ИТМО

TOXICOLOGY OF MATERIALS: THE BASICS. PRACTICAL GUIDE

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ITMO UNIVERSITY

TOXICOLOGY OF MATERIALS: THE BASICS. PRACTICAL GUIDE

RECOMMENDED FOR USE AT ITMO UNIVERSITY
within the Bachelor's degree program12.03.04 and Master’s Program 12.04.04 "Biotechnical Systems and Technologies" as a methodological guide for foreign students

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Рецензент: Уколов Антон Игоревич, доктор химических наук, зав. отдела Токсикологии Федерального государственного унитарного предприятия «Научно-исследовательский институт гигиены, профпатологии и экологии человека Федерального медико-биологического агентства» (ФГУП НИИ ГПЭЧ ФМБА России).

Данное практическое пособие предназначено для студентов старших курсов высших учебных заведений, обучающихся в области биотехнологии, технологии лекарственных средств, физико-химических методов и устройств контроля качества продукции, биоинженерии и биоэкологии. Практическое руководство состоит из восьми основных разделов, которые включают лабораторную работу или задание. Каждый раздел сопровождается краткой лекцией-введением, что позволяет успешно выполнять задания, предложенные в данном руководстве.

ИТМО (Санкт-Петербург) — национальный исследовательский университет, научнообразовательная корпорация. Альма-матер победителей международных соревнований по программированию. Приоритетные направления: IT иискусственный интеллект, фотоника, робототехника, квантовые коммуникации, трансляционная медицина, Life Sciences, Art&Science, Science Communication.

Лидер федеральной программы «Приоритет-2030», в рамках которой реализовывается программа «Университет открытого кода». С 2022 ИТМО работает в рамках новой модели развития — научно-образовательной корпорации. В ее основе академическая свобода, поддержка начинаний студентов и сотрудников, распределенная система управления, приверженность открытому коду, бизнес-подходы к организации работы. Образование в университете основано на выборе индивидуальной траектории для каждого студента.

ИТМО пять лет подряд — в сотне лучших в области Automation & Control (кибернетика) Шанхайского рейтинга. По версии SuperJob занимает первое место в Петербурге и второе в России по уровню зарплат выпускников в сфере IT. Университет в топе международных рейтингов среди российских вузов. Входит в топ-5 российских университетов по качеству приема на бюджетные места. Рекордсмен по поступлению олимпиадников в Петербурге. С 2019 года ИТМО самостоятельно присуждает ученые степени кандидата и доктора наук.

Content

Introduction

This practical guide is addressed to undergraduate students studying at ITMO University in the field of training 12.03.04 and 12.04.04 "Biotechnical systems and technologies", as well as teachers conducting theoretical and laboratory classes for the "Fundamentals of Materials toxicology" course. This course features the elements of competencies formed in such cources as "Biochemistry", "Anatomy", "Biomedical fundamentals of life safety", "General and Inorganic Chemistry", and "Organic Chemistry".

The practical guide consists of 8 main sections. Each section includes a lab work or a task and is accompanied by a short lecture introduction, which makes it possible to successfully complete the tasks proposed in this guide.

The plan of the course "Fundamentals of Materials toxicology" includes lectures, practical and laboratory classes, as well as self-study materials. The lecture course is designed for a systematic study of the fundamentals of general toxicology and includes such sections as "Basic concepts of toxicology", "Synthetic and natural toxicants", "Basic concepts of toxicokinetics", "Mechanisms of cytotoxicity", " Basic concepts of toxicodynamics", "Antidotes and mechanisms of their action", and "Planning of a toxicological experiment." Practical and laboratory works are aimed at consolidating the knowledge gained in lectures and acquiring practical skills in calculating toxicological parameters, applying analytical methods to determine the toxicant and acquiring practical skills in using antidotes.

Completing the lecture course, students should know: basic concepts of toxicology; basic classifications of poisoning; basic classifications of toxicants; main routes of entry and elimination of harmful substances; mechanisms of biotransformation of toxicants; basic principles of action of antidotes; mechanisms of toxic effects of harmful substances on the body.

Completing the practical course, students will be able to determine the hazard class of toxicants based on experimental parameters, will know the main routes of entry of toxicants into the body and ways of their elimination, will be able to predict the mechanisms of toxic effects of harmful substances on the body, will master the skills of chemical and toxicological analysis.

The competencies acquired during the course will be further developed in the discipline "Fundamentals of toxicology of nanomaterials".

Topic 1. Basic concepts of toxicology

Toxicology is the science that studies the interaction between toxic substances and living organisms.

Toxicology may be viewed as the forerunner of medicine, given that humans encountered toxic plants and animals well before acquiring the knowledge to treat diseases. In ancient times, poisons were mystified, shrouded in secrets, and used in hunting and various rituals. The history of the study of toxic substances is a part of science that represents a fascinating blend of human curiosity, centuries of experience and mistakes, and great discoveries alongside profound losses. The Ebers Papyrus, also known as the first pharmacopoeia, is considered to be the first source of knowledge about toxicology. It was there that the first references to the toxic properties of opium, arsenic, aconite, eserine, and strophanthin appeared.

Today, toxicology is a separate science that includes a wide variety of different fields of medicine, chemistry, biology, and pharmacology. There are several main areas of scientific research and their subdivisions in toxicology, including theoretical, preventive, and clinical toxicology.

Clinical toxicology is mostly about clinical treatment. It studies the full spectrum of human diseases caused by exposure to toxic chemical agents in the environment. This includes accidental or intentional poisonings, occupational exposures, adverse drug reactions, and health impacts of environmental pollutants. By understanding the mechanisms of toxicity, clinical toxicologists develop diagnostic protocols, treatment strategies (such as antidote administration and supportive care), and preventive measures to reduce morbidity and mortality from toxin-related illnesses.

Clinical toxicology studies various **side effects** (short-term functional disturbances) caused by medications and various toxic substances (see Table 1).

Table 1. Classification of side effects

Type	Side effects	Causes
A	Reactions that occur in most patients taking the drug in high doses	Excessive therapeutic effect Pharmacological and toxic secondary effects
B	Adverse side effects that do not depend on the dose	Immunoallergical reactions, which mechanisms are unknown
C	Adverse side effects due to prolonged therapy	Tolerance Addiction Withdrawal syndrome Cumulation Effects of hormone production suppression
D	Long-term effects of pharmacotherapy	Carcinogenesis, teratogenesis, mutagenesis

Preventive toxicology focuses on assessing the hazards posed by chemical compounds in the human environment and developing scientifically based measures to prevent their harmful effects. It is about determining the degree of danger of various chemicals, establishing safe exposure limits, and devising protective strategies. This part of the discipline has a distinctly environmental character and can also be subdivided into several main sections according to the setting and source of exposure. **Industrial toxicology** deals with chemicals in occupational settings, including solvents, heavy metals, and industrial byproducts. Considered one of the most outstanding Russian scientists in the field of industrial toxicology, **N. V. Lazarev** authored the seminal reference book "Harmful Substances in Industry." In this work, he classified all organic compounds into nine groups according to their increasing oil/water partition coefficient. **Agricultural toxicology** focuses on pesticides, herbicides, fertilizers, and other agrochemicals, assessing their impact on farmworkers and surrounding communities. **Food toxicology** investigates contaminants, additives, naturally occurring toxins, and residues in food products, ensuring food safety across the supply chain.

Theoretical toxicology is one of the most diverse and extensive disciplines, as it addresses the problem of identifying the basic laws of the interaction between the body and poisons. This field of study includes mathematical modeling, toxicometry, toxicokinetics, and toxicodynamics.

Chemical and toxicological analysis can also be considered a separate field of toxicology, which is about the isolation, identification, and quantification of toxic, potent, psychotropic, and narcotic substances and their metabolites in biological samples (such as blood, urine, organ tissues, hair, etc.).

What do all these areas have in common? They share a common goal: to study the patterns of the toxic process development, its qualitative and quantitative characteristics, its dependence on the substance structure, effective doses, and the conditions of interaction with biosystems.

Toxicology in general solves the following tasks:

1. Establish quantitative characteristics of toxicity and the correlation between the effects of a chemical substance on the body. This branch of toxicology is known as **toxicometry**.
2. Examine the intoxication symptoms, the course of toxic processes, the mechanisms underlying toxic effects, and the patterns of pathological conditions development. These are the objectives addressed by **toxicodynamics**.
3. Identify the routes of toxicant entry into the body, their distribution, processes of metabolism and elimination. These objectives are addressed by **toxicokinetics**.

The main concept in toxicology is the dose. It is the dose that distinguishes a toxic substance from a non-toxic one. The term **"dose" (D)** refers to the quantity of a substance that acts on a biological system. As the dose increases, the extent of damage to the biological system also increases. This principle underlies the **"dose-effect"**

concept. Furthermore, it is well known that various living systems differ considerably in their biochemical, physiological, and morphological parameters (for example, humans, fish, and mice). In most cases, these differences are attributed to their genetic traits. Consequently, the dose of the same toxicant that can cause harm varies among different organisms. Therefore, the dose-effect relationship reflects not only the characteristics of the toxicant but also the properties of the organism it affects. The dependence of the effect (R) on the substance dose can be visually depicted as a dose–effect curve. The effect observed can vary: the mortality rate, the number of allergic reactions or the drop in a specific blood parameter.

The kinetics describing the interaction of a ligand (L) with a receptor (R) with the subsequent formation of a cellular response can be represented as the equation:

$$L + R \leftrightarrow LR \rightarrow \text{cellular response} \qquad (1)$$

This equation describes the formation of a monophase dose-effect relationship (Fig.1). It is characterized by stimulation of the cellular response until the maximum physiological function is reached. At first, small doses have almost no effect. Then, as the dose increases, the effect grows rapidly. Finally, the growth slows down and the maximum effect is reached when all the target molecules are occupied. However, in some cases, biphasic dependencies can be found; they are characterized by stimulation of the cellular response with low doses and its subsequent suppression by large doses.

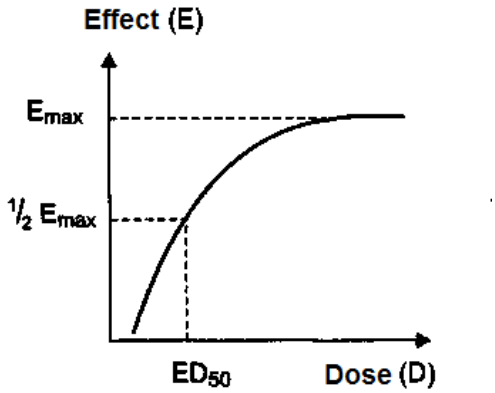


Figure 1 - Monophase dose-effect relationship

The assignment of chemicals to the respective hazard class (see Table 2) is based on the values of lethal toxodose and effective toxodosis.

Lethal toxodose LD is the amount of a toxicant that causes death with a certain probability if ingested.

Effective toxodose ED is the amount of a toxicant that causes a certain effect when ingested with a certain probability.

Table 2. Classification of industrial harmful substances by degree of danger.

Parameter	Toxicity classes			
	I (extremely dangerous)	II (highly dangerous)	III (moderately dangerous)	IV (mildly dangerous)
LD_{50} (peroral), mg/kg	less than 15	15–150	151–5000	more than 5000
LD_{50} (applicational), mg/kg	less than 100	100–500	501–2500	more than 2500
CL_{50} (inhalational), mg/m^3	less than 500	500–5000	5001–50000	more than 50000

Lab work «Determination of the hazard class of substances»

Legend: The toxicometric laboratory received a soil sample from an oil refinery. It is known that 15 kg of the sample contains 700 mg of sulphur, 1 kg of chromium (III) oxide and 3 kg of lead.

Task: using the tables, calculate the hazard index for each substance.

Table. Maximum permissible concentrations (MPC) and solubility of substances

Substance	**MPC, mg/м^3**	**Solubility, g**	**Equivalent LD_{50} mg/kg**
Chromium (III) oxide	1	Non soluble	>5000
Sulphur	6	Non soluble	4.1
Lead	0.05	4.5	5000

Table. Classification of toxicants by hazard index

Calculated value of the general hazard index	**Hazard Class**	**Danger level**
<1.2	I	Extremely dangerous
от 1.2 до 2.2	II	Highly dangerous
от 2.2 до 10	III	Moderately dangerous
More than 10	IV	Low danger

For each of the three substances, the hazard index is calculated using the formula:

$$K_i = \frac{\lg(LD_{50})}{(S_i + 0.1F_i + C_{вi})}$$

where S_i – is substance solubility coefficient (for insoluble $S_i = 0$), F_i – is volatility coefficient (for non-volatile F_i= 0), $C_{вi}$ – is substance content in the total mass of waste.

The substance content in the total mass of waste is calculated using the formula:

$$C_{вi} = \frac{C_i}{\sum C_в}.$$

Determine the total hazard index using the formula:

$$K_{sum} = \frac{1}{n}\sum_{i=1}^{n} K_i$$

Create a table with the parameters obtained, enter the data into the table, use the total hazard index to determine the hazard class, present the results visually (in the graph format) and draw a conclusion about the results obtained.

Topic 2. Basic concepts of toxicokinetics

Toxicokinetics is the branch of toxicology that examines the qualitative and quantitative aspects of how xenobiotics are absorbed, distributed, metabolized, and eliminated by the body (Fig. 2). As a substance moves through these stages, it is affected by such processes as dissolution, diffusion, convection, osmosis, and filtration through biological barriers.

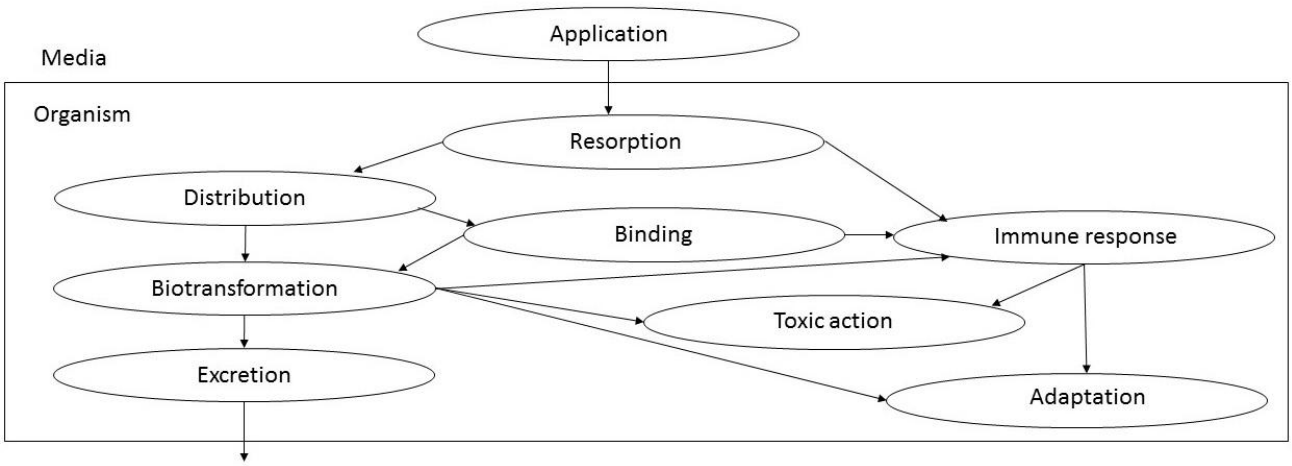


Figure 2 - The stages of xenobiotic interaction with the body

Key toxicokinetic parameters include the **constants for absorption, transfer, excretion, and elimination**. Other critical indicators are the half-lives for absorption, distribution, and elimination from plasma. The elimination half-life, specifically, is the time required for the plasma concentration of a substance to decrease by half. The degree of systemic uptake is quantified by the volume of distribution, while the extent of distribution is characterized by the percentage of toxicant bound to plasma proteins, primarily albumin. Furthermore, **bioavailability**—the fraction of an administered dose that reaches systemic circulation—is a fundamental toxicokinetic property.

Toxicokinetic curves are used to model changes in blood toxicant concentration, based on experimental data. These curves are generated using physical and chemical and microbiological assays, with High-Performance Liquid Chromatography (HPLC) being the most common method. Additionally, detecting the toxicant's active metabolites is typically an integral component of this analysis.

Toxicokinetic curves are graphs showing changes in the concentration of a toxicant and its metabolites over time (Fig. 3). The graph shows the characteristic areas: 1 – the stage of increasing concentration, 2 – the stage of reaching the maximum (constant concentration), 3 – the stage of decreasing concentration (elimination from the body), 4 – the stage of cessation of concentration changes due to elimination.

The most important indicators are the following:

C_{max} is the maximum concentration achieved;

t_{max} is the time to reach maximum concentration.;

$t_{1/2}$ is the half–life of the substance from its maximum concentration;

AUC is the area under the pharmacokinetic and toxicokinetic curve.

The area under the toxicokinetic curve (AUC) shows the total exposure of the drug over time and allows to calculate the constants of the absorption rate and elimination of the toxicant:

$$Cl = \frac{D}{AUC}.$$

where D – is a dose of toxicant, Cl – is the constant of elimination.

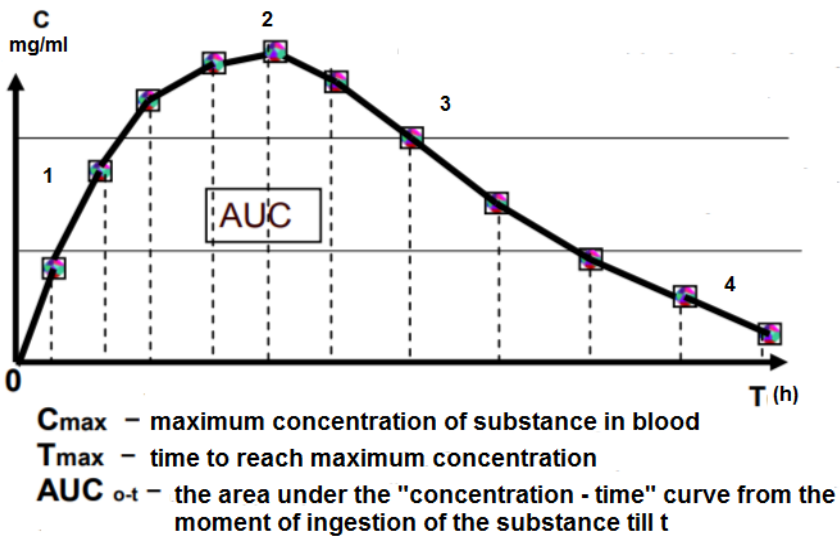


Figure 3 - Pharmacokinetic curve (example)

When a substance enters the body intravenously, absorption is not taken into account, since the substance enters the bloodstream evenly and simultaneously. Thus, this process is subject to the following law:

$$\frac{dm}{dt} = -k_{эл}m,$$

$$\frac{dm}{dt} = -k_{эл}m,$$

where m is a mass of the substance and $k_{эл}$ is the elimination rate constant.

$$m = m_0 \exp(-k_{эл} t),$$

where m_0 is the initial mass of the substance.

Consider the volume to be constant; therefore, the equation can be transformed:

$$C = C_0 \exp(-k_{эл} t).$$

The logarithm can be applied to this equation:

$$lnC = lnC_0 - k_{эл} t.$$

The slope of the line, determined from the graph, is equal to the elimination constant (Fig.4).

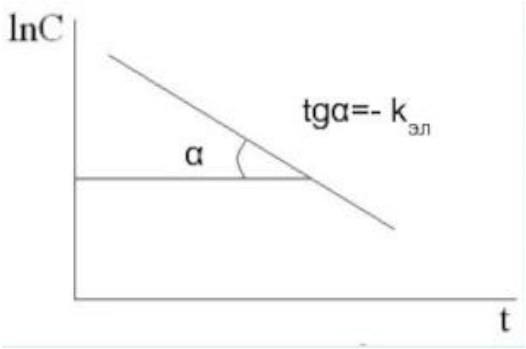


Figure 4 – Determination of the elimination constant

Consider a case where the toxicant was ingested orally. In this case, the substance is assumed to be absorbed in proportion to its mass. According to the law of acting masses, the change in mass over time is represented as:

$$\frac{dm}{dt} = k_1 m_1 - k_2 m_2,$$

where m_1 is the mass of the substance at the application site, k_1 is the suction rate constant, and $k_{эл}$ is the elimination rate constant.

Thus, the concentration is presented as:

$$C = C_0(\exp(-k_{эл} t) - \exp(-k_1 t)),$$

where C_0 is the apparent initial concentration of a substance at the initial time.

The constants of the rate of absorption and elimination can be found by the tangents of the angles of inclination of the asymptotes drawn to the initial and final sections of the graph (C/C_0, t).

To find C_0, it is necessary to plot a graph in the coordinate system (lnC, t) and find the necessary parameter at the intersection of the ordinate axis with the asymptote.

The toxicokinetic characteristics of a substance are determined by its properties and features of the structural and functional organization of cells, organs, tissues, and the body as a whole.

The properties of the toxicants that determine their toxicokinetic parameters are:

- aggregate state;

- the coefficient of distribution in the oil/water system (fat-soluble toxicants accumulate in lipids; water-soluble toxicants accumulate in the aqueous phase of blood plasma, intercellular and intracellular fluids);

- the size of the molecule (the larger the molecule, the lower the rate of its diffusion, the more difficult the filtration processes are, etc.);

- the presence of charge in the molecule (charged molecules do not penetrate ion channels well, do not penetrate lipid membranes, do not dissolve in the lipid phase of cells and tissues);

- the value of the dissociation constant of salts, weak acids and bases;

- chemical properties.

The rate and extent of a substance's absorption into the bloodstream is determined not only by its route of administration but also by multiple factors, including the administered volume, its physical form (shape), temperature, pH, degree of protein binding, and other parameters.

The absorption, distribution, and elimination of a toxicant require its dissolution in body fluids (e.g., blood, mucus) and translocation across biological barriers via mechanisms such as diffusion, filtration, cytosis, or active transport. Nonionic diffusion primarily facilitates the passage of nonionized, lipophilic molecules. Simple diffusion is characteristic of small nonpolar molecules and small, uncharged polar compounds. Conversely, the transport of high-molecular-weight toxicants is typically mediated by specific transporter proteins (translocases).

Most of the toxicants enter the body **orally** (Fig. 5). Some fat-soluble compounds, such as phenols and cyanides, are already absorbed in the oral cavity and enter the bloodstream, bypassing the gastrointestinal tract.

The gastrointestinal tract exhibits a pH gradient along its length, which significantly influences the absorption rate of toxicants. Gastric juice, with a pH close to 1, creates an acidic environment. Consequently, acidic compounds entering the stomach remain largely non-ionized and are readily absorbed. In contrast, non-ionized bases, such as morphine, become ionized in the stomach's acidic milieu, limiting their absorption there and facilitating their passage into the intestines.

The rate of absorption in the stomach depends on the intensity of blood circulation in its mucous membrane, peristalsis, mucus formation, etc. In the stomach, toxicants can also be absorbed and diluted by food masses. This means that the contact of the toxicant with the gastric mucosa is difficult.

Most toxicants are absorbed in the small intestine, where the pH is neutral to slightly alkaline (7.5–8.0). Lipid-soluble substances diffuse easily across the intestinal wall. For electrolytes, absorption depends on their ionization state. Compounds that resemble natural body chemicals are well absorbed through active transport or pinocytosis. However, metal-protein complexes are poorly absorbed. In contrast, organophosphorus compounds (OPs) are absorbed from the gut very quickly, with blood levels peaking anywhere from a few hours to several days later. OPs distribute

unevenly in the body, but after oral intake, high concentrations are typically found in the liver and lungs.

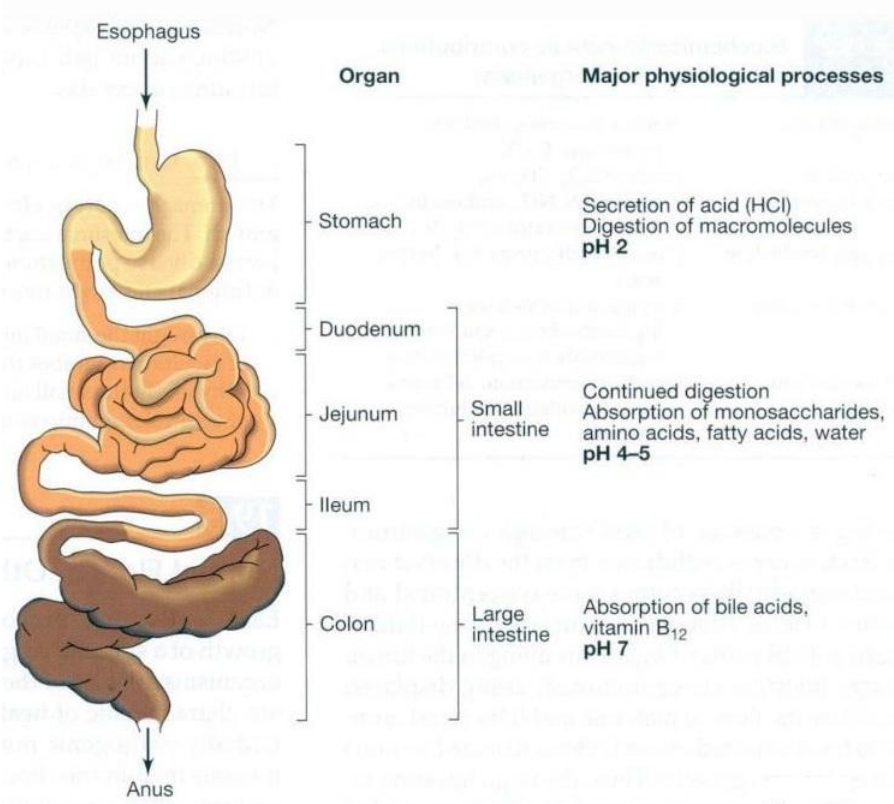


Figure 5 – The scheme of the human gastrointestinal tract

Inhalation poisoning is characterized by the fastest release of the toxicant into the blood. From the perspective of pulmonary absorption, the alveoli represent the most significant site (Fig. 6). The alveolar wall is composed of a thin alveolar epithelium, supported by an interstitial framework that includes a basement membrane, connective tissue, and a capillary endothelium.

The toxicity of gases and vapors depends on their water and fat solubility (hydro-/lipophilicity) and chemical reactivity. Water-soluble gases are absorbed in the moist lining of the upper airways. Fat-soluble gases and vapors travel to the alveoli, are absorbed there, and cause damage. Their absorption rate is influenced by solubility in blood, blood flow speed, and metabolic rate. For instance, mercury vapor is almost fully absorbed in the lung alveoli after inhalation, while only about 50% of inhaled cadmium vapor or inorganic lead compounds is absorbed there.

Gaseous toxicants, highly soluble in blood, are easily absorbed by the lungs. Gaseous toxicants with low solubility in the blood are easily excreted from the lungs with exhaled air.

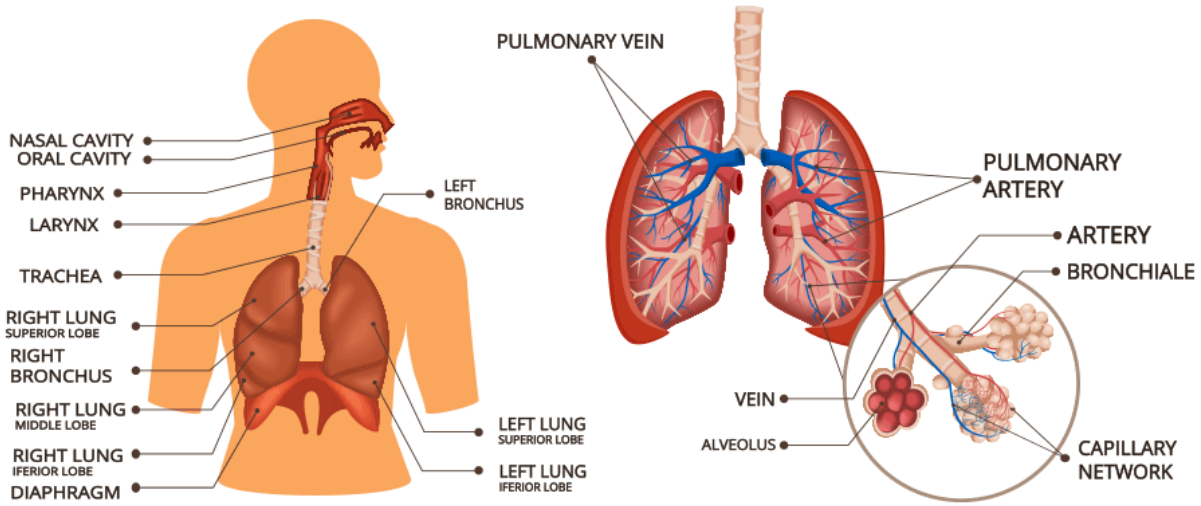


Figure 6 - The scheme of the human respiratory tract

The sorption of toxicants through the lungs depends on their chemical nature. Vapors and gases of non-reactive substances (aromatic and aliphatic hydrocarbons) do not change in the body, or their changes occur more slowly than accumulation in the blood. Vapors and gases of reacting substances (ammonia, sulfur dioxide, nitrogen oxides), quickly dissolving in body fluids, easily enter into chemical reactions.

Toxicants can cross the **skin** through three main routes: the epidermis (typical for non-electrolytes), hair follicles (for both electrolytes and non-electrolytes), and the ducts of sweat glands (Fig. 7).

The quantity of a toxicant that enters the systemic circulation via the dermal route is directly influenced by its hydrophilicity and lipophilicity, the surface area of skin exposure, and the local cutaneous blood flow. Furthermore, mechanical damage to the skin, as well as thermal or chemical burns, significantly enhances the percutaneous penetration of toxins.

Volatile liquid organic toxins rapidly evaporate from the skin surface, preventing significant dermal absorption. In contrast, for substances like organophosphorus compounds (OPs) entering via the transdermal route, the extent of absorption depends on factors such as dose, exposed surface area, skin integrity, and the compound's hydrophobicity. Many lipophilic OPs, in particular, exhibit high percutaneous absorption rates.

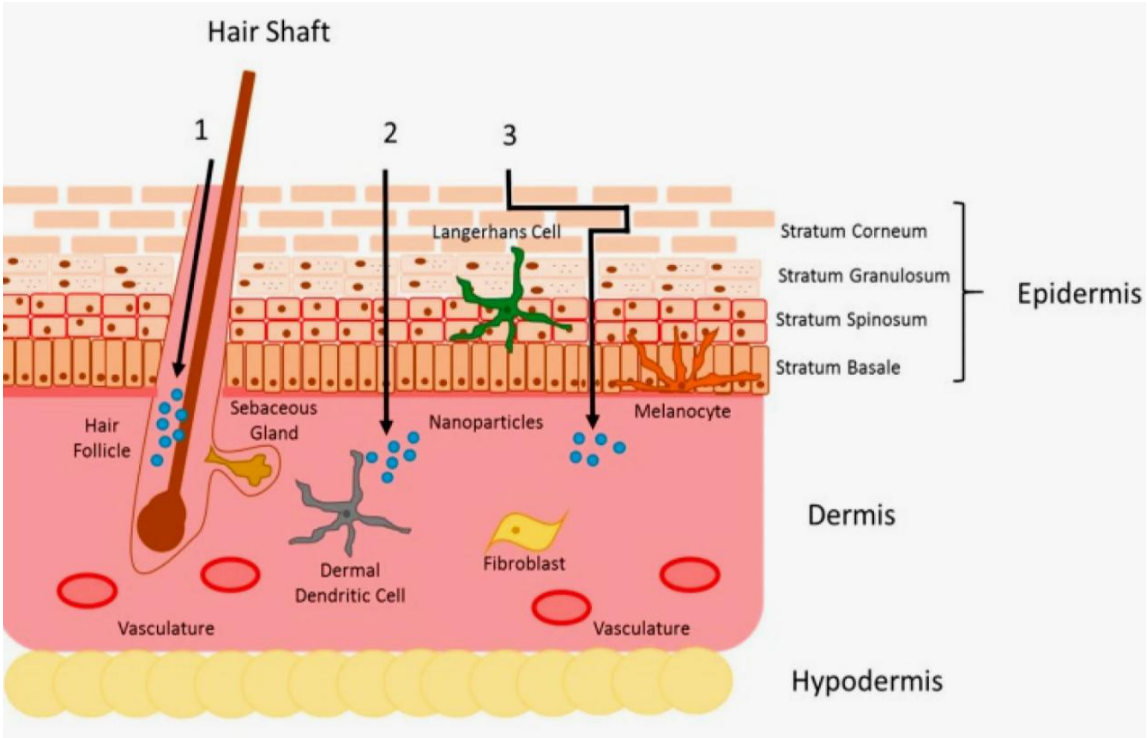


Figure 7 – Routes of penetration of toxicants through the skin

Solid and crystalline organic toxins can be absorbed percutaneously, leading to slow, cumulative poisoning. The most hazardous are low-volatility, oily substances due to their efficient skin penetration and prolonged retention. Furthermore, salts of mercury and thallium can react with skin lipids—such as fatty acids and sebum—forming lipophilic complexes that readily cross the epidermal barrier.

Upon dermal contact, liquid sulfur mustard rapidly dissolves in the skin's lipid layer, penetrates the epidermis, and accumulates in sebaceous glands and hair follicles. This leads to systemic poisoning and the formation of severe cutaneous lesions and ulcers.

Metabolism, or biotransformation, is the process by which the body chemically alters exogenous substances (xenobiotics). The biological purpose of this process is to convert chemicals into a form that is easier to excrete, which shortens the time they can act in the body. Microsomal (involving monooxygenases) and non-microsomal (involving cytosolic, mitochondrial, and other enzymes) mechanisms of xenobiotic metabolism are distinguished.

The first phase of biotransformation is carried out mainly by a large group of cytochrome P450 family enzymes responsible for the metabolism of foreign organic compounds. Enzymes of the cytochrome P450 family are functionally diverse, catalyzing reactions such as oxidation, reduction, and hydrolysis. They exhibit both monooxygenase and oxidase activities and are therefore classified as mixed-function oxidases. During microsomal oxidation, reactive intermediates are often formed, some of which are unstable and may undergo further transformations. The enzymes of the

monooxygenase system during biotransformation of lipophilic xenobiotics can not only detoxify them, but also participate in the formation of more toxic metabolites.

Metabolites produced in Phase I of biotransformation can undergo further changes in Phase II. In Phase II, a water-soluble molecule from the body attaches to the chemical, forming a conjugate. This pairing makes the substance more water-soluble and easier to excrete. The second phase of biotransformation includes conjugation reactions such as glucuronidation and sulfation. Other major Phase II pathways are acetylation, methylation, conjugation with glutathione and amino acids (e.g., taurine, glycine, glutamic acid), as well as the conversion of epoxides to diols catalyzed by epoxide hydrolases. The enzymes that activate the Phase II biotransformation process are: UDP-glucuronosyltransferase, sulfotransferase, glutathione-S-transferase, quinone reductase.

The elimination of toxicants from the body can be carried out in various ways – with urine, feces, bile, sweat, vomiting, or exhaled air (by diffusion). Cleansing the body of exogenous substances includes various types of detoxification, which together determine the so-called "total clearance". The body's defense systems are divided into:

- systems of limited toxic effects of xenobiotics (barriers, tissue depot);
- systems used to eliminate the toxic effects of xenobiotics (transport, enzymatic);
- systems of excretion of xenobiotics and their metabolites from the body.

The primary physiological barriers are the organs of entry: the respiratory tract, skin, gastrointestinal tract, and mucous membranes. Among tissue-level barriers, the alveolar-capillary membrane in the lungs and the mucosa of the small intestine possess the greatest surface area. The liver serves as the principal secondary defensive barrier. Internal protective systems include the blood, lymph, connective tissue, and the histohematic barrier. Specialized barriers encompass the blood-brain barrier (BBB), the blood-aqueous barrier (between blood and intraocular fluid), and the blood-testis barrier, among others.

In case of inhalation poisoning, the main part of the toxicant enters the kidneys and is excreted in the urine. In most cases of oral poisoning, the toxicant accumulates in the liver and is excreted in the feces. Fat-soluble compounds are usually excreted in sweat and breast milk.

The kidneys eliminate toxicants through three main processes: filtration, secretion, and reabsorption. Filtration removes small, dissolved molecules from the blood through the kidney's glomerular filter. Secretion moves specific compounds—like organic acids, uric acid, and some organic bases—from the blood into the urine via the tubule walls. Reabsorption pulls useful substances, such as fat-soluble compounds and electrolytes, from the urine back into the blood through the tubule cells.

Task

Legend: Max, the carpenter, got the pills mixed up and instead of aspirin he took orally an unknown substance with a dosage of 40μg/kg. To identify the drug Max had taken, identical blood aliquots were collected in the laboratory at regular intervals (every 10 minutes). The laboratory was able to calculate the quantitative content of the drug in each aliquot (see the table).

Task: Calculate elimination and absorption coefficients of the drug taken by Max based on the formulae given above and present the results in the report format.

Table - The analysis data to determine the quantitative content of the drug in each aliquot.

Time, min	lnC, μg/mg	Time, h	lnC, μg/mg
10	0.45	70	1.38
20	0.36	80	1.37
30	0.69	90	1.21
40	1	100	0.96
50	1.2	110	0.69
60	1.3	120	0.48

Topic 3. The importance of lipophilicity in toxicology

Lipophilicity (LogP) is a fundamental parameter that directly affects such important stages of toxicokinetics as the rate of overcoming skin barriers, mucous membranes and the blood-brain barrier, the volume of substance distribution, the degree of binding to plasma proteins, interaction with biotransformation enzymes, as well as the mechanisms and rate of elimination of substances from the body.

The penetration of substances through the membranes can be carried out in three ways: active transport (with the participation of Z-glycoproteins), passive diffusion (through the lipid bilayer without energy expenditure) and facilitated diffusion (through ion channels or along a concentration gradient using carrier proteins). Biological membranes consist primarily of lipids. For effective passive penetration, the substance must be sufficiently lipophilic to overcome the hydrophobic barrier and moderately hydrophilic for further transport in the blood. High lipophilicity promotes better passage through cellular barriers and thus provides a potent effect on target receptors. However, too high lipophilicity of the substance can lead to an increase in the intensity of metabolism and, consequently, to a decrease in the concentration of the active substance in the blood. The binding of a substance to proteins is of great importance, since it determines the intensity of its action and the ability to penetrate into tissues. Highly lipophilic compounds bind better to plasma proteins, thereby reducing the effectiveness of the drug. Due to their high affinity for adipose tissues, lipophilic compounds accumulate better in these tissues, while increasing the risk of side effects.

Lipophilicity is characterized by duality, which is caused by nonpolar and polar interactions. The contribution made by nonpolar interactions is related to the physical properties of the molecules, limiting their hydrophilicity and contributing to their lipophilicity. These factors include molecular volume, polarity, and molar refraction. An increase in these indicators contributes to an increase in the lipophilic ability of compounds. Polar interactions include various types of intermolecular interactions, for example, electrostatic interactions between charged groups, dipole-dipole interactions, and hydrogen bonds. The polarity negatively affects the lipophilicity of the compound by increasing the interaction of the substance molecules with water, reducing the possibility of penetration through biological membranes.

In chemistry, lipophilicity is commonly expressed as the logarithm of the distribution coefficient (LogP), shown in Table 3, or as the dissociation coefficient (LogD). LogP is included in equations used for quantitative structure–property relationship (QSAR) calculations, which are often employed in the modeling of new molecules. Additionally, this parameter is part of the Lipinski rule of five, which states that for a compound to be considered "drug-like," it should have no more than five hydrogen bond donors, a molecular weight under 500 Da, a LogP value below 5, and no more than ten nitrogen and oxygen atoms in total.

Table 3. Dependence of a substance's properties on the LogP value

Value	**Effect**
Log P < 0	the substance is hydrophilic and dissolves well in water; the compounds do not penetrate membranes well
Log P ≈ 1-3	the substance demonstrates moderate lipophilicity, optimal value for most drugs, good penetration through membranes without excessive accumulation
Log P > 3-4	highly lipophilic substances that tend to bind strongly to membranes and accumulate in lipid tissues
Log P > 5	the substance demonstrates high ability to penetrate the blood-brain barrier (BBB); the compounds have low solubility and poor absorption

There are several ways to assess the lipophilicity of a substance: chemical and analytical methods, and computational methods. Typically, these methods involve quantifying LogP or LogD.

The simplest and most reliable approach for assessing lipophilicity is the direct separation (shake-flask) method. This principle involves mixing an aqueous and an

organic solvent with the test compound, followed by monitoring phase separation. However, despite its apparent simplicity, this method is labor-intensive and time-consuming, and it suffers from limited sensitivity at very low concentrations. It is also unsuitable for unstable compounds. For ionized compounds, LogP measurement requires multiple experiments at different pH buffers to capture the pH-dependent distribution. For these reasons, analytical chemists often opt for more accurate methods, such as high-performance liquid chromatography, titration, mass spectrometry, and fluorescence spectroscopy.

The two-phase potentiometric titration method is well suited for ionized compounds. This method is based on measuring the chemical potential with a specialized electrode while adding acid or base to the test solution. To determine the logarithm by acid-base titration, the aqueous pKa of the compound must be known. For monoprotic acids, the distribution coefficients are calculated from the difference in pKa values using the equation provided below:

$$P = \frac{(10^{poK_a - pK_a - 1}) \times V_{H_2O}}{V_{org}} .$$

For a monoprotonated base the equation is:

$$P = \frac{(10^{-(poK_a - pK_a)_{-1}}) \times V_{H_2O}}{V_{org}} .$$

Despite the well-known disadvantages of classical methods of LogP assessment, it is possible to partially mitigate them and increase the effectiveness of research.

Reversed-phase high-performance liquid chromatography (RP-HPLC) has proven to be applicable in modeling the octanol-water separation process and is considered the most popular alternative to empirical methods used for lipophilicity assessment. This method offers several practical advantages, including high-speed analysis, reproducible results, insensitivity to impurities or degradation products, a wider dynamic range, prompt detection, and a relatively small number of samples required.

The analysis of the substance distribution between the stationary and mobile phases of a chromatographic column allows for a quick estimation of LogP. The lipophilicity index measured by HPLC is calculated from the retention time, which can be converted to the logarithm of the retention coefficient, log k.

To calculate LogP, the logarithm of the retention rate should be substituted:

$$LogP_{oct} = alogk + b ,$$

where a and b are the constants of linear regression.

Predicting lipophilicity is challenging because molecules are rarely simple. For complex molecules with intricate conformations, different prediction systems often yield varying results that deviate from the true LogP value. Many empirical approaches

to determine lipophilicity rely on neural network analysis of compound databases. For example, ALOGPS 2.1 program was specifically developed to predict LogP and evaluate water solubility of neutral compounds. It is based on an associative neural network (ANN) model and takes into account atomic electronic state indices, bond types, as well as the numbers of hydrogen and non-hydrogen atoms. This application enables simultaneous calculation and comparison of lipophilicity and aqueous solubility using multiple methods.

Lab work "Computational determination of the lipophilicity coefficient (LogP) of organic compounds"

The purpose of the lab work is to calculate LogP for ethanol using the Leo-Hansh method.

For ethanol, calculate LogP using the method of atomic and fragmentary Leo-Hansh constants and tables:

$$LogP = a_i f_i + F_{i,}$$

where f_i is the constant of the fragment (atom or group of atoms), F_i is the corrective factor.

Table - Fragmentary LogP constants (from Rekker)

Fragments	**Structure**	**f_i constant**
$–CH_3$	terminal methyl	0.70
$–CH_2–$ (methylene)	in chain	0.53
–CH<	tretiary	0.46
–C<	quaternary	0.39
–OH	alcohol	–1.16
–O–	ether	–0.86
–CHO	aldehyde	–0.77
–C(O)– (ketone)	carbonyl	–0.77
–COOH	carboxyl	–0.79

The Leo-Hansh method is used to predict the biological activity of compounds and to calculate hydrophobicity of a substance.

Table – F_i constants to Leo-Hansh method

Type of interaction	**F_i constants**
The hydrogen bond O–H…O (dimer)	–0.42

Intramolecular H-bond	+0.30

Topic 4. Basic concepts of toxicodynamics

Toxicodynamics answers the question "How and why does this substance cause poisoning?" in contrast to toxicokinetics, which answers the question "What happens to the substance?".

To understand how a particular substance affects the body, it is necessary to understand the specifics of its interaction with certain systems. It is known that many toxicants are divided into different groups based on their mechanism of action (Table 4). This means that each substance acts on a specific "favorite area" (a receptor) of the body.

Table 4.Classification of toxicants due to their toxicodynamics

Group	Disorder	Toxicant
cardiotoxicants	cardiac arrhythmias, myocarditis	glycosides, quinine
neurotoxicants	nervous system disorders, psychosis, seizures, and coma	sleeping pills, phosphorous organics, carbon monoxide, alcohol, and its substitutes
hematotoxicants	hemolysis, methemoglobinemia	aniline, nitrites
nephrotoxicants	kidney diseases	ethylene glycol, heavy metal salts
hepatotoxicants	liver diseases	chlorinated hydrocarbons, poisonous mushrooms, phenols
pulmanotoxicant	fibrosis, obstructive lung diseases	phosgene

The idea of a "favorite area" was put forward by scientists back in 1878 and the term "receptor" was proposed in 1910 by W. Ehrlich. Ehrlich believed that a substance was ineffective if it was not fixed. He presented the idea of receptors in the form of sections of the body's molecules with a "complementary" structure to the poison. The receptors can be enzyme sites, mediators, hormones, proteins and various blood components. For example, the oxy group of serine, in the molecule of the enzyme acetylcholinesterase, serves as a receptor for organophosphorus compounds. Amino acids, for example, have a high ability to bind metals. Opioid receptors are proteins embedded in the membranes of nerve cells. Currently, there are four types of specific receptors. The first type of receptor includes those that control the function of ion channels (ionotropic receptors). The second type includes receptors that are affected by G-proteins (adrenergic receptors). The third type includes those that control the

functions of tyrosine kinases (insulin receptors). The fourth type includes nuclear proteins that control DNA transcription (steroid hormone receptors).

The history of narcotic analgesics dates back to ancient times. However, it was not until 1805 that F. Sertürner isolated morphine from opium, the most controversial toxicant. It was not until more than a century later that opioid receptors were discovered. This discovery was a significant milestone not only in toxicology but also in pharmacology, as it provided insights into the mechanisms of pain perception and various behavioral responses. The first opioid receptor toxicants (ligands) that were discovered were of natural origin. Synthetic agonists were discovered many years later.

Currently, there are 5 known types of opioid receptors. However, many ligands for these receptors can interact with different types of opioid receptors rather than a specific one. For example, μ-receptors are the target for most analgesics and drugs, while κ-receptors are involved in the action of some hallucinogenic opioids. All substances that interact with opioid receptors are divided into three groups: antagonists (substances that block the action of opioids without interacting with the receptor), agonists (substances that bind to opioid receptors and activate them, causing a physiological response), and analgesics. For example, morphine and codeine are both considered narcotic agonists.

For toxicology, the nature of the toxicant-receptor bond is of great importance. Most toxic substances bind weakly to receptors through ionic, hydrogen and van der Waals bonds. These bonds are fragile and can be broken, for example, by a more favorable interaction with a chemically stronger antidote. Covalent bonds between toxicants and receptors are very strong and difficult to reverse, that makes them more dangerous; these substances include, for example, organophosphorus compounds.

Electrostatic (ionic) interactions are considered the most labile. However, they are dangerous because they can precede the formation of covalent bonds. This is how phosphorous organics are fixed on the surface of cholinesterase.

There are several ways in which toxicants can affect the body: antifermentative, non-electrolyte and membranotropic effects; effects on the genome; effects on energy metabolism; effects on neurotransmitter systems.

The toxicity of substances depends on their state of aggregation (liquid, gaseous, or solid), solubility (water-soluble or fat-soluble), and chemical structure (alcohols, glycols, esters, etc.). The formation of the substance-receptor complex also depends directly on the affinity of the substance to the receptor: the higher the affinity (the more active sites have reacted), the higher the toxic effect.

For example, most **organophosphorus compounds** are highly volatile and soluble in water and fat. Therefore, they can easily penetrate biological membranes and the blood-brain barrier. Their main targets are acetylcholinesterase, butyrylcholinesterase, carboxylesterase and phospholipase B. The basis of the toxic mechanism of action of organophosphorus compounds is the inhibition of acetylcholinesterase, an enzyme that catalyzes the hydrolysis of acetylcholine. The

inhibition of this enzyme leads to accumulation of acetylcholine. This excess acetylcholine overstimulates cholinergic receptors, initially causing intense excitation of nerve cells, followed by paralysis of organ function.

The effect of **blood poisons** is completely different. Their damaging effect is directed at blood cells, bone marrow, and the hemostasis system. Depending on their specific effects, blood poisons are divided into hemolytic, hemostatic, and coagulant poisons (Table 5).

Table 5. Mechanism of action of blood poisons.

Type	Action	Example
hemolytic	hemolysis (a poison damages the lipid bilayer of the erythrocyte membrane); conversion of hemoglobin to methemoglobin	benzene, aniline, acetic acid
hypoxicants	formation of persistent carboxyhemoglobin	carbon monoxide
hemostatic (anticoagulant)	disrupt blood clotting, causing either thrombosis or bleeding	viper poisons

Lab work «Determination of antimony in a sample»

Legend: You have received a sample for analysis. Presumably, the victim was poisoned with antimony.

Task: Conduct the reaction and tell whether this prediction is true.

Attention! When trivalent antimony reacts with sodium thiosulfate in an acidic environment, an orange precipitate forms when heated.

You will need a sample (with pre-dissolved antimony salt) and a control sample with water, a magnetic stirrer with heating, two heat-resistant cups, measuring cylinders, and a 10 ml automatic pipette.

Course of work: A weighed portion of $Sb_2(SO_4)_3$ is added to the first glass of water, and 1 ml of concentrated sulfuric acid is slowly added. After that, 5 drops of a saturated sodium thiosulfate solution are added, and the mixture is then boiled for 1-2 minutes. The formation of an orange precipitate of Sb2 S3 indicates the presence of antimony in the mineralization. Repeat the same steps (except for adding antimony salt) for the second sample.

Compare the results and write a report.

Topic 5. Mechanisms of cytotoxicity

Test systems using cell cultures are the most common *in vitro* method for evaluating toxicity. If a substance produces a toxic effect on a cell culture, it is

considered cytotoxic. **Cytotoxicity** refers to the ability of a chemical or physical agent to cause pathological changes in cells, including cell death or disruption of the cell cycle.

Chemicals at the cellular level can have three types of toxic effects:

- general cytotoxicity (affects at least one of the vital cellular functions, processes, organelles);
- selective cytotoxicity (damages certain types of differentiated cells that are more sensitive to a certain toxic compound than other cells);
- specific cytotoxicity (due to the effect of a toxic substance on structures or processes that are not vital for the target cell itself, but are critical for the body as a whole).

The toxic effect of many substances is associated with their influence on the state of membrane structures and, in terms of the form of impact, can be direct or indirect (Figure 8).

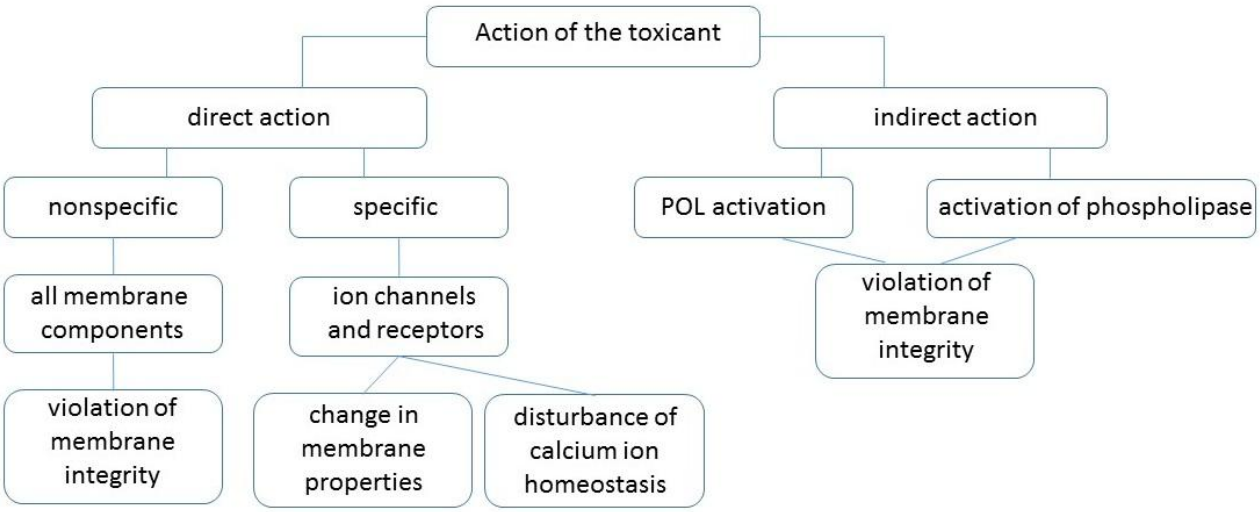


Figure 8 - Action of the toxicant onto the membrane state

Cytotoxicity is expressed in damage to the integrity of biological membranes and cytoskeleton, impaired synthesis, degradation or release of important biomolecules, ion homeostasis (Table 6), energy status of cells and cell division.

Many mechanisms can disrupt cellular energy metabolism, ultimately leading to cell death. Cells of the nervous system, kidneys, and myocardium are particularly sensitive to this disruption. An indirect damaging mechanism involves the impairment of the body's oxygen transport systems and a consequent drop in tissue partial pressure. Certain toxicants, like chlorophenols, act directly by binding to the active sites of enzymes responsible for biological oxidation and phosphorylation, thereby disrupting these vital processes. Inhibition of tricarboxylic acid (TCA) cycle enzymes is especially detrimental. Similarly harmful are toxicants, such as cyanides, that block enzymes within the respiratory chain.

Cell death during acute poisoning is often mediated by the disruption of intracellular calcium homeostasis, characterized by a pathological increase in

cytoplasmic Ca^{2+} concentration. This homeostatic failure can result from multiple insults, including damage to biological membranes (leading to increased ion permeability), impaired cellular bioenergetics, and modifications to the state of the protein complexes that regulate Ca^{2+} channel function.

Table 6. Violation of calcium homeostasis by toxicants

Disruptions	**Toxicants**
Release of Ca^{2+} from mitochondria	Dinitrophenol, quinones, dinitrocresol, peroxides, iron, cadmium
Release of Ca^{2+} from EPR	Bromobenzene, peroxides, quinones, aldehydes
Increased Ca^{2+} influx through the cytoplasmic membrane	Carbon tetrachloride, dimethylnitrosamine, paracetamol
Inhibition of Ca^{2+} from the cell	Quinones, cystamine, vanadium, peroxides

The cytotoxic effect is associated with damage to the integrity of the cytoskeleton, activation of phospholipase, protease and endonuclease.

Toxicants can damage membrane structures through two primary mechanisms: the activation of lipid peroxidation and the activation of phospholipases (Fig. 9). When phospholipases are activated, they hydrolyze phospholipids, which destroys membrane integrity and releases arachidonic acid. This arachidonic acid is then converted into eicosanoids, driving the activation of inflammatory processes in tissues.

Following entry into the body, some xenobiotics undergo biotransformation, yielding free radical metabolites. These reactive species are available for interaction with target biomolecules. The pathological impact of such radicals is governed by their chemical stability and migratory range from the site of generation. A representative example is the hepatic metabolites of pyrrolizidine alkaloids, which can migrate to induce lung damage. The free radical potential varies significantly between compounds: chloroform is a weak source due to its low reactivity, whereas carbon tetrachloride is efficiently metabolized to a trichloromethyl radical.

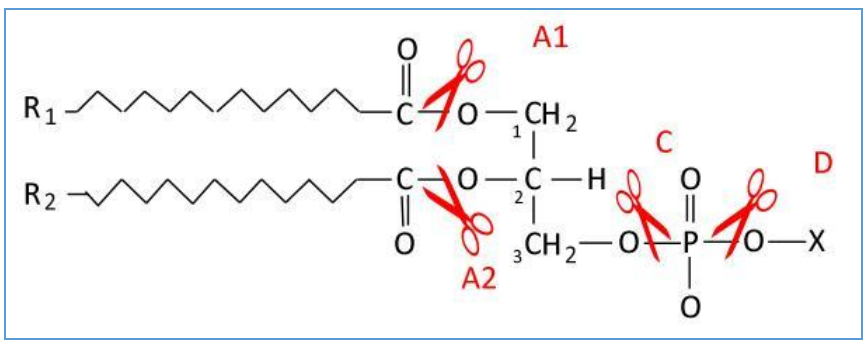


Figure 9 - A phospholipid and the position of ester bonds hydrolyzed by various classes of phospholipases

Damage to RNA and DNA molecules is at the heart of disorders of protein synthesis, cell division, and transmission of hereditary information by toxicants (Table 7). For example, thiol poisons interacting with SH groups can damage the mitotic spindle and disrupt cell division.

Table 7. Disruption of protein synthesis and cell division processes.

Disruptions	**Process**
DNA synthesis. Replication	DNA conformation change Disruption of the DNA polarization process Disruption of nucleotide synthesis Disruption of the DNA repair process Dysregulation of DNA synthesis
RNA synthesis. Transcription	RNA disruption Disruption of RNA polymerization Disruption of the RNA procession Disruption of nucleotide synthesis Disruption of RNA regulation mechanisms
Protein synthesis. Translation	Disruption of the organization and procession of ribosomes and polysomes Disruption of amino acid polymerization Violation of protein conformation and its tertiary and quaternary structures Violation of the mechanisms of translation regulation

The result of the toxic effect of a substance on DNA is mutagenesis. Some substances cause mutations in cells at a certain phase of the cycle (cyclo-specific). Cyclone-specific mutations are characteristic of toxicants acting on the genetic apparatus regardless of the cycle.

Since the features of the structure and function of cell groups forming various organs and tissues are extremely significant, and the sensitivity of these cell types to toxic compounds can vary greatly, cell lines are used in the *in vivo* tests, depending on the study aims.

In vitro cytotoxicity assays can be categorized into major groups based on the mechanism of cellular damage they assess. The first one includes the tests that measure the loss of membrane integrity, a hallmark of cell death. Viability assays measure the percentage of live cells remaining after exposure to a damaging agent, which can be any chemical compound. Most of these tests rely on detecting compromised membrane

integrity, as non-viable cells become permeable to dyes that normally cannot enter. Trypan blue is the most common *in vitro* dye used for this purpose (Fig.10a). When added to a cell suspension, it stains cells with damaged membranes, leaving viable cells unstained. A key limitation of this method is its low accuracy, as it assesses cell viability based solely on membrane integrity rather than functional activity. Another common marker, propidium iodide, specifically identifies late apoptotic or necrotic cells (Fig.10b). It enters through damaged membranes and fluoresces upon binding to DNA. This property makes it particularly useful in flow cytometry for distinguishing live from dead cells during immunofluorescence staining of unfixed samples.

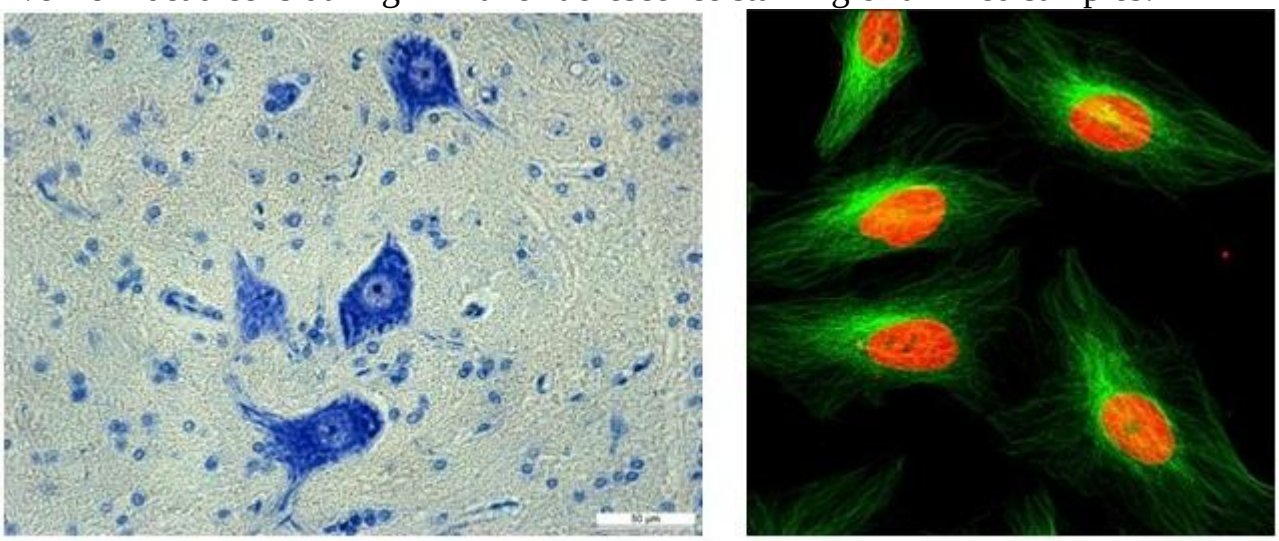


Figure 10 - Determination of plasma membrane damage using: a – trypan blue; b - propidium iodide

A second major class of tests includes histochemical assays that evaluate the metabolic activity of cells. In these assays, tetrazolium salts (e.g., MTT, XTT) are frequently employed due to their role as substrates for mitochondrial reductase enzymes. The reduction of these salts generates vivid formazan dyes, the concentration of which can be measured spectrophotometrically. The underlying chemical mechanism involves the cleavage of the positively charged quaternary tetrazole ring (containing four nitrogen atoms) during reduction, yielding the negatively charged, colored formazan products.

Lab work "Effects of toxicants on the integrity of the cell membrane and assessment of cell survival"

Legend: The cell membrane is one of the main targets for most toxic compounds. The cytotoxic effect of a substance is most often manifested in a violation of the cell membrane integrity. As a result of this violation, the contents of the cell escape into the intercellular space, causing cell death.

Most cytotoxicity tests rely on the principle that dyes can only enter cells when the plasma membrane is damaged, as intact membranes are impermeable to them. Based on their optical properties, these dyes are categorized as either visible-light dyes or fluorochromes. Chemically, they are classified as acidic (e.g., phenol red, cyanosine, trypan blue, lithium carmine) or basic (e.g., neutral red, methylene blue). Some of these dyes stain the cytoplasm diffusely upon entry, while others accumulate as granules in the region of the Golgi apparatus, leaving the nucleus and cytoplasm unstained.

In this study, cell viability was assessed using trypan blue exclusion. The method is straightforward: when an aqueous trypan blue solution is introduced to a cultured cell suspension, cells with compromised plasma membranes take up the stain and appear blue, while viable cells remain unstained. An additional practical advantage is that non-viable cells often detach from the culture plate surface, which simplifies both the counting and visualization of the surviving cell population.

Task: to evaluate the effect of methanol on the integrity of the cell membrane.

Equipment, reagents and materials: cell culture in a culture vial, pipettes for 10 and 200 µl, 0.4% trypan blue solution in saline solution, slides for a cell counter, methanol.

Course of work: Add a drop of methanol to the cells in the culture vial. After a 3-hour incubation, transfer the culture medium to a centrifuge tube and centrifuge. Aspirate the supernatant (spent medium) and wash the cell monolayer with 1 mL of warm saline solution. Add a dissociating solution (50 µL) to the wells. Incubate until cells detach from the substrate. Harvest the detached cells using PBS (2 x 1 mL washes), pooling them in the same centrifuge tube. Gently resuspend the cell pellet in the centrifuge tube. Transfer an aliquot (1000 µL) of the cell suspension to a microcentrifuge tube and add trypan blue solution (100 µL). Incubate the cell-dye mixture for 5 minutes. While incubating, prepare a Goryaev (hemocytometer) chamber: clean it with ethanol and polish the coverslip until Newton's rings are visible. At the end of incubation, gently resuspend the cells using a vortex mixer. Load 30 µL of the cell suspension into the prepared Goryaev chamber. Count both unstained (viable) and stained (non-viable) cells across 16 large squares of the chamber.

Write a report.

Topic 6. Antidotes and mechanisms of detoxication

The causes of acute poisoning can be divided into accidental (when the toxicant enters the body as a result of an error or accident) and intentional (such cases are usually investigated by criminology experts). When it comes to accidental poisoning, alcohol and its substitutes, drug intoxication, unsuccessful self-medication, and fires are the most common causes. At the same time, the developing toxic process manifests itself in clear clinical symptoms.

Natural body detoxication is a physiological process that has been shaped by evolutionary development and is aimed at neutralizing and eliminating toxic substances

directly from the body of a healthy person. The natural detoxification system includes the immune system of the blood (proteins and blood cells), the liver (involvement of the P-450 enzyme), and the excretory organs (kidneys). For example, proteins and blood cells can deposit many toxic substances by blocking their interaction with receptors. If the natural detoxification system is functioning properly, the body is effectively cleared of exogenous and endogenous toxins. However, there may be situations where natural detoxification requires assistance.

Antidote therapy is a measure taken when natural detoxification and its stimulation (forced diuresis, gastric lavage, etc.) are not effective. It is a critical component of acute poisoning treatment, which integrates both natural detoxification mechanisms and specific pharmacological interventions.

An antidote is a therapeutic agent that neutralizes a toxicant or prevents its toxic effects. Primarily administered by clinical toxicologists and emergency medical services prior to hospital transport, antidotes are also used in civilian settings during industrial accidents or in chemical exposure zones, both in peace- and wartime. Antidote therapy is only effective during the early toxicogenic phase (typically within the first day), as toxicants often become deposited—forming strong bonds with cellular and tissue structures. This sequestration makes subsequent elimination difficult, necessitating prolonged treatment, as exemplified in poisonings by "thiol poisons." Furthermore, when a toxicant employs multiple mechanisms of toxicity, simultaneous administration of antidotes with distinct modes of action may be required to address individual symptoms.

Antidotes include the following groups: toxicotropic antidotes, toxic kinetic antidotes, immune antidotes, and symptomatic antidotes (Table 8).

Table 8. The most common antidotes and indications of their use

Antidote	**Indications for use**
Group 1. Chemical Contact Antidotes	
Enterosorbents; *Ascorbic acid;* *Enterodesum*	barbiturates, antidepressants, cardiac glycosides, bacterial toxins (staphylococcal, botulinum), mushroom poisons (Amanita), ethyl alcohol, methanol
Complexones: *Cuprenil (D-penicillamine)*	copper, lead, arsenic
Group 2. Biochemical antidotes	
methylene blue; *amyl nitrite*	cyanids
vitamin B6	rocket fuel components
Unithiol *sodium thiosulfate*	mercury (organic and inorganic), arsenic, lead, bismuth, chromium, cadmium, cobalt, and copper

Ethyl alcohol	ethylene glycol, methanol
Oximes	organophosphorus compounds
Group 3. Pharmacological antagonists	
Naltrexone	opiates
Atropine	organophosphorus compounds
Group 4. Immunological antidotes	
Anti-snake serum	snake bites

Toxicotropic antidotes are a group of antidotes that affect the physico-chemical state of a toxicant that entered our organism. They can be divided to chemical antidotes of contact action and antidotes of specific physico-chemical action.

Chemical antidotes, unlike physical ones, have a fairly high specificity. This specificity is related to the nature of the ongoing chemical reaction between a chemical antidote and a toxicant: neutralization reactions, reactions with the formation of insoluble compounds, oxidation and reduction reactions. For example, it can be an acid-base reaction that converts a toxicant into a neutral less harmful salt. It can also be a conversion of a soluble toxicant into an insoluble or non-absorbable complex. It can be a formation of stable water-soluble complexes that are perfectly excreted by the kidneys.

Chemical antidotes also include complexing agents (**complexones, chelators**). Chelators are the most clinically relevant chemical antidotes, particularly for heavy metal poisoning. They form stable, low-toxicity, water-soluble complexes with metal ions, shifting the metal from tissues into the bloodstream and promoting its renal excretion. They can be divided into several groups: derivatives of polyamine-polycarboxylic acids (bind lead, zinc, cadmium, nickel, chromium, copper, manganese, cobalt), dithiols (remove arsenic, mercury, antimony, cobalt, zinc, chromium, nickel), monothiols (N-acetylpenicylamine) and other complexones. Another example of a chemical antidote is cobalt chloride, because cobalt forms strong bonds with the cyanide ion. This gave rise to the use of EDTA cobalt salt as an antidote for cyanide poisoning. One of the most well-known chelators is Unithiol, a dithiol antidote that contains two sulfhydryl groups (Fig. 11). Its principle of action is based on the chemical reaction of stable complex formation.

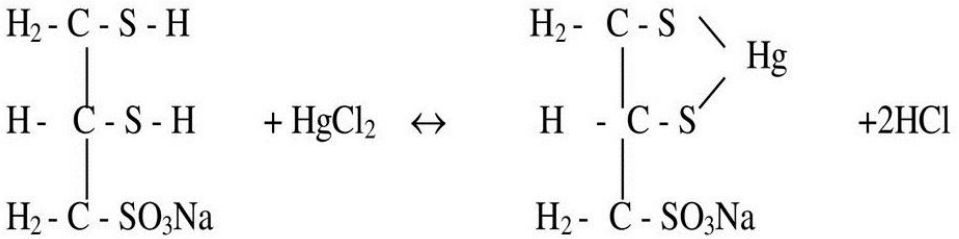


Figure 11 – Detoxication of mercury salt by Unithiol

Physical antidotes primarily function by adsorbing toxicants. Sorbents, such as activated charcoal, bind to toxin molecules, thereby preventing their systemic absorption (see Table 9). A critical consideration when using these antidotes is the subsequent need to eliminate the resulting adsorbent-toxin complex from the body. The best effect is achieved when taken within 1 hour after poisoning. This is due to the peculiarities of our gastrointestinal tract. The average time for food/toxicants to remain in the stomach is no more than 1.5-2 hours.

Table 9 – Examples of sorbents used for detoxication

Class of sorbent	Examples
Carbon sorbents	Activated carbon
Silicon-containing	Polysorb MP, Enterosgel
Lignin	Lactofiltrum, Polyphepan
Low molecular weight	Enterodesum
Smektites	Smekta

Pharmacological antidotes function not by directly neutralizing the toxicant, but through pharmacological antagonism. They counteract the effects of the poison by acting on the same physiological systems that the toxicant disrupts. Classic examples include the antagonism of atropine against acetylcholine in organophosphate poisoning, and the use of potassium chloride to oppose the effects of cardiac glycosides. For example, Naltrexone reduces opioid cravings by blocking μ-opioid receptors. It is metabolized in the liver to form several metabolites, including 6-β-naltrexol. This metabolite is involved in the treatment of drug addiction. It is believed that 6-β-naltrexol acts as a competitive antagonist of opioid receptors. Naloxone (as Naltrexone) also binds to the same receptors in the CNS as opioids (μ-receptors, κ- and δ-receptors). Instead of activating these receptors (as opioids act), it blocks them. Since it has very high affinity for these receptors, Naloxone displaces opioid molecules from their binding sites, instantly reversing their effects.

Immunological antidotes, administered as antitoxic sera, are the primary treatment for envenomation by snakes and certain insects. However, their efficacy is significantly reduced if administered late, typically beyond a 3–4-hour post-exposure. These sera are produced through a multi-stage process involving the hyperimmunization of horses with native venom, often in conjunction with a non-specific adjuvant. According to the spectrum of action, serums are divided into monovalent and polyvalent. **A monovalent serum** is effective against the bite of a single specific species of snake. For example, the viper venom serum is the only specific antidote used to treat bites from the common viper. It is an immunoglobulin fraction of horse blood serum. Horses are immunized with microdoses of viper venom. Antibodies are produced in their blood. Then, immunoglobulins are isolated from this immunized blood. After that it can bind and neutralize the venom. **A polyvalent serum** is effective against the bite of several species of snakes that live in the same

region. Not all types of snakes have their own serums. For example, there is no specific monovalent serum for the black mamba, but there are polyvalent antidotes that are effective against the venom of this species.

Some antidotes can change the metabolism or biochemical interactions between the toxicant and the body. For example, by creating non-functional ("false") receptors that divert the toxicant away from its target; this is how antidotes for cyanide and sulfide poisoning, such as sodium nitrite, amyl nitrite, or methylene blue, work. Other antidotes may rely on their ability to not only break the existing bonds between the toxicant and vital molecules, but also restore their function. This group includes cholinesterase reactivators (oximes), which are used in cases of organophosphorus poisoning. Oximes are antidotes that contain an oxime group. They bind the free organophosphorus toxin or cleave the phosphate group from the receptor, thereby reactivating it and restoring normal functioning of the nervous system.

It should be noted that antidote therapy is effective only until the toxicant binds to the receptor. Depending on the toxicant, the effective time for antidote administration can range from a few minutes to an hour. After a few hours, antidote therapy loses its effectiveness and becomes more of a symptomatic treatment.

Task

Give examples of different groups of antidotes, depending on their mechanism of action.

Group of antidotes	Name	Mechanism of action	Examples
1 group	Compounds whose action is based on physical and physico-chemical processes		
2 group	Compounds that neutralize the effect of poison by chemical reaction		
3 group	Biochemical (toxicokinetic) antidotes		
4 group	Physiological antagonists		

Topic 7. Planning of a toxicological experiment

A fundamental principle of well-designed experimental research is the appropriate selection of animal models for toxicological studies. The choice of species is guided by key characteristics, including the animal's size and specific physiological traits. Laboratory mice are the most commonly used mammalian research models, popular because of their availability, size, relatively low cost, and ease of handling. In experimental toxicology, rats are standard models for assessing chemical toxicity. To

investigate toxicant-induced allergic reactions, guinea pigs are usually the preferred models.

Every toxicological experiment is regulated by a large number of regulatory documents, the study of which is obligatory. Toxicants can enter the body mainly via inhalation, oral ingestion, dermal penetration, and intravascular injection. Therefore, special toxicity testing protocols have been developed for each route of entry of a toxicant. All toxicology studies require an approved plan, a protocol, and a final report that records every result. The research protocol should be approved by the head of the organization conducting the research and includes: the aim and objectives of the study, available information about the studied objects (its physical, chemical, biological and toxicological properties), storage and use conditions, information about the control material, the research scheme and justification of the chosen scheme research, research methods, research results, statistical processing of the results, conclusion, list of references. The number of animals in a group depends on the objectives of the study, but there should not be less than 10 test subjects in a group. The variation in the initial body weight of the animals in the group should not exceed ± 10%. Animals should have free access to food and drinking water.

The introduction of the test sample to laboratory animals is carried out by the way in which it is supposed to enter the body or in which it affects humans.

Two main methods are used for inhalation toxicity testing in animals under laboratory conditions (Fig. 12):

1. **Static Exposure:** This method is for initial toxicity screening. A volatile substance is introduced into a sealed chamber (like a desiccator) to create a constant concentration for the duration of the test.
2. **Dynamic Exposure:** Here, the test substance is continuously supplied to the chamber. This allows for precise control of concentration and maintains necessary air flow, keeping the concentration relatively stable over time.

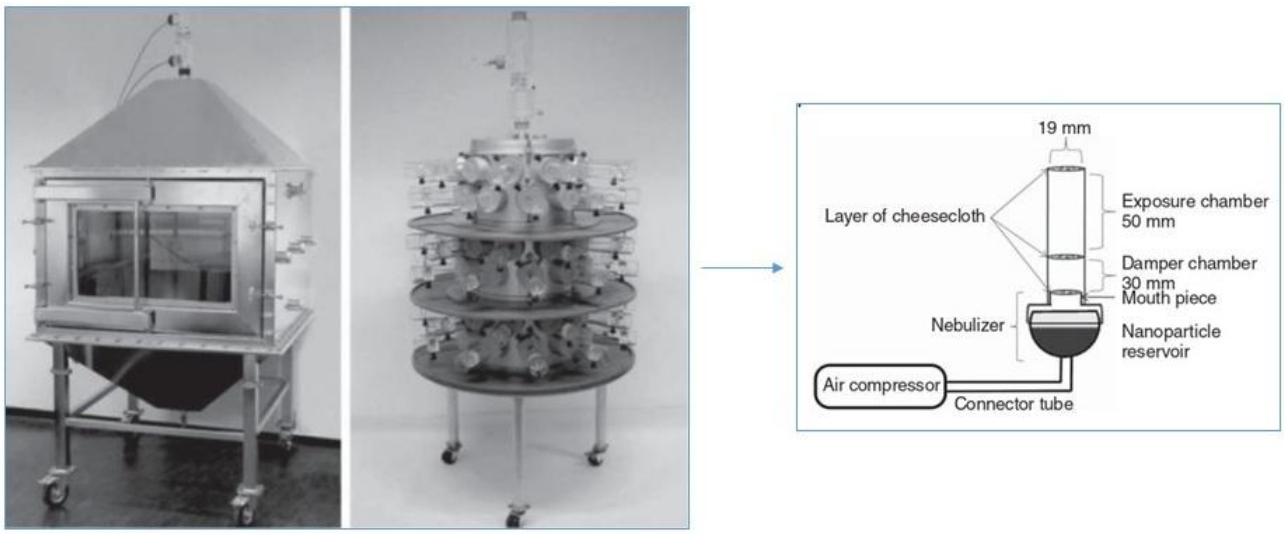


Figure 12 - Diagram of the nebulizer used in toxicological experiment

The functional state of experimental animals is assessed 4 hours after seeding, and then on the 2nd, 4th and 8th days of the experiment. At the same time, the maximum deviations of the value of the studied indicator are taken into account.

In oral tests, animals receive the substance 3 hours after being fed. The maximum dose volume is 1 ml for mice and 5 ml for rats (Fig. 13). Feeding resumes 3 hours post-administration. Substances are usually given in water. If they do not dissolve well, they are prepared in vegetable oil or suspended in a 1-2% starch solution.

Figure 13 - Oral administration of the sample with a probe

For acute toxicity testing, animals are observed for 14 days. The following are monitored and recorded: overall health, signs of poisoning and deaths, activity and movement, any seizures, muscle tone, reactions to stimuli, breathing rate and depth, heart rate, coat and skin condition, tail position, urination frequency and urine color, food/water intake, and weight changes. The exact time of death is noted, and all animals that die are examined internally (post mortem), both visually and under a microscope.

The total duration of the subacute toxicity study is 90 days for rats, while the general condition of the animals, the nature of motor activity, the condition of the hair and skin, feed and water intake, diuresis, and changes in body weight are recorded.

The extrapolation of experimental findings from animal models to humans represents a central and challenging objective in preventive toxicology. The primary

difficulty arises from interspecies differences in metabolism. Addressing this issue of species-specific sensitivity requires experiments conducted on multiple animal species with statistically adequate group sizes.

Chemical and toxicological analysis (CTA) is a critical tool for the rapid confirmation or exclusion of toxic substances in biological specimens from both animals and humans. Common biological matrices include blood, urine, saliva, sweat, vomit, hair, nails, and hand rinses. In contrast, non-biological evidence such as powders, tablets, and liquid residues are also subjected to laboratory examination. A defining feature of CTA is its focus on detecting trace amounts of substances within complex biological materials. The analytical process is structured into distinct phases: the pre-analytical stage, preliminary testing, the core chemical study (involving isolation, qualitative identification, and quantitative measurement), and finally, the interpretation of the findings.

For example, barbiturate analysis uses chemical, physicochemical, and biological methods. Confirming their presence requires 2-3 independent techniques, such as chemical (chromogenic) tests, immunochemical tests, UV-Vis spectrophotometry, IR spectrometry, and chromatography. Hybrid methods like gas chromatography-mass spectrometry (GC-MS) are also frequently employed.

Preliminary tests in CTA are important primarily for planning further research. Preliminary tests include determining the color and odor of biological and non-biological objects, determining their pH, conducting a number of color reactions and immunochromatographic analysis. Sometimes, preliminary extraction of a substance (solid-phase, liquid, distillation method, hydrolysis) and its concentration are required.

The CTA itself includes the detection of a toxic substance in a bioassay, its isolation and identification (determination of their nature), determination of the substance concentration and its metabolites, and interpretation of the results.

Lab work "Effects of $PbSO_4$ on Albumin"

Task: to evaluate the effect of heavy metals salts on albumin.

Equipment, reagents and materials: test tubes, penicillin tubes, beaker, 1 ml and 5 ml pipettes, filter paper, 0.5M PbSO4, distilled water, albumin, ethyl alcohol.

Course of work: Prepare solutions of 0.1M lead sulfate. Prepare two test tubes with 1 ml of a 1 mg/ml albumin solution. Then add 1 ml of one of the salt solutions to each tube. Then pour 1 ml of ethyl alcohol into each tube and mix. A precipitate forms at the interface of the protein/alcohol solution.

Write a report and explain the result.

Topic 8. Identification of substances isolated from biological objects

Forensic chemistry is a part of applied chemistry that deals with forensic chemical examination and is closely related to toxicology.

In forensic chemical analysis (FCA), it is often necessary to isolate the toxicant from various substances: viscera, food, and air. The FCA classifies isolated substances into five main groups.

Group 1 includes substances isolated by steam distillation of acidified objects. In this way, a large number of organic compounds and minerals (for example, yellow phosphorus) are isolated.

Group 2 includes objects that can be isolated by destroying the organic substances containing various toxicants (for example, protein complexes with heavy metal salts).

Group 3 includes objects extracted from biological material by water (for example, alkaline salts).

Substances of **group 4** are extracted from a biological material with acidified ethyl alcohol. In this way, it is possible to isolate substances of a basic nature (alkaloids). The higher the concentration, the more substances of a protein nature are precipitated by alcohol.

Group 5 consists of gaseous substances. Their isolation occurs by displacement from the object by air and other gases, followed by their absorption by sorbents.

Once substances are extracted from biological samples, they need to be identified. Most drugs and narcotics are identified using chemical analysis techniques. The ideal identification method should be: highly sensitive and selective, reliable and reproducible, fast, able to work with tiny samples, simple to prepare, automatable, and versatile. The best known method is chromatography-mass spectrometry (MS). It consists of three main parts: a chromatograph (usually gas or liquid), a mass spectrometer, and a single interface device. Chromatography-mass spectrometry is a hybrid analysis method that allows the separation of substances by gas or liquid chromatography, and identification and quantification are carried out by mass spectrometry. In a chromatograph-mass spectrometer system, the chromatograph separates the test mixture into individual components. These components are then analyzed by the mass spectrometer, which acts as a detector. The separation in chromatography is based on the repeated redistribution of molecules between a mobile phase and a stationary phase. The mobile phase can be a gas (as in gas chromatography) or a liquid (as in liquid chromatography). Within the mass spectrometer, sample substances undergo ionization to produce ions. These ions are subsequently separated based on their mass-to-charge ratio. The final mass spectrum conveys data regarding the ion population created by ionization of the substance. Chromatography-mass spectrometric systems mainly use two types of analyzers: analyzers with a sector magnetic field and quadrupole mass filters. The main factor limiting the sensitivity of the determination is interfering endogenous compounds with similar analytical characteristics.

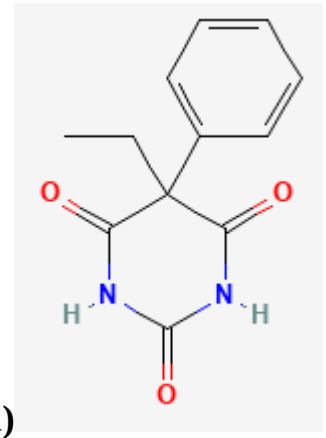
Pure chromatographic methods also make it possible to determine several substances in a single sample quickly and accurately, while gas-liquid chromatography and HPLC are laborious for wide practical applications.

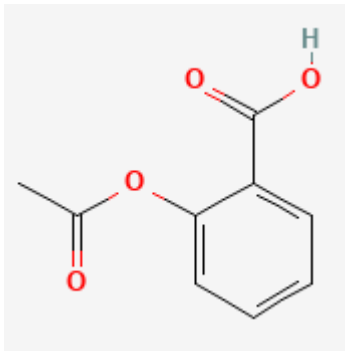
Task

Three jars of solutions were found at the crime scene. They were taken to a testing laboratory for analysis. From the analysis, it became known that one sample contains Phenobarbital, Aspirin and ethanol. However, during the work, the labels on the samples were accidentally mixed up. Help the laboratory technician identify substances using the known spectra and physico-chemical properties of the samples.

1) Identify the suspected poison using the obtained chromatography-mass spectrometry spectra of the tested substances;

2) Justify the answer.

a) 

b) 

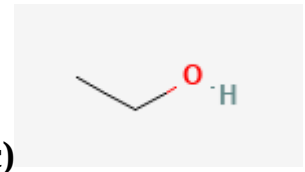
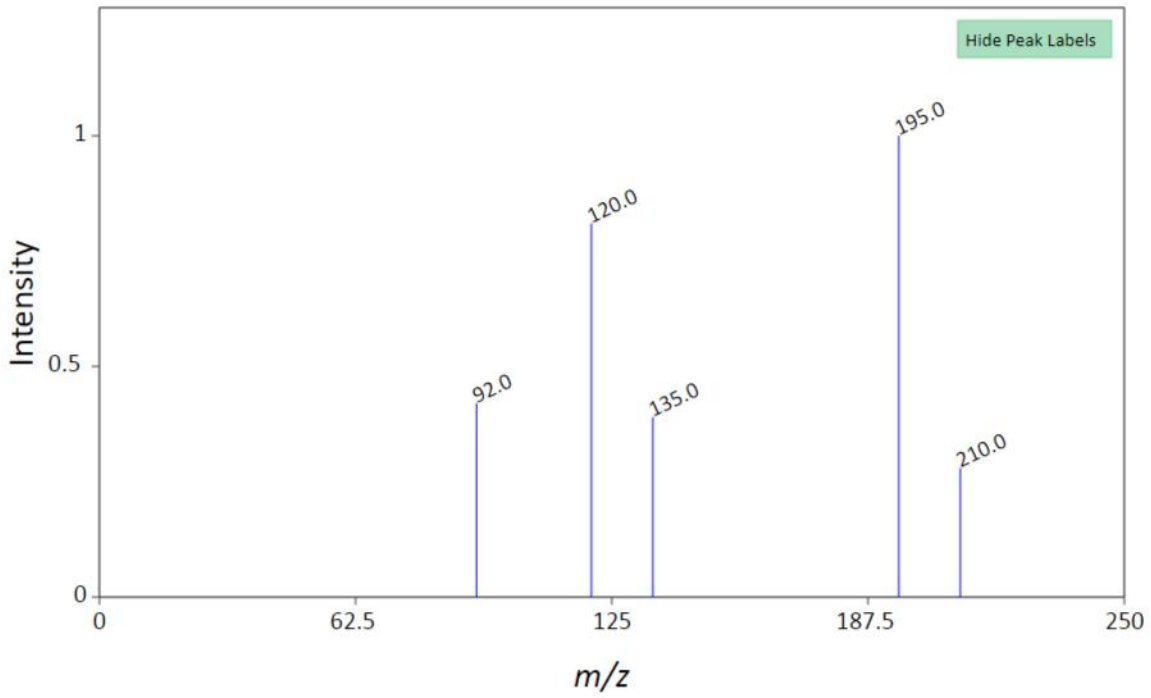
c) 

Figure – Structure of substances: a - Phenobarbital, b – Aspirin, c - ethanol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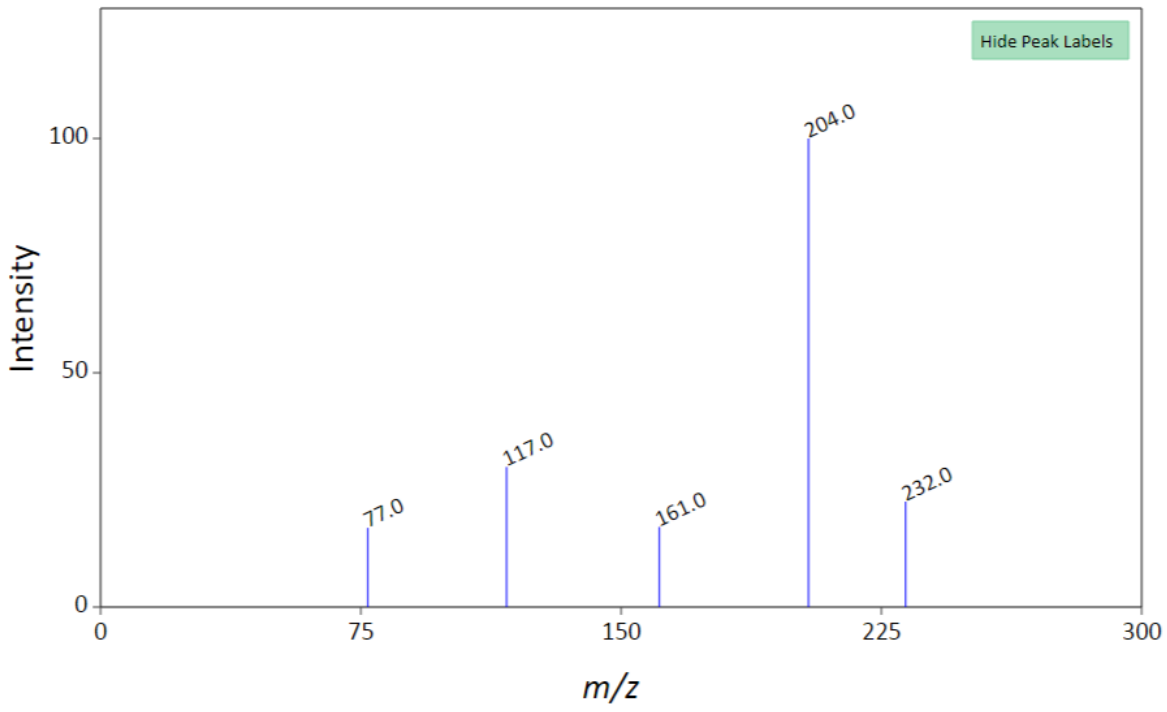
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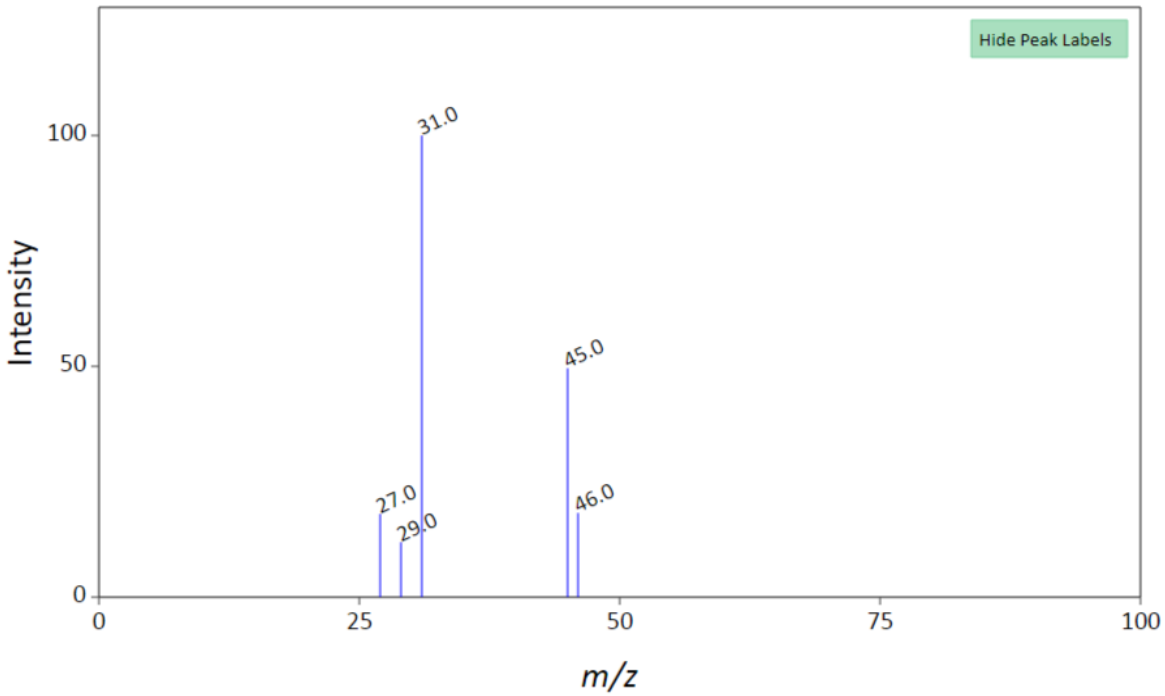
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Elena Fedotova

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