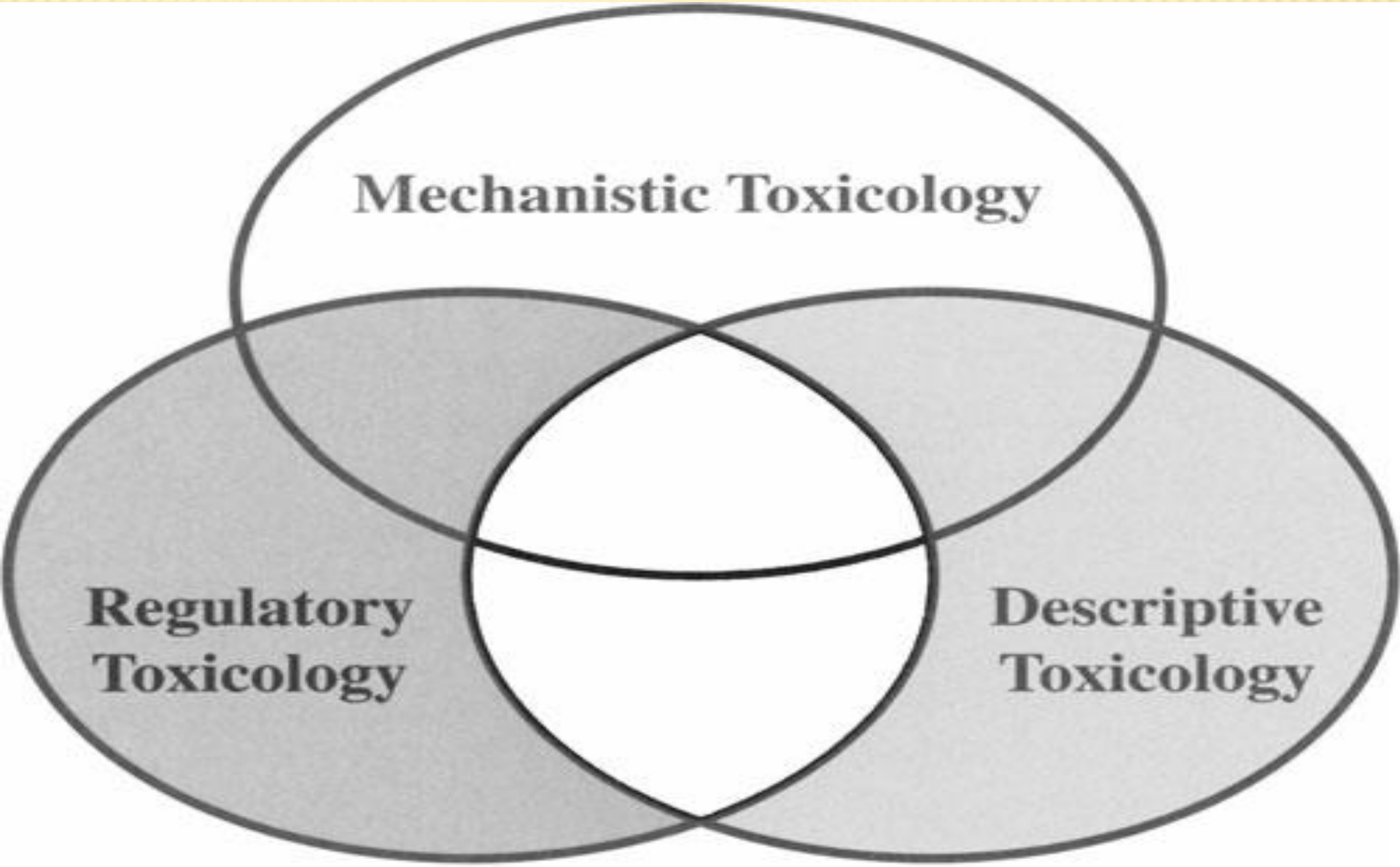

TOXICOLOGY

Toxicology is the study of the adverse effects of chemical or physical agents on living organisms. A *toxicologist* is trained to examine and communicate the nature of those effects on human, animal, and environmental health.

Risk assessment is the quantitative estimate of the potential effects on human health and environmental significance of various types of chemical exposures (e.g., pesticide residues on food, contaminants in drinking water).

In risk assessment, mechanistic data may be very useful in demonstrating that an adverse outcome (e.g., cancer, birth defects) observed in laboratory animals is directly relevant to humans. For example, artificial **sweetener saccharin** to cause bladder cancer in rats may not be relevant to humans at normal dietary intake rates??.

The professional activities of toxicologists fall into three main categories: descriptive, mechanistic, and regulatory



A *mechanistic toxicologist* is concerned with identifying and understanding the cellular, biochemical, and molecular mechanisms by which chemicals exert toxic effects on living organisms.

A *descriptive toxicologist* is concerned directly with toxicity testing, which provides information for **safety evaluation** and **regulatory requirements**. The appropriate toxicity tests in cell culture systems or experimental animals are designed to yield information to evaluate risks posed to humans and the environment from exposure to specific chemicals.

A *regulatory toxicologist* has the responsibility for deciding, on the basis of **data** provided by **descriptive and mechanistic** toxicologists, whether a drug or other chemical poses a **sufficiently low risk to be marketed** for a stated purpose.

The Food and Drug Administration (FDA) is responsible for allowing drugs, cosmetics, and food additives to be sold in the market according to the Federal Food,

In addition to the above categories, there are other specialized areas of toxicology such as

Forensic toxicology is a hybrid of **analytic chemistry and fundamental toxicological** principles. It is concerned primarily with the medicolegal aspects of the harmful effects of chemicals on humans and animals.

The expertise of forensic toxicologists is invoked primarily to aid in establishing the cause of death and determining its circumstances in a postmortem investigation

Clinical toxicology designates an area of professional emphasis in the realm of medical science that is concerned with **disease caused by or uniquely associated with toxic substances**

Environmental

toxicology focuses on the impacts of **chemical pollutants** in the environment on biological organisms.

Ecotoxicology is a specialized area within **environmental** toxicology that focuses more specifically on the impacts of **toxic substances** on population dynamics in an ecosystem.

General Characteristics of the Toxic Response

One could define a ***poison*** as any agent capable of producing a deleterious response in a biological system, seriously injuring function or producing death.

All things are poison and nothing[is] without poison. Solely the dose determines that a thing is not a poison.

SPECTRUM OF UNDESIRED EFFECTS

The spectrum of undesired effects of chemicals is broad. Some effects are deleterious and others are not.

- In therapeutics, for example, each drug produces a number of effects, but usually only one effect is associated with the **primary objective of the therapy**;

- all the other effects are referred to as *undesirable* or *side effects* of that drug for that therapeutic indication.

Allergic Reactions

Chemical allergy is an immunologically mediated adverse reaction to a chemical resulting from previous sensitization to that chemical or to a structurally similar one.

- The term *hypersensitivity* is most often used to describe this allergic state, exposure to the chemical results in an antigen–antibody interaction, which provokes the typical manifestations of allergy. The manifestations of allergy are numerous.

- They may involve various organ systems and range in severity from minor skin disturbance to fatal anaphylactic shock. The pattern of allergic response differs in various species.

Idiosyncratic Reactions

A classic example of an idiosyncratic reaction is provided by patients who exhibit prolonged muscular relaxation and apnea (inability to breathe) lasting several hours after a standard dose of succinylcholine.??

- **Succinylcholine** usually produces skeletal muscle relaxation of only short duration because of its very rapid metabolic degradation by an enzyme that is present normally in the bloodstream called **plasma butyrylcholinesterase**.

- Patients exhibiting this idiosyncratic reaction have a **genetic polymorphism** in the gene for the enzyme butyrylcholinesterase, which is less active in breaking down succinylcholine.

molecular genetic analyses have demonstrated that the presence of low plasma butyrylcholinesterase activity is due to the presence of one or more single nucleotide polymorphisms in this gene

Whats net result?

Reversible versus Irreversible Toxic Effects

Some toxic effects of chemicals are reversible, and others are irreversible. If a chemical produces pathological injury to a tissue, the ability of that tissue to regenerate largely determines whether the effect is reversible or irreversible.

- Thus, for a tissue such as **liver,** which has a high ability to regenerate, most injuries are reversible,

- whereas injury to the **CNS** is largely irreversible because differentiated cells of the CNS cannot divide and be replaced. Carcinogenic and teratogenic effects of chemicals, once they occur, are usually considered irreversible toxic effects.

Immediate versus Delayed Toxicity

Immediate toxic effects can be defined as those that occur or develop rapidly after a single administration of a substance, whereas delayed toxic effects are those that occur after the lapse of some time.

- Also, delayed neurotoxicity is observed after exposure to some organophosphorus insecticides that act by covalent modification of an enzyme referred to as *neuropathy target esterase* (NTE), a neuronal protein with serine esterase activity.

- Binding of certain organophosphates (OP) to this protein initiates degeneration of long axons in the peripheral and central nervous system.

- Ex:, daughters of mothers who took **diethylstilbestrol** (DES) during pregnancy have a greatly increased risk of vaginal cancer,, in young adulthood, some 20 to 30 years after their in utero exposure to DES??

Local versus Systemic Toxicity

Another distinction between types of effects is made on the basis of the general site of action.

Local effects are those that occur at the site of first contact between the biological system and the toxicant. Such effects are produced by the ingestion of caustic substances or the inhalation of irritant materials. For example, chlorine gas reacts with lung tissue at the site of contact, causing damage and swelling of the tissue, with possibly fatal consequences, even though very little of the chemical is absorbed into the bloodstream.

systemic effects require absorption and distribution of a toxicant from its entry point to a distant site, at which deleterious effects are produced. Most substances except highly reactive materials produce systemic effects. For example**, tetraethyl lead** produces effects on skin at the site of absorption and then is transported systemically to produce its typical effects on the CNS and other organs.

Most chemicals that produce systemic toxicity do not cause a similar degree of toxicity in all organ. The target organ of toxicity is often not the site of the highest concentration of the chemical. For example, **lead** is concentrated in bone, but its toxicity is due to its effects in soft tissues, particularly the brain. **DDT(**Dichlorodiphenyltrichloroethane) is concentrated in adipose tissue but produces no known toxic effects in that tissue.

Interaction of Chemicals

Chemical interactions are known to occur by a number of mechanisms, such as **alterations in absorption, protein binding, and the biotransformation and excretion of one or both of the interacting toxicants**. In addition to these modes of interaction, the response of the organism to combinations of toxicants may be increased or decreased because of toxicology responses at the site of action.

A number of terms have been used to describe pharmacologic and toxicology interactions.

An additive effect occurs when the combined effect of two chemicals is equal to the sum of the effects of each agent given alone (example: 2+3=5). For example, when two organophosphate insecticides are given together, the cholinesterase inhibition is usually additive.

A synergistic effect occurs when the combined effects of two chemicals are much greater than the sum of the effects of each agent given alone(example:2+2=20).For example, both carbon tetrachloride and ethanol are hepatotoxic compounds, but together they produce much more liver injury than the mathematical sum of their individual effects on liver at a given dose would suggest.

Potentiation occurs when one substance does not have a toxic effect on a certain organ or system but when added to another chemical makes that chemical much more toxic (example: 0+2=10). Isopropanol, for example, is not hepatotoxic, but when it is administered in addition to carbon tetrachloride, the hepatotoxicity of carbon tetrachloride is much greater than when it is given alone.

Antagonism occurs when two chemicals administered together interfere with each other's actions or one interferes with the action of the other (example: 4+6=8; 4+(−4)=0; 4+0=1).

Antagonistic effects of chemicals are often very desirable in toxicology and are the basis of many antidotes. There are four major types of antagonism: functional, chemical, dispositional, and receptor.

Functional antagonism occurs when two chemicals counter balance each other by producing opposite effects on the same physiologic function. Advantage is taken of this principle in that the blood pressure can markedly fall during severe barbiturate intoxication, which can be effectively antagonized by the intravenous administration of a vasopressor agent such as norepinephrine .

Similarly, many chemicals, when given at toxic dose levels, produce convulsions, and the convulsions often can be controlled by giving anticonvulsants such as the benzodiazepines (e.g., diazepam).

Chemical antagonism or inactivation is simply a chemical reaction between two compounds that produces a less toxic product. For example, dimercaprol (chelates with metal ions such as arsenic, mercury, and lead and decreases their toxicity. The use of antitoxins in the treatment of various animal toxins is also an example of chemical antagonism.

The use of the strongly basic low-molecular-weight protein protamine sulfate to form a stable complex with heparin, which abolishes its anticoagulant activity, is another example.

Dispositional antagonism occurs when the disposition—that is, the **absorption, distribution, biotransformation**, or **excretion** of a chemical—is alter that the concentration and/or duration of the chemical at the target organ are diminished. Thus, the prevention of absorption of a toxicant by **ipecac or charcoal** and the increased excretion of a chemical by administration of an osmotic diuretic or **alteration of the pH** of the urine are examples of dispositional antagonism.

If the parent compound is responsible for the toxicity of the chemical(such as the anticoagulant warfarin) and its metabolic breakdown products are less toxic than the parent compound, increasing the compound's metabolism (biotransformation) by administering a drug that increases the activity of the metabolizing enzymes (e.g., a "microsomal enzyme inducer" such as phenobarbital) will decrease its toxicity

General Characteristics of the Toxic Response

One could define a poison as any agent capable of producing a deleterious response in a biological system, seriously injuring function or producing death

Among chemicals there is a wide spectrum of doses needed to produce deleterious effects, serious injury, or death. This is demonstrated in Table 1, which shows the dosage of chemicals needed to produce death in 50% of treated animals(LD50).Some chemicals produce death in **microgram doses** and are commonly thought of as being **extremely poisonous**.

Other chemicals may be relatively harmless after doses in excess of **several grams**. It should be noted, however, that measures of acute lethality such as LD50 may not accurately reflect the full spectrum of toxicity, or hazard, associated with exposure to a chemical.

For example, some chemicals with **low acute toxicity** may have carcinogenic, teratogenic, or neurobehavioral effects at doses that produce no evidence of acute toxicity. In addition, there is growing recognition that **genetic factors** can account for individual susceptibility to a range of responses.

Approximate Acute LD_{50}s of Some Representative Chemical Agents

AGENT	LD_{50}, MG/KG*
Ethyl alcohol	10000
Sodium chloride	4000
Ferrous sulfate	1500
Morphine sulfate	900
Phenobarbital sodium	150
Picrotoxin	5
Strychnine sulfate	2
Nicotine	1
d—Tubocurarine	0.5
Hemicholinium-3	0.2
Tetrodotoxin	0.10
Dioxin (TCDD)	0.001
Botulinum toxin	0.00001

*LD_{50} is the dosage (mg/kg body weight) causing death in 50% of exposed animals.

CHARACTERISTICS OF EXPOSURE

Toxic effects in a biological system are not produced by a chemical agent unless that agent or **its metabolic breakdown (biotransformation) products reach appropriate sites** in the body at a concentration and for a length of time sufficient to produce a toxic manifestation. Two major factors that influence toxicity as it relates to the exposure situation for a specific chemical are **the route of exposure, and the duration, and frequency of exposure**.

Route and Site of Exposure

The major routes (pathways) by which toxic agents gain access to the body are the gastrointestinal tract (ingestion),lungs(inhalation), skin (topical, percutaneous, or dermal), and other parenteral (other than intestinal canal) routes.

Toxic agents generally produce the greatest effect and the most rapid response when given directly into the **bloodstream (the intravenous route**). An approximate descending order of effectiveness for the other routes would be inhalation, intraperitoneal, subcutaneous, intramuscular, intradermal, oral, and dermal..

In addition, the route of administration can influence the toxicity of agents. For example, an agent that acts on the CNS,

why oral less toxic than inhalation?

. Occupational exposure to toxic agents most frequently results from breathing contaminated air (inhalation) and/or direct and prolonged contact of the skin with the substance (dermal exposure),

whereas accidental and suicidal poisoning occurs most frequently by **oral ingestion**. Comparison of the lethal dose of a toxic substance by different routes of exposure often provides useful information about its extent of **absorption**. In instances when the toxic dose after oral or dermal administration is similar to the toxic dose after intravenous administration , if the assumption is that the toxic agent is absorbed readily and rapidly. Conversely, in cases where **the toxic dose by the dermal route is several orders** of magnitude higher than the oral toxic dose, it is likely that the skin provides an effective barrier to absorption of the agent.

Duration and Frequency of Exposure

Toxicologists usually divide the exposure of experimental animals to chemicals into four categories: acute, subacute, subchronic, and chronic.

Acute exposure is defined as exposure to a chemical for less than 24 hours, and examples of exposure routes are intraperitoneal, intravenous, and subcutaneous injection; oral intubation; and dermal application.

Whereas acute exposure usually refers to a **single administration, repeated exposures may be given within a 24-hours** period for some slightly toxic or practically nontoxic chemicals.

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Acute exposure by inhalation refers to continuous exposure for less than 24 hours, most frequently for 4 hours.

These three categories of repeated exposure can be by any route ,but most of ten they occur by the oral route, with the chemical added directly to the diet.

Repeated exposure is divided into three categories: subacute, subchronic, and chronic.

Subacute exposure refers to repeated exposure to a chemical for 1month or less,

sub chronic for 1to 3months,

chronic for more than 3 months, although usually this refers to studies with at least 1 year of repeated dosing

For many chemicals, the toxic effects that follow a single exposure are quite different from those produced by repeated exposure. For example, the primary acute toxic manifestation of benzene is central nervous system (CNS) depression, but repeated exposures can result in bone marrow toxicity and an increased risk for leukemia.

Acute exposure to chemicals that are rapidly absorbed is likely to produce immediate toxic effects but also can produce delayed toxicity that may or may not be similar to the toxic effects of chronic exposure.

The other time-related factor that is important in the temporal characterization of repeated exposures is the **frequency of exposure**.

The relationship between elimination rate and frequency of exposure is shown in Fig. 1. A chemical that produces severe effects with a **single dose** may have no effect if the **same total dose is given in several intervals**.

For the chemical depicted by line B in Fig. 1, in which the half-life for elimination (time necessary for 50% of the chemical to be removed from the bloodstream) is approximately equal to the dosing frequency, a theoretical toxic concentration(shown conceptually as two Concentration Units in Fig. 1) is not reached until **the fourth dose**, whereas that concentration is reached with only **two doses for chemical A**, which has an elimination rate much slower than the dosing interval (time between each repeated dose).

Conversely, for chemical C, where the elimination rate is much shorter than the dosing interval, a toxic concentration at the site of toxic effect will never be reached regardless of how many doses are administered.

it is possible that residual cell or tissue damage occurs with each dose even though the chemical itself is not accumulating. The important consideration, then, is whether the interval between doses is sufficient to allow for complete repair of tissue damage.

if the chemical accumulates in the biological system (rate of absorption exceeds the rate of biotransformation and/or excretion), if it produces irreversible toxic effects, or if there is insufficient time for the system to recover from the toxic damage within the exposure frequency interval.

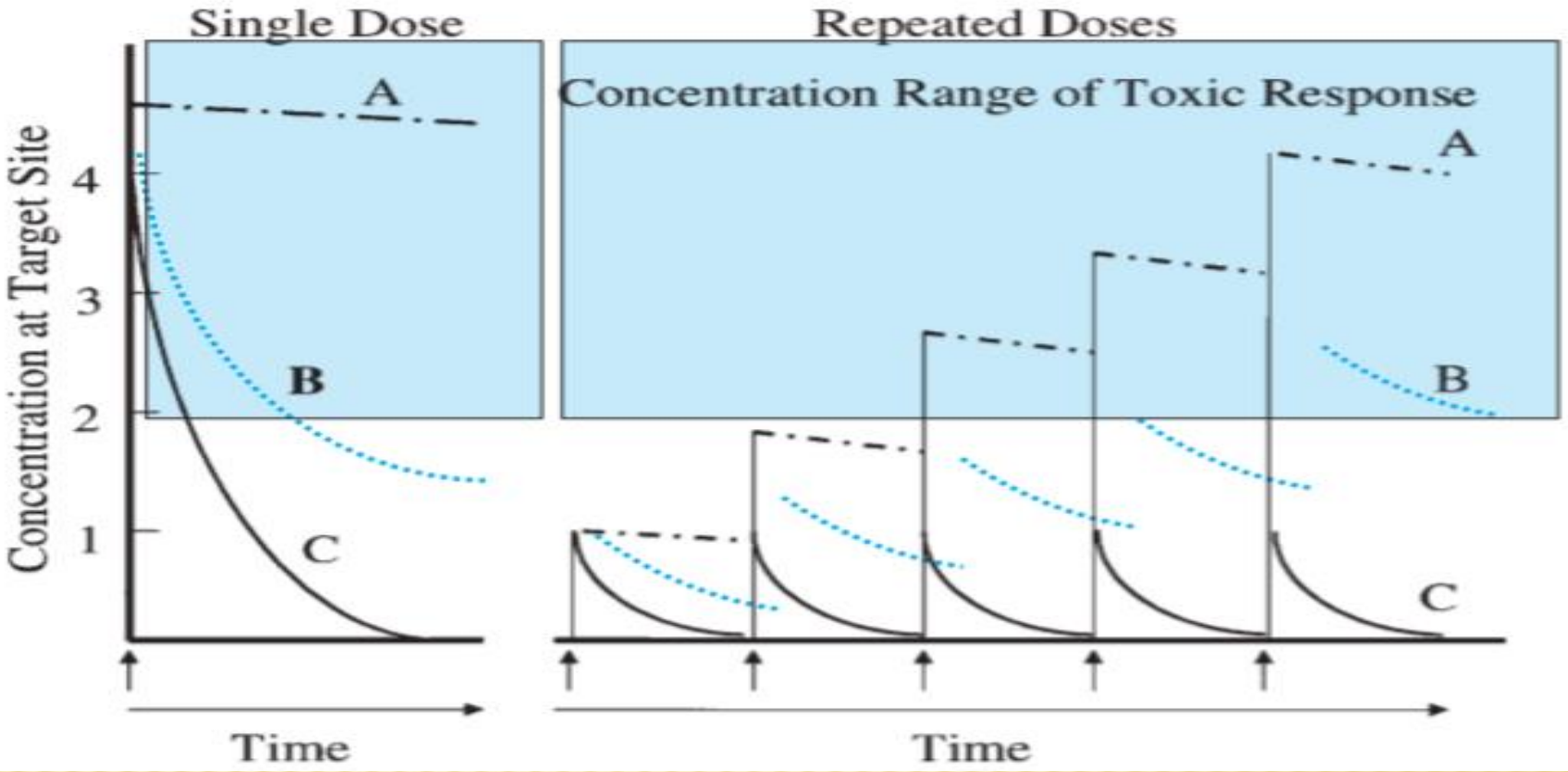


Figure 1. Diagrammatic view of the relationship between dose and concentration at the target site under different conditions of dose frequency and elimination rate. LineA.A chemical with very slow elimination(e.g.,half-life of1year).LineB.Achemical with a rate of elimination equal to frequency of dosing (e.g., 1 day). Line C. Rate of elimination faster than the dosing frequency (e.g., 5 h). Blue-shaded area is representative of the concentration of chemical at the target site necessary to elicit a toxic response.

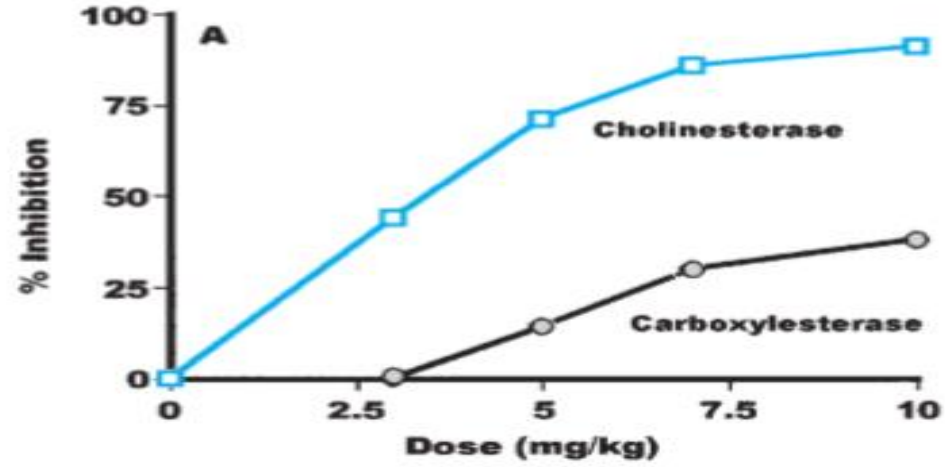
DOSE–RESPONSE RELATIONSHIP

response is selected for measurement, the relationship between the degree of **response of the biological system** and **the amount of toxicant administered** assumes a form that occurs so consistently as to be considered the most fundamental concept in toxicology.

Fig. 2.shows the dose–response relationship between different dietary doses of the **organophosphate insecticide chlorpyrifos** and the extent of inhibition of two different enzymes in the brain and liver: **acetylcholinesterase and carboxylesterase.** In the brain, the degree of inhibition of both enzymes is clearly dose-related and spans a wide range, although the amount of inhibition per unit dose is different for the two enzymes. From the shapes of these two dose–response curves it is evident that, in the brain, cholinesterase is more easily inhibited than carboxylesterase.

The toxicologic response that results is directly related to the degree of cholinesterase enzyme inhibition in the brain. Thus, clinical signs and symptoms for chlorpyrifos would follow a dose–response relationship similar to that for brain cholinesterase.

However, for many chemicals, more than one effect may result because of multiple different target sites in different tissues. Thus, the observed response to varying doses of a chemical in the whole organism is often complicated by the fact that most toxic substances have multiple sites or mechanisms of toxicity,



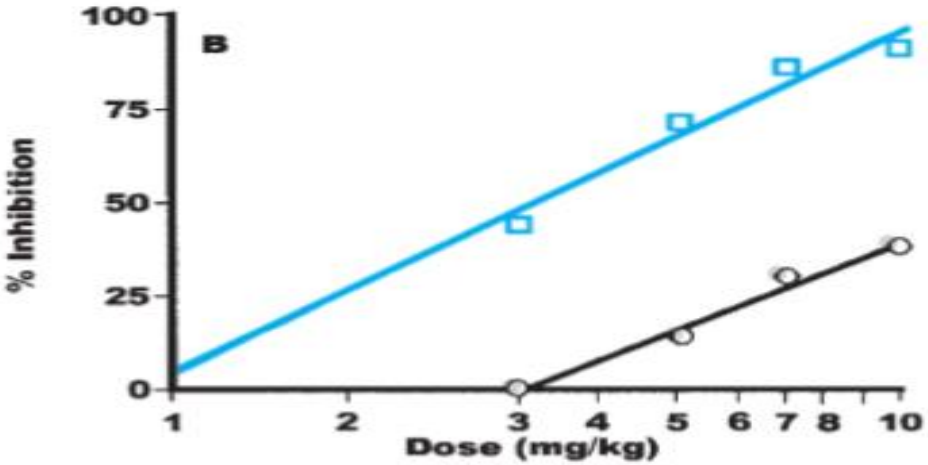


Figure 2-3. Dose–response relationship between different doses of the organophosphate insecticide chlorpyrifos and esterase enzyme inhibition in the brain. Open circles and blue lines represent acetylcholinesterase activity and closed circles represent carboxylesterase activity in the brains of pregnant female Long-Evansratsgiven5dailydosesofchlorpyrifos. A. Dose–response curve plotted on an arithmetic scale. B. Same data plotted on a semi-log scale. (From Lassiter et al., Gestational exposure to chloryprifos: Dose response profiles for cholinesterase and carboxylesterase activity. Toxicol Sci 52:92– 100, 1999, with permission.

Evaluating the Dose–Response Relationship

Comparison of Dose Responses Figure 3 illustrates a hypothetical quantal dose–response curve for a desirable effect of a chemical **effective dose(ED**) such as anesthesia, **a toxic dose(TD)**effect such as liver injury, and the **lethal dose (LD).**

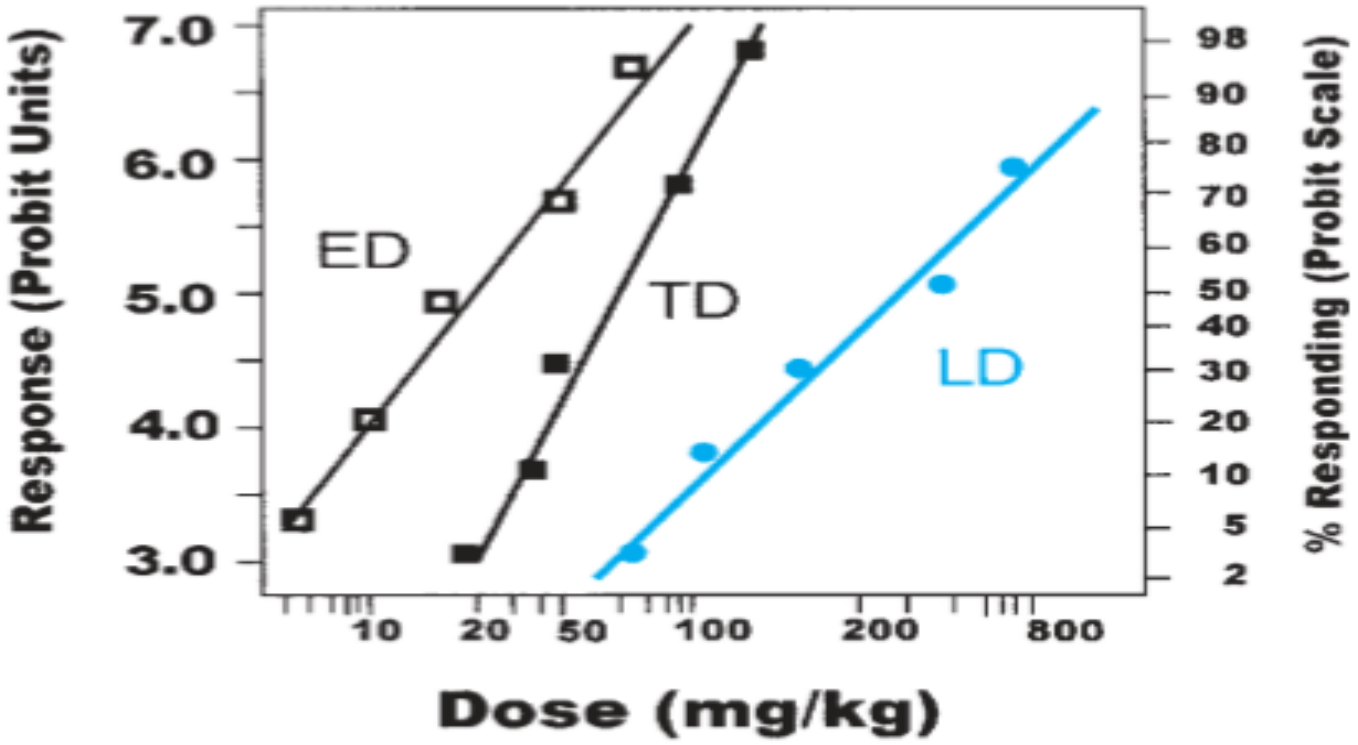


Figure 2-9. Comparison of effective dose (ED), toxic dose (TD), and lethal dose (LD).

The plot is of log dosage versus percentage of population responding in probit units.

Therapeutic Index.

The **therapeutic index** (TI) in its broadest sense is defined as the ratio of the dose required to produce a **toxic effect** and the dose needed to elicit the **desired therapeutic response**. Similarly, an index of comparative toxicity is obtained by the ratio of doses of **two different materials** to produce an identical response or the **ratio of doses of the same material** necessary to yield different toxic effects

The most commonly used index of effect, whether beneficial or toxic, is the median effect dose (ED50). The therapeutic index of a drug is an approximate statement about the relative safety of a drug expressed as the ratio of the adverse end point or toxic dose (historically the lethal dose) to the therapeutic dose:

Therapeutic Index=TD50/ED50

Margins of Safety and Exposure

One way to is to use the ED99 for the desired effect and the TD1 for the undesired effect. These parameters are used in the calculation of the margin of safety (MOS)

Margin of safety=TD1/ED99.

The quantitative comparisons described above have been used mainly after a single administration of chemicals. However, for chemicals for which there is no beneficial or effective dose and exposures are likely to **occur repeatedly,** the ratio of TD1 to ED99 has little relevance.