The disifectant and irritant activity of a concentrated disinfectant detergent conditioning

Activitatea dezinfectantă și iritantă a unui detergent dezinfectant concentrat

Taysuke Fujiwara, Mihai Folescu, Romeo T. Cristina
Faculty of Veterinary Medicine Timisoara

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Abstract

The present research aims to study the efficacy and toxicity of an indigenous cationic surfactant product, DEO-SEPT®, a concentrated disinfectant detergent (Manufacturer: Pasteur S.A., Filipesti Romania branch), an association of alkyl dimethyl benzyl ammonium chloride C12–C18 (C.A.S. 68391-01-5, E.C. 269-919-4) 5%, Didecyl dimethyl ammonium chloride (C.A.S. 7173-51-5, E.C. 230-525-2), 5% and excipient q.s. ad 100 ml. The work is designed as a harmonious combination between the continuing education part, where brief information is presented about: the morpho-histological structure of the skin, how medicinal substances penetrate the skin, the physico-chemical properties of the active substance and the effect of the excipient, the skin tolerance assessment (with the description of the standard patch test I - version I and II; standard patch test II - version I and II), then the toxic effects of the product in the studio are evaluated, according to the manufacturer's technical sheet. The objectives of the testing were: a bibliographic synthesis of the components of the DEO-SEPT® product. Evaluation of the disinfectant effect of DEO-SEPT® in field conditions (Test I), Evaluation of the skin irritant effect of DEO-SEPT® solutions on the skin (Test II). The histological investigation was carried out to determine the degree of damage to the skin layers. The results revealed that the disinfectant product used is suitable for application in animal shelters and other enclosures, with possibilities of airtight closure, being easy to handle, without particular risks. The efficiency of decontamination is conditioned by appropriate mechanical and hydrosanitary cleaning of the surfaces, ensuring a temperature of at least 10ºC, a relative humidity of at least 50% and the tightness of the enclosure in which the decontamination is carried out. The tested product has proven to be tolerable on epilated skin at solution concentrations of 0.5% and/or 2%. Skin contact with solutions with high concentrations (10% and 20%) causes severe and long-lasting irritations, as well as skin lesions, a situation confirmed by histological examination.

Rezumat

Prezenta cercetare are ca obiect studierea eficacității și toxicității unui produs tensioactiv cationic indigen, DEO-SEPT®, un detergent dezinfectant concentrat (Producător: Pasteur S.A., filiala Filipesti Romania), o asociere de clorură de alchil dimetil benzil amoniu C12–C18 (C.A.S. 68391-01-5, E.C. 269-919-4) 5%, Clorură de didecil dimetil amoniu (C.A.S. 7173-51-5, E.C. 230-525-2), 5% si excipient q.s. ad 100 ml. Lucrarea este concepută ca o îmbinare armonioasă între partea de educație continuă, unde sunt prezentate informații succinte despre: structura morfo-histologică a pielii, despre cum penetrează substanțele medicamentoase pielea, proprietățile fizico-chimice ale substanței active și efectul excipientului, evaluarea toleranței pielii (cu descrierea patch test standard I - varianta I și II; patch test standard II - varianta I și II), apoi se face evaluarea efectelor toxice ale produsului din studio, conform fișei tehnice a producătorului. Obiectivele testării au fost: o sinteză bibliografică a componentelor produsului DEO-SEPT®, Evaluarea efectului dezinfectant al DEO-SEPT® în condiții de teren (Test I). Evaluarea efectului iritant cutanat soluțiilor DEO-SEPT® asupra pielii (Test II). Investigația histologică a fost efectuată pentru a determina gradul de afectare al straturilor cutanate. Rezultatele au relevat Produsul dezinfectant utilizat este adecvat pentru aplicarea în adăposturi de animale și alte incinte, cu posibilități de închidere etanșă, fiind ușor de manipulat, fără riscuri deosebite. Eficiența decontaminării este condiționată de o curățare mecanică și hidrosanitară corespunzătoare a suprafețelor, asigurarea unei temperaturi de cel puțin 10ºC, a unei umidități relative de cel puțin 50% și a etanșeității incintei în care se realizează decontaminarea. Produsul testat s-a dovedit a fi tolerabil pe pielea epilată la concentrații ale soluției de 0,5% și/sau 2%. Contactul cutanat cu soluții cu concentrații mari (10% și 20%) determină iritații severe și de durată, precum și leziuni cutanate, situație confirmată prin examinarea histologică.

1. Introduction

1.1. Morpho-histological structure of the skin

The skin or cutaneous covering is an organ of the human and animal body. It separates the internal environment and internal organs from the external environment.

Its protective role is provided to the body by the stratified squamous epithelium whose structure is constantly evolving both morphologically and histochemically.

Only organisms with a morphofunctional integrity of the skin can maintain the "*crasis*" of their internal environment and can use the energy resulting from metabolic oxidation processes, especially the caloric one. For these reasons, the skin represents the mirror of the animal's health.

Embryologically, the skin originates from the ectoderm and mesoderm. The *ectoderm* gives rise to the epidermis and its accessory formations (glands and phanerae), and the *mesoderm*, to the dermis and hypodermis. It has an epithelial-connective structure adapted to its multiple and complex functions.

The main anatomical structures of the skin are: the epidermis, the dermis, and the hypodermis, to which are added the accessory structures (sebaceous and sweat glands), the appendages (hair, nails, horns, plumage), the nerve formations and the blood vessels through which it performs other functions ensuring the normal development of life.

Most authors agree on the following structure of the skin:

1. *Epidermis*, with the *stratum corneum, stratum lucidum, granular stratum, stratum spinosum, and stratum basale*;

2. *Dermis*, with the papillary layer and the reticular layer;

3. *Hypodermis*.

The development of the skin over time differs from one species to another. In cattle and horses, it is generally already formed by the third month of gestation, but glands and hair lacks, which are structured in the fourth and fifth month In the third month, the migration of melanoblasts from the neural crest to the epidermis also takes place, while the development of the enzymatic apparatus specific to the melanogenic function ends in the month fifth and sixth.

In general, in species with a long gestation period and large body development of the fetus (in horses and cattles esspecially), the skin is completely formed at birth, while in species with a short gestation period and reduced body development of the fetuses (e.g. rabbits, mice, etc.) the structure of the skin and especially its appendages continues after birth.

The epidermis is the thinnest component of the skin, has a variable thickness ranging between 0.1 mm and 3.0 mm, depending on the species, being made up of five layers of cells, whose evolution is from deep to the outside.

Epithelial cells are in a continuous keratinization process, the epidermis, on the outside, being composed of the *stratum corneum* formed by flattened and degenerated cells, impregnated with fat, keratin and pigments, the development of this layer being very varied depending on the anatomical region concerned.

This layer has the role of protecting the skin and constitutes a barrier to the penetration of medicinal substances that are not fat-soluble. The resorption of water-soluble substances through normal skin can only be achieved by prior maceration or degreasing with water, alcohol, chloroform, soap or detergents. Resorption is also favored by vigorous massage or by treatment with keratolytic, hyperemic, vasodilator, hyaluronidase substances, etc. if the epidermis is damaged, the resorption of hydrophilic substances is easy.

The *stratum corneum* cells, desquamated, formed by the most flattened and keratinized cells, located in an advanced layer of dehydration, constitute the disjunct layer.

The *stratum lucidum* is a thin, homogeneous, transparent layer formed by cells that are not well delimited, with a flattened nucleus and with inclusions of keratin and elaidin.

The *acanthous layer* (spiny, Malpighian mucous bodies) due to its structure, protects the dermis from external agents. The thickness of the layer is variable depending on the species,

its cells are polyhedral in shape, flattening as they approach the granular layer.

The *basal layer* is formed by a single layer of cells, tightly interlocked with the underlying dermal layer. Although it lacks blood vessels, the epidermis plays an important role in the percutaneous diffusion of drugs, through the ducts and pores of the sweat glands and hair follicles and the sensory nerve endings found at this level.

The time required for the renewal of the epidermis represents the time required for a single cell of the basal layer to cross the epidermis to the surface. In fact, it is an expression of the mitotic activity of the cells which corresponds to a period of 25-28 days.

The morphological process of the migration of cells from the basal layer to the surface of the stratum corneum is called keratinization.

According to Popovici cit. Cristina (11), in this process, an initial cell with a width of 6 nm can reach 30 nm in the *stratum corneum*.

Depending on the body area, large differences were found in the formation of the horny material, the degree of desquamation of the cells being higher at the level of the autopodium, knees than at the level of the armpits, face or scrotum.

The quantitative evaluation of the desquamated cells of the stratum corneum in different areas of the body are expressed in human medicine (with applicability also in veterinary medicine) by the Strippings index.

The dermis (*chorion*) is the most resistant layer of the skin, being made up of connective tissue. Due to the fact that it is very well vascularized, it ensures the nutrition function of the epidermis. In turn, the dermis is formed by a superficial layer rich in collagen (a hydrophilic colloid with an important role in the resorption of water-soluble substances), elastic fibers and well vascularized, which gives the skin its characteristic elasticity.

Between the fibrillar elements of collagen, an amorphous substance called the fundamental substance composed of an amorphous gel formed by muco-polysaccharides is interspersed. Elastic fibers are less numerous than connective ones but somewhat thicker, they are not organized into bundles but anastomos and branch, constituting a shiny, refractive mass (due to the content of elastin and albuminoid substances that have a different structure than that of collagen). The deep layer of the dermis or the chorion, well vascularized and innervated (most of the skin's sense organs are found at this level), at which the sebaceous and sweat glands are located.

The hypodermis *(tella subcutaneousa),* formed by fibrous tissue and loose connective tissue containing numerous adipose panicles with a role in limiting heat loss and storing thicknesses. At this level, the fats (triglycerides) are in an emulsified form of the A/U type, due to cholesterol. At the level of this last layer, the sweat glands with excretory ducts are found. These ducts represent potential pathways for the penetration of medicinal substances applied to the epidermis.

The number of *sweat glands* varies from one species to another, generally being of the order of 2-3 million. Two types of sweat glands are distinguished:

a) *eccrine sweat glands* which are constituted in their glomerular portion by contractile (myoepithelial), secretory (clear and dark cells) and transitional cells (whose functional significance is still unknown). The secretion of these glands has a strongly acidic reaction (pH = 3.8) with a role in defending the skin against pathogens;

b) *apocrine sweat glands* are made up of a single layer of cells in which mitochondria and the Golgi apparatus are well developed. The secretion of these glands has an alkaline reaction (pH = 6.9), which favors microbial growth.

Sweat secretion is a clear liquid, generally with an acidic reaction (pH = 4.5) with a specific gravity of 1.004, which normally contains 97-99% water and 1-3% soluble substances (sodium chloride, potassium chloride, urea, ammonia, glucose, albumins, uric acid, creatinine, water-soluble vitamins as well as volatile fatty acids). Also at this level are the *hair follicles* provided with muscle fibers and sebaceous glands (acinous glands), the latter being responsible for the secretion of sebum.

Sebum is an oily, yellowish, semi-fluid substance with a specific gravity of 0.9. The

amount excreted by the sebaceous glands is 1-2 g/day and is composed of unsaturated and saturated fatty acids, triglycerides, fatty acid esters, cholesterol and hydrocarbons.

An important role is played by unsaturated fatty acids (C_7-C_{22}). Linoleic and linolenic fatty acids are also present in small quantities.

Before secretion, all free fatty acids are found in the form of triglycerides, which are subsequently split into mono- and diglycerides and fatty acids due to the activity of enzymes with lipolytic activity. The pH of the skin varies from one individual to another and even depending on the body area, being the result of metabolic functions, the buffering property of the skin being attributed to amphoteric amino acids, bicarbonates and the acidity of sebum derivatives. After degreasing, the skin's lipid mantle is restored in approx. 30 minutes.

By massaging the ointments on the skin, follicular absorption will be accelerated due to the elimination of air from the follicular cavity, resulting in a negative pressure that absorbs the ointment spread on the surface of the epidermis. In the case of prolonged action of water on the skin, fats emulsify and keratin “swells”, thus making it possible for water to penetrate through the epidermis.

The most common ***enzymes*** found in the skin are hydrolases and oxidoreductases. Among hydrolases, proteases that convert albumins into polypeptides and amino acids, amylases that degrade polysaccharides, and lipases that act on fats play an important role.

Oxidoreductases are represented by dehydrogenases, cytochrome oxidase and catalases that catalyze oxidations and redox reactions. In addition to these enzymes, the skin also contains cholinesterase, tyrosinase, phosphatase and hyaluronidase.

The skin contains vitamins: A and D (provitamin D) in the epidermis, B1, B2, B6, pantothenic acid, vitamin H and ascorbic acid in the dermis, and vitamin P in the capillary wall.

The state of cutaneous eutrophy is achieved due to the specific functions of the skin and the cutaneous functions related to the general physiological processes of the body. The specific functions are constituted by the epidermal and dermal functions:

- protective (formation of the superficial hydrolipidic film, melanogenesis and keratinization);
- secretory (sweat and sebaceous secretion);
- pilogenesis;
- mechanical function (elasticity, plasticity, resistance);
- metabolic function.
- cutaneous functions related to the general physiological processes of the body:
- thermoregulation;
- local metabolism;
- reactivity (exteroceptor, neurovascular, immunological).

2. Penetration of medicinal substances through the skin

The release of a drug substance from a preparation is called the release of the active substance. This is possible only when the active substance does not interact with the vehicle.

Penetration is the intradermal penetration of active substances without their resorption through lymphatic or blood vessels.

Absorption is the crossing of the skin barrier and the participation of active substances in the metabolic process. Absorption is the basic function of all living cells. It can be selective (when it is achieved by diffusion, osmosis, inhibition) and active (when it is achieved by vital processes that use the energy produced by respiration). The notion of absorption includes interrelations between the vehicle and the possibility of the release of the active substance into the skin layers, from a dermatological aspect being, in fact, the affinity of the stratum corneum for topical substances.

Resorption is a process of passage of a liquid or gas from a body cavity (peritoneal, pleural), from a natural body duct or from the interstitium of a tissue into the blood or lymphatic circulation.

Resorption of liposoluble substances can be achieved by dissolving in the superficial lipid layer of the epidermis and then penetrating into the deeper layers. Drugs can also pass through the skin through the excretory ducts of the sweat or sebaceous glandular system, as well

as along the hair follicle (transfollicular). When substances are amphiphilic they can be resorbed through the skin because they can solubilize both in the lipid layer of the skin and in tissue fluids.

Percutaneous absorption defines the process of penetration of substances from the outside, through the skin, into the bloodstream and implies that the transfer of the active substance occurs through the entire thickness of the skin.

Penetration defines the phenomenon of the release of the active substance into the intradermal layers without systemic effects, and percutaneous resorption is the process of penetration of active substances into the body through the skin.

These two processes are practically included in the notion of percutaneous absorption. The percutaneous absorption process is very complex in which diffusion processes, electrostatics, capillary forces, chemical and biological reactions intervene.

In the modern concept, the skin is made up of a protein gel protected by a double barrier: the superficial lipid film and the hydroelectrolytic barrier.

The superficial lipidic film is located at the "*edge*" of the *stratum corneum* and *lucidum*, being the result of the emulsification of the aqueous and lipid components originating from the secretion of the sebaceous and sweat glands, the emulsifying agents in these situations being the lipoids (as a result of keratinization). An emulsion of the oil/water type (excess water) or water/oil (excess oil) will be formed.

The spontaneous alternation, possible between the two types of emulsions, will determine a behavior of accumulation, or on the contrary, of elimination of water, of course depending on the needs and existing conditions. In fact, a "*buffer system*" will be created that will ensure the homeostasis of the skin surface.

This film has the capacity to regulate the permeability of the skin ensuring: the hydration state of the skin, waterproofing against water-soluble substances and increased resistance of the cutis to acids, bases, water and hydrophilic substances. Due to the uneven distribution of sebaceous and sweat glands as well as the uneven thickness of the stratum corneum, the hydrolipidic film shows regional variations.

Research has revealed differences related to sex, age, and constitutional type of animals (dry, sebostatic skins need fats and W/O emulsions in contrast to seborrheic ones)(16, 20, 29, 33, 43).

In the case of desquamative dermatoses, seborrhea, acne, the proportion of fatty acids with less than 14 carbon atoms is higher. It seems that this proportion is the basis of the pathogenesis of the respective dermatoses. Also in the case of vesicular dermatoses, the hydrolipidic film is destroyed, the resulting exudates being coagulated and very rich in fibrin, with a tendency to form crusts, otherwise good microbial culture media.

Restoring the balance in these situations is achieved by choosing the most appropriate active substance and excipient (19, 23, 47).

The *hydroelectrolytic barrier* or the *acid mantle (or Marchionini acid mantle).* This barrier, without histological existence proper, is actually a hydroelectrolytic barrier that opposes the passage of hydrophobic substances.

The essential constituent element of this barrier is the water coming from cutaneous perspiration (formed from perspiratio sensibilis: sweat secretion visible to the naked eye and from perspiratio insensibilis: water coming from the dermis that passes the epidermis through physical forces).

The acid mantle, together with the hydrolipidic film, ensures the protection of the skin against chemical noxious agents and microbial attack.

The stratum lucidum also participates in the defense of the cutis, which, due to its chemical structure, participates in achieving the acidic pH of the skin as well as in the transit of water through the epidermis, being a regulatory factor of cutaneous permeability. The mechanism of cutaneous absorption is closely linked to the functionality of cell membranes.

The cell membrane is made up of bilayered phospholipid chains having hydrophilic ends oriented towards a protein, and hydrophobic ends oriented in opposite directions.

This structure ensures both the elasticity and plasticity of the membranes.

The membranes are surrounded by a shell rich in hyaluronic acid, chondroitin-sulfuric acid, elastin, collagen, etc., functioning as a true ion pump and as a molecular filter, thus managing to maintain the internal cellular environment intact.

The most important properties of the membrane are:

- *permeability,*
- *surface tension* and
- *electrical properties*.

The penetration of fat-soluble substances and gases is possible due to the lipid part of the membrane. The penetration speed will be directly proportional to the lipid / water coefficient, small ions (4Å) crossing the cell membrane. Lipoids with acidic groups allow the passage of cations, those with basic groups, of anions, neutral lipids being impermeable.

Permeability can also be achieved through membrane pores through which particles with a size of up to 10A can pass, positively charged (cations cannot pass, and bound protein molecules favor the passage).

The cell membrane has a high electrical potential due to lipoproteins. This membrane potential is located between 50-100 mV and compensates for the differences in concentration between chloride ions (Cl^-) and potassium (K^-).

The inner face of the membrane, at rest, has a negative electrical potential, which, in the presence of stimuli on the cell (pressure, light, active medicinal substances, heat) becomes positive, modifying the permeability.

After the cell is excited, the change in potential acts as a stimulus, transmitting impulses to other membranes that reach the nerve endings where mediator discharges (noradrenaline, acetylcholine) can occur.

In conclusion, it can be stated that the factors that favor percutaneous absorption are:

- the physicochemical properties of the active substance,
- the effect of the ointment base,
- the condition of the skin and
- the method of application of the ointment.

2.1. Physico-chemical properties of the active substance

Thermodynamic properties.

Higuchi was the first to mathematically express the dependence of the active substance on the thermodynamic properties in achieving the penetration process (considering that the excipient containing the active substance does not affect the skin):

$$\frac{dq}{dt} = \frac{(P \times C)(\text{Drug's concentration}) \times D \times A}{L}$$

Where:
dq/dt = absorption rate;
P x C = distribution coefficient of the active substance between the vehicle and the skin barrier;
Drug concentration = drug concentration in the vehicle;
D = diffusion of the active substance in the barrier phase;
A = cross-sectional area;
L = thickness of the barrier phase.

According to this equation, the degree of skin penetration is determined by the effective distribution coefficient and the diffusion in the barrier phase. The variable factor of this constant is the effective distribution coefficient, since the diffusion of a substance with a similar molecular weight and shape differs very little.

A substance can easily penetrate membranes when the distribution coefficient is small (10, 47).

In a complex structure such as the skin (where the membrane may be nonpolar and the fluids of the receptor tissue polar, a substance whose partition coefficient between polar and nonpolar solvents is close to 1.0 will have the highest degree of penetration) permeability is more accentuated if there is an affinity between a penetrating molecule and the membrane (which will attract the penetrating molecule) but not to such an extent that it fails to release it.

It is also known that liposolubility is a decisive factor in absorption, especially when associated with the property of hydrophilicity. For such systems, the degree of percutaneous absorption will be approximately constant only under the condition of a constant thermodynamic activity in the vehicle. Thus, ointments containing micronized suspensions will have the same degree of penetration as the

solid drug only if the thermodynamic activity of the two forms is the same.

Molecular size of the active substance

The degree of resorption is influenced by the molecular size of the active substances. Active substances with a molecular weight below 20,000 are resorbed through the blood capillaries, and those with a higher weight will be resorbed into the lymphatic vessels.

The effect of pH

The absorption rate of active substances, whether acidic or basic, is strongly influenced by the pH of the skin. Thus, histamine is absorbed 10 times more if incorporated into an ointment base buffered to a pH of 7.5 than if it is incorporated into an ointment base with a pH of 5.5. The maximum local activity of benzocaine has been established (as measured by the pain threshold) at a pH between 6.0 and 7.0, with efficacy decreasing significantly outside these limits. Systemic alkalosis usually increases the percutaneous absorption and excretion rate of various active substances, while acidosis decreases the percutaneous absorption rate.

The degree of dispersion of the active substances incorporated into the ointment base. Resorption depends very much on the physical state of the active substance. The best absorbed are the medicinal substances that are incorporated into the ointment base in the form of molecular dispersion, dissolved in solvents or emulsified. Large crystals, agglomerates slow down the resorption by reducing the contact surface, and can even be irritating. In pharmaceutical technology, the most recommended size of solid particles is 5-10 micrometers for ophthalmic ointments, their size can increase up to a maximum of 200 micrometers for the rest of the ointments.

Concentration of the active substance

Increasing the active concentration modifies the resorption of the drug. It was found that, in the case of concentrations between 1 and max. 10%, the resorbed proportion does not increase, this phenomenon being explained by the fact that, in the case of penetration into the cutis, even in low concentrations, the difference between the external and internal concentration has such a large value that the substance penetrates the skin at an increased speed (this is the case for substances insoluble in water, therefore hydrophobic). In the case of hydrophilic substances, resorption will increase with concentration. The role of the concentration of the active substance on its degree of absorption from an ointment results from Higuchi's relationship:

$$\frac{d_q}{d_t} = \frac{A \times D \times C_s}{2t}$$

Where:
A = drug concentration expressed in units/cm3;
CS = drug solubility in units/cm3 in the external phase of the ointment;
D = drug diffusion constant in the external phase;
dq/dt = absorption rate. The release rate of drugs from this type of preparation is a function of A, D and CS.

2.2. The excipient effect

It seems that the preponderance of one of the two components (active substance and excipient) in the penetration and pharmacological efficacy of drugs has not yet been established. However, most researchers lean towards excipient bases.

These have the role of facilitating contact between the active substance and the skin, in choosing excipients taking into account the therapeutic purpose pursued, the type of skin, the location and stage of the disease, the physicochemical properties of the active substance.

A good excipient should not influence the metabolic processes, secretion and respiration of the skin. The therapeutic idea is to treat “gently” an acute disease and more “aggressively” the chronic ones.

In the case of animals where the hair layer is more abundant, occlusive applications will be avoided. For oily skin (with high sebum secretions) lipogels, O/W emulsions and pastes are not tolerated due to their activity in preventing secretions. On the contrary, for skin with low sebum secretion, the use of oily ointments (O/W) and lipogels is required.

The most well-known classification of pharmaceutical forms according to the clinical characteristics of dermatoses is:

- *Acute, inflammatory, secretory dermatoses:* wet compresses, lotions, pastes, liniments, emulsions, emollient, absorbent ointments with anti-inflammatory action. These allow the passage of secretions, are easy to apply, emollient and refreshing.
- *Subacute or chronic mildly inflamed dermatoses:* lotions, pastes, ointments, creams, liniments, emulsions. They have anti-inflammatory and emollient activity.
- *Dry conditions with thick crusts:* ointments, pastes, liniments, emulsions, lotions. They remove crusts, can be applied easily and are not irritating.
- *Generalized rashes:* lotions, liniments, emulsions, ointments. They are applied easily, soothe the skin.

The degree of transepidermal penetration varies, not exceeding one third of the depth of the stratum corneum, with animal fats penetrating most effectively, followed by vegetable oils, mineral oils not favoring penetration.

O/W emulsions are less occlusive than fatty vehicles, while O/W emulsions tend to reverse (as the aqueous phase evaporates), leaving an oily layer on the skin.

Fatty and oily vehicles have the highest occlusive capacity, produce increased hydration and favor the accumulation of sweat. Wetting agents reduce the degree of hydration of the *stratum corneum*. Topicals can also be combined with other pharmaceutical formulations, becoming biphasic or triphasic systems depending on the stage of the disease.

The increasing order of the degree of release of the active substance from the excipient is: *hydrocarbons, vegetable fats, animal fats, W/O emulsions, W/O emulsions, hydrophilic bases.*

The drugs for which percutaneous absorption is more pronounced from fatty bases are: salicylic acid, acetylcholine oleate, aconitine, benzocaine, deoxycorticosterone, iodine, diiodofluorescein, ezerine, hydroquinone, methyl salicylate, morphine, nicotine, estrogens, phenol, phenolsulfophthalein, pilocarpine, progesterone, pyrogallol, resorcinol, sulfadiazine, sulfathiazole, strychnine, testosterone, vitamins A, D and K.

Particular attention should be paid to the physiological compatibility of excipients with the skin, the acanthosis index. Depending on this criterion, the drug substances are classified into three categories (Table 1).

Table 1.
Classification of excipients according to acanthosis index

non-acanthogenic	mild-acanthogenic	strongly-acanthogenic
silicone oil, cetacea, sesame oil, methylcellulose, stearyl alcohol, cetyl alcohol, paraffin, glycerin, propylene glycol, stearin, hydrated lanolin (50%), wax, PEG 400, 1500, 4000	vaseline, animal fats	Eucerin anhydrous and hydrated, yellow petrolatum, axungia, olive oil, paraffin oil, sorbitol (70%), undecylenic acid, cocoa butter 70%

The histological changes observed after applying the emulsions are strictly limited to the treated area in the case of U/A emulsions, but much more intense in the case of A/U emulsions (which induce distant changes in the epidermis due to deep diffusion).

Studies have shown that U/A emulsions have stimulating capacities on the sebaceous glands that A/U emulsions do not possess, causing an increase in reactive seborrheic activity.

On the contrary, A/U emulsions are miscible with fatty secretions and emulsify with aqueous ones. An artificial surface lipid film is formed, which is capable of inhibiting the activity of the sebaceous glands. The use of surfactants in external applications produces side effects, either by themselves or due to the potentiation of the toxic action of the substances present. Soaps and detergents, due to their degreasing effect, produce degreasing of the skin and

dermatitis. Soaps with short chains are more irritating than those with long chains.

Cationic substances cause irritation at concentrations above 1%, and anionic ones at concentrations between 0.5 and 5%. Toxicity decreases from cationic to anionic, with the highest toxicity occurring in nonionics. Ionic and nonionic surfactants are frequently used in the preparation of ointments with increased resorption.

Increasing resorption can be achieved (except for complexing incompatibilities) by reducing surface tension, wetting the skin and solubilizing active substances.

The penetration capacity of ointments is closely dependent on the nature of the surfactant. Thus, anionic and cationic surfactants have a higher penetration capacity compared to non-ionic surfactants.

A high degree of penetration can also be achieved through the synergistic activity of wetting agents, organic solvents and solubilizers (e.g. polyethylene glycol in the ointment base is a good emollient, humectant, has the property of inhibiting the development of microorganisms, while being at the same time a good solvent for active substances).

Of particular importance in facilitating skin penetration and resorption is the use of emulsifier mixtures that provide the achievement of hydrolipid balance (HLB) values of excipient bases suitable for each active substance. An important role in the resorption of the active substance is played by the water content of the *stratum corneum*.

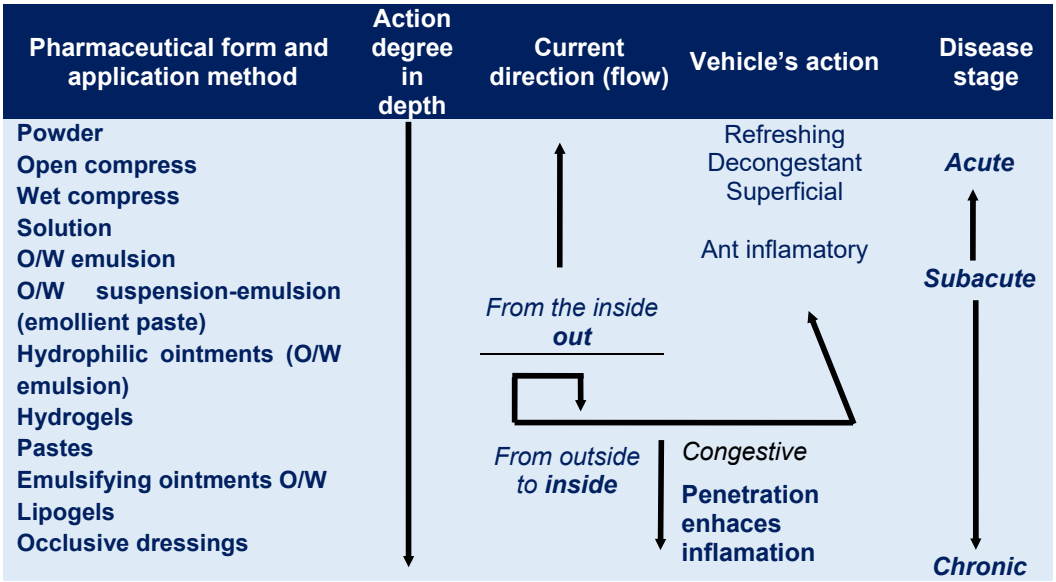
Anhydrous fatty excipients accelerate the hydration of the stratum corneum by preventing the evaporation of skin moisture (*occlusive effect*).

Humectants (sorbitol, glycerin) produce opposite effects, and hydrophilic excipients do not cause changes in the hydration of the stratum corneum. A layer covered with an occlusive dressing will retain sweat and provide additional hydration. The thickness of the applied ointment film directly affects the hydration of the stratum corneum. Increased moisture favors transfollicular absorption. Application of histamine to subsequently covered skin had a prolonged effect (due to prevented evaporation).

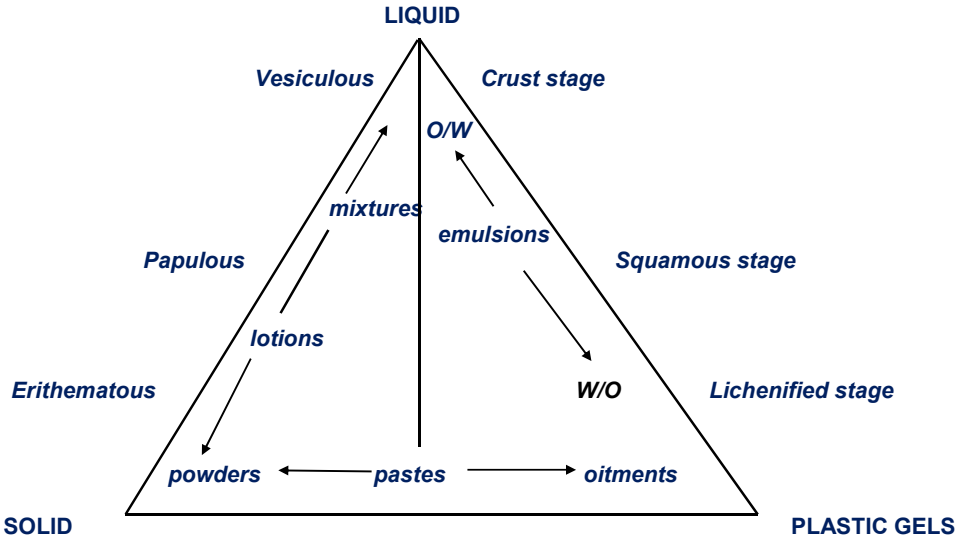
It is likely that the transfer properties of the skin layers are strongly influenced by the presence of water because it is absorbed by skin proteins.

The greatest increase in the degree of penetration through moisture was found for the substance with the lowest oil-water partition coefficient.

Scheme 1.
Classification of pharmaceutical forms by penetration, vehicle action and disease stage (10,11).



Scheme 2.
Correct use of preparations consisting of two-phase or three-phase systems in external treatment (10,11)



If the epidermal barrier presents discontinuities due to traumas of various origins (blisters, eczema, skin parasites, wounds), all active substances will pass into the dermis.

This results in the importance of choosing the vehicle in the case of a normal stratum corneum, since the differences in the penetration of active substances due to different vehicles are more pronounced.

The skin of old animals or those in a poor state of maintenance will determine significantly lower absorption rates due to atrophic changes in the pilosebaceous apparatus. Occlusive applications will produce intensification of cutaneous circulation, vasodilation and an increase in skin temperature which will enhance the absorption rate. The resorption through the skin of water-soluble substances is favored by maceration of the skin as a result of steam baths or with hot water (when there is a decrease in sebum viscosity), the sebum becoming miscible with ointments.

Application method

The rheological properties of the excipient bases allow the active medicinal substance to be applied in a continuous layer on the skin surface. The amount of the drug in the dermis is proportional to that used, as well as to the duration and number of applications.

Prior degreasing of the skin with solvents (alcohol, acetone, gasoline, chloroform) increases absorption in most cases. In general, the application of many solvents, excluding water, can cause notable changes related to the resistance of the skin barrier. It seems that this phenomenon is caused by the changes produced by these solvents on the activity coefficient and the diffusion constant. The addition of some enzymes (e.g. hyaluronidase) facilitates penetration.

Assessing skin's tolerance

The most recommended method for assessing skin tolerance is the patch test, known in two variants.

a. Standard patch test method I - variant I

The skin will be shaved and 0.5 ml of the solution being examined or 0.5 g of ointment will be applied to it. The area will be covered with a compress on an area of 6/4.5 cm^2.

The animals will be restrained from movement and the compress will be fixed with adhesive tape. Readings will be taken 24 hours after the applications. The compress will be removed and the results will be evaluated based on the values in Table 2. The following readings will be taken after 72 hours, the final value expressed representing the average from 24 to 72 h.

Table 2

Skin reaction assessment according to identified reactions

Identified reactions	Mark
1. Formation of erythema and bedsores	
No erythema	**0**
Slight erythema (visible pain)	**1**
Highly visible erythema	**2**
Moderate – severe erythema	**3**
Severe erythema (dark red to the formation of a slight eschar with lesions in depth)	**4**
Maximum possible value	**4**
2. Edema formation	
No edema	**0**
Mild edema (visible pain)	**1**
Mild edema (at the limit of visibility)	**2**
Moderate edema (1 mm)	**3**
Severe edema (over 1 mm)	**4**
Maximum possible value	**4**

b. Standard patch test method I - variant II

The procedure is similar to the first variant but the skin after epilation will also be scarified. Scarification lesions will be light incisions that do not touch the dermis. The values recorded at 24-72 hours (time required for erythema or edema to occur, as appropriate) are adjusted by averaging the values for intact and scarified skin. The value obtained will represent the primary irritation index and is used to classify products with acanthogenic potential according to this parameter (Table 3).

Table 3.

Primary irritation index determined by potentially irritating topical products

Identified reactions	Mark
Slightly irritating to the skin	**2**
Moderately irritating to the skin	**2-5**
Very strongly irritating to the skin	**< 6**

c. Standard patch test method II - variant I

This method consists of applying a swab soaked with 0.5 ml of the test solution, for 30 minutes, to a hairless surface on an area of 2/2 cm square. The skin reaction will be monitored at 30 minutes, 8 hours, 24 hours, 36 hours and 48 hours after application.

d. Standard patch test method II, variant II

It is a variant of the above, with the difference that, in this case, the skin after epilation was also scarified on an area of 2/2 square cm. The assessment of the skin reaction was made according to the score presented in Table 4.

Table 4.

Evaluation of skin reaction on epilated and scarified skin

Identified reactions	Mark
No visible reaction, possibly slight peeling	**0**
Slight congestion that disappeared after 24 hours	**1**
Congestion and inflammation that decreases and disappears within 36 hours	**2**
Congestion and inflammation that does not decrease within 36 hours	**3**
Congestion and pustules, lymph extravasation, prolonged healing	**4**

The final quantification of the patch test

It will be done by taking into account the grades obtained by the subjects in the study, adding them up and dividing them by the number of individuals, which will then be assessed according to Table 5.

Table 5.

Final quantification of the skin test

Identified reactions	Limits
Tolerable	**0 – 0.99**
Average tolerability	**1.0 – 2.79**
Irritant	**2.80 – 3.69**
Severely irritating	**3.70 – 4.0**

3. Toxic effects of the product in the proposed study

(According to the manufacturer's data sheet)

Concentrated disinfectant detergent (TP2) **(DEO-SEPT®)**

Manufacturer: Pasteur, Branch Filipesti S.A. Romania,

It is a transparent, golden yellow solution with a pleasant odor, perfectly soluble in water https://farmavet.ro/images/catalog/nomenclator-pasteur-ro.pdf

User category:

- professional,
- industrial.

Composition:

C12-C18 alkyldimethyl benzyl ammonium chloride (C.A.S. 68391-01-5, E.C. 269-919-4).....5%

Didecyl dimethyl ammonium chloride (C.A.S. 7173-51-5, E.C. 230-525-2) 5%

Excipients q.s. ad.....100 ml

ECHA field of use: Category TP2:

Products used for the disinfection of surfaces, materials, equipment and furniture and not used in direct contact with food or feed.

They are mainly intended for walls and floors in private, public and industrial premises and other premises intended for professional activities (https://echa.europa.eu/ro/regulations/biocidal-products-regulation/product-types).

Combats:

- Gram-positive
- Gram-negative bacteria,
- mycobacteria,
- lipophilic and hydrophilic viruses (with and without pericapsid),
- avian influenza (H5N1),
- foot-and-mouth disease virus,
- fungi (see Table 6).

How to use: The product is used diluted in water, at room temperature, by spraying on surfaces or by immersing objects in the disinfectant solution, respecting the specified concentrations and contact times, depending on the nature of the contaminant and the degree of contamination.

Table 6.

General activity by species, concentrations and action time of the product

Activity	Species	Concentration	Time of action
Virulicide of necessity	*BVDV (HCV), Vaccinia Virus, HBV, HIV, H5N1, H1N1*	2%	5 minutes
Prophylactic bactericide	*Staphylococcus aureus* *Pseudomonas aeruginosa*	2% 2,5%	10 minutes 5 minutes
Bactericide of necessity		2%	15 minutes
Prophylactic antifungal	*Candida albicans* *Aspergillus niger*	2%	15 minutes
Antifungal of necessity		2% 2.5%	60 minutes 30 minutes

Mold removal (TP2): prepare fresh solutions with a concentration of 2.5% (5:200) of DEO-SEPT® in water.

Apply the solution so that it completely covers the surface affected by the mold. Allow to dry and repeat the treatment after 7 days if mold reappears. No rinsing is necessary.

Surface disinfection (TP2):

The amount of disinfectant solution applied depends on the nature of the surfaces:

- 100 ml DEO-SEPT® solution / m^2 for smooth surfaces;

- 300 ml DEO-SEPT® solution / m^2 for porous surfaces.

Before using the disinfectant, the surfaces must be properly cleaned.

Storage: At temperatures below 25 °C, in original containers.

Shelf life: 2 years. Approval no. 1439BIO/02

Presentation: Bottles x 1 liter / canisters x 5 liters.

3.1. Toxicity of the cationic detergents

Synonyms: *Cationic detergents, Cationic surfactants, Quaternary ammonium compounds, Pyridium quaternary germicides, QAC*

The main structures of cationic surfactants on the market:

1) Quaternary ammonium compounds:

- alkyl-dimethyl-benzyl-ammonium chloride (C_{12}-C_{18});
- diisobutyl-phenoxy-ethoxy-ethyl-dimethyl-benzyl-ammonium chloride;
- cetyldimethyl-benzyl-ammonium chloride;
- benzyl-hexadecyl-dimethyl-ammonium chloride.

2) Pyridinium compounds:

- 1-hexadecyl-pyridinium chloride;
- stearyl dimethyl-benzyl-ammonium chloride);
- cetyl-trimethyl-ammonium bromide;
- 2,3-epoxy-propyl-trimethyl-ammonium chloride;
- didecyl-dimethyl-ammonium chloride

3) Quinolinium compounds

- qualinium chloride

The general and local toxic clinical effects caused by cationic detergents are shown in Tables 7 and 8.

Table 7.

General toxic and clinical effects caused by the cationic detergents (Synthesis: 2,3,7,9,12,29,38)

General clinical effects	General treatment in poisoning
Many consumer products and other products containing small amounts of cationic detergents are being monitored for effects, primarily due to their potential for eye and skin irritation. Cationic detergents are somewhat more toxic than anionic/amphoteric or nonionic detergents. If ingested, concentrated solutions (above 7.5% concentration) of quaternary ammonium compounds can result in corrosive burns of the mouth, pharynx, and esophagus. Medical evaluation is generally warranted, except for patients who have inadvertently ingested a mouthful of dilute cationic detergent solution (below 1%). The following have been observed: vomiting, diarrhea, dermal necrosis, dermatitis, pulmonary edema, hypotension, metabolic acidosis, and CNS depression.	Gastric lavage and emetics are not recommended due to possible corrosive effects and the potential for rapid onset of CNS depression.However, prompt administration of activated charcoal is highly recommended because cationic detergents are well absorbed by charcoal. Initial treatment should be dilution with milk or water. If ingested, the patient should be evaluated for esophageal or gastrointestinal tract burns by esophagoscopy. If burns are present, treatment should be based on clinical assessment. Patients should be monitored for the development of seizures, hypotension, or pulmonary edema and treated appropriately. Forced diuresis may be contraindicated. Eyes and skin of exposed individuals should be flushed with copious amounts of water.

Table 8.

Local toxic effects caused by cationic detergents (Synthesis: 2,6,8,12,16,23,24,29,33,40)

Local toxic effects	Specific treatment in poisoning
Local ingestion Ingestion of concentrated solutions may cause caustic burns of the lips, tongue, mouth, pharynx and esophagus ranging from mild to severe.	Following ingestion of diluted solutions: gastric evacuations as a priority, dilute immediately with 120-240 ml of milk or water (no more than 15 ml/kgb. in children). Activated medicinal charcoal (240 ml water/30g charcoal). *Dose:* 25-100g in adults/adolescents, 20-50 g in children (1-12 years) and 1g/kg in children under 1 year.
On the eye Exposure can cause effects ranging from mild discomfort (0.1% solutions) to very serious corneal damage (10% solutions).	Eye exposure ranges from mild discomfort (0.1% solution) to severe corneal damage (1-10%) depending on concentration. *Decontamination:* Exposed eyes should be irrigated with copious amounts of lukewarm water for at least 15 minutes. If irritation, pain, swelling, tearing or photophobia persist, seek medical attention.
Ototoxicity It has been observed in experimental animals when such agents have been instilled into the inner ear; instillation of dilute solutions into ears with intact tympanic membranes is not expected to be irritating.	-
Cardiovascular effects Hypotension and cardiac arrest have been observed rarely.	*Monitoring* for: hypotension, dysrhythmias, respiratory depression, and the need for endotracheal intubation.

	Assess for hypoglycemia, electrolyte disturbances, and hypoxia. *Hypotension:* infuse 10-20 ml/kg of isotonic fluid. If hypotension persists, administer dopamine (2-20 mcg/kg/min) or norepinephrine (0.1-0.2 mcg/kg/min) until desired response is achieved.
Respiratory effects Respiratory muscle paralysis, pulmonary edema, asthma, and hypoxemia have been observed	*Decontamination:* Remove patients to fresh air and observe for respiratory distress. If cough or difficulty breathing develops, evaluate for respiratory tract irritation, bronchitis, or pneumonia. Administer 100% humidified oxygen with assisted ventilation as necessary. *Pulmonary edema (noncardiogenic):* Maintain ventilation and oxygenation. Treatment should include recommendations in the oral or parenteral exposure section as appropriate.
Neurological effects CNS depression progressing to coma, seizures, shock, and paralysis of the respiratory muscles have been observed in experimental animals.	*In seizures:* administer i.v. benzodiazepines; Diazepam (adult 5-10 mg, repeated every 10-15 if necessary); in children (0.2-0.5 mg/kg repeated every 5 minutes if necessary) or Lorazepam (adults 4-8 mg; children 0.05 - 0.1 mg/kg). If seizures cannot be controlled or recur after administration of 30 mg diazepam in adults or 10 mg in children over 5 years, phenobarbital and/or fosphenytoin should be considered
Gastrointestinal effects Vomiting, diarrhea, and abdominal pain may occur. Hemorrhagic necrosis of the gastrointestinal tract and peritonitis have also been observed. **Toxicity limits** Esophageal or gastrointestinal burns are possible if only a few milliliters of concentrated solution are ingested. Death has been reported in an adult following ingestion of 30 mg/kg of quaternary The fatal dose in humans for ingestion of cationic detergents has been estimated to be between 1-3 g.	Following ingestion of concentrated cationic detergents (over 7.5%). Vomiting cannot be induced. Concentrated solutions should be treated as corrosive solutions with dilutions of milk or water.
Hepatic effects Hepatic necrosis and elevated transaminases have been observed. Metabolic acidosis has also been observed occasionally.	Liver function should be monitored.
Hematological effects Hemolysis and methemoglobinemia have been observed following peritoneal irrigation with cetrimonium bromide.	Blood gases should be monitored due to the possible development of acidosis.
Dermatological effects Dermal necrosis has resulted from exposure to cetrimonium bromide in concentrations ranging from 2-17.5%. Some of these agents have caused irritant or allergic contact dermatitis	*Decontamination:* Wash the exposed area with large amounts of water. If irritation or pain persists, have the area examined by a doctor.

3.2. Summary of toxicological properties of quaternary ammonium halides

Based on the reliable data in the literature, it can be stated that quaternary ammonium halides are gradually toxic in the case of oral administration at relatively low levels of exposure to different animal species and to humans. Contact, irritating effects on the skin and mucous membranes have been demonstrated for both components of the association introduced in the study.Long-term or multiple external exposure can lead to allergic reactions mainly in people who use these agents. Mutagenic, teratogenic and cytotoxic effects have been demonstrated after the application of the substances. In order to avoid too long contact with the particular agent, it is necessary to wear protective equipment and apply an adequate ventilation system.

4. The field study

4.1. Objectives

The objectives of the testing were:

- A bibliographical synthesys of the DEO-SEPT® product's components.
- The disinfectant effect of DEO-SEPT® under field conditions **(Test I)** and
- The skin irritant effect of DEO-SEPT® solutions on the skin **(Test II)**.

4.1. A bibliographical synthesys of DEO-SEPT® product's components

The identified data were presented synthetically in Tables 9 to 18, and it is a synthesis by sources: 1, 4, 5, 13, 17, 18, 22, 26, 31, 32, 45, 49, 50 and 51 inserted in the bibliography. The product under observation is a transparent golden yellow solution with a pleasant odor, completely soluble in water.

4.1.1. Pharmacological action

The product is considered a broad-spectrum disinfectant, with activity against bacteria, both Gram-positive and Gram-negative, viruses, fungi, mycoplasmas and even protozoa.

Table 9 demonstrates the synergistic effect of the product components QAC(C12-C18 alkyl dimethyl benzyl ammonium chloride (component I) and Didecyl-dimethyl-ammonium chloride (component II) when combined in the product. The table compares the phenolic coefficients of DEO-SEPT® and its separate components, the sum of the components being significantly lower than the comparative phenol coefficient for DEO-SEPT®.

Table 9.
Comparative synergistic activity of the components (multiple authors synthesis)

Microrganism	Coloration	DEO-SEPT®	Component I.	Component II
Bacteria				
H. paragallinarum	G -	120	1.2	30
P. multocida	G -	40	1.2	20
B. bronchiseptica	G -	10	0.2	5
K. pneumoniae	G -	41	5.4	24
E. coli	G -	50	2.4	38
S. aureus	G -	110	4.8	56
Fungi				
C. albicans	F	17	0.42	4
A. flavus	F	19	0.36	9
Viruses				
Por. Ent.	Hidro	1:200	1:100	1:10
T.G.E.	Lipo	1:400	1:200	1:100

4.1.2. Posible main interactions

- interaction with nucleic acids in specific types of viruses;
- interaction with proteins in metabolic enzymes;
- blocks amino acids, reacting with R-NH_2 groups
- inhibits viral penetration of host cells;
- lipophilicity;

All reactions are enhanced by the synergistic effect of the quaternary ammonium combination (QAC) = in interaction with diferent viruses like: *Arena, Bunya, Corona, Hepadna, Herpeto, Irido, Orthomyxo, Paramyxo, Retro, Rhabdo, Toga.*

Handling: all chemicals, if used incorrectly, can present risks to the user. Used correctly, they should not present any danger to the person handling them[1].

Hazard class UE: "*Irritant*" U.N. Code 1760

Environmental protection: Residues and packaging will not be used following disinfection, respecting the laws in force, avoiding the spread of packaging and residues in the environment.

Code C: Brown.

Risk phrases R:

R 20/22 hazardous if swallowed and inhaled

R 34 causes burns

[1] In order to assist the user, the following handling information is recommended:
Respiratory: Use in a well-ventilated area, avoid inhalation of vapors.
Eyes: Wear safety glasses, especially when diluting the concentrated solution.
Skin: Wear overalls and gloves. Food and drink should not be consumed where the material is stored, handled or used.
Smoking: Wash hands before smoking. Wear gloves.
Exposure to direct light: No risk.
Application by fumigation. When fumigating, it is recommended to use a Nord respirator with an organic vapor cartridge RP-3 or similar. 20,000 m^2 unit will last approximately 10 minutes using 6.75-7.50 l of product diluted 1:200.

R 42/43 causes sensitisation by inhalation or in contact with skin

Safety phrases S

S 1/2 keep closed and out of reach of children
S 36/37/39 protective equipment must be worn
S 45 case of accident, call a doctor. Show label!
S 61 should be avoided. Do not dispose of the product in the environment!

Instructions for use:

For general disinfection in animal breeding units for the disinfection of shelters, walls, shelter inventory, means of transport, as well as in the food industry. The application will be made on dry surfaces once to be able to see the areas covered with disinfectant and on the other hand to not dilute the solution.

The product will be diluted 1:200 (sol. 0.5%) and will be sprayed at a rate of 100 ml of diluted product per square meter for non-porous surfaces (metal, concrete, plastics, glass, etc.) and 300 ml/m^2 for porous surfaces (wood, soil, brick).

The minimum time for the solution to appear after application is 15 minutes.

For maximum efficiency, disinfection will be done with the doors and windows closed starting from the ceiling of the room, the operator taking the necessary protective measures (gown, mask and goggles). It is also recommended to spray the outer walls of the shelters. After spraying, the solution is allowed to dry. In the food industry, the application time is 15 minutes for 0.5% solutions and 5 minutes for 1.5% solutions, after which the solutions can be removed by simply washing with plenty of water, especially carefully in areas of direct contact with food raw materials. In the case of emergency disinfection, a 1:67 dilution will be used until the outbreak is declared extinguished. For the control of fungi, (e.g. trichophytosis) a 1:67 dilution will be used.

The solution for hoof bathing will be made in dilutions of 1:67. For aerosolization, a dilution of 1:200 will be used and 1 liter of diluted product/100 m2 of floor will be used - normally 2 applications per week is sufficient. Regular use of the product will prevent the accumulation of pathogens and ensure a cleaner environment for animals and birds. Shelf life: in original and unopened packaging for 2 years.

Table 10
Effectiveness on microorganisms (Synthesis)

Activity on bacteria	
	Effective concentration
Gram negatives	
Salmonella typhimurium	1:267-1:4666
Klebsiella pneumoniae	1:4666
Pseudomonas aeruginosa	1:6665
Escherichia coli	1:2000
Salmonella choleraesuis	1:2000
Salmonella enteriditis	1:200
Brucella suis	1:80
Pasteurella multocida	1:4266
Haemophilus pleuropneumoniae	1:2133
Yersinia enterolytica	1:267
Proteus mirabilis	1:267
Proteus vulgaris	1:133
Gram pozitives	
Streptococcus agalactiae saitama	1:2670
Staphylococcus aureus	1:2000
Streptococcus faecalis	1:667 -1:6665
Bacillus anthracis	1:80
Erysipelothrix rhusiopathiae	1:10666
Corynebacterium pyogenes	1:9333
Micobacterium smegmatis	1:667
Listeria monocytogenes	1:267

Activity on mycoplasmas, chlamydia and eimeria	
Organism	**Effective concentration**
Mycoplasma hyopneumoniae	1:1067
Mycoplasma hyorhinis	1:2133
Mycoplasma gallisepticum	1:2133
Mycoplasma synoviae	1:2133
Eimeria tenella	1:133
Chlamydia psittaci	1:1333
Activity on fungi	
Penicillium verrucosum	1:1333
Absidia corynbifera	1:66,7
Cladosporium caldosporoides	1:66,7
Candida albicans	1:133 – 1:1067
Trichophyton mentagrophytes	1:66,7
Saccharomyces cerevisiae	1:267
Aspergillus flavus	1:1333

Activity on viruses			
Virus	**Dilution**	**Type**	**Group**
Parvovirus- canine	1:66.7	H	*Parvo +*
Parvovirus canine	1:240	H	*Parvo 0*
Paravyccinia	1:1333	H	*Pox*
TGE	1:1180	L	*Corona*
Parainfluence type 3	1:1000	L	*Paramyxo*
Aujeski	1:1867	L	*Herpes*
Equine influenza virus	1:200	L	*Orthomyxo*
SVD	1:400	H	*Picorna*
FMD	1:107	H	*Picorna*
Newcastle disease	1:200	L	*Paramyxo*
Enterovirus (Polio 1)	1:133	H	*Picorna*
Orthopoxvirus	1:667	H	*Pox*
Classical swine fever	1:267	L	*Toga*
Bovine viral diarrhea	1:267	L	*Toga*
Swine enterovirus	1:133	H	*Picorna*
Rabies	1:200	L	*Rhabdo*
Adenovirus H5	1:133	H	*Adeno*
Rheovirus	1:133	H	*Reo*
Gumboro	1:267	H	*Rota*

Table 11
Efficacy in poultry diseases (Synthesis)

Viruses			
Family	**Genus**	**Disease**	**Effective concentration**
Poxvirus	*Avipox virus L*	Avian diphtheria	1:200
Herpesvirus	*Herpesvirus L*	Marek's disease	1:1867
Adenovirus	*Adenovirus H*	Oat drop syndrome	1:667
Rheovirus	*Rotavirus H*	Infectious tenosynovitis	1:267
Paramyxovirus	*Paramyxovirus L*	Newcastle disease	1:200
Orthomyxovirus	*Virusul Influenţei L*	Avian influenza	1:200
Rheovirus	*Reovirus H*	Rheovirus	1:267
Bacteria			
Microorganism	**Coloration**	**Affection type**	**Effective concentration**
Mycoplasma spp.	G -	Respiratory infections	1:267
Escherichia coli	G -	Colibacillosis	1:2000
Salmonella enteriditis	G -	Salmonellosis	1:200
Chlamydia psittaci	G -	Chlamydia	1:1333
Pasteurella spp.	G -	Pasteurellosis	1:267
Listeria monocytogenes	G +	Septicaemia	1:267
Erysipelothrix rhusiopathiae	G +	Avian rubella	1:10666
Staphylococcus spp.	G +	Staphylococcia	1:2000
Streptococcus faecalis	G +	Streptococcia	1:667
Fungi and coccidia			
Microorganism	**Type**	**Affection**	**Effective concentration**
Aspergillus Flavus	Fungi	Micotoxicosis	1:1333
Eimeria Tenella	Protozoa	Coccidiosis	1:133

(H) = Hydrophilic virus (L)= Lypophilic virus

Table 12
Efficacy in swine diseases (Synthesis)

Viruses			
Family	**Genus**	**Disease**	**Effective concentration**
Picornavirus	Porcine enterovirus H	SVD (Swine vesicular disease)	1:400
Parvovirus	Porcine parvovirus H	Parvovirosis	1:240
Adenovirus	Porcine adenovirus type 1-4	Respiratory disease, meningitis	1:667
Herpetovirus	Herpes virus L	Aujeski disease	1:1867
Coronavirus	TGE virus L	Transmissible gastroenteritis	1:1180
Picornavirus	Porcine enterovirus H	Swine poliomyelitis	1:400
Togavirus	Pestivirus L	Classical swine fever	1:267
Thabdovirus	Vesiculovirus L	Vesicular stomatitis	1:2000
Orthomyxovirus	Influenza virus L	Swine influenza	1:2000
Poxvirus	Suipoxvirus	Swine pox	1:667
Reovirus	Rotavirus H	Diarrhoea	1:267
Bacteria			
Microorganism	**Coloration**	**Affection type**	**Effective concentration**
Pasteurella spp.	G -	Septicaemia, atrophic rhinitis	1:267
Streptococcus spp	G +	Streptococcia	1:66.7
Staphylococcus spp.	G +	Staphylococcia	1:2000
Escherichia coli	G -	Swine colisepticemia	1:2000
Klebsiella pneumoniae	G -	Localized infections	1:4666
Salmonella spp.	G -	Salmonellosis, septicaemia	1:200
Corynebacterium pyogenes	G +	Porcine pyobacillosis	1:9333
Mycoplasma spp.	G -	Pneumonia, atrophic rhinitis, polyarthritis	1:267
Actinobacill. pleuropneumoniae	G -	Swine pleuropneumonia	1:2133
Pasteurella spp.	G -	Septicaemia, atrophic rhinitis	1:4266
Erysipelothrix rhusiopathiae	G +	Erysipelas	1:10666

Table 13.
Efficacy in cattle diseases (Synthesis)

Viruses			
Family	**Genus**	**Disease**	**Effective concentration**
Adenovirus	*Adenovirus H*	Pneumonia, pneumoenteritis	1:133
Herpetovirus	*Herpesvirus bovin L*	Bovine rhinotracheitis	1:1867
Picornavirus	*FMD virus H*	Foot and mouth disease	1:107
Poxvirus	*Orthopoxivirus L*	Smallpox	1:667
Paramyxovirus	*Parainfluenta virus 3 L*	Acute respiratory infections	1:1000
Togavirus	*Pestivirus L*	Mucosal diarrhoea	1:267
Rheovirus	*Rotavirus H*	Diarrhoea	1:133
Bacteria			
Microorganism	**Coloration**	**Affection**	**Effective concentration**
C. pyogenes	G +	Mastitis	1:9333
Escherichia coli	G -	Toxaemia	1:2000
Salmonella dublin	G -	Septicaemia	1:2000
Haemophilus spp.	G -	Septicaemia	1:2133
Bacillus anthracis	G +	Anthrax	1:80
Brucella abortus	G -	Brucellosis	1:4266
Pasteurella multocida	G -	Haemorrhagic septicaemia	1:4266
P. aeruginosa	G -	Mastitis	1:6665
Klebsiella pneumoniae	G -	Pulmonary lesions	1:4666
Staphylococcus aureus	G +	Mastitis	1:2000
Streptococcus agalactiae	G +	Mastitis	1:2670
Fungi			
Microorganism	**Type**	**Affection**	**Effective concentration**
T mentagrophytes	Spore-forming fungus	Ringworm	1:66.7
Aspergillus spp	Fungus	Aspergillosis	1:1333

Table 14.
Efficacy in sheep diseases (Synthesis)

Viruses			
Family	**Genus**	**Disease**	**Effective concentration**
Porvirus	*Capripovirus H*	Smallpox	1:200
Paramyxovirus	*Moribillivirus L*	Pest of small ruminants	1:1000
Rheovirus	*Rotavirus H*	Bluetongue	1:267
Flavivirus	*Flavivirus L*	Encephalomyelitis	1:267
Bacteria			
Microorganism	**Coloration**	**Affection**	**Effective concentration**
Chlamydia spp.	G -	Sheep enzootic abortion	1:1333
Salmonella spp.	G -	Enteritis	1:200
Escherichia coli	G -	Diarrhoea	1:2000
Staphylococcus aureus	G +	Dermatitis	1:2000
Streptococcus agalactiae	G +	Mastitis	1:2670
Fungi			
Microorganism	**Type**	**Affection**	**Effective concentration**
Trichophyton verrucosum	Fungus	Ringworm	1:66.7

Table 15.
Efficacy in leporidae diseases (Synthesis)

Viruses			
Family	**Genus**	**Disease**	**Effective concentration**
Poxvirus	Orthopoxivirus	Smallpox	1:667
Poxvirus	Myxoma virus	Myxomatosis	1:667
Parvovirus	VHD virus	Haemorrhagic disease	1:240
Bacteria			
Microorganism	**Coloration**	**Affection**	**Effective concentration**
Pasteurella spp.	G-	Tularaemia	1:4266
Pasteurella multocida	G-	Septicaemia	1:4266
Treponema spp.	G -	Treponemosis	1:667
Corynebacterium spp.	G +	Mastitis	1:9333

Escherichia coli	G -	Mastitis	1:2000
Klebsiella spp.	G -	Mastitis	1:4666
Mycoplasma spp.	G -	Mastitis	1:667
Pasteurella spp.	G -	Mastitis	1:267
Pasteurella multocida	G -	Mastitis	1:4261
Ps. aeruginosa	G -	Mastitis	1:6665
Staphylococcus spp.	G +	Mastitis	1:2000
Streptococcus	G +	Tularaemia	1:667
			Fungi
Microorganism	**Type**	**Affection**	**Effective concentration**
Aspergillus spp	Fungus	Mastitis	1:1333
Candida spp.	Yeast	Mastitis	1:533
Saccharomyces spp.	Yeast	Mastitis	1:267

Table 16.
Efficacy in feline diseases (Synthesis)

			Viruses
Family	**Genus**	**Disease**	**Effective concentration**
Parvovirus	*Parvovirus - feline H*	Feline pan leukopenia	1:240
Coronavirus	*Coronavirus - feline L*	Feline infectious peritonitis	1:1180
Picornavirus	*Feline classification H*	Feline influenza	1:400
Herpetovirus	*Herpes - feline L*	Feline viral rhinotracheitis	1:1867
Retrovirus	*Virus of leukaemia L*	Feline leukaemia	1:667
Rhabdovirus	*Lyssavirus L*	Rabies leukaemia	1:200
			Bacteria
Microorganism	**Coloration**	**Affection**	**Effective concentration**
Chlamydia psittaci	G -	Pneumonia, Encephalomyelitis	1:1333
Mycoplasma spp.	G -	Respiratory infections	1:267
Escherichia coli	G -	Pyometra	1:2000
L. monocytogenes	G +	Listeriosis	1:667
Streptococcus canis	G +	Septicemia	1:667
			Fungi
Microorganism	**Type**	**Affection**	**Effective concentration**
T. mentagrophytes	Spore-forming	Ringworm	1:66.7
Aspergillus	Fungus	Osteomyelitis	1:1333

Table 17.
Efficacy in canine diseases (Synthesis)

			Viruses
Family	**Genus**	**Disease**	**Effective concentration**
Paramyxovirus	*Canine parainfluenta L*	Kennel cough	1:200
Paramyxovirus	*Morbilivirus L*	Distemper	1:200
Parvovirus	*Parvovirusul canin L*	Parvovirus	1:240
Rhabdovirus	*Lyssavirus L*	Distemper	1:200
Adenovirus	*Adenovirusul canin -1 H*	Infectious canine hepatitis	1:667
			Bacteria
Microorganism	**Coloration**	**Affection**	**Effective concentration**
Bordetella bronchiseptica	G -	Kennel cough	1:333
Escherichia coli	G -	Pyometra	1:2000
Klebsiella pneumoniae	G -	Carre's disease	1:267
Staphylococcus spp.	G +	Demodectic mange	1:2000
Streptococcus canis	G +	Septicaemia	1:667
			Fungi
Microorganism	**Type**	**Affection**	**Effective concentration**
Tr. mentagrophytes	Spore-forming fungus	Ringworm	1:66.7
Aspergillus	Fungus	Osteomyelitis	1:1333

Table 18
Efficacy in equine diseases (Synthesis)

Viruses			
Family	**Genus**	**Disease**	**Effective concentration**
Adenovirus	*Adenovirus Mast*	Equine adenovirus	1:667
Herpetovirus	*Herpes equine L type 2,3,4 etc.*	Rhino pneumonia	1:1867
Orthomyxovirus	Influenta virus	Equine influenza	1:667
Picornavirus	Rhinovirus	Rhinitis, pharyngitis	1:400
Togavirus	Alphavirus	Viral arteritis, encephalitis	1:267
Coronavirus	Torovirus	Acute respiratory infections	1:1200
Paramyxovirus	Virus of parainfluence 3 L	Acute respiratory infections	1:1000
Poxvirus	Orthopoxvirus H	Smallpox	1:667
Rotavirus	Rotavirus H	Viral diarrhoea	1:267
Bacteria			
Microorganism	**Coloration**	**Affection**	**Effective concentration**
Bacillus anthracis	G +	Septicaemia	1:60
H. equigenitalis	G -	Contagious equine metritis	1:2133
Salmonella spp.	G -	Salmonellosis	1:667
Staphylococcus spp.	G +	Staphylococcus	1:200
Corynebacterium equi	G +	Demodectic mange	1:2000
Streptococcuse equi	G +	Pneumonia	1:9333
Fungi			
Microorganism	**Type**	**Affection**	**Effective concentration**
Aspergillus	Fungus	Fungal abortion	1:1333
Trichophyton mentagrophytes	Spore-forming fungus	Ringworm	1:66.7

4.2. Testing I - disinfectant effect of Deo-Sept® solution in field conditions

The testing was carried out with the aim of assessing the decontamination capacity of the product, through official tests (NTGMA, coliforms bacteria test, staphylococci test and microbiological field test -TMT) (14, 15, 25, 34-36, 44).

Three experiments were carried out, one of which in a maternity pen for dairy cows from the Didactic Station of ULS Timişoara and two in two hen houses for laying eggs that are to be populated with replacement chicks, both from Variaş, Timiş County.

The samples were processed in the Animal and Environmental Hygiene Laboratory within the Animal Hygiene and Pathology Research Center, (Veterinary Sanitary Authorization for operation no. 7/2003), belonging to F.M.V. Timişoara.

4.3. Testing II - irritating effect of Deo-Sept® solution on the skin

The irritating effect and possible undesirable effects of disinfectant solutions on the skin were clinically monitored through the classic patch test, coupled with histological examination performed on rats.

5. MATERIALS AND METHODS

5.1. Testing I

To establish the efficiency of surface decontamination with Deo-Sept®, the following experiments were designed:

5.1.1. Experiment 1.

In a box with an area of 25 m^2, with an air temperature of 22 ºC and a relative air humidity of 50%, mechanical and hydro-sanitary cleaning was carried out.

After the water evaporated from these surfaces, the 0.5% working solution was sprayed, 100 ml / m^2 each.

After the contact time expired, sanitation samples were collected by scraping 100 cm2 of surface with sterile swabs.

From these samples, the presence or absence of indicators for assessing the effectiveness of disinfection was determined. The inoculation was done on:

- Kessler medium for coliform bacteria (figure 1),

- Chapmann medium for staphylococci (6) (figure 2),
- Fraziere medium with TTC for enterococci (figure 3) and
- in the bottle with the concentrated TMT medium for hydrogen sulfide-producing enterobacteriaceae (21, 35) (figure 4).

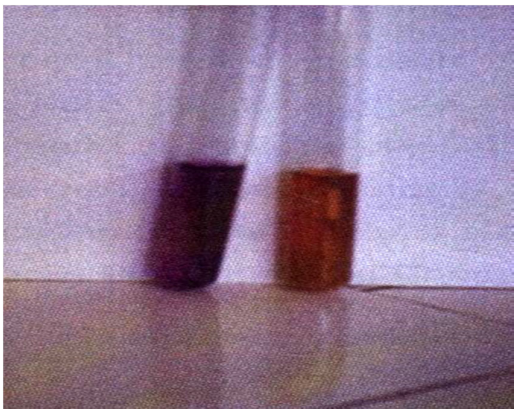


Figure 1. Test tubes with Chapman. Kessler-Swenarton medium on which colonies developed: staphylococci (left) and positive sample (right).

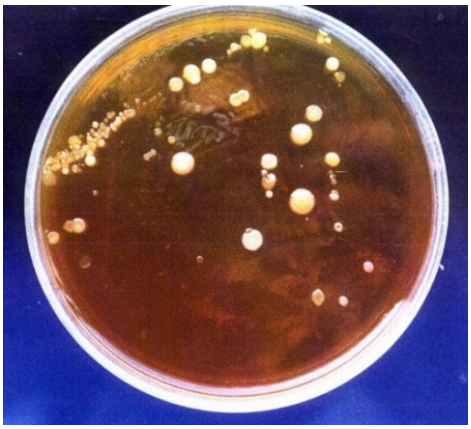


Figure 2. Petri dish with medium on which staphylococcal colonies have developed

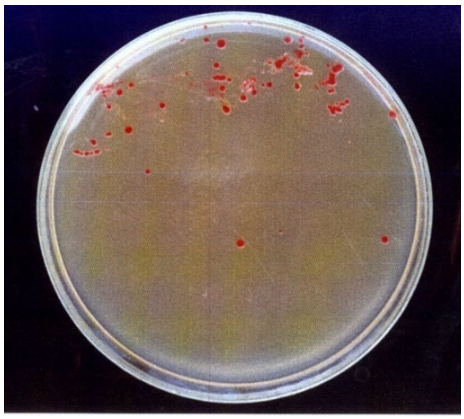


Figure 3. Petri dish with Frasiere medium on which staphylococcal colonies developed

Figure 4. Microbiological test
(left – negative sample; right – positive sample).

5.1.2. Experiment 2/a

Place

It took place in a shelter prepared for population with 3000 laying hens, with an area of 580 m^2.

The air temperature in the shelter was 25ºC and the relative humidity was 60%.

After a good mechanical and hydrosanitary cleaning, sanitation samples were collected, then disinfection was done by spraying with Deo-Sept®, after which sanitation samples were collected again.

The microbiological indicators and the working technique were the same as in the first experiment.

5.1.3. Experiment 2/b

Location

It was carried out in a 280 m^2 shed prepared for stocking with 1500 replacement chicks.

The temperature was 22ºC and the relative humidity was 60%. The same mechanical cleaning, washing and fumigation operations were carried out as in the second experiment.

Methods

The assessment of decontamination efficiency was carried out on 12 sanitation samples, collected after fumigation, through

official tests: coliform bacteria test, staphylococci test and TMT.

5.2. Testing II

To evaluate the irritating effect of **Deo-Sept®** solutions on the skin, three groups of three white rats and a control group were formed. They were shaved on the back on a 2 cm^2 square.

Method

On the shaved areas, disinfectant solutions with different concentrations of **Deo-Sept®** of 0.5% (working concentration), 2% (maximum recommended concentration), 10% (20 times the working concentration) and 20% (concentrated solution, 40 times the recommended one) were applied by swabbing.

Assessment of skin tolerance

The assessment method chosen for this test was the standard Patch test method II - variant II considered to be the most applicable for rat skin.

This method consists of applying a swab soaked with 0.5 ml of the solution to be investigated, for 30 minutes, on a 2/2 cm2 epilated and scarified surface.

The skin reaction will be monitored at 30 minutes, 8 hours, 24 hours, 36 hours and 48 hours after application.

The assessment of the skin reaction on the epilated and scarified rat skin was made according to the score presented below:

Identified reactions	Mark
No visible reaction, possibly slight peeling	**0**
Slight congestion that disappeared after 24 hours	**1**
Congestion and inflammation that decreases and disappears within 36 hours	**2**
Congestion and inflammation that does not decrease within 36 hours	**3**
Congestion and pustules, lymph extravasation, prolonged healing 48 hours	**4**

The final quantification of the ***patch test*** will be done by taking into account the scores obtained by the subjects in the study, which are added up and divided by the number of individuals, who are then assessed according to the table below:

Identified reactions	Limits
Tolerable	**0 – 0,99**
Average tolerability	**1 – 2,79**
Irritant	**2,80 – 3,69**
Severely irritating	**3,70 – 4,0**

The citohistological investigation

Was performed to determine the degree of damage to the cutis layers.

Skin samples were collected from the rats in the study, previously euthanized with chloroform, according to the ethical norms in research and the flaps were introduced into 5% formalin solutions.

Processing and staining of the samples was performed at the Histology discipline within our faculty

The staining method was Hematoxylin-Eosin (H.E.) and visualization was performed under the electron microscope at the x 400 objective.

6. RESULTS AND DISCUSSIONS

6.1. Testing I

6.1.1. Experiment I

The collected sanitation samples were examined by qualitative microbiological tests (coliform bacteria test, staphylococci test, enterococci test and TMT) and were found negative, which denotes good disinfection.

Analysis of the results for NTGMA determination highlighted a reduction of over 106 colony-forming units (CFU), which signifies good disinfection, according to SR EN 1040/2000 (10) (Table 20).

Table 2.
NTGMA analysis results in assessing the effectiveness of surface decontamination

Sample No.	Before desinfection	After desinfection	Reduction degree
1.	$2,2 \times 10^6$	9	**244**
2.	$1,9 \times 10^6$	12	**158**
3.	$2,9 \times 10^6$	13	**223**
4.	$2,7 \times 10^6$	12	**337**
5.	$2,6 \times 10^6$	8	**325**
6.	$2,1 \times 10^6$	7	**300**
7.	$3,1 \times 10^6$	10	**310**
8.	$3,8 \times 10^6$	11	**345**
9.	$1,8 \times 10^6$	13	**138**
10.	$3,5 \times 10^6$	7	**500**
11.	$3,8 \times 10^6$	8	**475**
12.	$2,3 \times 10^6$	16	**143**

Based on the results obtained, it can be stated that the Deo-Sept® product used according to the instructions, under optimal temperature and relative humidity conditions, achieves good decontamination.

According to SR EN 1040/2000 and taking into account the logarithmic reduction of over 105 of the number of FCU/ml, at the concentrations used and at a contact time of 15 minutes, the sanitation samples prove a good antimicrobial activity of the tested product.

The results of the sanitation samples collected from the two replacement chicken houses for consumption eggs were negative in the official tests.

This allows us to conclude that the Deo-Sept® product in the concentrations and working method prescribed in the leaflet has a good microbicidal effect.

6.2. Testing II

Table 21 shows the values registrated for the assessment of skin tolerance according to the standard Patch test method II - variant II for the tested conditioning.

As can be seen, the low concentrations, the recommended and the maximum for current disinfections (0.5% and 2%) had low values of the scores and averages of the skin reaction value, while in the case of high concentrations of 10% and 20%, determined reactions that included skin tolerance in categories:

I = *irritan*t, the average value for the concentrated solution (20%) being at the limit of declaring the category

S.I. = *strongly irritant.*

Histological examination of the skin on which different concentrations of **Deo-Sept®** were applied are presented in figures 1, 2, 3 and 4.

Table 21
Values ascertained for assessing skin tolerance to Deo-Sept®

Deo-Sept®	0,5%			2%			10%			20%			Control
Used concentration	1	2	3	1	2	3	1	2	3	1	2	3	
Individual	0	0	0	0	1	0	3	3	3	4	3	4	---
Identified reaction	0	0	0	0	Yes	0	Yes	Yes	Yes	Yes	Yes	Yes	
Average	0 = T			0.33 = M.T.			3.0 = I			3,66 = S.I.			

Note: *Tolerable* ***- T****, Medium tolerability* ***- M.T.****, Irritating -* ***I****, Strongly irritating -* ***S.I.***

Figure 1.
In the control group, microscopic examination of histological sections taken from the skin revealed that the epidermis had a normal appearance, with intact stratified epithelium, in which vacuolated keratinocytes were also found, but in a reduced proportion (→). The thickness of the epidermis is maintained within normal limits. A reduced number of inflammatory cells was evident in the dermis. (→). Also, glandular structures (in particular, the sebaceous gland) (→) and the horny formations (hairs do not show significant histological changes.

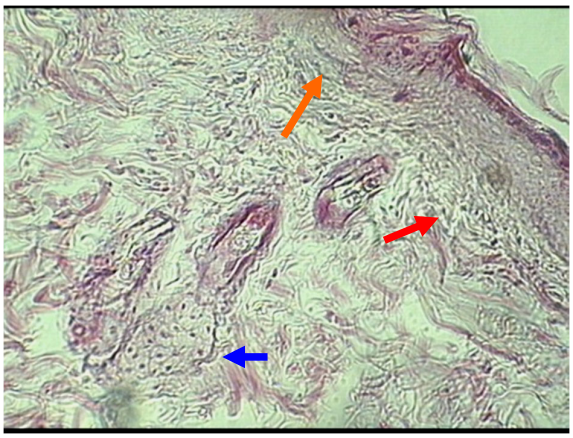


Figure 1 (col. H.E. × 400)

Figure 2.
The histological examination of the skin treated with the **Deo-Sept®** disinfectant solution at a concentration of 2% did not reveal the appearance of significant histolopathological changes, the epidermis did not show structural or thickness changes, but, compared to the control group in the dermis, an increased number of inflammatory cells was observed (→)

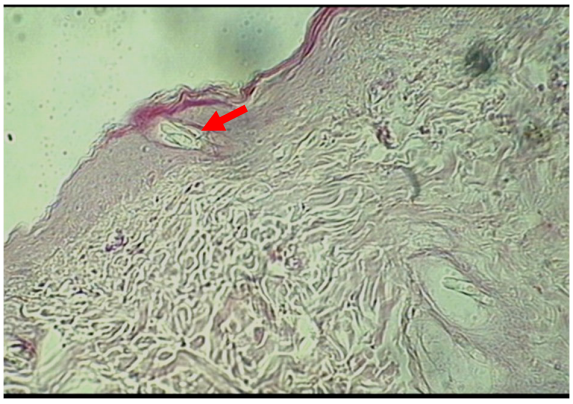


Figure 2 (col. H.E. × 400).

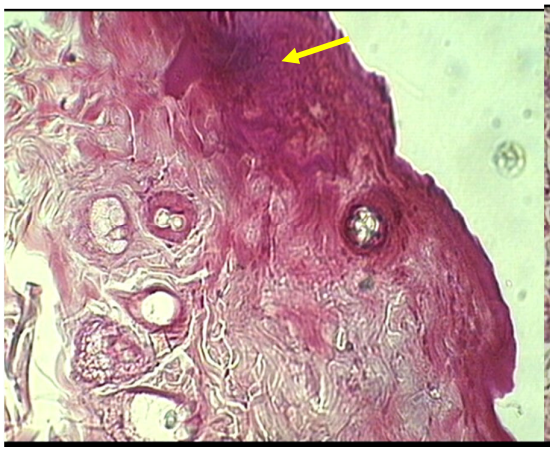


Figure 3 (col. H.E. × 400).

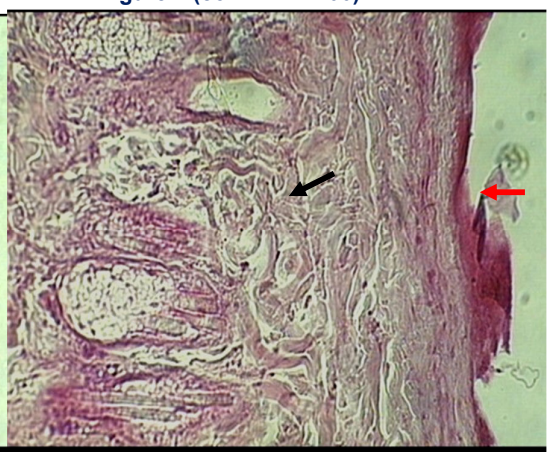


Figure 4 (col. H.E. × 400).

Histological examination of the skin treated with Deo-Sept®, in a concentration of 10%, revealed the onset of phenomena characteristic of contact dermatitis. Necrosis phenomena are evident in the epidermis (→), dyskeratosis, hypo - (→) or parakeratosis (→).Also, in certain areas, alteration of the basement membrane, which separates the epithelial tissue from the connective tissue, was noted, posing the risk of dissemination of bacterial and viral agents into the deep skin structures. In the dermis, the appearance of cellular infiltrations specific to an inflammatory process was noted.

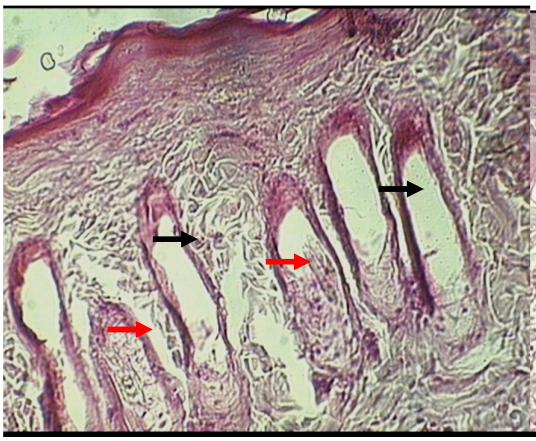


Figure 5 (col. H.E. × 400)

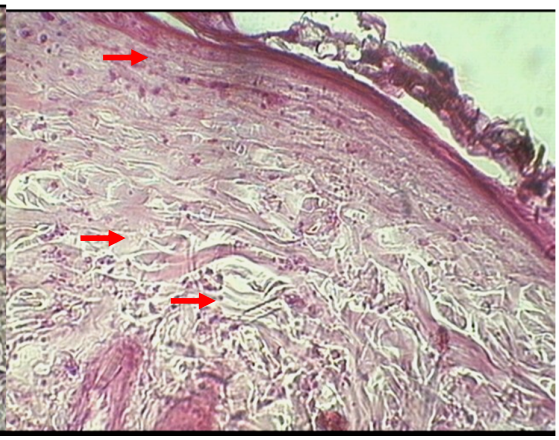


Figure 6 (col. H.E. × 400)

Histological examination of the skin treated with Deo-Sept®, concentrated solution (20%) revealed worsening of contact dermatitis. In addition, most of the sebaceous glands showed necrosis in various stages, from partial to complete (→) until complete (→), of secretory cells and basal proliferative cells, which become flattened. The alveolar sacs are emptied of cells and secretion product. These aspects indicate a severe reduction in the functional capacity of the sebaceous gland. Thus, the amounts of sebum secreted are greatly reduced, a phenomenon that explains the dryness and friability of the skin when using concentrated solutions of the product (figure 5). In the dermis, the appearance of massive cellular infiltrations was noted, specific to inflammatory processes (→) (figure 6).

7. Conclusions

The disinfectant product used is suitable for application in animal shelters and other enclosures, with possibilities of airtight closure, being easy to handle, without particular risks.

Considering the results obtained in the test, as well as the data from the specialized literature, the product is recommended for:

- prophylactic disinfection by spraying and
- aerosolization of animal shelters, veterinary clinics,
- incubation stations and cages for animals and birds.

The efficiency of decontamination is conditioned by good mechanical and hydrosanitary cleaning of surfaces, ensuring a temperature of at least 10ºC, a relative air humidity of at least 50% and ensuring the tightness of the enclosure where the decontamination is carried out.

The tested product proves to be bearable on epilated skin at solution concentrations of 0.5% and/or 2%.

Skin contact with high concentration solutions (10% and 20%) leads to serious and long-lasting irritation and skin lesions, a situation also revealed by histological examination.

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