Distribution of AChE and BuChE – positive nerves and alterations in the rabbit thymus and bone marrow after bendiocarbamate administration

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Introduction

Recently, a large amount of chemicals are produced, which come into close contact with the human body. Agrochemicals are certain part of modern agrotechnical procedures despite the fact that a great number of them are on the list of risk substances (Anwar, 1997). Many agrochemicals have toxic and genotoxic effects and, subsequently, after cumulative somatic mutations, also carcinogenic effects. Uncontrolled and thoughtless use of various pesticides resulted in health problems involving nervous, endocrine, reproductive and immune systems of animals and humans (Hayes and Lawes, 1991). Although some pesticides have been restricted or banned because they pose risks of cancer, birth defects, or neurological damage, little attention has so far been given to what may be their greatest health risk: impairment of the immune system. Hundreds of experimental studies on human cell cultures and animal models provide strong evidence that many pesticides are immunotoxic and may affect immune status and function. The thymus is an organ located in the upper anterior portion of the chest cavity and plays an important role in the development of the immune system in early life, and its cells form a part of the normal immune system (Belak et al., 1990). Thymus is a central lymphatic organ with an important endocrine function. It is a place where the T cells precursors, responsible for the cell-mediated immunity, proliferate and mature. The thymic microenvironment constitutes a unique environment for the differentiation, maturation and selection of the T cells (Varga et al., 2009; Varga et al., 2010). Histologically, the thymus can be divided into a central medulla and a peripheral cortex which is surrounded by an

outer capsule. The cortex and medulla play different roles in the development of T-cells. Cells in the thymus can be divided into thymic stromal cells and cells of hematopoietic origin (derived from bone marrow resident hematopoietic stem cells). Developing T-cells are referred to as thymocytes and are of hematopoietic origin. Stromal cells include thymic cortical epithelial cells, thymic medullary epithelial cells, and dendritic cells (Gartner and Hiatt, 2006). Bone marrow contains hematopoietic stem cells (HSCs) which produce all the blood cells and mesenchymal stem cells (MSCs) differentiate into mesenchymal lineages (Gu et al., 2009). The activity of rabbit bone marrow is a function of the lipide-free dry weight. From the results of the correlation of the various components, it may be stated that the activity of marrow is a direct function of the nitrogen, residue, water, and log of the lipide nitrogen content, and an inverse function of the lipide concentration. With the composition of marrow as a criterion of its activity, it is apparent that there are variations in activity at different points of the long bones. The distal ends are less active than the proximal. Such comparisons tend to make all marrow appear to be of the same composition, and fundamental changes in the composition of animals coincident with growth and aging may be obscured. Especially, if "fat-free" implies freedom from all lipid substances (Dietz, 1946). Carbamates generally are excreted rapidly and do not accumulate in mammalian tissue. If exposure does not continue, cholinesterase inhibition and its symptoms reverse rapidly. In this study we evaluated the genotoxic potential of bendiocarbamate after exposure in rabbits through an erythrocyte bone marrow assay. A range of pesticides belongs to the group of cholinesterase inhibitors involved in morphogenesis (Kozarova et al., 2001). Cholinesterase inhibitors are a group of chemical substances precluding hydrolysis of acetylcholine and thus causing accumulation of acetylcholine in reactive sites of live organisms. Inhibition of brain acetylcholinesterase has been the major therapeutic target for Alzheimer disease and other patients with dementia. One of the cholinesterase inhibitors, bendiocarbamate, is used to control insect populations. Animal cholinesterases are widespread enzymes present in cholinergic and non-cholinergic tissues as well as in their plasma and other body fluids. They are divided into two classes according to differing in their substrate specificity, behavior in excess substrate and susceptibility to inhibitors: acetylcholinesterase or *true cholinesterase* (AChE) and butyrylcholinesterase (BuChE). BuChE is also known as non-specific cholinesterase or simply cholinesterase (Smulders et al., 2003).

Target of this study was to describe distribution of acetlycholine and butyrylcholine – positive nerves and quantify structural and functional changes in the rabbit thymus and bony marrow after an experimental administration of bendiocarbamate.

Materials and methods

The study was carried out on 50 European rabbits (*Oryctolagus cuniculus*), hybrid Hyla, of both sexes. The rabbits were 54 days-old with mean BW of 1250 g. The experimental animals were kept under standard conditions in animal quarters at 15–18°C and natural light regimen. They were fed granulated mixed feed and were supplied drinking water *ad libitum*. The rabbits were placed into large litter less cages, 6 animals in each. The experimental animals were administered bendiocarbamate (96% Bendiokarb tech., Bayer, Leverkusen, Germany) in the form of capsules *per os* at a dose of 5 mg/kg BW daily for 13 days. Since the animals showed a strong response to the daily administered dose (diarrhoea, dehydration and alopecia in some animals), after 13 days the exposure was decreased by administering the same dose in 48 h intervals. During the experiment, control animals were fed standard granulated mixed feed intended for rabbits. On days 3., 10., 20., 30., 60. and 90. of the experiment, groups of experimental animals consisting of 6 rabbits were euthanized by ether together with control group (8 rabbits) of animals.

Detection of AChE- and BuChE-positive nerves in thymus

The experiment was conducted in compliance with the rules set by the Ethical commission of University of Veterinary Medicine in Kosice, Slovakia and conditions for experiments on animals. Samples taken from the thymus were processed for demonstration of AChE-positive and BuChE-positive nerve fibers. During two hours the samples were fixed in 4% formaldehyde at 4°C. Sections were prepared on freezing micro-tome thick 20 μm and incubated in the incubation solution during the period of 2–4 h at 37°C. The incubation medium (according to method of Karnovsky and Roots, 1964 and of El Badawi and Schenk, 1967) contained acetylthiocholine iodide needed for the visualization of specific AChE, tetraisopropylpyrophosphoramide (iso-OMPA) for inhibition of nonspecific AChE was used. In this method acetylcholinesterase present in the cholinergic nerves releases thiocholine from acetylthiocholine which reduces potassium ferricyanide to potassium ferrocyanide, capable of producing insoluble copper ferrocyanide with copper ions (Hatchett's brown).

The method for visualization of butyrylcholinesterase is the same, but the incubation medium contained butyriltiocholine iodide as an inhibitor of specific AChE instead of acetylthiocholine iodide. The exact composition of incubation solution can be found in Table 1, the final pH of solution was 5.6 to 6.0.

Elaborated samples were assembled on slides and were examined under a light microscope JENALUNAR 2 (Zeiss, Jena, Germany).

We assessed visually the density of the nerve fibers in thymuses of both experimental and control rats. The histological preparations were evaluated qualitatively under an optical microscope (Olympus Provis AX, Japan). We compared the histochemical localization of acetlycholine- and butyrylcholine-positive nerves in rabbit's thymuses after administration of bendiocarbamate.

Histological analysis of the rabbit thymus

Samples of thymus were fixed in 10% formalin. After fixation the samples were dehydrated in a graded series of ethanol (70, 80, 90 and 100%), saturated in benzene, benzene-paraffin and embedded into paraffin. Blocks of samples were sectioned on a microtome into 7–12 μm thick sections and stained with heamatoxylin and eosin. Qualitative and quantitave parameters were analyzed on microphotographs (Olympus Provis AX, Japan) and for quantitative analysis morphometrical image analyser software (Image ProPlus, Media Cybernetica, NY) was used according to micromorphological criteria (Weibel et al., 1966). In thymus the relative volume (%) of cortex, medulla, interlobular space and blood vessels; number of tymocytes and reticular cells per 1000 μm^2 as well as the diameter (μm) of tymocytes and reticular cells was determined. From final data, basic characteristics were calculated (mean, SD, coefficient of variance, median) and differences by Student's t-test were completed for each variable (software SPSS 11.0.1; ID 11-2502-02348).

Micronucleus assay in the rabbit bony marrow

A portion of bone marrow from the thoracic rib was flushed and resuspended in 1.5 ml of foetal bovine serum (Sigma, St. Louis, MO, USA). The bone marrow suspension was spun and smears were prepared and stained with May-Grünwald and Giemsa. Each slide was assessed for the presence of micronucleated polychromatic erythrocytes (MNPCEs) among 1000 polychromatic erythrocytes (PCEs). The ratio of PCEs to normocytes (NCEs) was determined among 1000 cells.

The frequency of MN in bone marrow was detected according to Mac Gregor et al., 1987. Data were summarized as the mean number of MNPCEs for 1000 PCEs. In addition, the toxicity in bone marrow was determined as ration between PCEs and NCEs. The staining must allow clear discrimination between PCEs and NCEs. However, this somewhat subjective since the transition in maturation is a continuous process. The normal ration is about 1 : 1. An increase in NCEs signals a cytotoxic effect. Similarly, at longer sample intervals, an increase in PCEs signals a stimulation of proliferative activity due to an early phase of cell depletion. Student's t-test was used to compare MNPCEs data and PCEs to NCEs rations between treated and control groups for statistical significance.

Results

Effect of bendiocarbamate on distribution of AChE- and BuChE-positive nerves in thymus

Rabbit thymus of control animals contained AChE- and BuChE-positive nerve fibers in two forms: i) nerve fibers joining to vessels. They formed networks around branches of vessels running in septa and, as a common bundle, headed to organ parenchyma. They entered the cortex layer of the thymus from subcapsular nerve network. The part of the cortex housing numerous lymphocytes was very poor in nerve fibers, while abundant nerve clusters could be observed at the level of cortico-medullary junction, i.e. the part which was a primary starting point of cells and early precursors; ii) nerve fibers independent on vessels. They were most abundant at the cortico-medullary junction and a little less frequent in the medulla.

Microscopic findings of BuChE-positive nerve fibers show the same density and the topography by the experimental and the control animals. On the other hand, AChE-positive nerve fibers in experimental animals after administration of bendiocarbamate is poorly identified, suggesting that bendiocarbamate inhibit AChE but not BuChE.

Microscopical findings related to AChE-positive innervations of the thymus of rabbits which were administered bendiocarbamate, the inhibitor of AChE, were rather indistinctive (Fig. 1 and 2). One could not identify nerve structures with certainty and AChE-positivity was exhibited only by non-neural cellular elements in all experimental materials examined (Fig. 3). In the control animals the AChE-positive nerve fibers were observed in the organ capsule in cortex layer originating from the interlobular septum. Fine nerve fibers terminated in the cortex and frequently came to close contact with lymphocytes while in the deep cortex layer and medulla they were located close to epithelial cells.

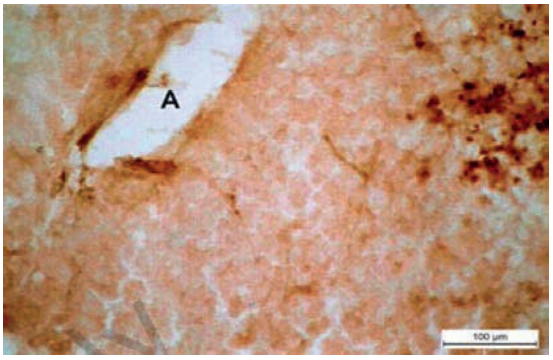

Fig. 1. Third day of the experiment; nerve fibers are not visualized after administration of bendiocarbamate. A – artery, Scale bar: 100 µm

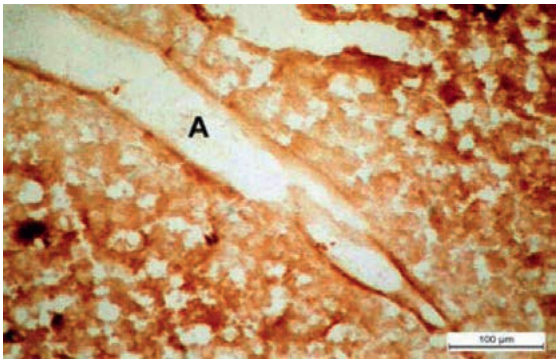

Fig. 2. Day 20 of the experiment. Thymus of an experimental rabbit with very poorly visible AChE-positive fibers in perivascular topography. A – artery, Scale bar: 100 µm

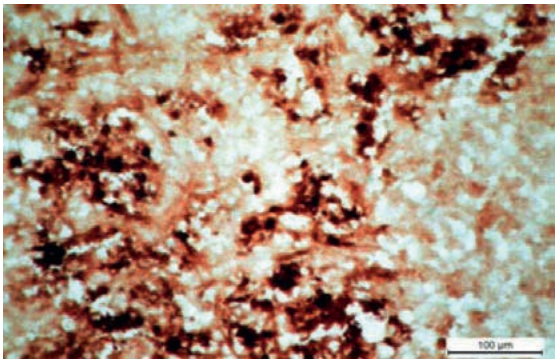

Fig. 3. Day 90 of the experiment. Abundant reaction product in the noncellular part of thymus of experimental animal lacking AChE-positive nerve fibers, Scale bar: 100 µm

The distributions of BuChE-positive nerve fibers after visualization were the same in experimental and control animals. Nerve fibers entered the thymus as a common bundle with arteries, as typical periarterial plexuses (Fig. 4). In the organ they were running in interlobular septa as a common bundle of branching off fibers. The highest density of thicker periarteri al nerve plexuses with abundance of reaction product was observed at the cortico-medullary junction (Fig. 5), while the finer fibers were less abundant in this area. A characteristic feature in this topography was the presence of non-neural cellular elements with high content of colored reaction product. The outer medullary layer contained numerous thicker and thinner nerve fibers. In the cortex layer we observed pre-domination of fine nerve profiles without evident direct connection to vessels. In the deep cortex topography we were able to observe thicker nerve fibers, particularly close to the cortico-medullary border. Fine BuChE nerve fibers were observed also in the thymic capsule.

Based on our findings we can state that BuChE-positive innervations of thymus in experimental and control animals show no differences, it follows bendiocarbamate in dose of 5 mg/kg BW daily not inhibit butyrylcholinesterase.

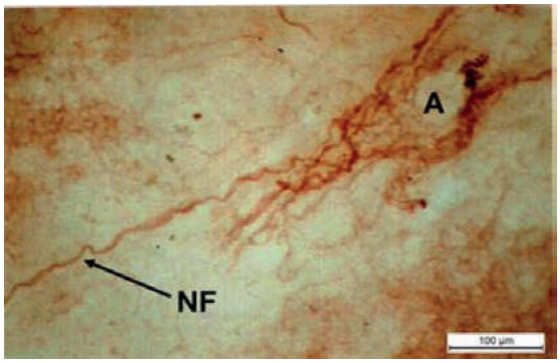

Fig. 4. Day 20 of the experiment. Abundant BuChE-positive nerve fibres (NF) in perivascular topography and solitary nerve fibres originating from them. A – artery, Scale bar: 100 µm

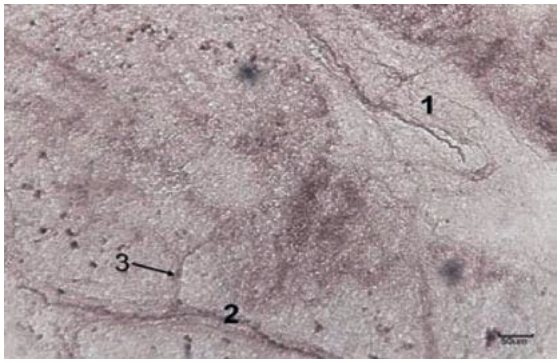

Fig. 5. Third day of the experiment. Fine BuChE-positive nerve fibers (NF) at the cortico-medullary junction. The distribution and the density of nerve fibers are similar to the control animals. A – artery, Scale bar: 100 µm

Bendiocarbamate inducted structural alterations in thymus

Qualitative observation of thymus showed peripheral formation of cortex and central location of medulla. Among all lobules interlobular space with blood vessels was present. The cortical portion was composed of lymphoid cells, supported by a network of finely-branched epithelial cells, which were continuous with a similar network in the medullary portion. In the medullary portion, the reticulum was coarser than in the cortex, the lymphoid cells are relatively fewer

in number, and there are found peculiar nest-like bodies, the concentric corpuscles of Hassall. Results of qualitative evaluation are listed in Table 1 and 2. In control group the relative volume of cortex is 57.94±7.10%. In all experimental groups with bendiocarbamate administration higher relative volume of cortex was detected. Except Day 30 all differences were significant ($p<0.05$). Medulla formed 35.94±7.38% in control group. In this parameter all relative volumes were significantly decreased ($p<0.05$) except animals after 30 days of administration. Relative volume of interlobular space was in the range of 3.30–5.46%, with a significant decrease ($p<0.05$) after day 60 in comparison with control. Analysis of the relative volume of the blood vessels showed weak non-significant differences (Tab. 2). Detail morphometric analysis detected a decreasing tendency of the number of tymocytes per constant area as well as the diameter of tymocytes in all experimental groups in comparison with control. Evaluation of the number of reticular cells per constant area and the diameter of these cells showed very similar data in all groups (Tab. 1). Our findings suggest alterations of the structure of thymus after bendiocarbamate administration that probably cause altered function of this organ.

Tab. 1. Cellular parameters in thymus after bendiocarbamate experimental administration; SD – Standard deviation; CV – variation coefficient (%)

	Day	X	SD	cv	minimum	maximum
Number of thymocytes per 1000 µm²	Control	**40.30**	25	62.03	25	50
	3	**30.45**	25	82.10	25	39
	10	**38.50**	30	77.92	30	47
	20	**37.93**	30	79.10	30	48
	30	**38.20**	31	81.15	31	47
	60	**29.53**	24	81.29	24	38
	90	**30.08**	24	79.80	24	37
Diameter (µm) of thymocytes	Control	**6.21**	4.89	78.74	4.89	7.58
	3	**5.00**	4.15	82.93	4.15	6.29
	10	**4.35**	2.15	49.40	2.15	5.88
	20	**4.44**	2.69	60.59	2.69	6.29
	30	**4.35**	2.35	54.05	2.35	6.87
	60	**5.43**	3.89	71.63	3.89	7.05
	90	**5.59**	2.98	53.29	2.98	7.09
Number of reticular cells per 1000 µm²	Control	**1.89**	1	52.94	1	5
	3	**2.76**	1	36.19	1	7
	10	**2.00**	1	50.00	1	5
	20	**1.71**	1	58.62	1	5
	30	**1.56**	1	64.29	1	4
	60	**2.26**	1	44.29	1	5
	90	**1.91**	1	52.31	1	4

Diameter (μm) of reticular cells	Control	**10.67**	6.23	58.36	6.23	15.12
	3	**11.06**	5.24	47.38	5.24	17.26
	10	**10.78**	6.21	57.62	6.21	18.24
	20	**11.06**	6.58	59.48	6.58	18.21
	30	**10.19**	5.24	51.43	5.24	13.67
	60	**11.64**	6.57	56.44	6.57	17.84
	90	**12.15**	7.42	61.06	7.42	16.32

Tab. 2. Relative volume (%) of basic structures of rabbit thymus in relation to bendiocarbamate administration; *p<0.05 (control vs. bendiocarbamate); SD – Standard deviation; CV – variation coefficient (%)

	Day	**x**	**Median**	**SD**	**cv**	**minimum**	**maximum**
Thymus cortex	Control	**57.84***	59.36	7.10	12.26	46.54	68.15
	3	**72.41**	74.46	8.86	12.15	54.02	86.51
	10	**68.18**	69.78	9.42	13.77	46.04	80.91
	20	**66.60**	66.93	3.11	4.67	60.69	70.63
	30	**61.40**	61.87	6.14	10.00	51.34	73.61
	60	**68.52**	70.56	5.22	7.58	59.28	74.88
	90	**78.20**	78.89	2.50	3.19	74.09	81.36
Thymus medulla	Control	**35.82***	35.24	7.38	20.52	26.06	45.92
	3	**23.15**	18.44	8.90	41.79	8.12	37.88
	10	**24.21**	26.23	11.38	43.42	8.89	51.34
	20	**27.26**	27.96	4.28	15.69	20.71	35.90
	30	**33.72**	32.41	7.18	21.25	23.97	45.96
	60	**27.08**	24.73	5.73	21.16	21.60	38.33
	90	**14.37**	13.18	4.03	26.94	11.16	22.86
Interlobular space	Control	**5.11**	4.92	2.30	44.58	1.52	8.65
	3	**5.46**	4.64	3.03	55.43	0.63	11.42
	10	**4.53**	3.26	2.82	62.34	1.85	11.25
	20	**4.96**	4.79	2.09	42.04	2.30	9.75
	30	**4.32**	3.74	2.40	55.60	1.94	9.37
	60	**3.30***	3.11	1.14	34.58	1.33	5.26
	90	**5.04**	5.23	2.01	39.94	2.01	8.46
Blood vessels	Control	**1.23**	1.24	0.73	59.82	0.28	2.60
	3	**0.98**	0.79	0.74	75.04	0.22	1.95
	10	**3.08**	4.37	2.23	71.03	0.66	5.09
	20	**1.18**	1.33	0.86	72.37	0.26	2.60
	30	**0.56**	0.43	0.33	58.85	0.09	1.11
	60	**1.10**	1.13	0.29	26.42	0.69	1.59
	90	**2.39**	2.54	0.84	35.03	0.87	3.26

The activity of bendiocarbamate in rabbit bony marrow micronucleus assay

The group sizes used in the micronucleus assay are shown in Table 3. The results of rabbit bone marrow micronucleus test after exposure to the bendiocarbamate are summarized in Table 4. For each group, the average incidences of MNPCEs as well as the PCEs/NCEs ratio are shown. *In vivo* administration of 5 $mg.kg^{-1}$ body weight of insecticide did not increase the incidence of MNPCEs in bone marrow of any tested groups. Cytotoxic insult to bone marrow frequently impairs the proliferating and maturational abilities of erythroid cells. Typically, a ratio of enucleate, immature polychromatic erythrocytes (PCEs) to mature normochromatic erythrocytes (NCEs) is used to assess cytotoxicity in the micronucleus (MN) assay. Depression of bone marrow proliferation was evident in the reduction of PCEs after three days of exposure and attained statistical significance ($p<0.05$).

Tab. 3. The groups of rabbits used in the micronucleus assay; F – female, M – male

Control		After 3 days		After 90 days	
No. of animal/sex	**Body weight (kg)**	**No. of animal/sex**	**Body weight (kg)**	**No. of animal/sex**	**Body weight (kg)**
28 F	2.43	10 F	1.29	3 F	3.16
31 M	2.78	11 F	1.35	35 F	3.34
41 M	2.67	12 F	1.31	44 M	3.95
42 M	3.00	23 M	1.50	47 M	4.05
		37 M	1.00	51 F	3.38
Group Mean±SD	2.72±0.24		1.29±0.18		3.58±0.39

Tab. 4. Frequencies of micronuclei in rabbit bone marrow treated with bendiocarbamate; MNPCEs – micronucleated polychromatic erythrocytes, PCEs – polychromatic erythrocytes, NCEs – normochromatic erythrocytes, SD – standard deviation; a – no significant difference; * – statistical significance ($p<0.05$)

N	**MNPCEs/ 1000PCEs**	**Control PCEs/ NCEs ratio**	**N**	**After 3 days MNPCEs/ 1000PCEs**	**PCEs/ NCEs ratio**	**N**	**After 90 days MNPCEs/ 1000PCEs**	**PCEs/ NCEs ratio**
28	0	1.071	10	0	0.450	3	1	0.607
31	1	0.680	11	1	0.394	35	0	0.872
41	0	0.933	12	0	0.473	44	1	0.841
42	0	0.904	23	0	0.607	47	0	0.966
			37	0	0.708	51	0	0.948
Group Mean±SD	0.25±0.5			0.2±0.5a			0.4±0.5a	
PCEs/ NCEs ±SD		0.89±0.16			0.53±0.13*			0.85±0.14a

Discussion

The thymus played a key role in the evolution of animals during development of an adaptive immune system; it is therefore an important element, distinguishing higher vertebrates from other animals (Massanyi and Uhrin, 1997; Varga et al., 2008; Bowden et al., 2005). Our observations of AChE- and BuChE-positive nerve fibers in rabbit's thymuses proved their presence in interlobular septa, capsule, cortex, cortico-medullary junction and in the medulla. Our results are in agreement with the observations of authors who investigated autonomic innervations of thymuses in rats and mice (Nieto-Cerón et al., 2006; Dorko et al., 1994, 1996).

Functional changes after administration of cholinesterase inhibitors are characteristic of disorders of metabolic processes responsible for regulation of acid-base balance. Accumulation of acetylcholine results in depolarization of biologic membranes and hypokalaemia (Ratner et al., 1983). It is well known that surface membranes of various subpopulations of lymphoid cells harbor a number of specific receptors of both conventional neurotransmitters and some peptides (Hrelia et al., 1996; Kocisova et al., 2002). According to Mignini et al., 2003 released neurotransmitters may affect functions of T and B lymphocytes as well as the process of entrapping antigens by cells. Our observations of BuChE-positive thymus innervations that bendiocarbamate blocks specific cholinesterases but will not accumulate in the body of mammals, as after 24 h it is released from mammal tissues either by urine or by lungs or faeces, are in agreement with the results of Ratner et al., 1983.

Long-term administration of bendiocarbamate caused a decrease in relative proportion of medulla at the expense of cortex which contained hyperplastic reticular cells. Our results allowed us to state that BuChE-positive innervations of thymus did not differ significantly between experimental and control animals, i.e. bendiocarbamate administered at a dose of 5 $mg.kg^{-1}$ BW failed to inhibit visualization of BuChE-positive nerve structures.

On the other hand, AChE-positive nerve profiles were not visualized in experimental animals after administration of this substance. Bendiocarbamate can affect not only the thymic innervations, but also the dividing of lymphocytes. Holeckova et al., 2009 investigated induction of the unstable chromosomal aberrations, sister chromatid exchanged and stable chromosomal aberration after bendiocarbamate administration in cultured peripheral bovine lymphocytes. They found increase of chromatin breaks frequency. Varga et al., 2009 found significant positive correlation in thymic size and number of lymphocytes in peripheral blood in newborns.

It has been assumed that suppression of the immune system by some pesticides forms the basis for increased number of allergies, hypersensitivity of organisms

and susceptibility to tumour growth (Gleichmann et al., 1989; Mojzisova et al., 2005). One of possible mechanisms of these observations is the oxidative stress: the action of xenobiotics is frequently associated with increased level of reactive forms of oxygen (Parthasaranthy et al., 2006; Agrawal, 1991; Holovská et al., 1996). Results of the study of the structural alterations detected significant changes in the thymus structure, mainly increased relative volume of cortex and decreased relative volume, after an experimental long-term bendiocarbamate administration. Data in relation to bendiocarbamate particularly describe the effect of this compound on insects (Zhao et al., 1995; Saxena et al., 1992; Casimiro et al., 2006; Wang et al., 2003; Mpofu et al., 1991).

In human beings bendiocarbamate is absorbed through all the normal routes of exposure, but dermal absorption is especially rapid. Carbamates generally are excreted rapidly and do not accumulate in mammalian tissue. If exposure does not continue, cholinesterase inhibition and its symptoms reverse rapidly. In nonfatal cases, the illness generally lasts less than 24 hours. Within two days after feeding doses of up to 10 mg/kg of bendiocarb to rats, 89 to 90% of the dose was eliminated in the urine, 2 to 6% was exhaled, and another 2 to 6% was eliminated in the feces. This same pattern of elimination was observed in a human subject given an oral dose of bendiocarbamate (Danko et al., 2005). In relation to bendiocarbamate exposure transfer to the developing fetus during pregnancy was reported (Whyatt et al., 2003). The carbamates alone weakly activate estrogen or progesterone responsive reporter genes in breast and endometrial cancer cells. All of the carbamates decreased estradiol or progesterone induced reporter gene activity in the breast and endometrial cancer cells. The carbamate insecticides may represent a class of chemicals which function through a mechanism separate from ligand binding and, therefore, may act as general endocrine modulators in mammalian cells (Klotz et al., 1997; Kacmar et al., 1999). In relation to immune system there is a serious lack of information about the effect of bendiocarbamate. In adult female mice the effects of aldicarb were studied. The absence of significant effects on any of immune parameters suggests that aldicarb at environmentally relevant exposure concentrations is not immunotoxic in rodents (Thomas et al., 1987). On the other hand, results of our study determined significant structural alterations in thymus structure after bendiocarbamate administration which probably cause alteration in immune system. To our knowledge, there are very few published reports available describing the cytogenetic or genotoxic effects of bendiocarbamate on animal cells. Our present genotoxicity data for the bendiocarbamate are a part of more extensive collaborative study (Mojzisova, 2004; Tuckova, 2004; Sulla et al., 2004). The micronucleus test in erythrocytes of mouse bone marrow has been proposed as a screening test by Boller and Schmid, 1970, and Heddle et al., 1991. The frequency of micronuclei can be most easily evaluated in young erythrocytes shortly after the main nucleus is expelled. These young erythro-

cytes are termed polychromatic (PCEs) and are distinguished from mature normochromatic (NCEs) erythrocytes by their different staining properties. With a combination of Giemsa and May-Grünwald staining the PCEs stain bluish to purple due to their high content of RNA in the cytoplasm. In contrast, the NCEs stain reddish to yellow and are also slightly smaller than PCEs. Published mutagenic, carcinogenic and teratogenic studies show that the bendiocarbamate is harmless (Baron, 1991). In micronucleus test, groups of male mice were treated with technical bendiocarbamate i.p. for two days. There was no significant increase in the frequency of polychromatic erythrocytes containing micronuclei or change in polychromatic/normochromatic ratio in any treated group (Houk and Waters, 1996).

In conclusion, the results presented in this paper show that bendiocarbamate is unable to raise the incidence of micronuclei in rabbit bone marrow significantly, at least in our tested conditions.

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