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Identification of selected cations and anions Supplementary materials

Veterinary practice requires not only clinical skills but also carrying out some quick laboratory analysis, and preparation of solutions for injections or disinfection. Therefore, chemistry exercises consisting in the detection of selected ions are devoted to the teaching of laboratory skills, using relatively easy examples and reactions. Additionally, these exercises give opportunity for the students to learn the properties and biological importance of ions that build the cells of a living organism. Some of the reactions carried out during these exercises occur also in living cells. Knowledge of these reactions will enable the understanding of metabolism mechanisms of and the recognition when these reactions run normally and when we deal with a disease. It should be remembered that each visible symptom of a disease appears because metabolic disorder has already affected the cells.

The periodic table is a list of all the chemical elements, ordered according to increasing atomic number, and periodically recurring chemical properties, based on which the groups of elements with similar characteristics are distinguished. Depending on the field of study, students perceive the period table from different perspectives. For veterinarians, the most important elements are those, which occur in specific chemical compounds in living organisms under physiological conditions, and also those which can be used as medicaments, disinfectants, or may enter an organism from the environment and cause disease symptoms.

The position of individual elements in the periodic table determines their chemical activity and the possibility to enter into chemical reactions that take place not only in the laboratory but also in the cells, based on the same mechanisms

The periodic table gives information about the number of valence electrons of individual elements, which determines the type of bonds in chemical compounds and the nature of the elements that can enter into a chemical reaction. The position of an element in the periodic table determines its activity in chemical processes, its metallic or non-metallic properties, and indirectly also the properties of its simple chemical compounds.

The elements with high atomic numbers (greater than 83) have unstable nuclei and radioactive properties.

Ions occurring in the cells and playing an important role in the metabolism of organisms:

Sodium ion (Na^{+})

Extracellular cation. Its transport across cell membranes and exchange for potassium ions is regulated by the so-called sodium-potassium pump. It is especially important in neurotransmission. Concentration of sodium ions is the indicator of fluid and electrolyte balance.

Potassium ion (K^{+})

Intracellular cation (in equilibrium with phosphate and protein anions). It participates in neurotransmission, and is essential for the proper functioning of the muscles of heart.

Calcium ion (Ca^{2+})

Calcium is essential for the proper functioning of the cells and the whole organism. It builds bones and teeth, is involved in blood clotting, muscle contraction and transmission of cellular signals. The calcium pump maintains the calcium gradient between the inside of the cell and extracellular space.

Iron ions (Fe^{2+} and Fe^{3+}) /Iron(II) and iron(III) ions

Most iron ions are bound to haemoglobin and myoglobin. The rest is bound to other proteins: in plasma iron is stored as transferrin and within the cells – as ferritin and hemosiderin (iron(III) phosphate) complexes. Free iron ions practically do not exist in organisms. They could generate a chain reaction leading to the formation of free radicals which could be dangerous for the cells.

Iron plays major roles in oxygen transport and metabolism, as well as in redox processes. Iron compounds can be found also in the active centres of many enzymes.

At neutral pH the compounds of Fe^{3+} are insoluble, so only Fe^{2+} ions can be absorbed in the alimentary canal. Iron deficiency is usually associated with insufficient dietary intake or poor absorption from the alimentary tract. Iron deficiency causes anaemia and general weakness of the organism.

Copper ion (Cu^{2+})

Copper ions occur in the active centres of many enzymes, and are essential for their proper functioning. Intracellular copper is usually located in the mitochondria and the nucleus. Ceruloplasmin protein acts as the transporter of copper ions. As in the case of iron, copper ions do not initiate free radical reactions because they are bound to the transporting proteins.

Both the observed synergism between copper and zinc concentrations and antagonism between copper and iron levels, have a favourable effect on haemoglobin synthesis. Copper has a beneficial effect on the metabolism of connective tissue through the participation in its "maturation" and formation of cross-linking bonds of proteins that build this tissue.

Copper deficiency can be manifested by disorders of the nervous system and impaired growth.

Magnesium ion (Mg^{2+})

It occurs mainly as intracellular cation. Magnesium not only plays a role in enzyme activation but it is a component of bones.

It participates in metabolic processes by affecting the permeability of cell membranes, regulation of redox processes, and stabilization of macromolecule structures, especially of nucleic acids. Magnesium deficiency leads to disturbances in muscle contraction. Magnesium has anti-stress effects.

Zinc ion (Zn^{2+})

Zinc occurs mainly as intracellular cation, it builds bones and muscles. Within the cells it is bound to metallothionein protein. Metallothionein, due to the presence of -SH group in its molecule, has the capacity to bind heavy metals (cadmium and lead) so it has detoxification functions. A small amount of zinc occurring in plasma is bound to proteins. Zinc is found in the active centres of enzymes. Zinc affects their normal activity, and consequently it has positive effects on metabolic processes.

Zinc deficiency is usually associated with disorders of its absorption, and can lead to disorders of bone structure, reproductive defects, inflammatory conditions of the skin, and hair loss.

Phosphate ion (PO_4^{3-})

1-st and 2-nd order salts of orthophosphoric(V) acid form buffer systems of blood. Calcium phosphate is a component of bones, and phosphate esters are essential components for the metabolism (e.g., phosphorylated glucose is "retained" in the cells).

Chloride ion (Cl^-)

It is generally extracellular ion, the main anion of plasma. It participates in the regulation of acid-base balance.

Iodide ion (I^-)

Iodide ions, taken up from blood by the thyroid, are oxidized to I2, which is essential for the synthesis of thyroid hormones. Biological importance of iodine is related to thyroid hormones. Iodine does not accumulate in the organisms so it must be constantly replenished through the diet.

Selenium and fluoride can interfere with iodine metabolism and absorption.

Iodine deficiency causes hypothyroidism, which has serious metabolic consequences leading to a slowdown of vital functions and all processes occurring in the cells.

Selenium (Se)

Selenium is an antioxidant due to its presence in the active centres of antioxidant enzymes as well as his own ability to scavenge free radicals.

Selenium deficiency can cause muscle damage, reduce immune system function and promote the development of cancer.

The excess of selenium is toxic and may lead to anaemia, bone stiffness, hair loss and disturbances in the functioning of internal organs.

Fluorine (F)

Fluorine is found in living organisms in small quantities. Its biological importance is related to the binding of magnesium, calcium and phosphorus ions in the process of bone mineralization. Both the excess of fluorine and its deficiency is harmful: in the first case the disorders of iodine metabolism may occur, while in the second case – bone mineralization may be disordered.

Carbon (C)

This element can be considered as inorganic and organic carbon. As a component of bicarbonates it takes part in the buffering of biological fluids. Carbon dioxide, removed from the organisms together with the exhaled air, is formed during the metabolism processes in the cells, and it also participates in gas exchange. Carbon monoxide is toxic – it replaces dioxide and blocks the respiratory functions.

Carbon atoms occur practically in all the organic compounds. Many properties of organic compounds occurring in living cells are conditioned by the kind of bonds between carbon and hydrogen atoms, as well as by the number of carbon atoms in a molecule.

Elements used in medical procedures

Silver ion (Ag^{+})

Silver occurs in pharmaceutical (see below) and diagnostic (films for X-ray) preparations.

Barium ion (Ba^{2+})

Insoluble barium sulphate is used in medicine as a contrast agent in diagnostic imaging.

Toxic elements

Mercury(I) and mercury(II) ions

Mercury compounds are strong poison. However, they are used in small doses in treatment of certain diseases in the form of calomel (mercury(I) chloride) or sublimate (mercury(II) chloride).

Cadmium ions

Cadmium compounds are toxic to living cells. Adverse effect of this element results from the fact that it is easily bound to the -SH groups of proteins, and replaces biologically important metals in the active sites of some enzymes. The cell's protein metallothionein, due to the presence of a large number of the -SH groups, can bind cadmium and thereby reduce its toxic effects. Accumulation of cadmium (taken from the environment) within the cells results in the disorder of kidney function and calcium metabolism, as well as favours the development of cancer processes.

Lead ions

The toxic effects of lead are associated with its ability to be bound to plasma proteins and to accumulate in the cells. Lead inhibits the activity of some enzymes by antagonistic action on metals building their active centres. In addition, combined with nucleic acids, amino acids and haemoglobin, it interferes with metabolic processes. The presence of lead leads to damage of kidney, liver and nervous system.

Disinfection

Properties of some elements allow the use of their compounds as the antibacterial agents both in living organisms and in the environment. Therefore, these compounds are the components of disinfectants but their mechanisms of disinfection are different. Theoretical knowledge on the subject is necessary to use the individual elements in veterinary practice in the right way and in accordance with their disinfection abilities. Some examples are given below:

Definition: disinfection is a procedure aimed at destroying of pathogenic microorganisms and their spore forms, using physical and chemical agents, to prevent infection. Disinfectants are mainly used outside the body or on the skin, and to a lesser extent in the body cavities.

Disinfectants – chemicals that destroy (kill) all microorganisms and their spores, and also prevent the reproduction of microorganisms. Disinfectants are used to create aseptic conditions.

There are two terms close in meaning, which must be distinguished: **antisepsis and asepsis**.

- **Antisepsis** is destruction of microorganisms occurring on tissues with the use of antiseptic agents. In historical perspective, antisepsis is also the destruction of microbes with the use of high temperature.
- **Asepsis** includes all procedures used to prevent infection by pathogenic microorganisms. Both physical and chemical methods are used in aseptic procedures. Aseptic conditions mean the environment free of any pathogenic forms of life. Asepsis is a broader term than antisepsis.
- **Antiseptic agents** include the substances used to disinfect tissues and prevent infections on the skin and mucous

membranes.

- **Antiseptic substance (antiseptic)** is any chemical compound (or element, eg. fluorine, ozone, chlorine) that destroys microorganisms and prevent their reproduction.
- The name **disinfectants** is related to the chemicals used to disinfect rooms, objects (surgical instruments, sanitary facilities, furniture, walls, floors) or fragments of the natural environment (e.g. soil, water).

The term *antiseptica* was first used in scientific and practical context by surgeon John Pringle (1707-1782) in 1750, in relation to the treatments and substances used to prevent wounds from festering. Pringle developed sanitary rules for army, and called for the observance of hygiene rules.

The etymology of the term antisepsis derives from the Greek words: *anti* – against, and *sepsis* – decomposition. Therefore, antisepsis literally means the prevention of decomposition. The major role of bacteria in the putrefactive or infectious processes was not known in the 18th century. Antiseptic treatment was also a heat treatment, including annealing tools and burning wounds.

The introduction of aseptic and antiseptic procedures to medicine was not a simple matter because the lack of knowledge of the pathogenic bacteria, fungi, and viruses. The famous promoter of antisepsis was obstetrician Ignaz Philipp Semmelweis (1818-1865), the author of „Die Aethiologie, der Begriff und die Prophylaxis des Kindbettfiebers" (1861). Semmelweis observed that antiseptics prevented lethal puerperal infections. He concluded that the majority of infections in maternity wards were caused by not disinfected hands and surgical instruments. He appealed to physicians to wash hands and instruments with aqueous solution of calcium chlorate(I) (formerly calcium hypochlorite) before each examination of pregnant women and each delivery of baby. He believed that puerperal fever was caused by „cadaveric contamination", i.e. microbes, invisible to the naked eye, which were transmitted on the clothes and skin of physicians who earlier were engaged in autopsies. Semmelweis's discoveries were not commonly accepted by the medical community, despite a huge decline in mortality and infections in the ward led by him. He was later removed and condemned for his pioneering ideas.

Particular emphasis was also placed on the necessity to observe antisepsis rules by the English surgeon Joseph Lister (1827-1912). In 1867 he published the method of application of aqueous solution of phenol (carbolic acid) for disinfection purposes. The experiments conducted by Louis Pasteur (1822- 1895) were also of great significance for the development of antisepsis. In 1862 Pasteur published a paper entitled "About organized bodies existing in the atmosphere", in which he invalidated the theory of spontaneous generation of bacteria (according to this theory, bacteria generate spontaneously, i.e. accidentally, directly from non-living matter). He was able to invalidate the theory of spontaneous generation based on an experiment, during which he observed the results of sterilization, i.e. complete destruction of any microorganisms occurring in the studied inanimate material.

Modern biocides should meet the following criteria:

- high activity at low concentrations against a broad spectrum of microorganisms,
- lack of antimicrobial resistance after repeated administration,
- insensitivity to water hardness and the presence of organic substances,
- high stability of used concentrates and solutions,
- good solubility in water,
- lack of destructive or colouring effects on the disinfected equipment and surfaces,
- lack of odour,
- lack of irritant effects on the skin and mucous membranes,
- high degree of biodegradability and low toxicity,
- favourable economic aspects.

The question of finding a substance that meets the above criteria is still topical. None of the groups of compounds used so far meets all of these expectations.

Inorganic antiseptics – chlorine

Chlorine compounds have been used to disinfect wounds since about 1822. Chlorine is yellowish-green gas, chemically very active and highly toxic to all organisms. Chlorine was and is still used for the production of chemical bombs. Chlorine chemical bombs not only kill all organisms, but also totally destroy the military equipment, especially metal. The following chlorine compounds are used as disinfectants: chloramine, sodium chlorate(I), calcium chlorate(I), chloric(I) acid, chloric(VII) acid, chlorhexidine, chloroxylenol.

The mixture of calcium chlorate(I), calcium hydroxide and calcium chloride is called chlorinated lime – calcium hypochlorosum (*calcaria chlorata*). It is white or greyish powder with characteristic odour. Aqueous solutions of chlorinated lime have bleaching effects. It has been used to disinfect toilets, floors, walls, sewage, dustbins/rubbish tips, and enamel appliances for a long time. It is used as 20% aqueous solution or as powder.

Chloric(I) acid (HClO), which exhibits high activity against bacteria, viruses, fungi, and protozoa, is formed from calcium hypochlorite under the influence of water (or humid air) and acids.[Hypochlorous *acid* is a weak *acid* which is typically *formed* when *chlorine* dissolves in water.] Chloric(I) acid is unstable and releases chlorine. Oxygen, which is also released during the decomposition of chloric(I) acid, has also antibacterial properties. Chlorinated lime is a protoplasmic poison that damages (kills) all living cells. It has a caustic effect on the skin and causes skin burns. Chlorine combines with amino groups of amino acids and proteins, which results in destabilization of proteins.

Chlorine is not suitable to disinfect textiles, leather (it fades the colours) and metal objects (it causes corrosion).

Calcium chlorate(I) and sodium chlorate(I) (*natrium hypochlorosum*) are components of many household detergents and cleaners (e.g. ACE, Blux, Clorox – liquids). Chlorine is released from these compounds under the influence of acids and carbons dioxide. Sodium hypochlorite reacts with NaOH to form antiformin – a strong caustic liquid disinfectant/detergent. Antiformin is used in dentistry for rinsing root canals.

Chloramine B – sodium toluolosulfonochloramide – is white or yellowish crystalline powder with a chlorine odour, which is soluble in 3 parts of water. It contains 25% of chlorine. It has disinfecting, antiseptic, and anti-odour (eliminates other odours) effects. Formerly it was used to rinse body cavities after the evacuation of pus; now it is used to disinfect medical equipment, sanitary facilities and hands.

Inorganic antiseptics – iodine

Iodine (*iodum*; chemical element with the symbol "I") occurs in the form of metallic crystals (platelets) of dark brown, grey-brown or brown colour. It is very poorly soluble in water, more soluble in alcohols and acetone, and highly soluble in the aqueous solution of potassium iodide. Iodine sublimes in the open air and in the sunlight. It is a strong antiseptic used to disinfect the intact skin. It exhibits activity against bacteria, fungi, and viruses. It causes irritation and even surface necrosis of tissues. Iodine, due to its irritant properties, was formerly used to improve blood supply to the skin and subcutaneous tissue, which sped up the resorption of inflammatory exudates. At present, iodine is also used as a 3-10% solution of iodine dissolved in 70% or 95% ethyl alcohol (*Solutio Iodi Spirituosa* known also as *Tinctura Iodi* – tincture of iodine). Iodine reacts with amino groups of proteins inhibiting their activity. It also releases oxygen from aqueous solutions, which complement its disinfectant effects. Alcoholic solutions of iodine cause denaturation of structural and enzymatic proteins of bacteria and fungi. Iodine preparations are not used to disinfect metal objects due to the corrosive properties of iodine.

Tincture of iodine and Lugol's solution are used to disinfect the skin. Undiluted preparations can only be used to disinfect the intact skin and the area around wounds and burns.

The complex of iodine with the polyvinylpyrrolidone polymer is highly soluble in water, exhibits long-lasting antiseptic effects and does not irritate the mucous membranes and skin. It does not wear out the medical instruments. It is known under the international name of Povidone-Iodine. It kills bacteria, viruses, and fungi. It is used to disinfect the skin, mucous membranes, and medical equipment. It can be used for washing the genital tract and oral cavity. Before use it should be dissolved in water in a ratio of 1:8 or 1:10.

Inorganic antiseptics – oxidizing compounds

Potassium manganate(VII) (*kalium hypermanganicum, kalium permanganicum;* $KMnO_4$) belongs to the group of oxidizing antiseptics. It is reduced during the reaction with organic compounds (e.g. proteins), and releases oxygen, which kills bacteria, fungi, and protozoa. Free manganese ions have also strong antiseptic properties.

Potassium manganate(VII) occurs in the form of deep violet crystals with metallic lustre. It dissolves easily in water. Its aqueous solutions, depending on the concentration, are pink or dark purple (blue-violet). Its taste is tart and bitter, with a metallic aftertaste. It emits a distinctive, fresh odour. Potassium manganate(VII) has astringent, anti-inflammatory, anti-pus, antibacterial, bacteriostatic, antifungal, fungistatic, antiviral and anti-protozoa properties. It exhibits deodorizing and dehydrating effects on tissues. Its concentrated solutions are caustic; they can stain and burn the skin. Under the influence of body fluids and enzymes, potassium manganate(VII) is gradually decomposed to dark brown or brown manganese dioxide (MnO_2). Potassium manganate(VII) neutralizes the venom of invertebrates and vertebrates.

Potassium manganate(VII) solutions (0.5-4%) are used are used to disinfect wounds and burns. They are also used to rinse out throat, oral cavity, conjunctivas, ear canals, nasal cavity, genitals, to wash skin, and for bath. Potassium manganate(VII) accelerates the healing of wounds and burns, inhibits bleeding by the constriction of small blood vessels, reduces swelling, exudation, and secretion of sebum, gently exfoliates the epidermis. It inhibits the formation of acne, dries-off ulcerations, erosions, oozing wounds, abscesses and acne. It heals acne and seborrheic dermatitis.

Potassium manganate(VII) solution is also used to rinse the stomach, because it oxidizes or absorbs cyanides, alkaloids and phosphorus compounds. Formerly it was placed on bite wounds to neutralize the venom.

Hydrogen peroxide (*hydrogenium peroxydatum*) is 3% solution of Perhydrol (30% solution of H_2O_2).

Under the influence of catalase (enzyme found in living organisms), hydrogen peroxide is decomposed into water and oxygen. Oxygen has antibacterial, antifungal and anti-putrefaction properties. It softens scabs, cleanses wounds, removes smells, whitens, and accelerates tissue regeneration. It is a weak antiseptic agent, but very good to cleanse skin and wounds. It has anti-acne properties and whitens skin stains.

Oxidized water can also be used to rinse out mouth and throat (1 tablespoon of hydrogen peroxide (3%) per 100-150 ml of boiled water). It exhibits activity against anaerobic bacteria, which most commonly cause inflammation of the gums.

Inorganic antiseptics – acids

Boric acid, i.e. boracic acid (*acidum boricum*) is white, crystalline substance, which dissolves in hot water, glycerol, and alcohol. It is a weak antiseptic substance. It has anti-inflammatory and astringent properties. Blue pus bacillus (*Pseudomonas aeruginosa)* is especially sensitive to this compound.

In medicine, an ointment with 10% boric acid in vaseline, 3% solution, and powder have been used for a long time. Boric acid does not irritate tissues. It easily penetrates through the skin and mucous membranes into the blood, so when applied to large areas of the body, it causes symptoms of poisoning. If it is used for a long time, it is accumulated in the body and damages the kidneys, nervous system and liver. Half of patients poisoned by boric acid die. The lethal dose for humans is 250-300 mg/kg body weight. Therefore, the use of boron preparations for children is prohibited. Boric acid in aqueous solutions was used (at present it is rarely used) as compresses, rinses, irrigations and washing to treat inflammation of the skin and mucous membranes. It is a component of some eye drops, complex disinfectant liquids and powders.

Inorganic antiseptics – metal compounds

Aluminium acetate (*aluminium aceticum*) dissolved in water (3% solution) has antiseptic, drying, astringent, anti-inflammatory, oedema-reducing and anti-exudative effects. It inhibits the formation of haematomas. It is used for rinses and compresses to treat inflammatory infiltrations, effusions, ulcerations, bruises and haematomas. It has anti-acne and antiseborrheic properties. Antiseptic properties of aluminium acetate are related to its denaturing effect on structural and enzymatic proteins, and to the effect of free aluminium ions. Aluminium acetate solution is recommended for compresses in the case of purulent or festering, painful pimples. It is a component of the formerly popular Burow's solution (*Liquor Burowii*) – both the original (3% aqueous solution of aluminium acetate) and modified (containing potassium aluminium sulphate). Before use the Burow's solution was diluted with water: 1 part of preparation with 2-4 parts of boiled water.

Aluminium sulphate (*aluminium sulfuricum*) is white, crystalline, water-soluble substance. It has antibacterial, astringent, anti-inflammatory, antihaemorrhagic, antiseborrheic, anti-acne, and antiperspirant effects. Its 3-5% solutions are used to rinse the genitals in the case of infections and inflammations, erosions, and ulcerations. It is also used to wash the skin and rinse the mouth.

Potash alum (potassium aluminium sulphate; *alumen, aluminii kalii sulfas*) has disinfecting, antihaemorrhagic (it inhibits bleeding from small blood vessels), antiseborrheic, antiperspirant, anti-odour (it eliminates odours from stinking lesions), dehydrating, and anti-inflammatory effects. Its 1-1.5% solutions are applied to the eyes as drops in the case of excessive lacrimation, inflammation, haematomas and effusions. Its 1% solution is used to rinse the mouth, throat and vagina.

At present, the preparations containing aluminium acetate or aluminium aceto-tartrate are still available on the market. Altacet – contains aluminium aceto-tartrate and boric acid.

Silver nitrate (*argentum nitricum;* $AgNO_3$) is whitish, crystalline substance, easily soluble in water. Silver nitrate solutions are slightly acidic. They easily darken on exposure to oxygen and light due to the precipitation of metallic silver. The concentrated solutions of silver nitrate (10-20%) are used for chemical cauterization of outgrowths (e.g. papillae), and also in dentistry. Silver nitrate (dissolved in double distilled water) is also used as 0.25-0.5% solutions to rinse the conjunctival sac in the case of inflammation and purulent conditions. Its 2% solution is used to paint hard-to-heal ulcerations and inflammatory foci in the mouth, throat and nasal cavity.

Silver and potassium nitrates (*argentum nitricum cum kalio nitrico*; $AgNO_3$+KNO_3) are used in the form of sticks to eliminate warts (the sticks are covered with a protective layer of wax or paraffin; this layer must be scraped before use, many patients do not know it). Before application, the tip of the stick should be moistened with lukewarm water. After application, 2-3 days later, the treated wart darkens and then gradually exfoliates.

Zinc sulphate ($ZnSO_4$) is a colourless, crystalline substance, which dissolves in water and glycerol. Its 5% aqueous solution is irritating to the skin and mucous membranes. Its 30-50% solutions have a caustic effect and are used for cauterization. Its 0.5-2% solutions have astringent and antiseptic effects. Zinc sulphate has anti-acne and anti-pus properties. It inhibits the activities of sebaceous, sweat and mucous glands.

In ophthalmology, the 0.2-0.5% solutions of zinc sulphate are applied to the eyes as drops in the case of inflammation, excessive lacrimation, and bacterial infections. Zinc sulphate may be a component (3-5%) of astringent powders that are applied on the skin. It is perfect for the treatment of seborrheic dermatitis.

Zinc oxide (*zinci oxidati;* ZnO) is white substance, insoluble in water. It has astringent, anti-inflammatory, antibacterial, drying, exfoliating, and anti-acne effects. It is a component of antiseptic pastes, ointments, and powders. The petrolatum-based ointments are used to treat wounds, herpes, acne, lichens, pimples, ulcerations, bedsores, burns, folliculitis, skin pruritus (with menthol). They are also used to form protective coatings during chemical cauterization of outgrowths, actinic keratosis, and warts. Zinc oxide was also administered orally at a dose of 100-400 mg 3-4 times per day in the case of inflammation of the digestive tract.

Zinc chloride ($ZnCl_2$) is white, crystalline, hygroscopic (absorbing moisture from air) substance, which is easily soluble in water and glycerol. Depending on the concentration of zinc chloride, its solutions have disinfecting or astringent effects.

Factors affecting the efficacy of biocides

- contact time of biocide with microorganisms (+++)
- concentration of biocide (++)
- temperature (++)
- water hardness and environmental pH (+/-)
- number of microorganisms (--)
- protein burden (large amount of organic matter in the environment: residues of food, faeces) (-)
- presence of surfactants (++)

Among the factors presented above, the contact time (t) and the concentration of preparation (c) are undoubtedly the most important. The product of concentration and time is the constant for a given group of compounds:

$c^{n} \cdot t = const$

The coefficient n for a given group of compounds is determined experimentally. For example, for 4-th order salts of ammonium n=1, for phenols n=6, and for alcohols n=10. It means that a twofold reduction in the concentration of a preparation based on alcohol requires a 1024-fold increase of contact time (210). Therefore, the proper dilution of these preparations is particularly important. It is unacceptable to dilute disinfectants "approximately" or "more or less". Too low concentration will not produce the desired results, and will carry biological (e.g. infection of people, infection of newly introduced group of animals, or recurrence of a disease after treatment), economic (need to repeat the procedure) and legal (professional responsibility of the supervising veterinarian) consequences. The use of too concentrated solutions is uneconomical, and moreover it can cause irritations, burns or poisoning of animals and people.

An increase in temperature generally supports the action of a chemical agent. This is due to an increase in the reactivity of active substances with an increase in the reaction environment temperature. Moreover, high temperature has biocidal effects.

The influence of water hardness and pH of the solution on the efficacy of biocides can be different, depending on the chemical nature of active substance. For example, an increase in pH results in lower activity of phenolic compounds, while the activity of 4-th order salts of ammonium increases.

In addition to disinfecting applications, some chemical compounds have been used and continue to be used in anaesthesia. Here are some examples:

Nitrous oxide (N_2O)

Nitrous oxide, known also as **laughing gas**, is an inorganic chemical compound, nitrogen oxide in the 1-st degree of oxidation. This compound is often used in anaesthesia. At room temperature, it is a colourless, non-flammable gas, with a faint odour and slightly sweet taste.

This gas was discovered by Joseph Priestley in 1772. Anaesthetic properties of nitrous oxide were utilized for the first time by American dentist Horace Wells.

Nitrous oxide is commonly used as one of the components in combined general anaesthesia, and also (mainly in the West) as inhalational anaesthetic in dentistry. Mixed with oxygen (up to 70% of N_2O), it is used as a carrier for other anaesthetic drugs. It is also used in combination with intravenous anaesthetics, opioids and relaxants. Nitrous oxide has a strong analgesic effect, significantly weaker hypnotic effect, and it practically does not relax the muscles. It has low euphoric effects, and that is why it is called **laughing gas**. Nitrous oxide inactivates the cobalamin form of vitamin B12, so prolonged use of large amounts can lead to the symptoms of vitamin B12 deficiency (anaemia, neuropathy). It can damage the bone marrow, and have a negative impact on the ovaries and testicles. However, sporadic use of this gas does not cause such effects. The use of nitrous oxide may cause the symptoms resembling alcohol poisoning.

Nitrous oxide is very quickly and well absorbed from the lungs into the blood, and penetrates into the tissues of an organism. It is also quickly removed through the lung. A very interesting characteristic of this gas is the reduction of organism tolerance to the same dose – unlike most drugs, after a prolonged use of N_2O, the reduced dose can have the same effect as the first dose.

The use of this gas causes the risk of tissue hypoxia (so it is only used in the form of mixture with oxygen), but other adverse reactions are very minor. On the contrary, some contaminants, which often occur in N_2O bottles (e.g. NO,) are dangerous.

Diethyl ether (ether; $C_2H_5-O-C_2H_5$**)**

Diethyl ether is a colourless, highly volatile, flammable liquid. Its boiling point is 34.6°C. It has a characteristic, pleasant odour, and its vapour has narcotic effects.

Diethyl ether is produced from ethyl alcohol by treatment with sulphuric acid at 140°C. It has been used since 1846 as a general inhalational anaesthetic (W. Morton was the first who demonstrated ether anaesthesia in Boston), at first by the drop method, later using the apparatuses for general anaesthesia. The induction of and recovery from ether anaesthesia are slow. Diethyl ether has a relatively wide margin of safety, gives good muscle relaxation in deep anaesthesia. However, deep anaesthesia is toxic.

Diethyl ether can be combined with other anaesthetics and with relaxants. At present it is almost not used.

Chloroform (trichloromethane; $CHCl_3$**)**

It is non-flammable substance with low reactivity. In contact with fire, chloroform vapours decompose to form chlorine, hydrogen chloride and phosgene. Similar slow decomposition occurs also under the influence of light, so chloroform is often contaminated with the above substances.

Chloroform is irritating to the skin and eyes, harmful to respiratory and digestive tracts. In case of long-term exposure or high concentration, it presents serious health risks. It may be sudden death due to arrhythmias and cardiac arrest and/or respiratory arrest. Chloroform has depressive effects on the central nervous system (including permanent changes), causes sleepiness and nausea, reduces mental ability, and causes loss of consciousness. It damages the liver and kidneys, and probably exhibits carcinogenic properties.

Taken together, chemical knowledge can be useful in many aspects of veterinary practice.