Unit-3: Basics of Toxicology

[Significance of toxicological findings. Techniques used in toxicology. Toxicological analysis and chemical intoxication tests. Lethal dose 50 and effective dose 50.]

The word "Toxicology" is came from the *Greek* word "*toxicon*" which was used as a poisonous substance in arrowheads. Conventionally, the toxicology may be defined as the science representing the character, source, knowledge, lethal dose, fatal effect, analysis of poisons and their curative measures. More precisely, Toxicology can be said as the study of antagonistic effects of chemical or physical agents on living organisms. A "toxicologist" is competent to examine and lead into the nature of those effects on human, animal, and environmental health. Toxicological study examines the cellular, biochemical, and molecular mechanisms of action as well as functional effects such as neurobehavioral and immunological, and assesses the likelihood of their event.

History of Toxicology [extra information]

The earlier Indian text ***Rig Veda*** (12th century BC) also describes several plant poisons. However, Toxicology dates back to the earliest humans, who used plant extracts and animal venoms for warfare, hunting, and assassination. The ancient book ***Ebers Papyrus*** contains information pertaining to many known poisons, including hemlock, aconite, opium, and metals such as arsenic, lead, copper, and antimony. Among mineral poisons, one of the earliest known elements was lead which was discovered as early as 3500 BC. In the literature of ancient Greece, there are numerous references to poisons and their use. Some explanations of *Homer* have *Odysseus* attaining toxins for his arrows. Theophrastus, a student of Aristotle, comprised many references to poisonous plants in ***de historia plantarum.*** Dioscorides, a Greek physician in the court of the Roman emperor Nero, did the first effort at a classification of poisons, which was supplemented by descriptions and diagrams.

Although, Organic chemistry was in its nascent stage till 1800, but by 1825 war gases like Phosgene ($COCl_2$) and Mustard Gas (bis [β-chloroethyl] sulphide) had been synthesized successfully. These two agents, along with Chlorine gas, were used by the German forces in World War I as chemical warfare agents. They were stored throughout World War II, and used by Iraq in the Iran–Iraq War in the 1980s (Marine Corps History). By 1880 over 10,000 organic compounds had been manufactured including chloroform, carbon tetrachloride, diethyl ether, and carbonic acid, and petroleum and coal gasification by-products were used in trade. Experimental toxicology accompanied the growth of organic chemistry and developed rapidly during the 19th century.

Some common terms in toxicology

1. **Toxicity:** The word "toxicity" describes the degree to which a substance is poisonous or can cause injury.
2. **Toxic:** This term relates to poisonous or deadly effects on the body by inhalation (breathing), ingestion (eating), or absorption, or by direct contact with a chemical.
3. **Toxicant:** A toxicant is any **chemical** that can injure or kill humans, animals, or plants; a poison. The term **"toxicant"** is used when talking about toxic substances that are produced by or are a by-product of human-made activities.

4. **Toxin:** The term "**toxin"** usually is used when talking about toxic substances produced naturally. A toxin is any poisonous substance of microbial (bacteria or other tiny plants or animals), vegetable, or synthetic chemical origin that reacts with specific cellular components to kill cells, alter growth or development, or kill the organism.
5. **Dose**: The dose is the actual amount of a chemical that enters the body. The dose received may be due to either acute (short) or chronic (long-term) exposure.

Classification of Toxicology

The field of toxicology can be further divided into the following sub-disciplines:

1) Environmental Toxicology	2) Occupational Toxicology	3) Regulatory Toxicology
4) Food Toxicology	5) Clinical Toxicology	6) Descriptive Toxicology
7) Analytical toxicology	8) Mechanistic Toxicology	**9) Forensic Toxicology**

Forensic Toxicology: Forensic Toxicology is used to help in establishing the cause and effect relationships between exposure to a drug or chemical and the toxic or lethal effects that result from that exposure. Forensic Toxicology talks about to the application of conventional toxicology for the purposes of the Criminal Investigation to assist legal administration. It can be considered as a hybrid of Analytical Chemistry and Fundamental Toxicology with advanced Forensic Medicine. Forensic toxicology is also primarily concerned with the medico - legal characteristics of the detrimental effects of chemicals on human and animals. The expertise of forensic toxicologists is primarily utilized in establishing the cause of death and interpreting its circumstances in post-mortem investigation.

Significance of toxicological findings

- Forensic toxicology integrates principles from multiple scientific disciplines including chemistry, pharmacology, and medicine, focusing primarily on the detection, identification, and quantification of drugs, poisons, and other potentially harmful substances in biological and environmental samples.
- The importance of forensic toxicology extends beyond mere academic interest; it is crucial in legal contexts where precise scientific analysis can be the determining factor in the outcomes of criminal and civil disputes.
- The forensic toxicologist's expertise is often called upon to answer complex questions such as whether a substance contributed to or caused a death, whether an individual was impaired by a substance at the time of an incident, and if a substance was present due to intentional administration or accidental exposure.
- Its scope has now expanded to include living subjects and a broader array of substances such as recreational drugs, environmental toxins, and industrial chemicals, postmortem forensic toxicology, human performance toxicology, and forensic drug testing.
- Forensic toxicology plays a crucial role in both criminal and civil law by providing scientific analysis that helps elucidate cases involving drugs, poisons, and alcohol.

Techniques used in toxicology

Toxicologically significant substances include a wide array of substances with diverse physico-chemical properties:

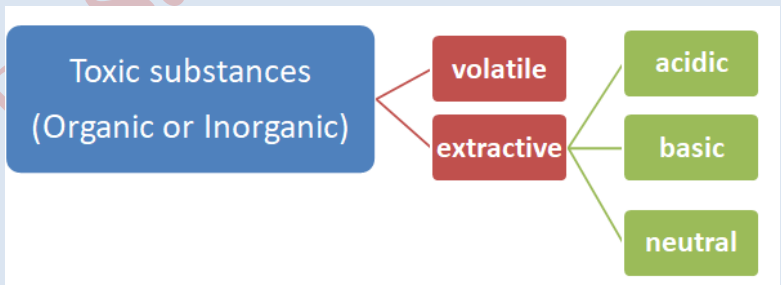
- Drugs, addictive substances;
- Volatile substances (e.g. ethanol, methanol, toluene, trichlorethylene);
- Gases (e.g. carbon monoxide, hydrogen cyanide);
- Inorganic acids and bases;
- Metals (e.g. arsenic, lead, mercury)

In toxicological analysis a diverse material is processed. It is important to send the material in sufficient amount so that in case of poisoning with an unknown toxin as many analyses as possible can be performed. The usually examined kinds of materials are the following:

- **Stomach content** – at least 50 ml of vomits or the first portion of gastric lavage.
- **Blood, or serum** (10 ml) – level in blood is affected by the time passed since entry of the harmful substance to the body, rate of its absorption and excretion.
- **Urine** – poison appears in urine later than in blood, but also persists longer, and so analysis of urine gains significance in later stages of intoxications.
- **Biological material obtained after death** – contents of the gastrointestinal tract, tissue samples, and body fluids.
- **Material secured in relation to intoxication** – includes pills, liquids, injection syringes, and food remnants.
- **Hair** – accumulates certain substances, and so enables detection of drug abuse or chronic exposition.
- **Exhaled breath** – contains volatile substances.
- **Saliva** – utilized especially for rapid screening tests of drugs in controls of car drivers or employees on duty.
- **Meconium** – can be used in suspicion of drug abuse in a pregnant woman.

Methods used in toxicology

The choice of analytical technique is affected by nature of the poison. The toxic substances can be classified to **volatile** and **extractive**; another division is to **inorganic** and **organic**. The extractive substances are further divided to **acidic, basic or neutral** according to the pH of medium to which they pass during extraction.



The subsequent toxicological analysis relies mostly on various types of chromatographic methods, such as thin layer chromatography (TLC), high performance liquid chromatography (HPLC), and gas chromatography (GC), both the latter often coupled to mass spectrometry (MS). Immunochemical methods also play a significant role. For estimation of **volatile substances, gas chromatography** is used. Estimation of ethanol is of special importance, because of high frequency of examinations. **Spectrophotometric methods** are employed for estimation of toxicologically significant derivatives of **hemoglobin (carbonylhemoglobin, methemoglobin, sulfonylhemoglobin, cyanmet hemoglobin)**. In analysis of **inorganic substances**

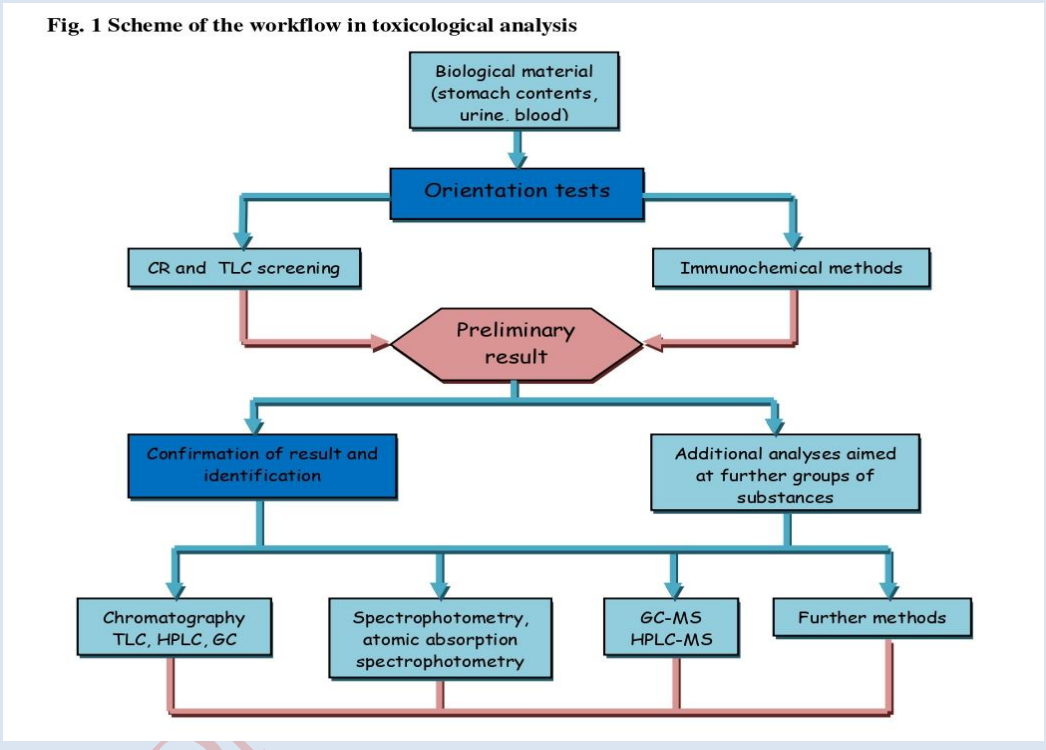
further methods find usage, such as **atomic absorption spectrometry, neutron activation analysis or polarography**.

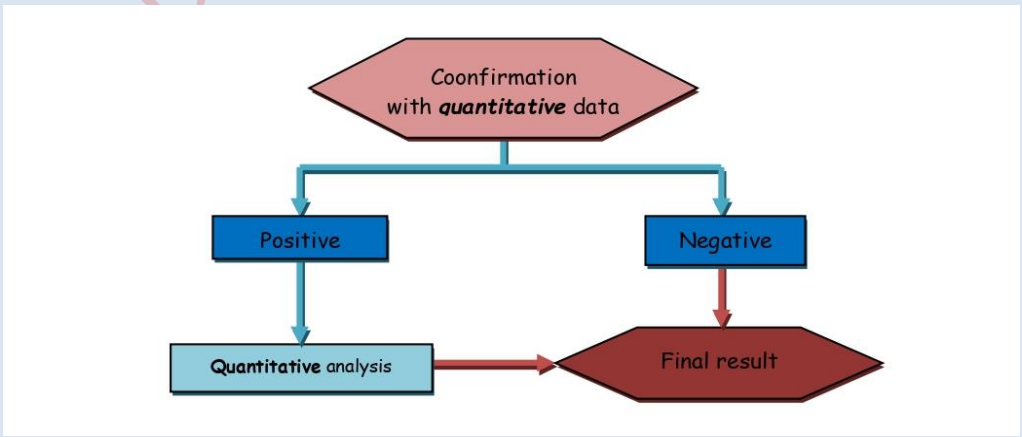
Workflow of toxicological analysis

The workflow of toxicological analysis includes several stages:

1. Screening – based on immunochemical methods, thin-layer chromatography together with the system of colour reactions (CR), gas and liquid chromatography.
2. Demonstrations (identification) of a chemical entity – more sophisticated methods are usually needed. The analysis employs liquid and gas chromatography with mass detection, thin-layer chromatography with various developing systems, sorbents and detection agents, and other methods.
3. Quantitative estimation of exogenous substance in biological material.
4. Evaluation of findings by a toxicologist and physician.

Fig. 1 Scheme of the workflow in toxicological analysis





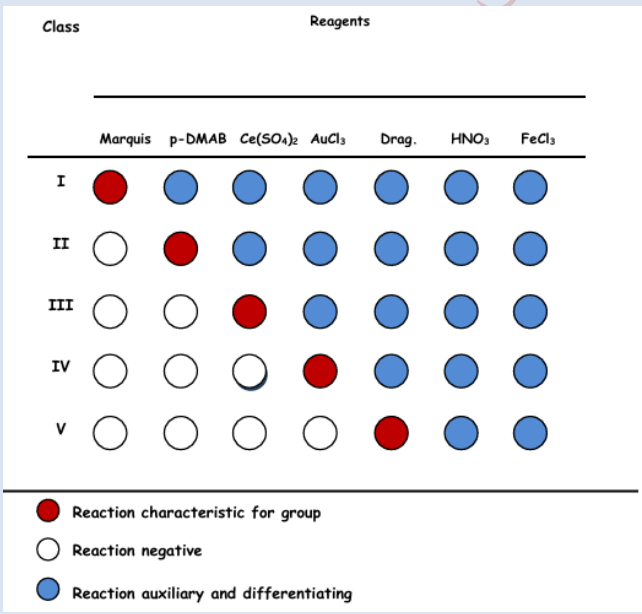
1. *Screening*

Screening of unknown substances represents an introductory step of toxicological analysis. The results obtained with screening tests are rather preliminary in nature and it should be understood that the screening tests might not detect substances that are unexpected. The preliminary tests need not be specific but they should provide sufficient sensitivity so that the negative result can be considered reliable. In the screening of unknown substances especially **immunochemical methods** and **thin-layer chromatography** are used.

Imunochemical methods: This approach is suitable especially in the introductory phase of toxicological examination. Immunochemical detection of a drug is based on its reaction with a specific antibody. For the purpose of screening the immunochemical techniques are targeted at the groups of substances known as significant and often abused drugs (narcotic and psychotropic substances). An advantage is that the sample needs no processing before the test. Immunochemical analysers are available for these methods.
In the form of diagnostic strips or plates, they are used to test for drugs in urine. Recently also tests applicable to analysis of saliva or sweat have appeared.

TLC -screening: The principle of the method lies in detection of an unknown drug and/or its metabolites in biological material by means of several criteria, each of which enables classification of the detected drug or metabolite to certain group. An advantage of TLC screening is detection of a fairly wide range of substances and their metabolites and also ability to resolve the particular components in analysis of mixtures. It is a simple and inexpensive technique. Unlike the immunochemical methods, when the TLC is used a sample processing prior to the chromatography is necessary. The TLC screening includes several steps. Each of them contributes to understanding of nature of the searched substance (*already discussed in Unit 1*).

CR (color reactions) screening: The actual chromatography can be preceded with a CR-screening. It employs reactions of many drugs with inorganic (acids, salts of heavy metals) or organic reagents, in which characteristic colored products are formed. The CR-screening is performed in a form of dot reactions, where drops of the analysed sample or its extract are combined with various reagents in certain order.



On the basis of colors produced with the group reagents and further auxiliary reagents the drugs are classified to several groups – classes of colored reactions. The first positive reaction with the given order of reagents is decisive for class determination. The CR-screening represents a typical screening method; its usage can exclude or suggest presence of certain drug in the analysed sample. A group of structurally related drugs can be identified in this way. The method is not suitable for detection of drugs in mixtures. Examination of biological material only by CR-screening has a limited significance.

In the stomach content an original form of the drug can be expected and the results are not affected by presence of certain interfering substances. Analysis of urine is complicated by the presence of ballast substances and metabolites, which can react differently from the original substance. However, if the CR-screening is combined with TLC, it provides quick and cheap preliminary information that guides further analysis. The CR-screening results suggest which colored reactions should be used for detection in TLC analysis.

Biotransformation of drugs

Majority of exogenous substances in the body undergoes biotransformation, in which metabolites originate. Especially lipophilic substances are intensively metabolized. Their conversion increases polarity and enables excretion of the resulting metabolites to urine. It often happens that the original form of the drug is not present in the sample. Then demonstration of metabolites gains importance. In evaluation of the toxicological findings the following situations should be taken to the account:

- Drug is present in its original unchanged form;
- Drug is demonstrated in original form together with a metabolite;
- Metabolites prevail.

The conversion of the original drug to metabolites complicates the toxicological analysis.

Examples of biotransformation

1. Biotransformation of acetylsalicylic acid

Acetylsalicylic acid is widely used analgesic and antipyretic remedy. In toxicological analytics, it is encountered in intoxications of children or suicidal attempts of adults, where it is usually detected together with other drugs, found in the combined analgesic-antipyretic mixtures. The core reaction in biotransformation of acetylsalicylic acid is its cleavage to acetic acid and salicylic acid (M_2). Part of the salicylic acid is excreted as such, whereas another part appears in a conjugated form with glycine as salicyluric acid (M_1). Yet another portion of salicylic acid is hydroxylated to gentisic acid (M_3).

Hydroxylation and conjugation of the original drug increases polarity of the resulting molecules. As a consequence mobilities of the products in chromatographic separation decrease. In TLC/CR-screening analysis with the basic developing system the acetylsalicylic acid and their metabolites remain on the start. In order to increase the mobility and separation of metabolites a different composition of the mobile phase must be chosen. Detection of the spots can employ either ferric chloride that forms a colored complex (violet-red) with derivatives of salicylic acid or UV light (254 nm).

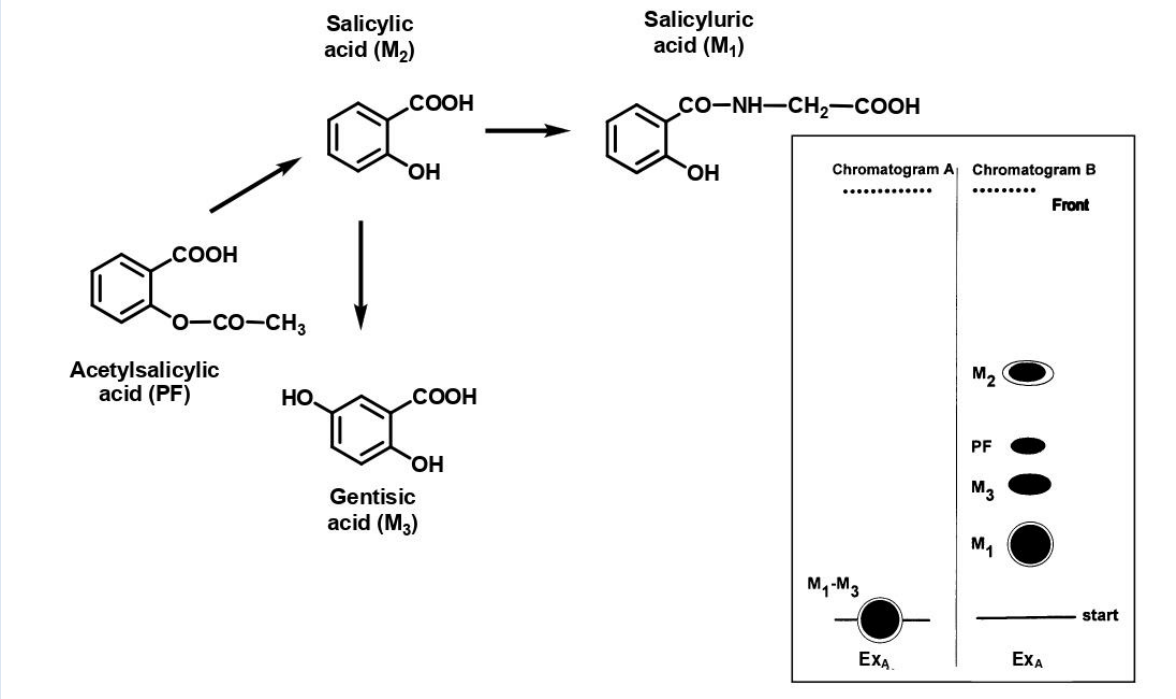


Fig. Biotransformation of acetylsalicylic acid and its detection in urine with TLC chromatography (chromatogram A – basic mobile phase, chromatogram B – mobile phase of composition cyclohexane: tetrachlormethane:diethyl ether:acetic acid in ratio 10:10:10:5; detection of chromatograms A and B with ferric chloride). M – metabolites, PF – pure form.

2. Biotransformation of codeine

Codeine is a remedy used to suppress cough; in addition it is a part of analgesic combinations. It is one of the substances abused by drug addicts. Interestingly enough, in biotransformation of codeine one of the metabolites is morphine. It is a serious fact that needs to be taken into account in interpretation of toxicological analysis. Morphine (M_1) originated through demethylation reaction on oxygen, called O-demethylation. Codeine in the body undergoes also another type of demethylation on nitrogen - N-demethylation, which yields norcodeine (M_2).

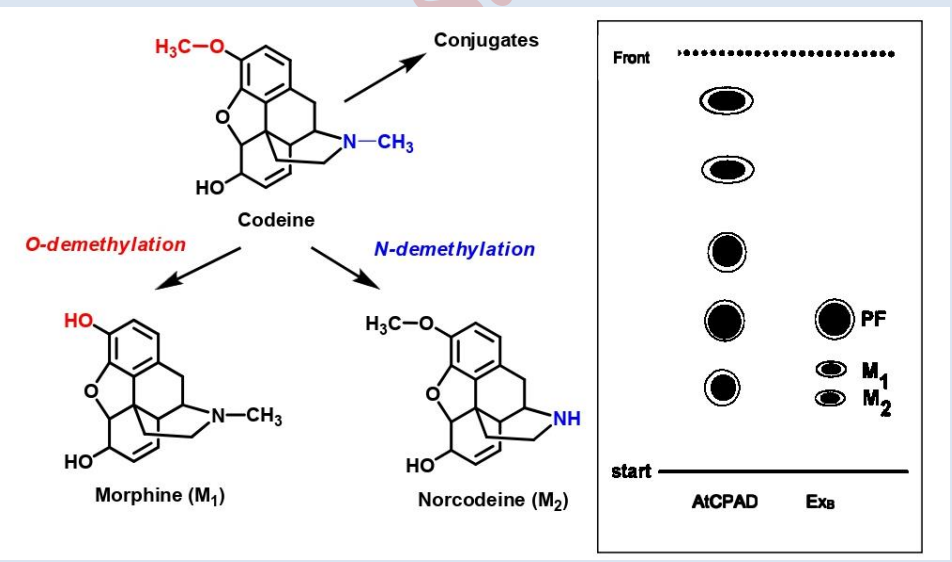


Fig. Biotransformation of codeine to morphine and norcodeine

3. **Ethanol in toxicology**

Estimation of ethanol in biological material is one of the commonest requests for examination in toxicological laboratory. The main pharmacological action of ethanol takes place in the Central Nervous System (CNS). Low doses induce euphoria and excitation. Ethanol at higher doses has narcotic effects; it can cause unconsciousness and even death in the most severe cases. The ingested alcohol is readily absorbed in the digestive tract. It is distributed to all organs. The metabolism of ethanol takes place mostly in the liver, where it is oxidized by means of the enzyme alcohol dehydrogenase to acetaldehyde, which is further converted to acetic acid. About 90 – 95 % of ethanol is oxidized; the remaining part is excreted unchanged to breath, sweat, tears, milk, and urine.

Preliminary estimation of ethanol in exhaled breath

Breath analyzers or detection tubings are available for examination of ethanol in exhaled breath. A negative breath test is sufficient as a proof that the breath does not contain ethanol.

a. **Breathe analyzers:** Some breathe analyzers utilize for determination of the presence of ethanol in exhaled breath sensitive electrochemical detectors on the basis of ion mobility spectrometry (IMS). The exhaled breath is exposed to radiation effect of americium (241Am), which causes its ionization. In an electric field the ions move with a characteristic speed that is recorded in a form of impulses and evaluated with a special software. Other types of breath analyzers, which serve only as screening devices, are semiconductor sensors.

b. **Detection tubings:** In the past the ethanol in exhaled breath was detected by means of detection tubings. The test for ehanol utilized a reduction of some substances ($K_2Cr_2O_7$, $KMnO_4$) with ethanol, seen as a color change. Reaction with potassium dichromate is used in the detection tubings Altest. The tubing is filled with silicate grains coated with a thin layer of potassium dichromate and sulfuric acid. The air containing ethanol vapors reduces the indication mixture. Reduction changes yellow $K_2Cr_2O_7$ to green $Cr_2(SO_4)_3$. Ethanol oxidizes to acetaldehyde. The reaction is unspecific, and can be turned positive also e.g. by fruit, candy, and mouth wash.

$$3CH_3CH_2OH + K_2Cr_2O_7 + 4H_2SO_4 \rightarrow 3CH_3CHO + K_2SO_4 + Cr_2(SO_4)_3 + 7H_2O$$

Estimation of ethanol in blood

a. **Gas chromatography**: Gas chromatography is considered as an objective method acceptable for the court. It enables a qualitatively specific and quantitative estimation of concentration of ethanol in the blood. One of its advantages is that ethanol is conclusively differentiated from other volatile substances such as methanol, acetone or toluene. (GC is discussed in Unit 1).

b. **Enzymatic method:** An enzymatic method can be used for estimation of ethanol as well. Ethanol is oxidized by NAD^+ with catalytic action of the enzyme alcohol dehydrogenase (ADH) to acetaldehyde. The amount of reduced NADH is proportional to the amount of ethanol in the sample. Absorbance of NADH is measured in UV region at 340 nm (the Warburg optical test). This method is not absolutely specific for ethanol, but it can be used for clinical purposes.

$$CH_3CH_2OH + NAD^+ \xleftrightarrow{ADH} CH_3CHO + NADH + H^+$$

Lethal dose 50 and effective dose 50

Lethal Dose 50 (LD_{50}) and **Effective Dose 50 (ED_{50})** are metrics used in toxicology and pharmacology to assess the potency and safety of substances, particularly toxic agents and drugs.

1. Lethal Dose 50 (LD_{50})

LD_{50} is the dose of a substance that causes death in 50% of a test population, usually measured in animals. It's typically expressed in terms of milligrams of substance per kilogram of body weight (mg/kg). LD_{50} is used to measure a substance's acute toxicity. The lower the LD_{50} value, the more toxic the substance is, since it takes a smaller dose to be lethal.

LD_{50} helps in assessing safety profiles of chemicals, drugs, and environmental toxins. LD_{50} tests provide a standard comparison between toxic substances and help in establishing regulatory guidelines for human and animal safety.

2. Effective Dose 50 (ED_{50})

ED_{50} is the dose of a substance that produces a desired therapeutic effect in 50% of a test population. Similar to LD_{50}, it's often measured in mg/kg. ED_{50} indicates the potency of a substance by showing how much of it is needed to achieve the intended effect in half of the subjects.

This measure is particularly useful in pharmacology, as it helps determine appropriate dosages for desired therapeutic effects. It also aids in calculating the Therapeutic Index (TI), a ratio of LD_{50} to ED_{50} which indicates the safety margin of a drug. The higher the TI, the safer the drug is.

Comparison between LD_{50} and ED_{50}

- LD_{50} focuses on toxicity, while ED_{50} focuses on efficacy.
- Both are essential for determining safe dosages for therapeutic compounds.
- By comparing LD_{50} and ED_{50} values, scientists assess the balance between effective and harmful dosages to ensure the substance is used within safe limits.