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Review Article
IMMUNOTOXICOLOGY AND VETERINARY MEDICINE

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Abstract

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Immunotoxicology investigates unwanted adverse effects of xenobiotics (pesticides, heavy metals from emissions, new DNA-recombinant products, immune response modulators, monoclonal antibodies, drugs and others) on the immune system of man and animals. Immunotoxicology in veterinary medicine deals mostly with the problems of dominant ecological toxicants, such as pesticides. Therefore, the veterinary immunotoxicology can be referred to as *ecoimmunotoxicology*. DNA-recombinant biopreparations and drugs are also important in this respect. Interactions of various xenobiotics with live organisms pose diverse immunological problems. The present study emphasizes that the assessment of immunotoxicological risk arising from xenogenous substances demands the development of new and more exact immunotoxical testing methods. Moreover, the thorough evaluation of the immunotoxic effect of a respective xenobiotic should be supported by a complex set of tests rather than limited number of them. The extent of functional and non-functional immunity tests should be determined on the basis of knowledge about the mechanism of the toxic effects of xenobiotics in animals. This knowledge can be supplied by biochemistry, toxicology, pharmacology and histopathology (histochemistry and immunohistochemistry). The up-to-date knowledge about immunosuppressive effects of pesticides and their possible interference with the genetic material of live organisms indicates that it is necessary to restrict gradually the extensive use of a broad spectrum of pesticides through accentuated application of scientifically justified agrotechnical procedures and the use of transgenic plants developed by molecular genetic methods. To minimize the immunotoxic risk of pesticides to farm animals and free-living animals a system of appropriate undergraduate and graduate education should be developed. This is a long-term process that can obviously be realised by establishing a joint scientific branch of toxicology and pharmacology within the veterinary medicine. A thorough study of mechanisms of immunotoxicity and immunopharmacology of various agents can result in the production of safer protective agrochemicals and more effective drugs.

Immunotoxicology, immunotoxicants, pesticides, immunosuppression, ecoimmunotoxicology, immunotoxicological tests

The initial studies of the susceptibility of the immune system and its possible use for detection of subclinical toxic states were published in the seventies and early eighties (Vos and van Genderen 1973; Vos 1977; Loose et al. 1978; Faith et al. 1980) when the attention began to focus on the immune system as an important object of toxic action, particularly in connexion with the experiments on rodents. The susceptibility of the immune system to toxic damage can result from several factors. Host resistance to infectious agents and spontaneous neoplasms depends on immunocompetent cells which are subject to continuous proliferation and differentiation and because of that they become excessively susceptible to various agents. The immune system is known for highly organized cooperation and regulation of various cells which is ensured on the one hand by soluble mediators (immunoglobulins, immunohormones, cytokines) and on the other by intercellular interactions on the level of membrane receptors and antireceptors. All agents

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that affect the fine balance mechanisms mentioned can cause agent-specific or species-specific immunity damage which, in majority of cases, results in immunosuppression (e.g. decreased resistance to infectious agents and development of tumours). Manifestations of immunosuppression were observed either on systemic or on the local level (e.g. in lungs or skin, Luster et al. 1996). Pathological increase in the immune response can also occur, such as hypersensitivity, manifested as respiratory tract allergies or contact allergic dermatitis, and some agents can participate in the development of autoimmune diseases. Industrial development increased considerably the risk of different xenobiotics in the outer environment to which they have been introduced incidentally (emissions, accidents) or intentionally (the use of various potentially toxic chemicals such as pesticides). It is the latter group of substances that includes chemicals which do not always have acute toxic effects. However, they exhibit considerable potential for chronic toxicity which is most frequently accompanied by impairment of the immune system (Saunders and Harper 1994). Individual immune status indices allow us to reveal the toxic action of a number of substances (Zavázal and Richter 1985) even in those cases in which the conventional toxicological examinations give frequently only negative results (Dési et al. 1986).

Immunotoxicology as a branch of science

Immunotoxicology is relatively new interdisciplinary scientific field focused on identification and analysis of chemical and, in a broader sense, also physical and biological factors of the environment which can cause unwanted and usually incidental immunomodulations (Dietert et al. 1996). Similar subjects are studied also by immunopharmacology. As opposed to immunotoxicology, immunopharmacology investigates the immunomodulative effects of various substances that are applied intentionally for therapeutic purposes. The objective of immunotoxicology is to protect humans and animals against the harmful effects of chemical factors present in the environment, to develop and check immunotherapeutic products and introduce and evaluate the methods intended for determination of interactions between the factors of the external environment and the immune system (Dietert 1996). Harmful effects of environmental factors can arise from (1) direct or indirect action of xenobiotics on the immune system or of products obtained by their biotransformation, (2) induction of the immune response to xenobiotics or their metabolites, (3) modification of self-antigens by chemicals or their metabolites (Berlin 1987). In addition to the protection of humans and animals against environmental risks, immunotoxicology also investigates the properties of new immunotherapeutic pharmacological products prepared via recombinant DNA techniques (interleukins, interferons, growth factors, anti-inflammation drugs, neuroendocrine hormones, neuropeptides) with regard to their immunotoxic potential and safety of their use (Cavagnaro et al. 1987; Sanders 1996). Another specific domain of immunotoxicology deals with immunotoxins, the substances isolated from bacteria and plants the general property of which is the inhibition of proteosynthesis (diphtheria toxin, *Pseudomonas* exotoxin, ricin, ribosome-inactivating protein and others), as well as with conjugates of monoclonal antibodies and their use mostly in antitumour immunotoxin therapy (Vitetta et al. 1993). Another not unimportant field of immunotoxicology is the development of methods which can be used to investigate the interactions between the external environment and the immune system.

Nature of substances exhibiting immunotoxic effects (immunotoxicants)

Immunotoxicants are factors of the external environment which cause significant changes (modulation) in the immune mechanisms in humans and animals (Dietert et al. 1996).

Zbinden (1987) used the term immunomodulators to characterize a group of substances of different origin which affect the immune system and include the following: environmental chemical contaminants (pesticides, industrial emissions and similar), drugs (including contaminants which have become particularly important as potentially sensitising impurities in products manufactured by recombinant DNA and other biotechnological technologies - D'Agnolo 1983) and physical factors (UV-B light, electromagnetic field - Luster et al. 1990). The action of immunotoxicants results in the reduced reactivity of the immune system - immunosuppression (e.g. PCB, lead, cadmium, Safe 1994; Procházková et al. 1990; Skokanová et al. 1993) or, on the contrary, in its excessive reactivity - hypersensitivity (polycyclic aromatic compounds, organophosphate insecticides and others - Luster and Rosenthal 1993). Some substances (e.g. nickel and mercury) can participate in the development of autoimmune diseases (Luster et al. 1996; Henry et al. 1988). The present interest focuses on possible immunologic effects of indoor pollutants. This involves not only chemicals but also bioaerosols, such as viruses, bacteria, moulds, algae and protozoa, as potential sensitizing agents or mediators of infectious diseases (Luster et al. 1996). Substances with immunomodulative effects are summarized in Table 1.

Table 1
Xenobiotics inducing immunosuppression or exerting positive immunomodulative effects
(Luster et al. 1996; Dietert et al. 1996)

- polycyclic aromatic hydrocarbons (dimethylbenzanthracene - DMBA)
- polychlorinated aromatic hydrocarbons (PCB, p-diazine – TCDD)
- heavy metals (e.g. mercury, lead, cadmium, arsene metalloids)
- pesticides (e.g. chlordane, organophosphates, carbofuran)
- organotins (tributyltin oxide)
- aromatic hydrocarbons (e.g. benzene, toluene)
- aromatic amines (e.g. benzidine, dimethylnitrosamine)
- oxidative gases (e.g. O_3, NO_2, SO_2)
- metabolites of toxigenic moulds (e.g. aflatoxins, ochratoxin, T_2 – toxin)
- drugs of abuse (e.g. cocaine, alcohol, marijuana)
- environmental particles (e.g. asbestos, silica, beryllium)
- drugs (e.g. cyclophosphamide, cyclosporine)
- inducers of allergic reactions (e.g. nickel, palladium) and autoimmune diseases (mercury)
- immunotoxins (diphtheria toxin, ricin)
- bioactive substances prepared by recombinant DNA techniques (interleukins, interferons)
- ultraviolet light (UV-B), electromagnetic field

Spectrum of the effect of immunotoxicants

The susceptibility of the immune system to xenogenous substances which can result in immunosuppression depends on both the properties of the respective chemical and complex nature of the immune system (antigen recognition and processing, cellular interactions - cooperation, regulation and amplification, activation and differentiation of cells and production of mediators by various cell types). The high diversity of cells and cellular interactions can induce changes in reactions in various components of the immune system and in the reactions controlled by this system in dependence on the character of immunotoxicants. The interactions of xenobiotics with the immune system can be affected also by additional factors, such as malnutrition, stress and genetic predisposition (Luster et al. 1993). From the point of view of respective mechanisms of immunotoxicants we recognize systemic (interaction with one or more components of the immune system) and local effects (e.g. decreased local lung immunity) and also some selective effects on target cells (B-lymphocytes, T-lymphocytes, macrophages). Moreover, they can participate in the development of allergic reactions and autoimmune diseases (Dietert et al. 1996).

1. Systemic immunosuppressive action

Systemic immunosuppression affects the activity of the immune system as a whole and develops frequently as a result of action of various chemical agents. The most detailed studies of this form of immunotoxicity were carried out in rodents. The systemic immunosuppression is indicated by the following: a) altered weight and histology of lymphoid organs b) quantitative changes in cellularity of the lymphoid tissue, peripheral blood leukocytes and bone marrow, c) impairment of cell function at the effector or regulatory level, d) increased susceptibility to infectious agents or transplantable tumours (Luster et al. 1996). Some examples of the immunosuppressive mechanisms are presented in Table 2.

Table 2
Possible mechanisms for xenobiotics – induced immunodepression
(Spreafico et al. 1987)

- Affect antigen distribution and/or persistence
- Influence production-differentation of precursors immunocytes
- Affect traffic and/or number of inducer, effector and/or regulatory immune cells
- Influence activation threshold of immunocytes to antigen and/or growth and differentation factors (e.g. responsiveness to lymphokines and monokines, expression of receptors)
- Affect production, release and/or metabolism of lympho-monokines and/or other substances (e.g. α-fetoprotein) modulating immune cell function
- Influence quantity, quality, persistence of effector products of immune cells (e.g. antibody)
- Affect number, traffic, functional capacity of other cells (e.g. platelets, neutrophils) and dependent mediators
- Affect indirect regulatory circuits (e.g. CNS-endocrinological homeostasis)

2. Local immunosuppressive action

Local immunosuppression induced by xenobiotics is most frequently demonstrated in lungs and on skin in both humans and animals.

a) Lungs

Pathogenesis of a number of pulmonary diseases, such as fibrosis, granulomatosis and bronchial asthma, is associated with inhalation of pollutants. The pulmonary diseases which are conditional upon local damage to the immune system can be induced by various agents, such as oxidizing gases (ozone, NO_2, SO_2) or fine solid particles (silica, beryllium, asbestos, coal dust, sheep wool and others). Numerous studies point to the fact that the progress of pulmonary diseases is related to the post-activation release of cytokines, predominantly interleukin 1 (IL-1), tumour necrotizing factor (TNF-α, TNF-β) platelet-derived growth factor (PDGF), growth transforming factor beta (TGF-β), by pulmonary macrophages (Luster et al. 1996). In addition to cytokines, alveolar macrophages also produce various short-term acting products which can contribute to a decreased resistance of lungs to infection and inflammation. This refers particularly to the reactive forms of oxygen, such as superoxide and hydrogen peroxide as well as to metabolites of arachidonic acid. Lungs also contain considerable numbers of NK cells (natural killers), probably as a result of evolutionary development, for the purpose of protection of lungs against pulmonary tumours the development of which may be induced by inhaled carcinogens. Several agents, such as fosgen, decrease the activity of pulmonary NK-cells. The suppression of above mentioned systems can also be caused by pollutant gases, such as ozone and nitrogen oxides, resulting in predisposition of an organism to infectious agents (particularly viruses) and development of tumours (Luster et al.1996).

b) Skin

Similar to the lungs, the skin is another potential place of entry of many xenobiotics. Skin reactions can acquire the form of specific immune response (contact hypersensitivity) or nonspecific inflammation response (contact irritation), both conditional upon several similar pro-inflammation cytokines. Immunologically active cells and their soluble mediators penetrate from the circulatory system into the skin as a response to the stimulus induced by xenobiotics. Besides that, other dermal cells (such as Langerhans cells) which participate in the immune reactions can be activated in response to dermal stimuli. Under the action of exogenous factors, such as ultraviolet radiation (UV) or some chemicals (dimethyl benzanthracene), they can disappear or lose their function (Luster et al. 1996). Keratinocytes as cells prevalent in epidermis also play important role in the immune and inflammatory response, mostly by producing cytokines in response to various stimuli by which they affect significantly the character of dermal responses. Keratinocytes induced by UV-B radiation, chemical irritants, sensitizing compounds and some pharmacological substances produce a wide scale of cytokines: IL-1, granulocyte and macrophage colonies-stimulating factor (GM-CSF), IL-6, IL-8, TGF-α, TGF-β, TNF-α and IL-3. The basic response of keratocytes consists in the production and secretion of IL-1α and TNF-α which results in the expression of leukocyte adhesive molecules on the surface of dermal endothelial cells (e.g.VCAM-1). Important is also their paracrine effect in which TNF-α and IL-1 also play role as autocrine mediators inducing release of IL-8 by keratinocytes. IL-8 is an important chemotactic and activating substance affecting polymorphonuclear neutrophils (PMNL). In case of antigenic character of the stimulant (e.g. nickel sulphate), an increased number of mononuclear cells and subsequent participation of T-helper cells is characteristic of the response. This leads to significant release of IFN-γ and TNF-α with a resultant enhancement of the response (Luster et al. 1996).

3. Exclusive effect on target cells

Some xenobiotics exhibit more intensive selective effect on certain type of cells or some cells exhibit primary susceptibility to the action of such substances. Table 3 shows the examples of the effect of immunosuppressive substances on the function of macrophages and monocytes (Spreafico et al. 1987).

Table 3
Selective immunosuppressive effect of some xenobiotics on monocyte – macrophage functions
(Spreafico et al.1987)

Direct cytotoxicity: Azathioprine, Steroids, Dacarbazine, Actinomycin D, Vinblastine
Responsiveness-production of lymphokines: Steroids, Azathioprine, Cyclosporin,Vinblastine
Antigen processing-phagocytosis: Steroids, Actinomycin D, Azathioprine
Chemotaxis-migration: Steroids, Azathioprine

4. Hypersensitivity mediated by xenobiotics

Chemically induced hypersensitivity, as a manifestative stage of immunotoxicity, aroused great interest of clinicians (Berlin 1987). Contact hypersensitivity reaction is the principal demonstration of hypersensitivity induced by xenobiotics (Asherson 1987). Such a reaction develops after the contact of various substances with body surface during which xenobiotics bind to skin proteins and change from incomplete antigens (haptens) to complete ones capable of inducing the immune response. The character of carrier skin proteins has not yet been elucidated. Haptens bound to proteins are further processed in dermal Langerhans cells and are presented to T_H1 - lymphocytes. The sensitization that

results from primary exposure to xenobiotics develops for several days, however, once it is established, it lasts for many years or for life. When the activated T-lymphocytes are repeatedly exposed to allergens, they release numerous cytokines (macrophage activating factor - MAF, macrophage chemotactic factor - MCF and macrophage inhibiting factor - MIF) which attract additional macrophages to the inflammation foci and activate them. The activated macrophages release proteolytic enzymes which can damage the tissues. Eczema are produced clinically in the place of contact with an allergen. The acute phase is manifested as erythema with swelling and formation of vesicles while production of papulae and scales and focal pruritus predominates in the acute phase (Buc 1997). Contact hypersensitivity depends on additional physiological and pharmacological factors, such as permeability of skin which affects directly the antigen dose uptake (Asherson 1987). The antigen concentration can also be affected by some irritants which damage the skin, increase its absorption and activate the antigen presenting cells. There are records of contact dermatitis caused by various components of paint coats (polycyclic aromatic compounds), pesticides (organophosphates, Luster and Rosenthal 1993), different chemicals (e.g. formaldehyde, epoxide, Peruvian balm, lanolin, stains and others), drugs (neomycin) and plants, such as poison ivy (Buc 1997). Particular attention was paid to contact dermatitis caused by nickel which is a frequent constituent of various articles (costume jewellery, jewellery, metal eyeglass frames, dog collars, etc.). Nickel dissolves in weakly acidic environment of sweat and its salts have high affinity to various proteins, including albumin and proteins of the complement system which creates conditions for the development of hypersensitivity reaction (Dolovich et al.1984; Fishelson et al. 1983; Asherson 1987). The development and course of contact hypersensitivity is also affected by genetic factors (particularly genes encoding MHC class II antigens) and additional factors that can affect the presentation of antigens (Asherson 1987). Speaking about immune mechanisms of contact hypersensitivity, one must distinguish them from pseudoallergies which resemble the allergic reaction, however, they do not proceed on the basis of immune mechanisms but as a result of direct activation of monocytes, as in the case of dextran (Berlin 1987), contrast substances used in radiography (Witten 1975) or intravenous anaesthetics (Lorenz et al. 1981). The group of diseases which, according to their etiopathogenesis, includes also type IV cellular hypersensitivity comprises also hypersensitive pneumonitis induced by inhalation of various allergens. The hypersensitive pneumonitis has been described under different names, such as farmer's lungs, furrier's lungs, pigeon breeder's disease, cheese washer's disease. The initial phase of hypersensitive pneumonitis is characterised by formation of immunocomplexes in the lung interstitium composed of IgA or IgG antibodies and the inhaled pathogen. In case of repeated exposure, T-lymphocytes are also involved in the immunopathogenic mechanisms and granulomatous interstitial pneumonia develops (Buc 1997).

5. Autoimmune diseases

One of the characteristic features of the immune system is the recognition and the development of immune response to various xenogenous antigens present in the external environment and the absence of immune response to the own tissues. Some degree of autoreactivity is a normal feature of the immune system physiology because somatic proteins, many carbohydrates, lipids and nucleic acids are potential antigens capable of inducing humoral and cellular immune responses (Klein 1991). The mechanism that prevents the induction of an immune response to own body components and prevents potential autoreactive lymphocyte clones from entering into reactions is called tolerance. An impairment of the immunotolerance caused by various factors results in autoimmune diseases (Klein 1991). One of the possible ways of the development of autoimmune

disease is the reaction of autoantibodies with xenogenous substances which are bound to own cells or structures of the organism. In this way, for example, autoimmune haemolytic anemia can develop as a result of interaction of antibodies with erythrocyte-bound penicillin (Jeffe 1979) or pesticide dieldrin (Hamilton et al. 1978). Autoimmune glomerulonephritis, which develops as a result of deposition of circulating immunocomplexes (mercury and gold) in the basal glomerular membrane (Pelletier and Druet 1995), can serve as another example. Autoimmune glomerulonephritis develops after the chronic exposure to low doses of mercury salts (Henry et al. 1988; Bigazzi 1988; Gleichmann H. and Gleichmann E. 1987). Experimental induction of an autoimmune disease caused by mercury ($HgCl_2$) was described in rats in dependence on the species and strain of experimental animals (e.g. out of 22 brown Norwegian rats tested by exposure to $HgCl_2$ only one animal developed autoimmune disease). Experimental studies point to the fact that the interaction of mercury with cellular thiols causes polyclonal activation of B-lymphocytes, most likely as a result of modification of T-helper lymphocytes (Druet 1989). Similar autoimmune disease was described also in rabbits (Roman-Franco et al. 1978) and humans (Druet et al. 1994). The autoimmune process was demonstrated as glomerulonephritis, lymphadenopathy, production of antinuclear antibodies and significant increase in the level of IgE. Some other autoimmune diseases, induced by HLA-molecule-associated xenobiotics, were also described: hydralazine induced systemic lupus erythematosus (Batchelor et al. 1980), D-penicilamine and aurothiomalate-induced nephropathy (Wooley et al. 1980), selective IgA deficiency induced by diphenyl hydantoin (Shakir et al. 1978), production of autoantibodies of various specifity induced by Spanish toxic oil (Vicario et al. 1982) and serious scleroderma-resembling lesions induced by vinyl chloride (Black et al. 1983).

Ways of determination of immunotoxic properties of xenobiotics

The immunotoxic mechanism of xenobiotics is implemented either through direct action of the chemical with the immune cells and their membrane receptors, affecting the production of cell mediators and antibodies, or indirectly, through secondary effects on important central organs such as liver (hepatotoxicity - production of hepatoproteins with immunomodulative properties) and the neuroendocrine system (neuroendocrine toxicity - chemically induced changes in hormones or neuroactive substances with immuno-modulative properties). The direct and indirect action can produce a combination of both effects (Dietert et al. 1996). Several methods were developed to determine the immunosuppressive or immunostimulative action of xenobiotics using the following: dose- and time-dependence of response, toxicokinetic, determination of direct and indirect effects on the immune system as well as biological relevance of *in vitro* tests to the effects of agents *in vitro*. The examinations should provide answers to the following 3 questions: 1. Whether the xenobiotic can alter the immune functions, 2. If yes, what concentration and exposure time is required to induce a response, 3. Whether the repeated exposure to the xenobiotic mentioned causes activation of the immune mechanisms resulting, for example, in an autoimmune reaction (Berlin 1987). In searching for the answers to the questions presented the following factors are of essential importance: (a) selection of species of experimental animals, (b) determination of exposure interval, (c) dose, and (d) setting up the panel of the tests which can be used to evaluate the immune status of experimental animals (Zbinden et al. 1987).

a) Model experimental animals, cells and cell cultures. The tests of immune function alterations have been carried out mostly on rodents (particularly inbred lines of mice, e.g. B6C3F1 - Dean et al. 1987; Blair et al. 1990) because of their defined genetic

characteristic, suitability for host resistance tests and resemblance between their immune system and the immune system of humans. Rats are animals most frequently used in conventional toxicology studies, however, they are less suitable from the genetic and immunologic points of view. Guinea pigs are frequently used as experimental models in testing the contact delayed hypersensitivity reaction (Berlin 1987). The selection of animals should correspond to the objective of the experiment, i.e. dogs should be selected for evaluation of drug effects, rabbits for teratogenesis and so on (Dean et al. 1987). In view of the fact that the majority of experiments have been focused on the man there is some controversy regarding the application of results obtained in rodents to humans. In this respect genetic factors play an important role because, as a rule, experimental animals are of the same genotype while the human population exhibits genetic diversity. For the purpose of application of immunotoxicological data obtained in mice to other animal species or to the man one can use the method of xenogenous extrapolation. It is necessary to consider the differences in the susceptibility of individual mice strains to immunotoxicants ranging from high susceptibility to relative resistance (Dietert et al. 1996). Immunotoxicological tests were carried out also on rabbits (Angle et al. 1980, Inns and Rice 1993), golden hamsters (Blair et al. 1990), pigs of small breeds (Dietert et al. 1996), dogs (Klimmek et al. 1993), sheep (Mikula et al. 1992; Pistl et al. 1995) and monkeys (Dietert et al. 1996). In addition to mammals, chickens (Bírešová et al. 1997) and some fish species (Sprague 1973; Svobodová 1987; Hodson 1988; Košuth and Legáth 1997) are also frequently used as models. The studies of direct immunotoxic effect employ also cell cultures (Dietert et al. 1996) and deal with *in vitro* effects of xenobiotics on leukocytes isolated from the peripheral blood (Kačmár et al. 1995). However, the latter method cannot be used to evaluate the immune system itself because of the absence of interactions with hepatic, endocrine and nervous systems. An effort has been made recently to prepare new animal models (knockout mice, transgenic animals and similar). Mice with severe combined immunodeficiency syndrome (SCID mice) were transplanted with human immune stem cells. Such a xenogenous mosaics provided mice containing intact human immune system cells. However, the question of neuroendocrine-immune and hepatic-immune interactions within the mice mentioned in comparison with humans remains unclear (Dietert et al. 1996).

b) Exposure interval i.e. the time needed to induce immune dysfunction, depends on the type of immunological damage, chemical characteristics of the substance tested, toxicokinetic of the compounds and functional reserve of the loaded immune parameters. In general, in case of subcutaneous repeated-exposure regimen used in animals at the beginning of sexual maturity, approximately 14-30 days are needed before the chemically induced effect on immunocompetence is recorded (Dean et al. 1987). Up to this date, only few chronic exposure experiments were carried out. The long-term acting low levels of xenobiotics used in them can have no effect on animals, however, on the other hand, more serious or persistent effects of chronic action could be demonstrated in comparison with the subacute action.

c) Selection of the dose is a principal step in immunotoxicological experiments. One should avoid to high doses causing apparent toxicity. The selection of the dose should be based on data about the effect of chemicals on general toxicological parameters (LD_{50}, LD_{10}, type of acute or subchronic toxicity in dependence on the dose). As a rule, three exposure levels are recommended for determination of the dose-dependent effect of xenobiotics. An ideal situation occurs when the lowest dose used is lower than LD_{10} and does not result in mortality. Under normal conditions, the lowest dose should not influence

the immune functions (Dean et al. 1987). The way of exposure should, if possible, be identical with the natural one. Peroral exposure is preferred for most environmental chemicals while inhalation exposure is the way of choice for atmospheric pollutants.

d) The methods commonly used to evaluate immunotoxical effect of xenobiotics recognize non-functional and functional tests (Vandebriel et al. 1995). Nonfunctional tests (Table 4) provide information mainly about the changes in the lymphoid

Table 4
Review of non-functional tests used in immunotoxicology
(Luster et al. 1993; Vandebriel et al. 1995; Dietert et al. 1996)

Weights of lymphatic organs
- thymus, spleen
- lymph nodes (after oral exposure the mesenteric and after inhalatory exposure the bronchial nodes)

Histopathology (hematoxylin-eosin staining)
- thymus, spleen, lymph nodes
- mucosal immune system (Peyers patches in the gut and lymphoid tissue in the respiratory tract)

Immunohistochemical methods (immunoperoxidase)
- differentiation cell antigens (CD)
- adhesion cell molecules (e.g. ICAM-1)
- activation cell markers (e.g. CD25 - IL-2R)

Basal immunoglobulin level
- total antibody levels
- total IgM, IgG, IgA and IgE levels

Bone marrow
- qualitative and quantitative analysis of immunocompetent cell populations

Enumeration of leukocytes
- peripheral blood
- bronchoalveolar and peritoneal lavage skin

Fluorescence-activated cell sorter (FACS) analysis
- the CD4/CD8 ratio
- T cells (e.g. CD3, $CD4^+$ $CD8^-$, $CD4^-CD8^+$, $CD4^+CD8^+$)
- B cells (surface Ig)

Solubile factors
- complement (total and fractions)
- cytokine determination
  = biological assays (cytokine-dependent cell lines, anticytokine monoclonal antibodies)
  = ELISA (comercially available for mouse and rat)
  = quantification of m RNA (blotting and subsequent hybridization or reverse transcription and subsequent PCR)

tissue, number of peripheral lymphocytes and monocytes, level of total globulins, cytokines, etc. (Vos 1977, 1987; Dean et al. 1987; Vandebriel et al. 1995; Luster et al. 1996). Functional tests (Table 5) reflect in greater detail the situation *in vivo* because they focus on the direct assessment of phagocytic and antigen-specific components of immunity. The evaluation of immunotoxicological action of xenobiotics requires a spectrum of methods that can provide as detailed as possible information about the immune system status. In this process one must consider that some immune status parameters are not necessarily affected by xenobiotics while others are in turn affected significantly. This is suggested by a range

of results obtained in the course of subchronic intoxication of sheep with some pesticides and during acute intoxication of sheep with heavy metals (Mikula et al. 1992a, 1992b; Pistl et al. 1995).

Table 5
Review of functional tests used in immunitoxicology
(Luster et al. 1993; Vandebriel et al. 1995; Dietert et al. 1996)

Functional tests of phagocytes
- phagocytic activity (PHA) and index of PHA
- metabolic and bacterial activity of phagocytes
Antigen-specific antibody responses
- sensitization to LPS, ovalbumin (ELISA)
- sheep red blood cells immunisation (ELISA, plaque forming cell assay PFC)
Mitogen responsiveness to B-cell mitogens
- the proliferative response of B cells to LPS
Mitogen responsiveness to T-cell mitogens
- the proliferative response of T cells to concanavalin A (Con A) and phytohaemagglutinin (PHA)
Mixed lymphocyte reaction (MLR)
- difference in cell proliferation between lymphocytes cocultured with allogeneic cells and those cocultured with syngeneic cells
Cytotoxic T-lymphocyte assay
- differentiation of T-cells into cytotoxic effector cells
Natural killer (NK) activity
- cell populations are cultured together with NK-sensitive target cells (e.g. YAC lymphoma cell line)
Delayed-type hypersensitivity responses (DTH)
- evaluating of cell-mediated immunity
- sensitization to PPD, ovoalbumin, keyhole limpet hemocyanin (KLH) and *L. monocytogenes*
Host resistance models
- *Listeria monocytogenes, Streptococcus pneumoniae, Pasteurella multocida*
- Cytomegalovirus, Influenza virus
- *Trichinella spiralis, Plasmodium yoelii*
Tumour models
- B16F10 melanoma, P4B6 fibrosarcoma, MADB106 adenocarcinoma
Autoimmune and allergy models

Animal health problems with respect to pesticide toxicology

The reality of today is that pesticides and heavy metals from emissions are dominant components of the chemical load on the environment of man and animals (Kačmár 1990). One can assume that further improvement in technology of trapping the industrial emissions will minimize gradually the problem of ecotoxicology of heavy metals, fluorine, nitrogen oxides, sulphur and other industrial pollutants. This involves analogy of toxicological risk of highly persistent and cumulative insecticides and acaricides based on DDT, HCH, HCB, heptachlor and other chlororganic pesticides. The residues of these chemicals show decreasing tendency in the majority of game species and cease to be toxicologically important risk factors for free living animals; at present, they are not subject to extensive monitoring in plant and animal commodities of the human food chain. Gradual decline in residues of chlororganic pesticides mentioned is related to the fact that insecticides-acaricides based on DDT and technical HCH were banned in 1975 and those based on HCB in 1980 (Breyl 1988; Kačmár 1990). However, this does not apply to some falconiform species, some of them containing DDE in concentrations by two orders higher than those found in DDT (Kottferová et al. 1995). Sporadic cases of acute intoxications of animals with pesticides could be prevented by strict observation of the valid legislative standards determining the application and storage of protective chemicals in the agriculture-forestry practice and in municipal hygiene (Bulletin of MP and VŽ, 1979; Decree No.

130/82, issued by FMZVŽ; Bulletin of MP and VŽ SSR, 1984, and others - In Slovak). However, it is obvious that chronical harmful effects of pesticides on the animal kingdom must also be considered with regard to distant future. To be specific, the protective chemicals are the principal means of protection of agricultural crops against pests and disease agents and controlling the weeds. According to data obtained by researchers in the former ČSSR, pests caused losses exceeding 5.5 billions Kčs (some 160 mil. US $) in plant production alone (Svítil 1986).

1. Use of pesticides in SR and negative consequences of their broad-spectrum applications

Total use of pesticides in SR in 1980-1995 ranged between 4 774 (1995) and 19 017 tonnes (1980). Out of that 7 % were zoocides, 27 % fungicides, almost 60 % herbicides and 8 % other pesticides (insecticides, acaricides, molluscocides and others). Thus herbicides have been the greatest source of risk to the environment. In 1996 altogether 931 pesticide preparations based on 350 active ingredients were registered in Slovakia (Dolinay and Moravčík 1997). The extensive use of pesticides poses many problems. Through their application thousands of tonnes of chemicals, most of them synthetic and included in various groups of organic compounds, enter the ecosystems. Different chemical structure of active ingredients present in respective pesticides is related to possible differences in the mechanism of their action and differences in symptomatology of acute poisoning (Kačmár 1980; Legáth et al. 1992). Many pesticides used currently in practice do not comply with the pressing requirement on high specifity, i.e. they are toxic not only to the target organisms, the pests, but to a certain extent also to productive animals and humans (Paulov 1984; Cremlyn 1985; Kačmár 1990). The long-term supply of pesticides to animal organisms, mostly through the feed and water but also *per inhalationem*, results in certain degree of their cumulation in various organs and tissues. The process of biotransformation of pesticide residues induces pathological changes in the respective tissues. From the point of view of regulation of physiological processes, this refers to serious pathological changes in internal secretion glands, negative interferences with the genetic material and immunosuppressive effects (Medveď 1973, 1974; Paulov 1984; Cremlyn 1985; Košutzký 1988; Vial et al. 1996). The most frequently observed practical consequences of chronic toxicity of pesticides in animals include sterility, embryotoxicity or even embryonic mortality, decreased vitality of offspring, total decrease in productivity and resistance of adults, and so on. The negative effects on animal production mentioned above are either not registered or ascribed to other factors than to their chemical etiology (Olefir 1973; Medveď 1974; Košutzký 1988; Kačmár 1990). The study of toxic effects of pesticides are carried out mainly on laboratory animals. The results obtained are then interpreted from the point of view of health disturbances in important farm animal species. In this respect, the physiological-anatomical differences of individual farm animals are the very cause of different toxicity and immunotoxicity and different qualitative and quantitative biotransformation changes of active pesticide ingredients in animal bodies. The same applies to biotransformation of drugs in animals (Šutiak and Šutiaková 1998). This is closely connected with the important question of residues in the products of animal origin (Ivie and Dorough 1977) and different susceptibility to infectious diseases. It remains to be established whether the immunotoxic results, obtained for example in model rodents, bear any relevance to the human population (Procházková 1992). Free living animals belong to the group of animals most affected by and immediately exposed to the harmful influence of pesticides. This refers not only to possible massive dying of animals but predominantly to so-called covert, clinically non-manifest damage to the organism and the real possibility of mutual potentiating effects of different active ingredients. Chronic pathological changes caused by pesticides are frequently manifested as deviations from the so-called normal situation and can involve damage to the immune system manifested for example as changes in resistance to the action of other relatively unharmful unfavourable factors (sufficient well-

developed greenery and vegetation in general, more or less extensive recreational attractivity of forests, extreme meteorological conditions and similar Sobocký 1974; Kačmár 1977). The present toxicology explains chronic harmful effects of pesticides on the cellular and molecular levels (Seiler et al. 1995; Lawrence et al. 1996). We have no substitute for the relatively new direction in toxicology - **immunotoxicology**. Its development in mid seventies (Vos 1977) was initiated by (1) errors in application of pesticides and accidents in chemical manufacturing plants; (2) necessity of investigating the unwanted effects of drugs, i.e. commercial interests of pharmaceutical companies; (3) the fact that toxic effects of xenobiotics on the immune system are manifested in lower doses and well before they can be detected by conventional chemical-toxicological analyses.

2. Veterinary immunotoxicology of pesticides

Immunotoxicology in veterinary medicine deals with the problems arising from dominant ecological toxicants, such as pesticides. From the medical point of view we can refer to it as **ecoimmunotoxicology**. At the present, 931 pesticide preparations based on more than 350 active ingredients are used in Slovakia (Dolinay and Moravčík 1997). According to reliable literary sources (Pesticide Manual 1994), almost none of the active ingredients present in pesticides that are used in our country were subject to immunotoxicological testing. The exposure of animals to residual concentrations of pesticides can lead to immunosuppression either directly or with participation of stress mechanisms (hunger, thirst, unfavourable microclimate conditions, long distance transport fatigue and others) and of the neuroendocrine system. Immunosuppression results in defective immunological supervision (e.g. defective maturation of cells) conducive to the development of tumours (Ferenčík 1993). However, with regard to the length of life of animals, more typical of them are changes in the length of life, increased susceptibility to infectious diseases and decreased immune response to vaccination. Potential consequences of immunotoxicity of pesticides can be divided to three groups: 1. Direct immunotoxicity (connected mainly with immunodepression), 2. Hypersensitivity reactions, 3. Autoimmune reactions (Descotes and Vial 1994). Majority of studies dealing with clinical immunotoxicity of pesticides in humans involves immune status alterations in occupationally exposed workers, farmers and accidentally pesticide-exposed humans. The alterations mentioned were suggested to be associated with infectious complications (Vial et al. 1996).

3. Pesticide-induced immunosuppressivity

Immunoinsufficiency was studied with regard to **chlororganic insecticides** (DDT, HCH, heptachlorine - Hermanowicz et al. 1982; Wysocki 1985; Kashyap 1986; Menconi et al. 1988; Broughton et al. 1990; McConnachie and Zahalsky 1992; Barnett and Rodgers 1994), **organophosphates** (Hermanowicz and Kossman 1984; Esa et al. 1988; Newcombe and Esa 1992; Thrasher et al. 1993), **pyrethroid insecticides** (allethrin, cypermetrin, fenpropathrin, permethrin - Descotes 1988), **herbicides** (atrazin 2,4-D, 2,4,5 - T - Descotes 1988; Fournier et al 1992; Wolfe et al. 1990), **fungicides** (pentachlorphenol - PCP - Descotes 1988; Lang Mueller - Ruchholtz 1991; Klemmer et al. 1980; Colosio et al. 1993; McConnachie and Zahalsky 1991; hexachlorbenzene - HCB - Vos 1986) and **carbamates** (aldicarb - Fiore et al. 1986; Barnett and Rodgers 1994). The up-to-date knowledge about **clinical immunotoxicity of pesticides** in humans can be summarized briefly as follows: Despite the fact that the epidemiological studies focused on direct immunotoxicity of pesticides revealed some immunity alterations, we lack direct evidence indicating that the occupational or environmental exposure of humans to pesticides results in clinical immunosuppression associated with increased incidence of infections or tumours. However, the possibility of long-lasting consequences of low doses of pesticides on the immune system in the course of chronic exposure to pesticides cannot be excluded (Pruell

1994). The selection of immunotoxicity biomarkers is one of the priorities of correct interpretation of minor changes in the immune system that are not easily reflected in measurable health indices (Descotes et al. 1995). If it is possible to provide biological proof of the immunosuppressive effect in the experimental model then the proof of clinical consequences requires simultaneous exposure e.g. to microbial or viral agents, or inoculation with neoplastic cells. Immunotoxic effects of pesticide preparations could result sometimes from by-products of the synthesis of active ingredients used in pesticides, or products of their biotransformation. The immunotoxic potential of pesticides is also related to the requirement on rational planning of epidemiological studies (Maroni and Pait 1993; Pruell 1994). Some partial results of epidemiological studies related to the extent impairment of to the immune system of humans exposed to chronic action of pesticides could provide preliminary information which can help in evaluation of the effect of residual concentrations of effective ingredients in pesticides, mainly on immunosuppression in animals which is most frequently associated with increased susceptibility to infectious diseases and decreased response to vaccination.

Veterinary medicine - research and practice: indication of application of immunotoxicology

Dominant toxicological problems in humans and animals are related mostly to chronic effects of pesticides and heavy metals released in emissions. The hidden, clinically latent effects allow us to reveal changes in individual immunological parameters also in those cases in which the conventional toxicological examinations provide most frequently only negative results (Desi et al. 1986). This view is supported also by our preliminary results obtained in experiments with subchronic intoxication of sheep with some pesticides and heavy metals. The investigations into the influence of herbicide Bentazon TP on the cells of the immune system in the course of 3-month subchronic intoxication of sheep (1/10 of LD_{50} 195 $mg \cdot kg^{-1}$ and 1/20 of LD_{50} of live weight), revealed decrease of responsibility of lymphocytes to mitogenic activation ($p < 0.05$) from the 8th week of the experiment. (Fig. 1,

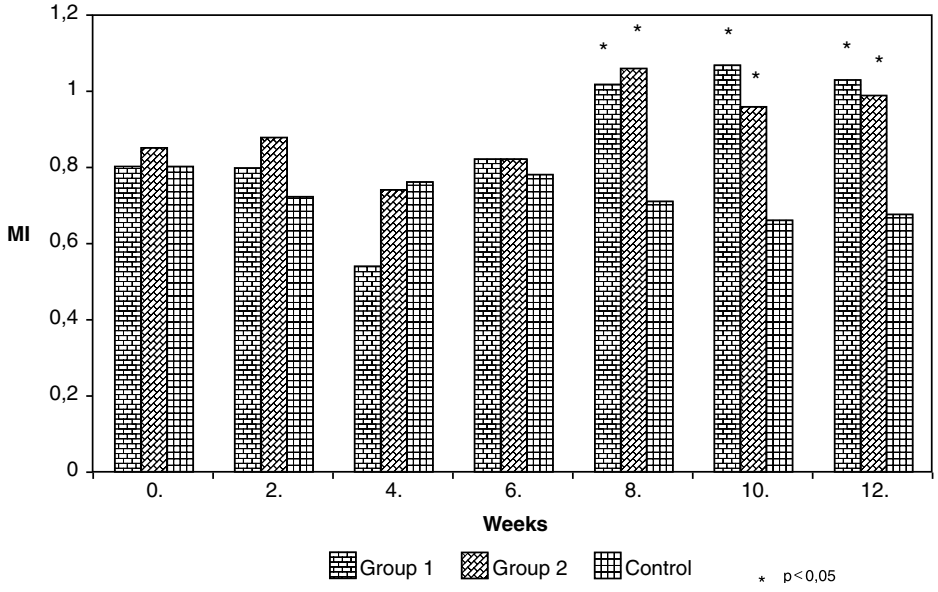

Fig. 1. Index of leukocyte migration in sheep after application of Bentazon

Mikula et al. 1992a). Results of additional tests (phagocytic activity, number of rosette-forming T-lymphocytes) were not affected by the pesticide. Additional tests were carried out on sheep using the model of subchronic intoxication (50, 200 and 300 mg·kg^{-1} live weight) to test pyrethroid insecticide supercypermethrin. Significant ($p < 0.05$) dose dependent (200-300 mg·kg^{-1}) decrease in the intensity of phagocytosis (index of phagocytic activity - IPA, Fig. 2, Mikula et al. 1992b) was detected in the 5th and 6th week of the

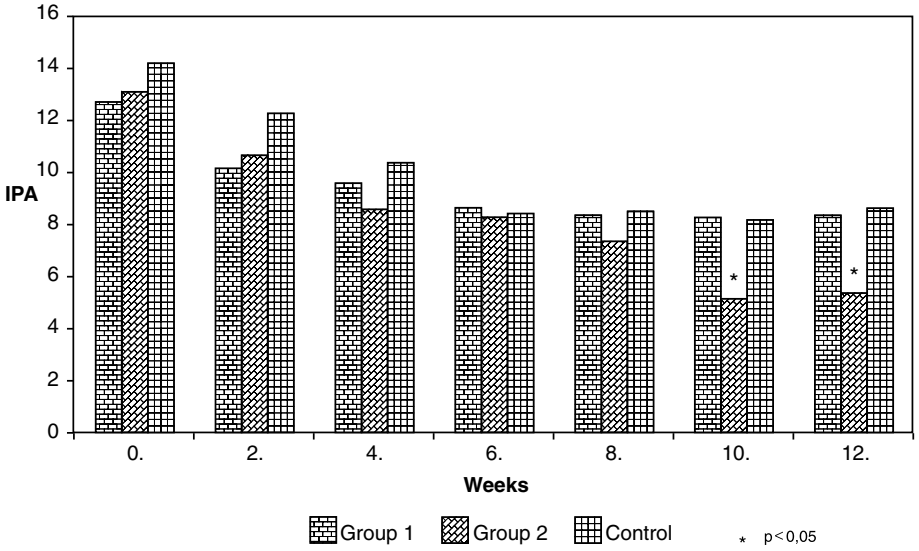

Fig. 2. Index of leukocyte phagocytic activity in sheep after administration of Supercypermethrine

experiment, however, per cent proportion of phagocytic cells, the number of E-rosette forming lymphocytes and mitogenic activation of lymphocytes were not changed significantly. *In vitro* leukocyte migration-inhibition tests were conducted to test the immunotoxic effect of p-chloraniline, the metabolite of herbicide monolinurone. The toxic effect of p-chloraniline was confirmed by total inhibition of leukocyte migration (1-0.1 mg.ml^{-1}). An immunotoxic effect was established on the basis of decreased response of lymphocytes to mitogenic activation in the presence of 0.01-0.001 mg·ml^{-1} p-chloraniline (Kačmár et al. 1995, Table 6). Experiments were carried out to test the effect of oral

Table 6
The effect of p-ClA on leukocytes of sheep in LMI assay

Le+p-ClA(mg·ml^{-1})	Leukocytes of 5 sheep			
	Con A-		Con A+	
	x ± sd	MI	x ± sd	MI
Control (without p-ClA)	15.3 ± 3.4	-	5.3 ± 1.2	0.35
1	1.7* ± 0.5	0.11	-	-
0.1	5.6* ± 1.8	0.37	-	-
0.01	10.7* ± 3.1	0.7	9.9 ± 3.2	0.65*
0.001	11.8 ± 3.6	0.77	8.6 ± 3.0	0.54*
0.0001	14.3 ± 3.2	0.93	4.4 ± 1.7	0.29

Le - leukocytes, MI- migration index, p-ClA - para-chloroaniline
* - $p < 0.05$, LMI - leukocyte migration inhibition assay, Con A - concanavaline A

15-day administration of heavy metals-containing emissions on the immune system cells in sheep. Two grams of emission contained 6 mg Hg, 44.4 mg Cu, 0.09 mg Pb, 0.0015 mg Cd and 0.29 mg Zn, which corresponded to the dose accepted by grazing at a 2 km distance from a copper producing plant (Pist1 et al. 1995). A 12-week experiment in animals immunized with the vaccine Listakol showed significant decrease in the index of metabolic activity of phagocytes ($p < 0.05$, $p < 0.01$, Fig. 3) from week 2 to week 8 and decreased response of

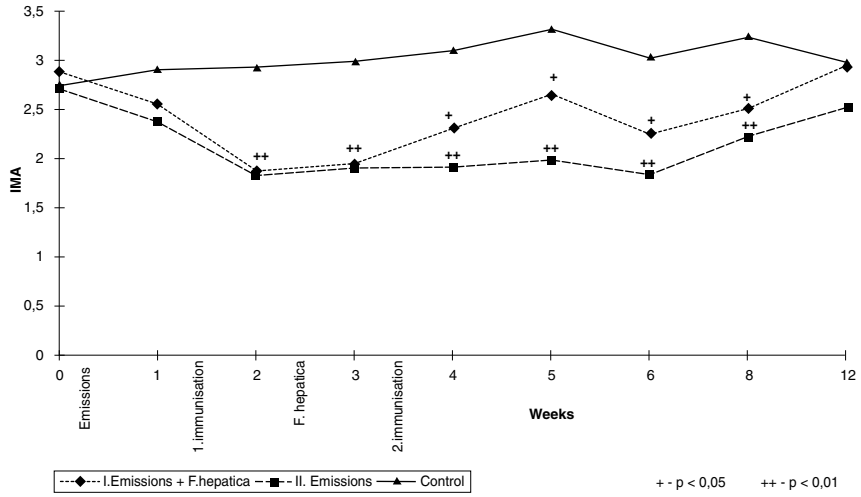

Fig. 3. Index of metabolic activity of phagocytes in sheep after application of heavy metal emmisions

lymphocytes to mitogenic (weeks 2-12, Fig. 4) and antigenic (*L. monocytogenes* - in sheep

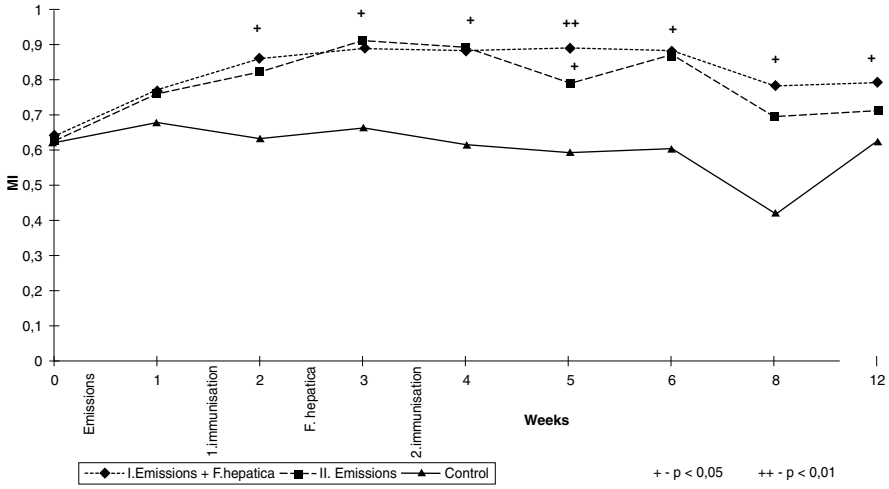

Fig. 4. Index of leukocyte migration in sheep after application of heavy metal emissions (tested with mitogen-PHA)

immunized with vaccine Listakol) stimuli (weeks 3-6, Fig. 5). Decreased titres of specific

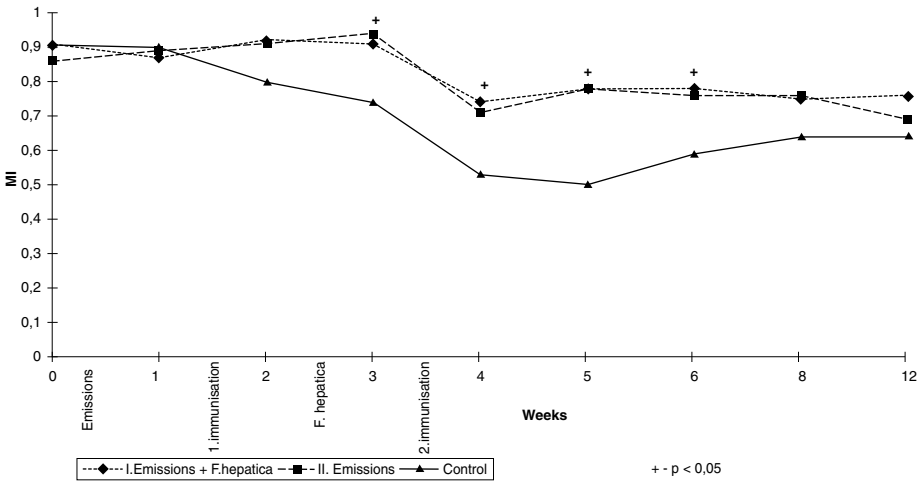

Fig. 5. Index of leukocyte migration in immunized with vaccine Liskanol after application of heavy metal emissions (tested with Ag L. monocytogenes)

antibodies to *L. monocytogenes* were observed in comparison to the control animals (Fig. 6). Changes in phagocytic activity of leukocytes, level of plasma Ig and albumine in

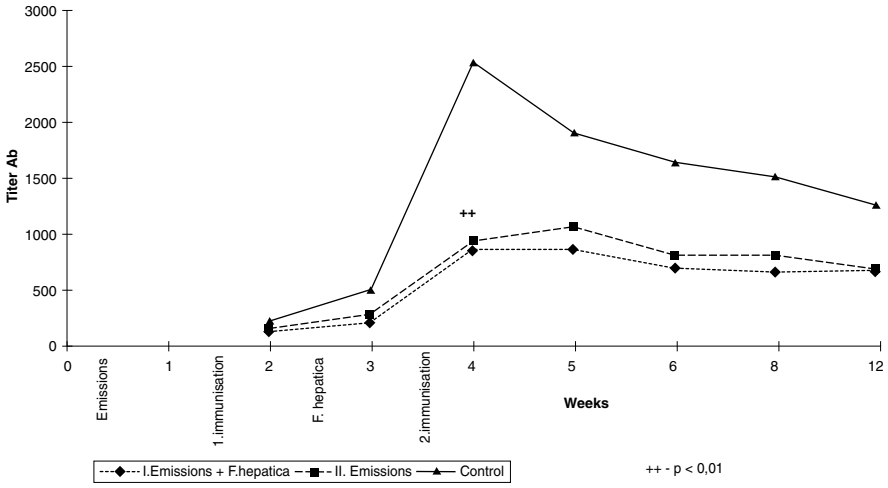

Fig. 6. Agglutination titres of antibodies to L. monocytogenes in sheep immunized with vaccine Liskanol after application of heavy metal emissions

the sheep tested were recorded after experimental feeding on the mixture of emissions from copper and zinc producing plant (Cu, Fe, Zn, Mo, Se, As, Cd and Pb) (Bíreš et al. 1990). Immunotoxicological problems caused by pesticides and heavy metals arise not only from

the presence of residues introduced by direct application of protective preparations in agriculture, forestry, water management and municipal practice but also from direct contamination of grass or crops with emissions. It was proved that they are also related to the quality of microclimate in animal housings which is significantly affected also by human activities (Anonymus 1979). Chemical analysis of the dust collected from ceiling fittings in pig houses (intensive pig fattening) showed that concentration of Cd in this material was 7-fold higher, Pb 3.4-fold, Zn 3.3-fold and Cu 3.3-fold higher than those in the granulated feed mixture. The examination of the same dust for the presence of chlorinated and organophosphate insecticides and herbicides revealed 10-fold higher concentration of lindane (gamma isomer of HCH) and 3-fold higher concentration of thiazine herbicides in comparison to he feed. The dust also contained PCB (up to $1 mg.kg^{-1}$) and the concentrations of aflatoxin B1 in this material were by 77% higher than those determined in the feed mixture (Raszyk et al. 1984). Thirteen years later, Raszyk et al. (1997) present some data about the influence of pollutants present in animal housings on the immune system of pigs and cows. Relatively high concentrations of gamma HCH ($0.139 mg \cdot kg^{-1}$), lead ($214 mg \cdot kg^{-1}$) and some other pollutants in the dust collected from stables (cow house N) were interpreted by the authors also from the point of view of possible immunosuppressive effect on the housed cows. The lymphocytic activity in cows in the respective herd was decreased in 35% of animals and corresponded to the lower limit of reference values. Water in rivers and lakes is another important reservoir of xenobiotics of different origin. This scope of problems was investigated by considerable number of studies that monitored first of all the presence of heavy metals, pesticides and PCB in relation to water fauna as a part of the food chain (Eisler 1987; Nriagu and Sprague 1987; Hodson 1988; Wester 1988; FAO Rev. 1991; Košuth and Breyl 1991; Košuth et al. 1992). Intensive investigation was carried out into the influence of mycotoxins on the immune system function. The studies dealing with the immunotoxic effect of aflatoxin B1 were the most detailed. Aflatoxin B_1 inhibits protein synthesis and division of cells which results in decreased resistance of the organisms to infectious and parasitic diseases (Sharma 1993; Corrier 1991). An immunosuppressive effect of aflatoxin B_1 on humoral activity of 21-day- old chickens was described by Bírešová et al. (1997). The importance of immunological testing has practical impact also on drugs. Unaltered application of only some drugs is associated on the one hand with immunological insufficiency (chloramphenicol, tetracyclins - Kanjuka and Šutiak 1990) and on the other with immunostimulation (levamizol, thiobenzol - Šutiak and Šutiaková 1988).

Urgent tasks of veterinary immunotoxicology

The testing of emergency immune system of animals in the production of ecological food of animal origin and increasing production-reproduction capabilities of animals takes priority over all other objectives of veterinary medicine. Because of that, out of all obligatory OECD tests determining the toxicological risk of pesticides to animals (Kačmár 1997), the greatest emphasis should be put on their possible immunotoxicity. To meet this objective the veterinary immunotoxicology must look for solutions to at least 3 principal tasks: (1) Development of standard and reliable methods (correct laboratory practice in veterinary immunotoxicology) or adaptation of existing immunotoxic tests considering those physiological-anatomical specifities of individual farm animal species which can result in different immunotoxicity. (2) Determination of the degree of immunotoxic potential of any pesticide to animals before it is introduced into practice. (3) Monitoring of the immune system of animals exposed to various pesticides, drugs and other xenobiotics. Specifities and functions of the immune system indicate that the immunotoxic effects of the respective pesticide cannot be evaluated on the basis of only limited number of tests but rather on the

complete set of them, provided that the set mentioned complies with some basic requirements (Table 7). In many countries the preparations for meeting the objectives have

Table *7*
Requirements on tests intended for determination of immunotoxic effects of xenobiotics
(Ferenčík 1993)

1. They should provide objective information about the changes in - individual components and factors of the immune system - complex immune functions (resistance to infections, response to vaccination) - multiple induction of immunopathological reactions (allergic and autoimmune processes).
2. They should be based on the fact that the immune system is a dynamic set of tissues, cells and molecules the activities of which are driven by antigens.
3. They should detect the largest possible number of functional changes in the immune system using minimum number of examinations.
4. They should determine the immunotoxic potential of the respective substance as soon as during the pre-clinical screening.
5. They should enable monitoring of abnormal immune functions in animals exposed to individual pesticides and drugs.

been going on for several years. For example, the national toxicological programme implemented in the USA has included immunotoxicology since late seventies. Twelve state organizations-agencies have been established in the USA and the study of immunotoxicological issues is part of their regular duties (Table 8). One must agree with the

Table 8
State agencies in the USA monitoring the influence of xenobiotics on the immune system
(Ferenčík 1993)

Environmental Protection Agency
Office of Pesticide Programs
National Institute for Occupational Safety and Health
Occupational Safety and Health Administration
Food and Drug Administration
Center for Food and Applied Nutrition
Center for Drugs and Biologics
National Center for Toxicological Research
National Toxicology Program/National Institute of
Environmental Health Science
Chemical Industry Institute of Toxicology
Consumer Product Safety Commission
Communicable Disease Center/Division of Environmental Health
Laboratory Science
National Cancer Institute
National Heart Lung and Blood Institute
National Institute of Allergy and Infectious Disease
National Research Council/National Academy of Science

statement of immunologists that the situation in our country is completely different. We have no organization in SR dealing with the influence of xenobiotics on the immune system. Only individual enthusiasts have been making an effort to solve some partial tasks without sufficient support from state bodies. In the education sphere, we are aware of the absence of an appropriate system for underegraduate and graduate education of immunotoxicologists. It is well a known fact that education of such specialists is a long-term process. Here we must emphasize immediate necessity of establishing a separate branch of science - **veterinary toxicology and pharmacology -** that could create the necessary space for immunotoxicology of pesticides and drugs in farm and free living animals. The

minimization of negative economical impact of pests in agriculture and forestry is related to the necessary broad-spectrum application of protective preparations and pharmacological substances. In this sense the immunotoxicological testing resembles "extinguishing of fire". One must consider the requirement "not to allow the fire to flare up". However, this is the task other specialists are faced with: correct agrotechnical practice (early and thorough ploughing, sowing and similar) efforts of genetics, breeders, entomologists - cultivation of crop varieties resistant to insect, pathogenic moulds and so on. Methods of molecular genetic - use of transgenic plants - are prospective in this respect (Vrtiak and Mikula 1998). This means integrated protection of plants **versus** application of tonnes of pesticides.

Imunotoxikológia a veterinárna medicína

Imunotoxikológia skúma neželané škodlivé vplyvy xenobiotík (pesticídov, ťažkých kovov z exhalácií, nové DNA-rekombinantné produkty, modulátory imunitnej odpovede, monoklonálne protilátky, liečivá a i.). Imunotoxikológii vo veterinárnej medicíne sú najvlastnejšie problémy dominantných ekologických toxikantov, akými sú pesticídy. V prípade veterinárnej imunotoxikológie teda ide o *ekoimunotoxikológiu*. Na význame tu nestrácajú ani DNA-rekombinantné biopreparáty a liečivá.

Interakcia rôznorodých xenobiotík s organizmom predstavuje rôznorodé imunotoxikologické problémy. V práci sa zdôrazňuje, že posúdenie imunotoxikologického rizika cudzorodých látok si vyžaduje vývoj nových a stále exaktnejších testovacích imunotoxikologických metód, pričom na dôkladné posúdenie imunotoxického účinku toho ktorého xenobiotika nestačí pár testov, ale je potrebný ich komplexný súbor. Rozsah funkčných a nefunkčných testov imunity má byť selektovaný poznatkami o mechanizme toxického pôsobenia xenobiotík u zvierat. Tieto poskytuje biochémia, toxikológia, farmakológia a tiež histopatológia (histochémia a imunohistochémia). Z doterajších poznatkov o imunosupresivite pesticídov a ich možného negatívneho zásahu do genetického materiálu živých organizmov vyplýva nutnosť postupne obmedzovať širokospektrálne používanie pesticídov cestou dôraznejšej aplikácie vedecky zdôvodnených agrotechnických postupov a využitie transgénnych rastlín ako výsledku molekulového šľachtenia.

Minimalizácia imunotoxických rizík pesticídov u hospodárskych a voľne žijúcich zvierat si vyžiada vybudovanie potrebného systému pre pregraduálnu a postgraduálnu výchovu. Ide o mnohoročný proces, ktorý sa zrejme bude môcť realizovať zriadením spojeného vedného oboru **toxikológia a farmakológia,** v rámci veterinárneho lekárstva.

Dôkladné štúdium mechanizmov imunotoxicity a imunofarmakológie rôznych agens by malo viesť k produkcii bezpečnejších protektívnych agrochemikálií a účinnejších liečív.

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Immunotoxicology has been defined as the study of adverse effects on the immune system resulting directly or indirectly from occupational, environmental or therapeutic exposure to chemicals (including drugs), biologic materials and, in certain instances, physiological factors, collectively referred to as agents. It encompasses studies of altered immunologic events associated with exposure of humans and wildlife species including immune regulation (suppression or enhancement), allergy and autoimmunity (Figure 1). In the former case, the systemic or local (e.g. skin, lung) immune system acts as