to cells for respiration. In the hydroxyapatite lattice matrix of bones, because of chemical similarities, F<sup>-</sup> may readily displace OH<sup>-</sup>, and lead or strontium may substitute for calcium, causing bones to lose flexibility.
  - Receptor agonist/antagonist - Toxic agents can act at specific cellular receptors to produce a chemical response, called agonism, or produce a suppressive effect or no effect at all, called antagonism. For example, some organochlorines when bound to estrogen receptors stimulate cellular responses similar to estrogen, but also increase the production of a metabolite that activates a gene responsible for increasing the risk of breast cancer. Another organochlorine, lindane, accumulates in female reproductive parts and blocks estrogen from binding to its receptors.
  - Direct binding to and inhibition of enzymes, other proteins, and essential substances - An example of this mechanism of injury involves DDT, which after acute exposure can bind to and inhibit Na<sup>+</sup>K<sup>+</sup> ATPases, the enzymes responsible for active transport of the two ions, thus disabling the nerve from establishing an action potential. Organophosphates bind to acetylcholinesterase, thus inhibiting the action of its substrate, the neurotransmitter acetylcholine. Cadmium and mercury can inhibit the activation of vitamin D, thus preventing Ca<sup>2+</sup> and Mg<sup>2+</sup> metabolism. An important case of this mechanism of action is the binding of toxic agents

to enzymes essential for cellular respiration, which is necessary for cell survival. For example, hydrogen cyanide inhibits cytochrome oxidase, the terminal enzyme of the electron transport chain, thus causing interference with cellular respiration. Arsenic inhibits succinic dehydrogenase, thus uncoupling oxidative phosphorylation and resulting in the fall of ATP levels.

- Interference with structural integrity - An important example of this toxic mechanism is narcosis, in which certain lipid soluble toxic agents such as ethanol become lodged in various parts of the cell membrane, thus altering its fluidity and the conformation of the proteins, preventing them from functioning properly. Similarly, carbon tetrachloride produces the reactive metabolite  $CCl_3$  which induces lipid peroxidation and subsequent destruction of lipid membranes. Another example in which structural integrity is disrupted is when a toxic source, such as cigarette smoke, alcohol or ionizing radiation, causes over-accumulation of free radicals called reactive oxygen species (ROS); photoreceptor phospholipids donate an electron to stabilize them, but subsequently undergo oxidation and damage in the process.
- **Alteration or incorrect expression of genetic material** - Toxic agents can harm DNA through strand breakage, oxidation, alkylation, large bulky adducts (between mismatched base pairs), or the induction of mutations. A mutation is a permanent alteration in DNA produced by base-pair substitutions, frame-shift mutations, aneuploidy/polyploidy (gain or loss of chromosomes), or chromosome aberrations (deletion, translocation, duplication, etc.). Incorrect expression can also occur when toxic agents bind to elements critical to the transcription and translation of genes, such as transcription factors.

Toxic agents can result in any of the following toxic endpoints:

- Inability of genetic material (DNA, RNA) to produce functional proteins - Both the covalent binding of chemicals to DNA and the formation of mutations cause nucleotide mispairing during replication, which, if not overcome by DNA repair mechanisms, may result in inaccuracy in every subsequent step in the transcription and translation processes. For example, covalent binding of aflatoxin 8,9-oxide (metabolite of aflatoxin) to guanine results in the pairing of guanine with adenine rather than cytosine, leading to the formation of an incorrect codon, incorrect translation, and finally a dysfunctional protein. Mercury is another example of a toxic agent that can bind to DNA and lead to the translation of dysfunctional proteins in the brain and kidneys.

Besides inducing toxic effects through direct contact with DNA, toxic agents may also bind to transcription factors and prevent the proper production of functional proteins. For example, PCBs bind to a ligand-activated transcription factor called the Ah receptor, leading to the increased induction of the cytochrome P450 enzyme, which forms reactive intermediates that bind to DNA. The toxic agent, dexamethasone, can bind to the glucocorticoid receptor (instead of its endogenous ligand, cortisol), forming a complex that tightly binds to DNA. This promotes the transcription of genes that increase gluconeogenesis at the expense of essential lipid and protein synthesis, thus leading to apoptosis of lymphocytes and teratogenesis.

- Carcinogenesis - Any site with which carcinogenic toxic agents make contact is a potential toxic site, including exposure sites (GI tract, skin, lungs),

biotransformation sites (liver), distribution modes (blood, lymph), storage sites (bones, fat) and elimination sites (kidneys). Carcinogens are responsible for up-regulation of oncogenes (e.g. *ras* genes), down-regulation of tumor-suppression genes (e.g. *p53*, which is mutated in 50% of all human cancer) or both processes. These occurrences can either be mutagenic or epigenetic. Mutagenic agents (e.g., ionizing radiation, reactive oxygen species, metals like arsenic, chromium, nickel) exert their effects through direct mutations in the DNA in either of the above-mentioned types of genes. Epigenetic agents (e.g., DES, TCDD, excess alcohol) alter the expression of oncogenes and repression of tumor-repressing genes instead of causing direct damage to DNA.

- Mutagenesis in germ cells - If mutations occur in germ cells, they can lead to teratogenic effects. For example, acrylamide found in some pre-cooked and processed foods can cause reduced fertility in males.
- **Direct death of cells, without affecting actual chemical processes** occurs via exposure to corrosive agents, radiation and heat which can actually coagulate the contents of a cell without inducing modification of any molecules or biochemical processes. This mechanism is discussed in more detail in the 'Trauma' section of the Cure Map.
- **Allergy<sup>1</sup>** - An allergy is a hypersensitive immune reaction caused by exposure to a specific substance. In this reaction, the body's immune system produces leukocytes and chemicals such as histamine, which causes an inflammatory response at the site where the allergen is detected. An example is contact dermatitis, which is caused by a variety of allergens such as chromium, mercury, formaldehyde, chloramine, etc.

Toxic damage can occur to cells in the exposed individual, developing embryo, or future offspring (teratogenesis). If not overcome by cellular repair mechanisms, the above-mentioned mechanisms can yield impaired functional capacity in cells (e.g. reduced ATP synthesis, ion channel dysfunction, impaired fatty acid metabolism, etc.), which, in turn, can lead to any of the following dysfunctional states. If enough functional cells are lost to these states of abnormality, the result can be tissue or organ failure, and in the extreme case, can lead to the death of the human (anaphylaxis).

- **Necrosis** - This is a progressive failure of essential metabolic and structural cell components usually in the cytoplasm, which causes excessive accumulation of fluid (swelling), ultimately resulting in death. Generally necrosis occurs in a group of contiguous cells or at the tissue level.
- **Apoptosis** - This is a programmed cell death that can occur in individual cells. In apoptosis the cell destroys its nucleus and disintegrates into fragments, which are then phagocytized by surrounding cells or local macrophages, without the onset of an inflammatory response.

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<sup>1</sup> Most toxicologists do not consider allergic reactions to be a toxic effect, as the immune reaction is not caused directly by the toxic agent, but instead is triggered by its presence. However, allergies are not relevant to any other section of the Cure Map, and since they do involve a foreign agent, we have included them here.

- **Metaplasia** - This is the conversion of one type of mature cell to another, often in response to chronic irritation or inflammation, for the purpose of forming a tissue more resistant to the external stress. The cellular changes, however, usually result in a loss of the function performed by the original cells. For example, the change in the cells of the trachea and bronchi of chronic cigarette smokers from ciliated columnar epithelium to non-ciliated stratified squamous epithelium causes the cilia to lose their defense function.
- **Fibrosis** - After necrosis, if the ability of the tissue to regenerate the same type of cells is exceeded, imperfect repair may occur through replacement by connective tissue, a process called fibrosis. Fibrosis is a form of metaplasia. Fibrosis causes diminished function of the organ, and in the extreme case, complete dysfunction. For example, chronic consumption of alcohol leads to fibrosis of the liver, called cirrhosis.
- **Neoplasia** - This is basically a new growth of tissue and is commonly referred to as a tumor. There are two types of neoplasias, benign and malignant. A malignant neoplasia, called cancer, exhausts the resources of its neighboring cells when it metastasizes, thus preventing proper function.

### **Dose response**

The medieval alchemist Paracelsus stated, “All substances are poisons...only the right dose differentiates a poison from a remedy”. This notion of dose is critical for understanding toxic effects. Based on the model we are looking at (threshold or hormesis), even some of the most toxic chemicals known will cause either no discernable effect or a positive effect on humans at very low doses. On the other hand, at very high doses, even essential substances like oxygen and water will harm or kill. In between, different quantities of toxic agents can induce different degrees of harm.

A ‘dose-response’ relationship is the quantitative relationship between the amount of a toxic agent exposed to and the extent of a specific effect that is induced. The units for dose are mg/kg, which represents the amount of toxic agent per amount of body weight.

The two main characteristics of dose response, its patterns (linear, threshold, hormesis) and range of effects (acute, subchronic, chronic) can be explained by how the toxic agent acts at the molecular level and the body’s disposition mechanisms for it.

- **Patterns** - The dose-response relationship of a toxic agent acting in an individual follows three different patterns – linear, threshold and hormesis – based on the strength of effect (either positive or negative) produced at different doses.
  - Linear - In this pattern, toxic response is directly proportional to dose and there are adverse effects at all doses, even low ones. This can happen when there are no defense mechanisms in the body that can overcome the toxic effects produced by an agent. Traditionally, this model has been applied to many mutagenic carcinogens in which damage of genetic material is assumed to have no lower limit—even one base pair deletion caused by a very small amount of a carcinogen can produce a mutation in a gene, which then spurs the formation of a tumor. However, currently there is substantial

controversy over whether the linear model is applicable to mutagenic carcinogens, based on the finding that most of our background radiation comes from radioactivity in our own body. We have at least 9,000 radioactive disintegrations in our body each second, and this number can significantly vary depending on where an individual was born and raised. The resulting radiation strikes billions of our cells each hour. Thus, the idea that radiation to one cell can initiate cancer may be viewed as illogical, as background radiation is proof that the body has defense or repair mechanisms against it.

In terms of non-mutagenic carcinogens (which bind to DNA, RNA or protein), the linear model definitely does not apply, because there are known mechanisms in the body that can overcome the damage caused. Nonetheless it is standard for many agencies to use the linear model for all carcinogens, so as to be on the safe side and lower the risk of harmful effects.

- **Threshold** - Toxic response is directly proportional to dose, but unlike the linear model, there is a dose, called a threshold, below which there are no apparent adverse effects from exposure to the toxic agent. In this situation the human body has defenses against the toxic agents that follow this model. Some organs, especially the liver and kidneys, can biotransform chemicals into non-toxic substances that can be eliminated from the body. However, if the dose is so large (over the threshold) that the body's defense mechanisms are exhausted, the body must absorb the toxic insult. This model applies to many non-carcinogenic toxic agents. For example, the threshold dose for DDT is 10 mg/kg.
- **Hormesis** - In this pattern, toxic substances may impart beneficial or stimulatory effects at low doses, but adverse effects at higher doses. The curve of a hormetic model is U-shaped or J-shaped. When a toxic agent challenges the body's adaptive capacity, if the dose of the toxic agent is small enough, the body is capable of biotransforming and/or eliminating the toxic substance and then returning to its normal balance point (homeostasis). In addition, the body over-compensates for the initial disruption and damage, leading to a stimulatory response such as growth, longevity, reduction in cancer risk and birth defect incidence. However, at any dose higher than the hormetic threshold, the toxic agent overwhelms the body's capacity to eliminate it and produces its negative damaging effects.

An example is Tylenol, whose active ingredient is acetaminophen. Taken at the suggested low dose, this chemical produces its therapeutic response, and then is rapidly biotransformed, with the metabolites eliminated. However, at a high dose, the normal level of biotransforming enzymes may not be sufficient and the excess acetaminophen is sent to a cytochrome P450 enzyme, which produces a reactive metabolite that is toxic to the liver. Some other examples of hormetic substances are metals (e.g. trace amounts of arsenic can lower body weight) and alcohol (controlled consumption can decrease the risk of cardiovascular disease). Recently, some studies have shown that radiation may follow the hormetic model, which has caused much controversy.

- **Range of effects** - The type of effect that occurs is dependent on the dose of the

toxic agent administered, and its duration and frequency of exposure, as well as the action of the toxic agent at the molecular level. The effects can either have an immediate impact (acute) or develop gradually over longer periods of time (subchronic or chronic):

- Acute - This type of effect takes place immediately after a single episode or incidence of a toxic agent that is very potent, or one that is administered in a larger dose. In either of these cases, when the toxic agent enters the body, it can begin to induce damage right at the site of exposure (GI tract, lungs, skin) or in areas where it is easily absorbed (blood, brain), because it produces cellular dysfunction or necrosis in the first cells that it comes in contact with. For example, acute skin exposure to many toxicants such as mercury and formaldehyde can induce contact dermatitis. An acute exposure to DDT causes paresthesia (tingling and numbness), which occurs as a result of rapid migration of the toxic agent into the CNS, where it reduces the permeability of the neuronal membrane and makes it extremely sensitive to even small stimuli.

In a few cases, acute exposure can also explain some systemic effects. For example, an important acute side effect of the drug ecstasy is unwanted muscle contraction in the jaws, arms and legs caused by the increased release of monoamine transmitters (serotonin, noradrenaline, dopamine). For the quantification of acute toxicity, the terms lethal dose, effective dose and toxic dose are used (See Appendix A).

- Subchronic - A subchronic effect occurs as a result of repeated exposure but of smaller doses over several weeks. This type of effect represents diseases caused by the cumulative damage of the toxic agent in specific organ systems. A small dose of a toxic agent can be absorbed and distributed to an organ where it is mostly biotransformed and/or eliminated. The amount that is not detoxified or excreted may cause minimal, inconsequential damage, and over a short period of time remains unnoticeable. However, with repeated exposure, the effect may become cumulative in the damaged organ. For example, workplace exposure to lead over a period of several weeks can result in anemia. Ingestion of coumadin tablets (blood thinners) for several weeks as a treatment for venous thrombosis (clumping of platelets and fibrin in the veins) can cause internal bleeding.
- Chronic<sup>2</sup> - A chronic effect occurs as a result of repeated exposure but of smaller doses over several months or years (as opposed to weeks in subchronic). This can be explained by the same reasoning for a subchronic effect, except that the extent of damage is more pronounced. Chronic effects tend to involve damage to organs where biotransformation and elimination occur, as well as the development of various types of cancer. Subchronic and chronic exposures can result in systemic effects as well as long-term local effects. For example, over a period of time, alcohol consumption can result in

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<sup>2</sup> The definitions of subchronic and chronic exposure used here are standard definitions. Often it is difficult to distinguish between the subchronic and chronic exposure, given that the duration of exposure is inferred from the toxic effects produced; these can be very similar in the two situations.

development of cirrhosis (fibrosis and nodular regeneration) of the liver, which is the site for alcohol detoxification. Workers who have been exposed to lead for years can develop chronic kidney disease (systemic toxicity). Coal miners can develop pulmonary fibrosis (local toxicity).

## Epidemiology

This section describes the population characteristics of toxicology. Epidemiology is especially important because it deals with the relationship between exposure to toxic agents and disease in actual populations rather than theoretical models. This section discusses how factors of variation in the population can lead to differences in exposure to toxic agents and the extent of the effects caused by them.

### Factors of variation in populations

These factors can lead to differences in exposure to certain toxic agents and susceptibility to the diseases caused by them:

- **Age** - This factor is important both for exposure and susceptibility. In terms of exposure, approximately 50% of all accidental poisoning fatalities in the U.S. involve preschool children, who do not yet understand proper usage and handling of prescription drugs and household chemicals.

In terms of susceptibility, age is important because it may affect the efficiency of biotransformation. In general, fetuses and newborns have limited abilities for toxic agent detoxification, due to inherent deficiencies in many of the biotransforming enzymes. The fluctuations in the activity of these enzymes may not stabilize until early adulthood, so young children and adolescents may also be more susceptible. For example, babies and young children are more vulnerable to arsenic poisoning because they are less efficient in converting inorganic arsenic to the less harmful organic form. Biotransformation capability may also be decreased in the elderly. Also, the cardiorespiratory systems of the elderly function with reduced capacity, so they are more prone to diseases caused by toxic agents that enter through the lungs.

- **Gender** - Gender is particularly important in the case of reproductive toxicants, because males and females secrete different hormones. For example, toxic agents such as DDT and DES are estrogen antagonists in females; the fungicide dicarboximide is a testosterone antagonist in males. Females are more susceptible to toxic reproductive insult because the ovaries do not have a protective mechanism like the blood-testis barrier of males. However, in other cases, such as for inorganic mercury or aniline, males may have a higher appearance of toxic symptoms.

During pregnancy, females have a lower capacity of overcoming the effects of certain toxic agents because the activity of many biotransforming enzymes decreases. This occurs because the increasing levels of female sex hormones during pregnancy, such as progesterone and pregnanediol, inhibit the functions of these biotransforming enzymes.

- **Genetics** - Genetic variation in biotransforming capability accounts for most of the large variation among humans. In particular, acetylation, a type of phase II reaction (see 'Modification- Biotransformation') is influenced by genetic differences in humans, so that in people with slow acetylation, the blood or tissue levels of certain drugs (or Phase I metabolites) exceeds their toxic threshold. For example, slow acetylation of isoniazid, an anti-tuberculosis drug, can yield nerve and liver damage. Other genetic variations can increase susceptibility to toxic agents as well. For instance, in humans with defective monooxygenase (an enzyme that catalyzes oxidation reactions), there is an excessive fall in blood pressure due to hydralazine (anti-hypertensive drug).
- **Diet** - There are several pathways of exposure to toxic agents through the diet. Some foods may contain excess doses of certain essential minerals. Fish can easily bioaccumulate toxic agents such as methylmercury, so a diet rich in fish can be detrimental. A diet consisting of more processed foods increases exposure to food additives and preservatives such as nitrates, antioxidants and synthetic sweeteners, which can be harmful in large doses. As a specific example of this, nitrates, which are used to preserve meat, react in the stomach with secondary amines from fish and certain juices to form carcinogenic nitrosamines. Some foods may produce toxic agents after preparation or cooking. For instance, when muscle meats such as beef, pork or fowl are cooked at a high temperature, carcinogenic heterocyclic amines are formed.
- **Health status** - Strength of immunity, dietary deficiency, pre-existing diseases, etc. are all aspects of health that can influence the extent of effect of a toxic agent. For instance, inadequate intake of folate results in impaired biotransformation ability in the case of arsenic, thus increasing body retention of the toxic agent. In another example, skin conditions such as skin burns and dermatitis enhance dermal absorption. The most important example of a pre-existing disease that affects the susceptibility to toxic agents is liver damage, because this organ is most crucial in detoxifying toxic agents.
- **Occupation** - This is probably the most important factor that determines exposure to certain toxic agents. Agriculture workers (farmers, pest controllers) are exposed to various insecticides, pesticides and herbicides such as DDT, benzene hexachloride and sarin. Workers in mining, smelting and metallurgy are exposed to metals and alloys (such as zinc, lead) through direct contact and inhalation. Workers in industrial settings (such as chemists, pharmacists) are exposed to reactants and products of reaction as well as hazardous wastes. Other occupations with high risk of exposure to toxic agents are sewage work, water treatment, undertaking, etc.
- **Environment** - This is an important consideration for exposure to toxic agents, particularly in the form of pollutants in certain areas. Areas with high numbers of motor vehicles can increase risk of exposure to various harmful gases such as carbon monoxide, sulfur dioxide and nitrogen oxides. Areas in proximity to industrial and power plants, which release harmful products both into the air and water supply, may have elevated pollutant levels. For example, more mercury is found in the form of methylmercury in fish living in water near industrial plants.

- **Geographic location** - Some regions of the world have a greater supply of naturally occurring toxic agents. For example, arsenic is naturally found in the underground water of South and East Asian countries such as Bangladesh and Thailand respectively. Foodborne botulism is a significant public health problem among Alaskan natives and is usually associated with consumption of fermented meat from aquatic mammals and fish.

Exposure to sunlight, and therefore the incidence of skin cancers, increases with proximity to the equator (as well as with decreasing shade of skin color). For example, Australia has one of the highest rates of skin cancer, in part because of its location near the equator.

## Appendix A: Quantal dose-response

Dose-response of a population is different from individual dose-response discussed under the 'Mechanisms' section because of the factors of variation mentioned above. Unlike individual dose-response, quantal dose-response is measured to show the relationship between the dose of a toxic agent and the percentage of the population in which the agent causes a specific effect. In creating the quantal relationship, the effect is considered as an all-or-none response (definition of quantal), i.e. at any given dose, an individual of the population is classified as either a responder or a non-responder.

Some standard measurements and calculations performed to determine the relative potency of different toxic agents and the risk associated with exposure to them are:

- **Tolerable daily intake (TDI)** - For substances that do not cause cancer or for the non-carcinogenic effects of carcinogens, through toxicological studies on laboratory animals and epidemiological studies a dose threshold can be estimated below which effects on human health are not expected. This threshold is established by either the no observed adverse effect level (NOAEL, the dose at which no adverse effects were observed), or the lowest observed adverse effect level (LOAEL, the lowest dose at which adverse effects were observed). This threshold dose is then converted to a dose considered tolerable by the average human population by using an uncertainty factor (UF), which takes into account the differences between individuals and between test animals and humans, and the type of experimental data available.
- **Lethal dose (LD)** - In setting the threshold dose for a toxic agent, we have to consider a response caused by the toxic agent that we would want to avoid in the population. Generally every studied toxic agent has a threshold dose for lethality, which represents the highest dose at which no individuals are expected to die, called lethal dose 0% (LD0). LD50 gives the statistically derived single dose of a toxic agent that can be expected to produce death in 50% of test animals, from which an adjusted dose for humans is estimated. Since the measurement of LD50 is for a single dose, it is useful in judging how acutely toxic one toxic agent is relative to others. For instance, the LD50 of botulinum toxin, one of the most powerful toxins, is 0.00001 mg/kg, while that of DDT is 113 mg/kg. However, LD50 is not very helpful when trying to determine the chronic long-term effects of exposure to lower non-lethal doses of toxic agents.
- **Effective dose/toxic dose (ED/TD)** - Toxic dose measurements apply to toxic agents that follow the linear or threshold model. As the names suggest, an effective dose measures the dose which produces a desired positive effect in a certain percentage of the population (e.g. ED0, ED50), and a toxic dose measures that which produces adverse toxic effects (e.g. TD0, TD50). A combination of both measurements are important in the case of hormetic (see 'Patterns- Hormesis' above) toxic agents, or other substances such as pharmaceuticals and nutritional supplements which have both beneficial and toxic doses. Because a range of effects can be produced by a toxic agent, we can establish multiple effective doses (e.g. anesthesia, pain relief) and toxic doses (e.g. organ damage, cancer) for it. The knowledge of the effective and

toxic dose levels help toxicologists and clinicians in determining the relative safety of pharmaceuticals.

- **Radiation doses** - Absorbed dose is a measure of the energy deposited in a medium by ionizing radiation. It is equal to the energy deposited per unit mass of medium, with units J/kg, also called gray (Gy). The absorbed dose is not a good indicator of the likely biological effect. For example, 1 Gy of alpha radiation would be much more biologically damaging than 1 Gy of photon radiation. Appropriate weighting factors can be applied reflecting the different relative biological effects to find the equivalent dose, which has units of sieverts.

The effective dose, another quantity calculated for radiation, is adopted by the US National Council for Radiation Protection and Measurement (NCRP). An effective dose is the weighted sum of the equivalent dose, using a complex determination of the weighting factors depending on the surface dose and the depth and position of the organ. The quantity is meant to equate the relative risk of inducing a fatal cancer from a partial body dose (such as radon progeny in the lungs) to the whole body dose that would have the same risk of inducing a fatal cancer.

## Appendix B: Government Regulations

The ultimate goal of studying dose-response in a population is for risk assessment. Based on this assessment, various regulatory bodies of the government try to set regulations on the exposure to toxic agents. The most important of such administrations is the **Environmental Protection Agency (EPA)**, which has the task of writing and enforcing specific regulations.

Examples of legislation dealing with the control of toxic agents include:

- **Occupational Safety and Health Act (OSHA)** - sets standards for worker exposure to various chemicals and air concentration values.
- **Federal Food, Drug and Cosmetic Act** - administered by the FDA, it establishes limits on food additives, cosmetic components and drugs.
- **Clean Air Act** - administered by the EPA, it sets standards on both national air quality standards, and standards for sources that create air pollutants, such as automobiles, power plants, etc.
- **Toxic Substances Control Act** - administered by the EPA, this covers almost all chemicals manufactured in the U.S. for industrial and other purposes. This act requires the evaluation of existing chemicals, and it has the authority to control or stop the production of substances deemed hazardous.

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