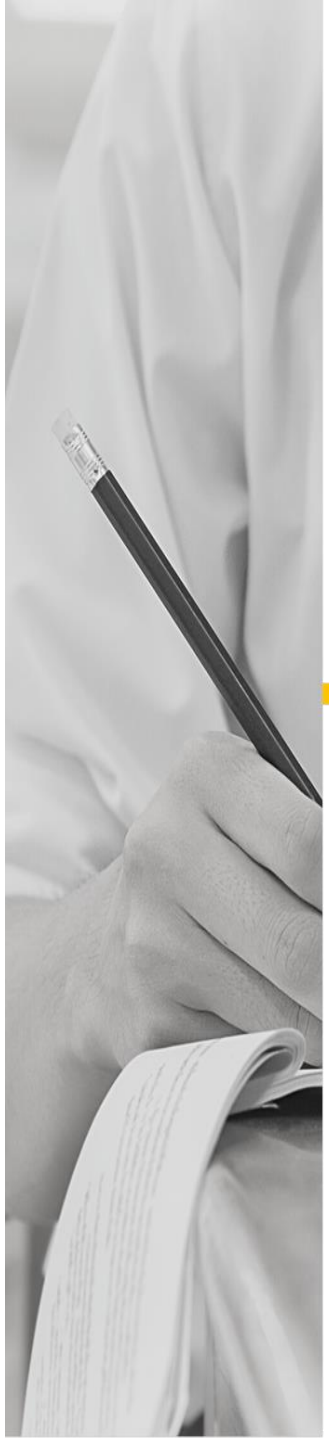

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PHARMACOLOGY MASTER – DRUG EVALUATION
TIKRIT UNIV. – VET. COLLEGE

LD 50

2nd Term

2024\2023

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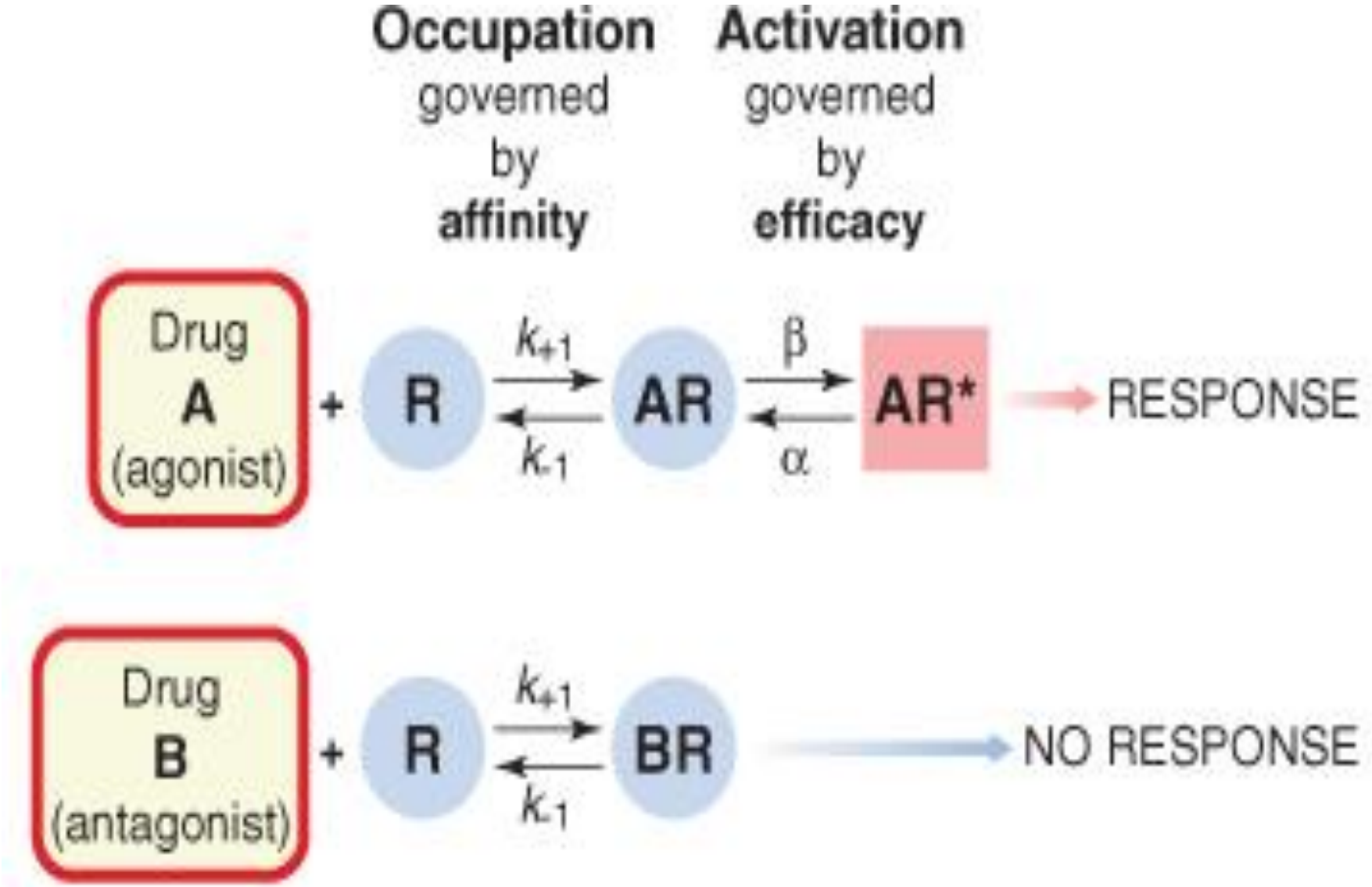
Drug-Receptor interaction
Full & Partial agonist & antagonist

- Drug-Receptor binding may or may not result in receptor activation → response.
- Occupation → affinity (tendency of the drug to bind the receptor)
- Activation → efficacy (ability of a drug, once bound, to initiate changes → effect.



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- **Agonist**: a drug that binds to the receptor" affinity" → activation of the receptor" efficacy".

a. **Full agonist**: possesses ↑ affinity & efficacy (+1). Large % of receptors reside in (R*) → maximal tissue response

b. **Partial agonist**: possesses ↑ affinity & intermediate efficacy (0-1) i.e.↓ no. of receptors are activated even at 100% occupancy → submaximal tissue response.

   They have low intrinsic activity (act as an agonist, if no full agonist is present, or as antagonist if full agonist is present, e.g. Pindolol

- **Antagonist**: a drug that binds to the receptor " affinity" without causing activation " zero efficacy" i.e. equal affinity for (R) & (R*).

Dose & Response in Patients

Graded Dose-Response Relations: To choose among drugs and to determine appropriate doses of a drug, the prescriber must know the relative pharmacologic **potency** and **maximal efficacy** of the drugs in relation to the desired therapeutic effect.

These two important terms, often confusing to students and clinicians, can be explained by referring to Figure , which depicts graded dose-response curves that relate the dose of four different drugs to the magnitude of a particular therapeutic effect.

FIGURE : Graded dose-response curves for four drugs, illustrating different pharmacologic potencies and different maximal efficacies

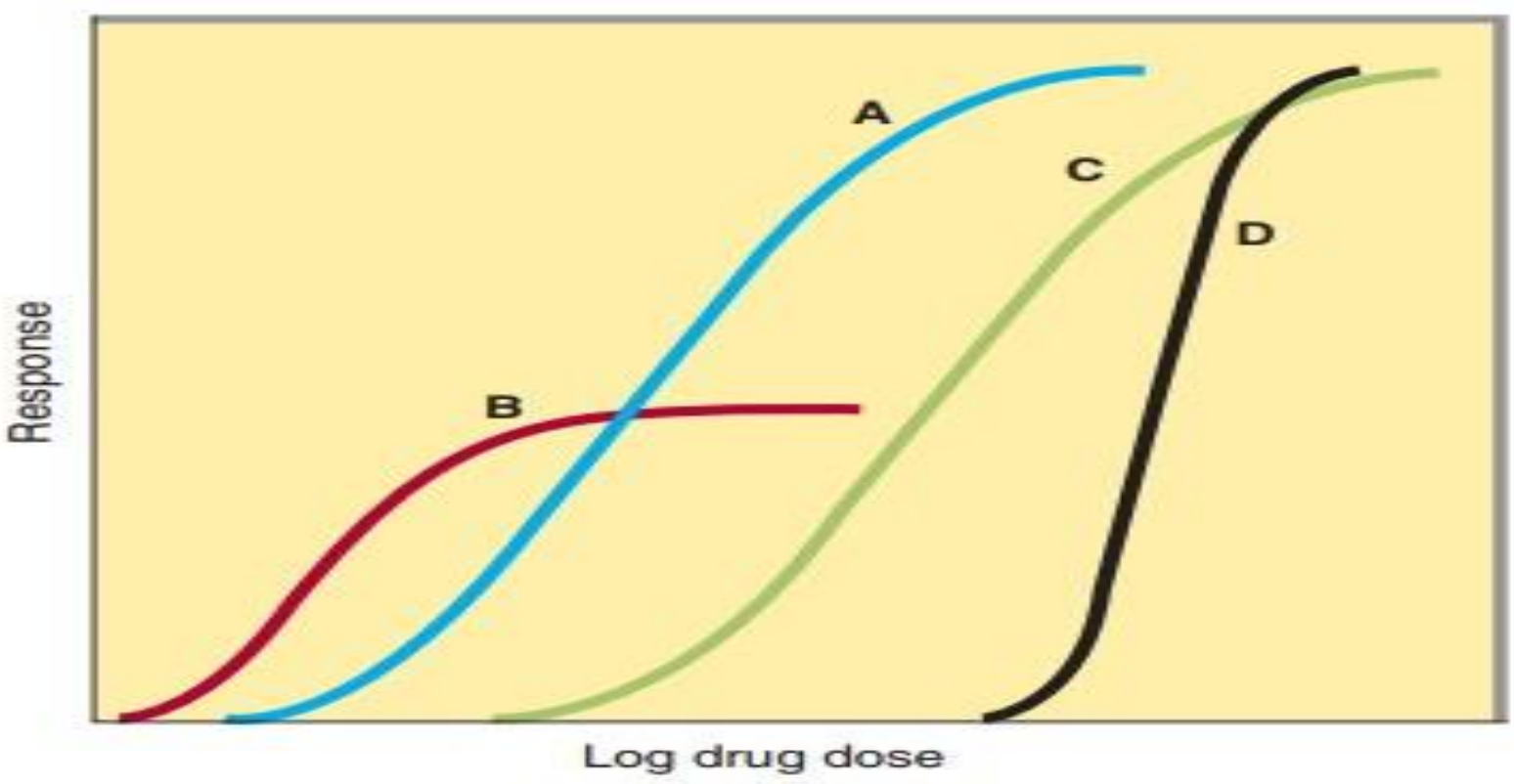


FIGURE 2–15 Graded dose-response curves for four drugs, illustrating different pharmacologic potencies and different maximal efficacies. (See text.)

1. Potency—Drugs A and B are said to be more potent than drugs C and D because of the relative positions of their dose-response curves along the dose axis.

Potency refers to the concentration (EC 50) or dose (ED 50) of a drug required to produce 50% of that drug's maximal effect. Thus, the pharmacologic potency of drug A in Figure is less than that of drug B, a partial agonist because the EC 50 of A is greater than the EC 50 of B.

Potency of a drug depends in part on the affinity (K d) of receptors for binding the drug and in part on the efficiency with which drug-receptor interaction is coupled to response.

Note that some doses of drug A can produce larger effects than any dose of drug B, despite the fact that we describe drug B as pharmacologically more potent. The reason for this is that drug A has a larger maximal efficacy

For clinical use, it is important to distinguish between a drug's potency and its efficacy.

The clinical effectiveness of a drug depends not on its potency (EC 50), but on its maximal efficacy and its ability to reach the relevant receptors.

This ability can depend on its route of administration, absorption, distribution through the body, and clearance from the blood or site of action.

In deciding which of two drugs to administer to a patient, the prescriber must usually consider their relative effectiveness rather than their relative potency.

Pharmacologic potency can largely determine the administered dose of the chosen drug.

2. Maximal efficacy— This parameter reflects the limit of the dose-response relation on the response axis.

Drugs A, C, and D in Figure have equal maximal efficacy, and all have greater maximal efficacy than drug B.

The maximal efficacy (sometimes referred to simply as efficacy) of a drug is obviously crucial for making clinical decisions when a large response is needed. It may be determined by the drug's mode of interactions with receptors (as with partial agonists ∗ or by characteristics of the receptor-effector system involved.

Thus, diuretics that act on one portion of the nephron may produce much greater excretion of fluid and electrolytes than diuretics that act elsewhere.

In addition, the practical efficacy of a drug for achieving a therapeutic end point (eg, increased cardiac contractility) may be limited by the drug's propensity to cause a toxic effect (eg, fatal cardiac arrhythmia) even if the drug could otherwise produce a greater therapeutic effect.

Median effective dose (ED 50), which is the dose at which 50% of individuals exhibit the specified quantal effect.

Similarly, the dose required to produce a particular toxic effect in 50% of animals is called the median toxic dose (TD 50).

If the toxic effect is death of the animal, a median lethal dose (LD 50) may be experimentally defined. Such values provide a convenient way of comparing the potencies of drugs .

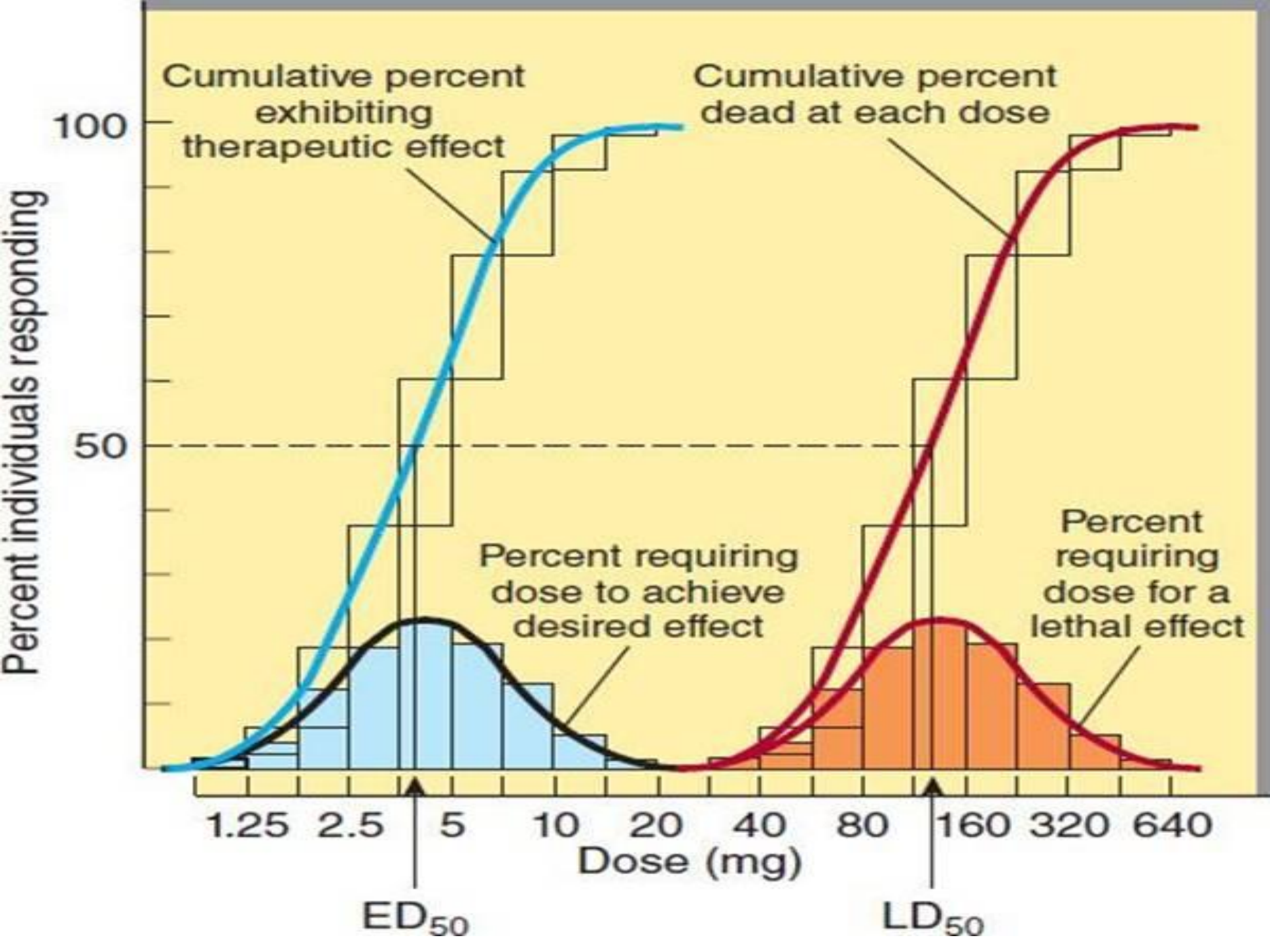
Experimental and clinical settings:

Thus, if the ED 50 s of two drugs for producing a specified quantal effect are 5 and 500 mg, respectively, then the first drug can be said to be 100 times more potent than the second for that particular effect.

Similarly, one can obtain a valuable index of the selectivity of a drug's action by comparing its ED 50 s for two different quantal effects in a population (eg, cough suppression versus sedation for opioid drugs).

FIGURE Quantal dose-effect plots. Shaded boxes (and the accompanying bell-shaped curves) indicate the frequency distribution of doses of drug required to produce a specified effect;

that is, the percentage of animals that required a particular dose to exhibit the effect. The open boxes (and the corresponding colored curves) indicate the cumulative frequency distribution of responses.


Cumulative percent exhibiting therapeutic effect
Cumulative percent dead at each dose
100
50
Percent individuals responding
Percent requiring dose to achieve desired effect
Percent requiring dose for a lethal effect
1.25 2.5 5 10 20 40 80 160 320 640
Dose (mg)
ED_{50}
LD_{50}

Variation in Drug Responsiveness

- Quantitative or qualitative variation:

A. Quantitative variation in drug response:

- **Tolerance:**

  Gradual decrease in drug response as a result of continuous or repeated drug administration. It takes days or weeks e.g. barbiturates, nitrates

- **Tachyphylaxis or desensitization:**

  The response of the drug is diminished rapidly (min.-hr) after repeated administration of the drug. e.g. amphetamine

- **Drug resistance:** loss of effectiveness of antimicrobial or antitumor drugs.

B. Qualitative variation in drug response:

- **Idiosyncratic drug response:**
- It is genetically determined abnormal & harmful drug response occurs, rarely and unrelated to the dose of the drug (may be due to immunological reactions or genetic abnormality)
- Examples:
- Penicillins → HSR

- **Genetic polymorphism**
- oxidation rate (↓ oxidation rate →TCA toxicity)
- acetylation rate (↓acetylation rate (slow acetylators)→ INH peripheral neuropathy).

  (↑acetylation rate (fast acetylators → INH hepatotoxicity).
- Suxamethonium → suxamethonium apnea due to the presence of abnormal type of cholinesterase that fails to inactivate suxamethonium rapidly leading to prolongation of suxamethonium duration of action.
- Primaquine, sulfonamides & dapsone cause hemolytic anemia in individuals with G-6-PD deficiency.
- Chloramphenicol → aplastic anemia.

The mechanisms for variation in drug response:

1. **Alteration in the numbers of receptors**:

- Down regulation: is gradual decrease in the numbers of the receptors (internalization) due to prolong exposure of the receptor to an agonist. e.g. β-agonists
- Up-regulation: ↑ numbers of the receptors e.g. thyroid hormones →up regulation of β receptors in the heart

2. **Change in receptors:**

Conformational change in the ion channel linked receptor (nAch) → desensitization

Phosphorylation of G-protein coupled receptor → desensitization

3. **Exhaustion of mediators:**

Amphetamine desensitization occurs due to depletion of the stored noradrenaline

4. **Increased metabolic degradation:**

Tolerance to some drugs (barbiturates & phenytoin) occurs due to auto-induction (↑ their own metabolism).

5. **Physiological adaptation:**

- The development of homeostatic response decreases drug response. Antihypertensive effect of thiazide diuretic is limited due to activation of renin-angiotensin system. Physiological adaptation decreases many side effects during drug administration.

6. **Active extrusion of the drug from the bacterial or parasitic cell:**

Results in resistance to antimicrobial agents e.g. tetracycline resistance

The most common test of acute toxicity is the LD50 test. LD50 means, if administered dose of drug to animal group, for experimental purpose for the estimation of therapeutic effectiveness kills 50% of animals, than it means that particular dose of drug is lethal dose 50 (LD50).

It was developed in 1920's and called "classical LD50" involved 100 animals for 5 dose-groups, later in 1981 it was modified by the Organization for Economic Co-operation and Development (OECD) and reduced number up to 30 for 3 dose-groups.

Methods to calculate LD50 values are - Litchfield and Wilcoxson, Reed-Muench, Miller-Tainter and Karber's method. But all these methods require large number of animals.

Factors which affect the results of LD50 are- Species, Age, Sex, Amount of food, Social environment etc.

LD50 study has some Limitations and results may vary greatly. Due to excess of animal sacrifice we should go to alternative methods which minimum number of animals is required.

Three alternative methods and these are: Fixed Dose Procedure (FDP)-OECD TG 420, Acute Toxic Class method (ATC)-OECD TG 423, Up-and-Down Procedure (UDP)-OECD TG 425. These methods only consider signs of toxicity in place of death.

Signs recorded during studies like; increased motor activity, anaesthesia, tremors, arching and rolling. Alternative methods save numbers experimental animals.

Toxicity test examine toxic effects when a chemical is absorbed into the body, via mouth, skin, lungs. The most common test of acute (short-term) toxicity is the LD50 test.

Many different substances are tested in this way, including all drugs, agricultural chemicals, cleaners, some cosmetics and their ingredients.

LD50 means if we administer any dose of drug to animal group for experimental purpose for the estimation of therapeutic effectiveness of that drug, and if 50% of animal get died than it means that particular dose of drug is lethal dose 50 (LD50).

The smaller the LD50 value, the more toxic is chemical. The opposite is also true: the larger the LD50 value, the lower the toxicity.

In 1987 further reduced to 20 animals . Mice, rats, rabbits, guinea pigs, cats, dogs, fish, monkeys and birds are use for LD50 study.

The LD50 values of a new drug are determined by various route of administration (intravenous, intraperitoneal, subcutaneous and oral) .

Results of LD50 study may affected by other factors as amount of food, Social environment, Route of exposure* (oral, dermal, inhalation) and Physical environment such as temperature and humidity.

Rout of exposure (example, some LD50s for Dichlorvos, an insecticide commonly used in household pesticide strips): - • Oral LD50 (rat): 56 mg/kg • Dermal LD50 (rat): 75 mg/kg • Intraperitoneal LD50: (rat) 15 mg/kg • Inhalation LC50 (rat): 1.7 ppm (15 mg/m3); 4-hour exposure .

There are also some Limitations for LD50 study like:-
The LD50 gives a measure of the immediate or acute toxicity, results may vary greatly, LD50 is not tested on humans, All relation to humans are only a guess.

((The LD50 test is neither reliable nor useful, because the human lethal dose can't be predicted from animal studies.))

(1) Arithmetical method of Karber method :

The sum of the product was divided by the number of animals in a group and the resulting quotient was subtracted from the least lethal dose in order to obtain LD50 value.

(2) Graphical method of Miller and Tainter

The observed percentage mortality was converted into probit referring to the probit table. The values thus obtained were plotted against log dose.

The deletion of the LD50 test from the OECD guidelines was due to three alternative methods being adopted which all involve more humane treatment of the animals and use fewer animals than the LD50 test. They record toxicity signs in place of death. 5 These three alternative tests are:

(1) Fixed Dose Procedure (FDP) — OECD TG 420. This method does not use death as an end point, instead it uses the observation of clear signs of toxicity developed at one of a series of fixed dose levels to estimate the LD50.

(2) Acute Toxic Class method (ATC) — OECD TG 423 This method does not use death as the only end points, it also uses signs of toxicity in its stepwise approach to estimating the LD50.

Principle: - It is based on the Probit model. Procedure: - The ATC method is a sequential testing procedure using only three animals of one sex per step. Depending on the mortality rate three but never more than six animals are used per dose level. This approach results in the reduction of numbers of animals used in comparison to the LD50 test by 40–70%.

(3) Up-and-Down Procedure (UDP) — OECD TG 425
This method does still use death as an end point, but doses animals one at a time to see if the dose needs to be put up or down to achieve an estimate of the LD50 therefore giving the minimum number of animals a lethal dose of the test substance.

In the up-and-down procedure, animals are dosed one at a time. If an animal survives, the dose for the next animal is increased; if it dies, the dose is decreased. Each animal is observed for 1 or 2 days before dosing the next animal. Surviving animals monitored for delayed death for a total of 7 days.

IN SUMMARY

What does LD_{50} mean?

LD stands for "Lethal Dose". LD_{50} is the amount of a material, given all at once, which causes **the death of 50% (one half) of a group of test animals.**

The LD_{50} is one way to measure the **short-term poisoning potential (acute toxicity) of a material.**

Toxicologists can use many kinds of animals but most often testing is done with **rats and mice**.

It is usually expressed as the **amount of chemical administered (e.g., milligrams) per 100 grams (for smaller animals) or per kilogram (for bigger test subjects) of the body weight of the test animal.**

The LD_{50} can be found for any route of entry or administration but **dermal (applied to the skin) and oral (given by mouth) administration methods are the most common.**

Why study LD_{50}'s?

Chemicals can have a wide range of effects on our health. Depending on how the chemical will be used, many kinds of toxicity tests may be required.

Therefore, to compare the toxic potency or intensity of different chemicals, researchers must measure the same effect. One way is to carry out lethality testing (the LD_{50} tests) by measuring how much of a chemical is required to cause death. This type of test is also referred to as a "quantal" test because it is measures an effect that "occurs" or "does not occur".

What are some other toxicity dose terms that are used?

- LD_{01} Lethal dose for 1% of the animal test population
- LD_{100} Lethal dose for 100% of the animal test population
- LDLO The lowest dose causing lethality
- TDLO The lowest dose causing a toxic effect

The LD_{50} value obtained at the end of the experiment is identified as the LD_{50} (oral), LD_{50} (skin), LD_{50} (i.v.), etc., as appropriate.

Researchers can do the test with any animal species but they use rats or mice most often. Other species include dogs, hamsters, cats, guinea-pigs, rabbits, and monkeys.

In each case, the LD_{50} value is expressed as the weight of chemical administered per kilogram body weight of the animal and it states the test animal used and route of exposure or administration; **e.g., LD_{50} (oral, rat) - 5 mg/kg, LD_{50} (skin, rabbit) - 5 g/kg.**

So, the example "LD_{50} (oral, rat) 5 mg/kg" means that 5 milligrams of that chemical for every 1 kilogram body weight of the rat, when administered in one dose by mouth, causes the death of 50% of the test group.

Which LD_{50} information is the most important for occupational health and safety purposes?

Inhalation and skin absorption are the most common routes by which workplace chemicals enter the body.

Thus, the most relevant from the occupational exposure viewpoint are the inhalation (LC_{50}) and skin application tests (LD_{50}-skin). Despite this fact, the most frequently performed lethality study is the oral LD_{50}.

This difference occurs because giving chemicals to animals by mouth is much easier and less expensive than other techniques. However, the results of oral studies are important for drug studies, food poisonings, and accidental domestic poisonings. Oral occupational poisonings might occur by contamination of food or cigarettes from unwashed hands, and by accidental swallowing.

How do I compare one LD_{50} value to another and what does it mean to humans?

In general, the smaller the LD_{50} value, the more toxic the chemical is.

The opposite is also true: the larger the LD_{50} value, the lower the toxicity.

The LD_{50} gives a measure of the immediate or acute toxicity of a chemical in the strain, sex, and age group of a particular animal species being tested.

Changing any of these variables (e.g., type animal or age) could result in finding a different LD_{50} value. The LD_{50} test was neither designed nor intended to give information on long-term exposure effects of a chemical.

It is also important to know that the actual LD_{50} value may be different for a given chemical depending on the route of exposure (e.g., oral, dermal, inhalation). For example, some LD_{50}s for dichlorvos, an insecticide commonly used in household pesticide strips, are listed below:

- Oral LD_{50} (rat): 56 mg/kg
- Dermal LD_{50} (rat): 75 mg/kg
- Intraperitoneal LD_{50}: (rat) 15 mg/kg
- Inhalation LC_{50} (rat): 1.7 ppm (15 mg/m3); 4-hour exposure
- Oral LD_{50} (rabbit) 10 mg/kg
- Oral LD_{50} (pigeon:): 23.7 mg/kg
- Oral LD_{50} (rat): 56 mg/kg
- Oral (mouse): 61 mg/kg
- Oral (dog): 100 mg/kg
- Oral (pig): 157 mg/kg

Differences in the LD_{50} toxicity ratings reflect the different routes of exposure.

The toxicity rating can be different for different animals. The data above show that dichlorvos is much less toxic by ingestion in pigs or dogs than in rats.

Using dichlorvos is moderately toxic when swallowed (oral LD_{50}) and extremely toxic when breathed (inhalation LC_{50}) in the rat.

Can animal LD_{50} data be applied to humans?

In general, if the immediate toxicity is similar in all of the different animals tested, the degree of immediate toxicity will probably be similar for humans.

When the LD_{50} values are different for various animal species, one has to make approximations and assumptions when estimating the probable lethal dose for man.

Where can I find LD_{50} and LC_{50} values?

The largest, single collection of LD_{50} and LC_{50} values is in the database Registry of Toxic Effects of Chemical Substances (RTECS) that is available by subscription on the Internet.

Two other databases available from CCOHS, CHEMINFO and the Hazardous Substances Data Bank (HSDB), are in the CHEMpendium collection.

THANK YOU