TECHNICAL REPORT

2. Toxicity study of Micro Aerosol of Electrochemically Activated Solutions in the conditions simulating it application for volumetric disinfection of enclosed environment

1. INTRODUCTION

The results generated during Phase I demonstrated high decontamination efficacy of Microaerosol of Electrochemically Activated Solution (MAEAS) toward a variety of bioagents, including G-positive and G–negative microbial cells, bacterial spores, and viruses. It was shown that the contact of different bioagents with MAEAS resulted in serious morphology damage and irreversible death of microbial cells, spores and viruses.

First MAEAS toxicity study was conducted with outbred white mice to observe toxicity of MAEAS. As the controls, microaerosols generated with dist water and with 1% NaCl were used. Minor and very similar variations were found in liver, spleen and lungs of the animals exposed to MAEAS and to the aerosol of 1% NaCl. That indicated that MAEAS was not more toxic than the aerosol of 1%NaCl. This study of MAEAS toxicity was conducted in the conditions simulating MAEAS application for volumetric disinfection of enclosed environment.

2. MATERIALS AND METHODS

2.1. Animals

Swiss Webster specific pathogen-free (SPF) female mice were purchased from "Pushchino" Animal Breeding Facility, Moskovskaya obl., Russia. Before the study, mice were quarantined for 14 days with daily observation for behavior and general condition, and twice per day - for sickness and death rates. After quarantine, only proper animals (healthy and with appropriate weight) were randomly split into the experimental and control groups. During the study mice were kept in cages in an animal room at 12-h light/dark cycle and 15 fresh air changes per an hour. The following conditions were controlled in the room with experimental and control groups: temperature 18-20^0 C, RH 50-70%; CO_2 concentration has not exceeded 0.15 % (v/v) and the ammonia concentration has not exceeded 0.001 mg/l. The experiments with animals were conducted in compliance with the Protocols and Procedures approved by IHPBP Animal Care and Use Committee.

2.2. Electro-activated solution (EAS)

EAS was prepared from 1%NaCl solution with a STEL device (NPO "Ekran", Moscow). The content of active chlorine in the EAS was (0.20 ± 0.02) % and pH =7.2 ± 0.1.

2.3. Protocol of MAEAS administration

Two hours before the test, mice were deprived of feed and water, weighed, and randomly distributed among seven groups of 10 animals each.

The mice were spread among the groups as follows:

1. Control group I– mice not exposed to any aerosol
2. Control group II – mice exposed to a microaerosol; of non-electrolyzed 2% NaCl during 20 hours
3. Mice exposed to MAEAS right after atomizing and remained inside the chamber for one hour
4. Mice exposed to MAEAS right after atomizing and remained inside the chamber for four hours
5. Mice exposed to MAEAS right after atomizing and remained inside the chamber for 20 hours
6. Mice positioned in the chamber 4 hours after MAEAS was dispersed inside the chamber and remained in the MAEAS atmosphere for 20 hours
7. Mice positioned in the chamber 20 hours after EAS was atomized inside the chamber and remained in the MAEAS atmosphere for 20 hours

Mice were placed to a closed container and positioned inside the aerosol chamber of 2.8 m^3 (~100ft^3). MAEAS was atomized with the VAG generator operating at ~90 ml/min. In each experiment ~650 ml of EAS was atomized in the chamber for 7.2 minutes to provide the initial MAEAS concentration 232 ml/m^3 (~6ml/ft^3). It was six times higher concentration of MAEAS than required for effective disinfection of enclosed environment.

Right after MAEAS generation was terminated (VAG turned off), the container with mice was opened with a tool located outside the chamber, and mice were directly exposed to MAEAS. The same protocol was used when microaerosol of non-electrolyzed NaCl was generated inside the chamber.

- Mice of Groups 3 to 5 remained inside a chamber during 1, 4, or 20 hours, respectively.

- Mice of Groups 6 and 7 were positioned in the chamber 4 or 20 hours after EAS was atomized inside the chamber and then remained inside the chamber during subsequent 20 hours.
- Control mice Group II were positioned in the chamber, non-electrolyzed 2% NaCl was atomized inside the chamber, and the mice remained within the chamber during subsequent 20 hours.

All groups were observed during 14 consecutive days. Mice were monitored for general condition, behavior, motor activity, convulsions, irritant reactions, state of hair and skin, and changes in body weight daily. On day 14, blood samples were obtained by retro-orbital puncture under anesthesia and animals were euthanized.

Pathology

Euthanized mice were inspected externally; subjected to necropsy, and abdominal and thoracic internal organs undergone extensive examination; the lungs were subjected to histological study.

Simultaneously, bronchoalveolar lavage fluid (BALF) was taken from mice to study the BALF cellular composition and neutrophils activities by zymosan-dependent chemiluminescence. The mice lungs were lavaged with 1 ml of PBS supplemented with 20 U/ml of heparin solution. The lavage samples were centrifuged at 200*g* for 10 min. at 4^0C and obtained pellet was re-suspended in PBS. The cell concentration was counted using Goryev's chamber. Simultaneously, the smear preparations (thick smear) were prepared, dried, fixed in 96% ethanol, and stained by Romanovsky using Azure-eosin. The BALF cell differentiation was examined under a light microscope (630 x). The number of monocytes/macrophages, neutrophils, and lymphocytes was counted per 100 cells. Data are presented in %.
The luminol-dependent chemiluminescence (CL) in BALF cells was measured using the Chemiluminometer Victor 2 (LKB Wallac). All measurements were made in 3 parallels in 96-well white non-transparent plates (Costar). Each well contained 20 µl of BALF sample, 40 µl of luminol at the concentration 10^{-4} M, and 20 µl of the opsonized zymosan used as a stimulant for CL reaction. CL was followed for 30 min. at 37^0C. Data were presented as cumulative counts.

Histological examination of the lung tissue

The lungs were stored in 4% paraform, washed with tap water for 24 hours and dehydrated in 70% ethanol. For histological study, lung pieces were cut from the diaphragmatic lobes and dehydrated in graded ethanol, incubated in celloidin-castor mixture for 24-hour, and embedded in Paraplast. Lung sections were cut at 6 μm thickness and stained with Hematoxylin and Eosin using a standard protocol, and analyzed using a light microscope Leica DMLB, a video camera Leica DC 300, and a software Leica IM 50 (Germany); magnification was 20x for the objective and 16x for the camera.

Hematological and immunological studies

On day 14 blood samples were collected from the orbital sinus under chloroform anesthesia. Total leukocyte count was recorded. Blood smears were air-dried, and blood formula was analyzed (lymphocyte, neutrophil, monocyte, eosinophil and basophile cell counts were estimated; data are presented in %). Blood smears were fixed in 96% ethanol and stained by May-Grunwald and Romanovsky with Azure-eosin.
The cellular differentiation was examined under a light microscope, using oil immersion objective, magnification 630x. The number of lymphocytes, neutrophils, monocytes, eosinophils, and basophils was counted per 100 cells. Data are presented in %.

Spleens were removed under aseptic conditions from euthanized mice. The spontaneous and Con A-stimulated proliferation of spleen T-cells (the reaction of blast transformation) and Con A-induced interleukin (IL)-2 synthesis were studied. For this purpose, spleens were pooled and cut with scissors. The cells were suspended in sterile PBS, filtered through double gauze and centrifuged. Then, the cells were washed twice with PBS (erythrocytes were preliminarily lysed with 0.83% solution of NH_4Cl) and re-suspended in RPMI-1640 (Sigma) supplemented with 2.0 mM of L-glutamine, 50 μM of 2-mercaptoethanol and 20 μg/ml of gentamycine (complete medium). The cell counting was performed. In 4 parallels, spleen T-cells (5×10^5cell/well) were incubated in 96-well flat-bottomed plates (Costar) with either 0 (spontaneous proliferation), 0.33 or 1.0 μg/ml of Con A (Sigma) in a complete medium, supplemented with 10% FCS (Sigma), in a CO_2 - incubator for 72 hours at 37^0C in absolute humidity. 24 hours prior to completion of incubation ^{3}H-labeled thymidine (the final concentration 5 μCi/ml) was added, the cells were harvested into fiberglass filters, type GF/C (Whatman) with a semi-automatic harvester (Flow Lab). The ^{3}H-labeled thymidine inclusion was measured with a scintillation liquid β-counter RackBeta 1217 (Wallac).

To evaluate the Con A-stimulated IL-2 synthesis, spleen T-cells (1 x 10^6 cell/well) were incubated in 96-well flat-bottomed plates (Costar) with either 1 or 5.0 μg/ml of Con A (Sigma) in a complete medium, supplemented with 5% FCS (Sigma). The cell supernatants were collected 24 hours after incubation, and IL-2 activity was measured using the IL-2-dependent CTLL-2 cell line in accordance with the well-known protocol (Gillis S. et al, 1982).

Statistical analysis

Statistical analysis of the data was performed using the Microsoft Excel 2003 program. The meanings were presented as mean ± StD and the level of significance was set at $p < 0.05$. The parameters measured in mice exposed to MAEAS were compared with corresponding parameters in mice of both control groups. The differences were calculated using two-way unpaired Student's *t*-test with unequal deviations. The correlation was considered to be significant if $p<0.05$.

3. RESULTS

Body weights, general conditions, and internal organs

Body weight of the mice exposed to MAEAS and of control mice are presented in Table 1.

Table 1. Body weight (g) of the MAEAS-exposed and control mice

Period of observation	Mice groups						
	Control group I Intact mice	Control group II Exposed to 2% NaCl aerosol	Directly Exposed to MAEAS			Positioned to the chamber:	
			1 hour	4 hours	20 hours	4 hours after EAS atomizing	20 hours after EAS atomizing
	1	**2**	**3**	**4**	**5**	**6**	**7**
Prior to exposure	22.82±1.30	23.06±1.02	23.04±1.45	19.82±1.26	22.20±0.92	20.89±1.85	21.77±1.42
Day 3	23.04±1.01	23.12±1.27	23.10±1.76	20.14±1.32	22.81±1.57	21.38±1.46	22.58±1.37
Day 7	24.08±1.32	22.85±1.49	23.02±1.55	21.36±1.72	2379±1.83	22.67±1.59	23.04±1.53
Day 14	24.70±1.13	23.22±1.04	23.79±1.83	23.18±0.94	24.21±1.41	23.22±1.74	23.53±1.26

One can see that mice body weight similarly and insignificantly increased in all groups, as compared to mice weight before the tests.

It was also observed that during14 days after exposure to MEAS, mice general health, hair and skin conditions, behavior, and appetite remained unchanged in all experimental groups and the Control group II, as compared to the mice from Control group I (non-exposed to microaerosol). In all groups mice mucous membranes were shine, smooth, pale, and without pathology.

In all groups mice necropsy revealed no exudates in thoracic and abdominal cavities, and the normal anatomic position of the internal organs.

In all mice groups:

- Submandibular lymph nodes and salivary glands were of spherical and oval forms, of white color and of moderate and dense consistency without changes
- Thyroid gland was of usual size and form, of reddish color, without pathology
- Thymic gland was of triangular form, of whitish color and slightly dense consistency, without pathology
- Aorta intima was shine and smooth.
- Heart was without changes in size and shape; the heart muscle was of moderate thickness and of brownish color, without pathology.
- Tachea and large bronchi lumena were uniformly wide. Pulmonary collapse easily occurred on thoracotomy. The lungs were of uniform pale pink color. The lung tissue seemed gas-filled by touch, without pathology.
- Esophageal mucosa was shine and smooth
- Stomach was of normal size with the lumen filled with food debris. The gastric mucosa was folded, of uniform pinkish color, none irritation or hyperemia was observed. The large and small intestine mucosa were smooth and shine of grayish and pale pink color, respectively. The straight intestine mucosa was smooth, shine of grey-pink color without irritation
- Pancreas was of pale pink color, segmental
- Liver and spleen were of normal color, size and fullness
- No pathological changes in mice digestive system were revealed
- A kidney capsule was easy to remove, the surface was smooth and of uniform brownish-grayish color. The cortical and medulla substances were well observed on incision.

- Adrenal glands were of spherical form, of whitish color, and of moderate dense consistency, without changes.
- Urinary bladder mucosa was shine, smooth and pale, without changes. The uterine body and horns were of normal appearance and size.
- Ovaries had uneven surface, were of reddish color and of moderate density. The urinary and genital systems organs were without pathological changes
- Brain tunics were shine, smooth and thin. On the front brain section, no brain ventricular ectasia was observed.

Conclusively, macroscopic examination has not revealed pathological changes in the internal organs of mice exposed to MAEAS.

Liver and spleen weight of the mice exposed to MAEAS and mice from the controll groups are presented in Table 2.

Table 2 Liver and spleen weight (g) in mice exposed to MAEAS and in control mice

Organs	Mice groups						
	Control group I Intact mice	Control group II Exposed to 2% NaCl aerosol	Exposed to MAEAS			Positioned in the chamber	
			1 hour	4 hours	20 hours	4 hours after EAS atomizing	20 hours after EAS atomizing
	1	**2**	**3**	**4**	**5**	**6**	**7**
Liver	1.11±0.09	1.03±0.14	0.99±0.60	1.08±0.13	1.15±0.13	1.10±0.08	1.00±0.14
Spleen	0.13±0.02	0.11±0.02	0.11±0.01	0.11±0.01	0.12±0.01	0.13±0.01	0.10±0.02

As evident from the results presented in the Table 2, no reliable difference was detected between the liver and spleen weight of the mice directly exposed to MAEAS (groups 3-5), indirectly exposed to MAEAS (groups 6-7), exposed to microaerosol of non-electrolyzed NaCl (Control group II), and non-exposed to microaerosol (Control group I).

Lung tissue histology

Lung tissue histology studies demonstrated no pathological changes in the lungs of mice exposed to MAEAS as compared to mice non-exposed to MAEAS:

- General lung tissue pattern was normal in all mice groups
- Bronchi lumena was clear, and none peribronchiolar, or perivascular cuffing was revealed. In most lung tissue specimens, the bronchial epithelium was of normal appearance.
- No pneumosclerosis foci or significant lung injuries were revealed.
- The airway tissue looked more plethoric than normal one.
- The number of monocytes/macrophages was slightly increased however no typical signs of the acute inflammation were revealed.

Among the mice from one group, the lung tissue pattern slightly differed in the airway area, most probably due to different perfusion. One mouse from Group 2 demonstrated a sharp increase in the RBC counting and in monocyte/macrophage infiltrating that might be attributed to individuality of this mouse.

Fig.1 - Fig.7 present the micrographs of the lung tissue of mice exposed to MAEAS and control mice.

Conclusively, the lung tissue histology studies demonstrated no pathological changes in the lungs of mice exposed to MAEAS.

Hematologic and immunological studies

The peripheral blood differential cell counts and formula were analyzed in mice. The results are presented in Table 3.

Table 3 Changes of mean blood cell counts in mice of experimental and control groups

Animal Group	Total WBC count (10^6/мл)	Differential cell count, %				Absolute Lymph. Number x10^6/ml	Absolute Neutroph. number x10^6/ml
		Lymphocytes	Neutrophils	Eosinophils	Monocytes		
1	5.9 ±1.7	86.2±3.44	12.1±3.52	1.31±1.02	0.43±0,56	5.07±1.41	0.74±0.32
2	6.1±1.24	86.60±2,0	12.3±2.31	0.32±0.4*	0.82±0.64	5.29±1.1	0.75±0.20
3	7.4±1.2	86.6±2.76	11.0±2.61	2.03±1.18	1.04±0.35	6.42±1.06	0.79±0.17
4	4.24±1.4	86.8±2.42	11.2±2.28	1.71±0.70	0.32±0.48	3.71±1.27	0.45±0.17
5	6.25±1.7	88.8±2.24	9.62±2.52	1.12±0.74	0.51±0.60	5.53±1.48	0.62±0.27
6	6.6±1.6	87.4±4.4	10.3±3.31	1.61±1.08	0.72±0.84	5.78±1.45	0.67±0.25
7	4.9±1.4	83.0±5.43	15.2±4.84	1.23±1.08	0.64±0.60	4.06±1.13	0.75±0.33

* $p < 0,05$

As can be seen from Table 3, no significant changes in the peripheral blood cell formula were found in the mice from experimental and control groups.

The eosinophil count in mice exposed to MAEAS seemed to be increased compared to that in mice of group 2 (exposure to NaCl aerosol), however did not significantly differ compared to that in intact mice. No other significant changes of peripheral blood parameters were revealed in mice of Group 2 compared to that in mice exposed to MAEAS and in intact mice. So, the difference in eosinophile count in mice of Group 2 may be ignored as insignificant for general results.

Based on our long-term experience, the morphological parameters of peripheral blood in healthy mice are the following: WBC - 7-15x10^6/ml, neutrophils – 10-40%, eosinophiles - 0-7%, basophils– 0-1%, lymphocytes – 35-90%, and monocytes – 0-3%. As follows from the data presented in Table 3, the peripheral blood counts were within physiological norm in all mice groups, though slight differences have been revealed among the groups.

The influence of MAEAS on the BALF cell differentiation and on functional activities of leukocytes was studied by the luminol-dependent chemiluminescence method. The results are presented in Table 4 and Table 5.

Table 4. BALF cell differentiation in experimental and control mice

Animal Group	Total cell count (10^6/ml)	Differentiation cell count, %		
		Monocytic cells/macrophages	Lymphocytes	Neutrophils
1	0.21±0.11	95.52±1.12	4.02±1.01	0.50±0.50
2	0.26±0.22	94.01±2.23	3.62±1.60	2.40±1.91
3	0.17±0.10	96.82±0.81	3.02±0.81	0.20±0.31
4	0.47±0.31	94.83±2.91	3.51±1.90	1.67±1.62
5	0.27±0.11	96.11±1.72	3.62±1.43	0.22±0.41
6	0.15±0.10	96.51±1.63	3.03±1.31	0.44±0.60
7	0.26±0.11	96.93±0.71	3.02±0.61	0.26±0.10

It has been reported (Kaufmann, 1999) that BALF cellular composition in healthy mice for the most part consist of monocytes and macrophages with the small number of alveolar epithelial and blood cells: $1x10^5$ - $5x10^5$ of alveolar macrophages, about 5% of neutrophils, and about 6% of lymphocytes. The results of this study demonstrated that the BALF cell differentiation in all mice was within the physiological range. No significant signs of inflammation were observed. No significant changes in BALF cell differentiation were found in mice exposed to MAEAS compared to that in control mice.

Table 5. Changes of the functional activities of leukocytes in mice exposed to MAEAS and control mice (10^{-4} pulses within 30 min)

Chemi-luminescence (CL)	Mice groups						
	Control I Intact mice	Control II Exposed to 2% NaCl aerosol	Directly Exposed to MAEAS			Positioned in the chamber	
			1 hour	4 hours	20 hours	4 hrs. after EAS atomizing	20 hrs. after EAS atomizing
	1	**2**	**3**	**4**	**5**	**6**	**7**
Spontaneous	13.80±0.32	13.72±0.33	11.52±0.40	14.05±0.44	14.26±0.49	14.38±0.54	11.28±0.35
Zymosan-stimulated	14.65±0.34	14.39±0.39	12.22±0.89	15.05±0.51	14.84±0.36	13.92±0.56	11.68±0.35

As can be seen from Table 5, no significant differences in spontaneous and zymosan-stimulated CL were revealed among the mice groups. Hence, no changes compared to the background indices were revealed in the functional activities of neutrophils.

The MAEAS effect on spontaneous and Con A-stimulated proliferation of spleen T-cells was studied. In addition, the MAEAS effect on the ability of these cells to produce IL-2 was also investigated. We used these tests as the routine immunotoxicity study and because of the high sensitivity of the immune cells to toxicants. Table 6 and Table 7 present the results.

Table 6. Spontaneous and Con A-stimulated proliferation of T-spleen cells in mice exposed to MAEAS and in control mice (pulses/min. x 10^{-3})

Con A (μg/ml)	Mice groups						
	Control I Intact mice	Control II Exposed to 2% NaCl aerosol	Directly Exposed to MAEAS			Positioned in the chamber:	
			1 hour	4 hours	20 hours	4 hrs after EAS atomizing	20 hrs after EAS atomizing
	1	**2**	**3**	**4**	**5**	**6**	**7**
0	84.8 ± 3.3	98.6 ± 5.0*	73.4 ± 6.1	80.7 ± 8.3	110.7 ± 2.8*	76.7 ± 6.6	85.9 ± 4.2
0.33	122 ± 3.0	127.7 ± 8.3	129.5 ± 8.6	123.1 ± 6.6	161.9 ± 5.1*	117.8 ± 4.9	150.5 ±5.7*
1.0	135 ± 6.3	175.4 ± 4.5	149 ± 15.0	203.7±6.3	183.5 ± 1.2*	128.8 ± 10.5	150.8 ± 9.7

* - p < 0,05 compared to intact mice

Table 7. Spontaneous and Con A-stimulated IL-2 synthesis by T-spleen cells in mice exposed to MAEAS and in control mice (IU/ml)

Con A (μg/ml)	Mice groups						
	Control I Intact mice	Control II Exposed to 2% NaCl	Directly Exposed to MAEAS			Positioned in the chamber	
			1 hour	4 hours	20 hours	4 hrs. after EAS atomizing	20 hrs. after EAS atomizing
	1	**2**	**3**	**4**	**5**	**6**	**7**
0	1.4 ± 1.0	2.4 ± 0.3	2.5 ± 0.4	1.8 ± 1.1	3.1 ± 0.1	1.1 ± 1.1	3.8 ± 0.4*
1.0	16.4 ± 2	15.9 ± 0.5	16.1 ± 1.6	10.5 ± 3.2	13.9 ± 0.5	18.2 ± 2.3	30.8 ±0.6*
5.0	170 ± 10	190.2 ± 30.2	146.4 ± 18.5	165.4±18.5	201.2 ± 17.5	166 ± 11.5	188.7± 4.7

*p < 0.05 compared to mice exposed to NaCl aerosol

According to the results outlined in the Table 6, significant increase of the spontaneous proliferation was revealed in mice of Groups 2 and 5, compared to that in intact mice. A significant increase of the Con A-stimulated proliferation was found in mice of Group 5 and Group 7, compared to intact mice. In mice exposed to MAEAS for different time intervals, the stimulated proliferation increased in accord with increased contact time time. However, no significant differences were revealed between mice exposed to MAEAS and those exposed to NaCl aerosol (Group 2), suggesting that the registered variations were nonspecific for MAEAS. These variations could be attributed to general stress effect of mice exposure that caused the proliferation increase.

We found difficult to interpret the results presented in the Table 7 because there were no real consistency between them. Increase of the spontaneous and Con A-stimulated (1 μg/ml) IL-2 synthesis by T-spleen cells was found in mice groups indirectly exposed to MAEAS compared to that in mice directly exposed to MAEAS and NaCl aerosol. The mechanism of this effect requires further investigations. However, it is hard to attribute to MAEAS toxicity. As known, toxic effects usually cause a reduction of spontaneous and Con A-stimulated (1 μg/ml) IL-2 synthesis by T-spleen cells

According to the data presented in Table 6 and Table 7, the MAEAS had no noticeable toxic effect on the immune cells because the values of spontaneous and Con A (1 μg/ml)-stimulated cell proliferation and of IL-2 synthesis have not reduced in mice exposed to MAEAS, which is characteristic for toxic effect.

4. CONCLUSION

The results of the study of the MAEAS toxicity conducted in the conditions simulating the MAEAS application for volumetric disinfection of enclosed environment, revealed no toxic effects in mice exposed to the MAEAS during different time and those positioned in the aerosol chamber after visual aerosol disappearance.

The results of the study demonstrated that: the animal behavior and general conditions, the state of skin and hair, the body and the internal organs weight were not distorted after a contact with MAEAS. Macroscopic and microscopic examination of lung tissues revealed no pathology. All hematological parameters measured in mice remained within physiological range.

No toxic effect of the MAEAS toward the immune system of mice was revealed. The mechanism, contributing to T-spleen cell proliferation and IL-2 production increase shown in mice exposed to MAEAS during 20 hours, is not clear and requires further investigations.

5. CONCLUSION

1. Gillis S., Ferm M.M., Ou W., Smith K.A. T cell growth factor: parameters of production and quantitative assay for activity // J. Immunol.-1982.-V.120.-P.2027-2032
2. Methods in microbiology. Ed by Kaufman S., 1999, Acad. Press, v.25, p.342-344

6. ILLUSTRATIONS

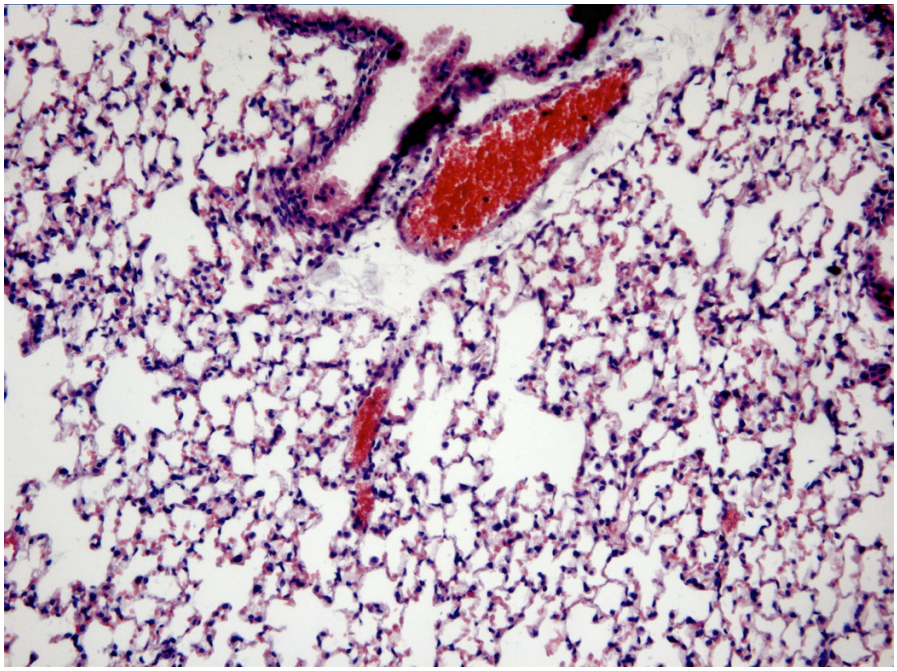

Fig.1. Lung cross-section (Control I - intact mice), cut at 5 μm, stained with H/E, magnification 160x.

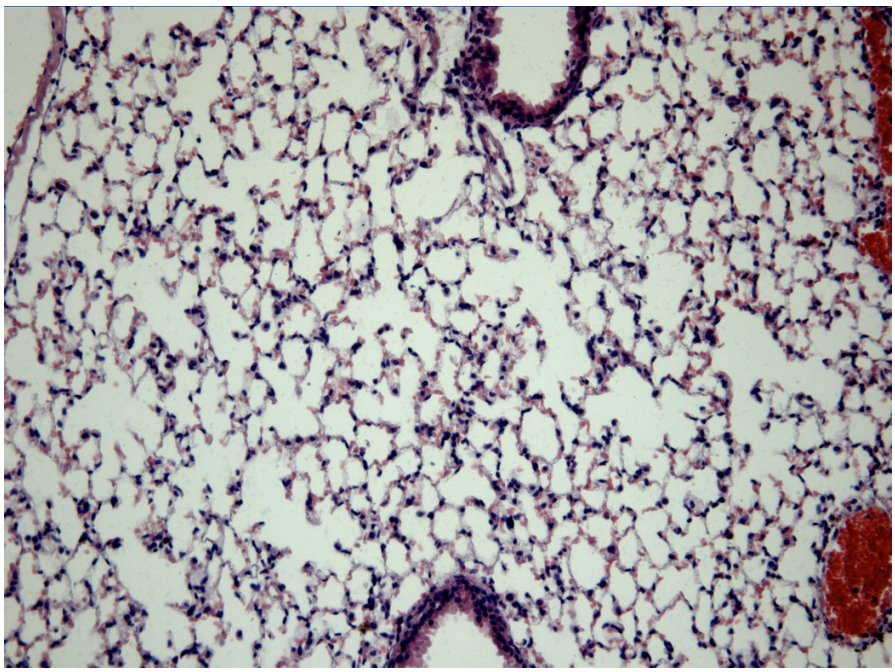

Fig.2. Lung cross-section (Control II – exposed to NaCl aerosol), cut at 5 μm, stained with H/E, magnification 160x.

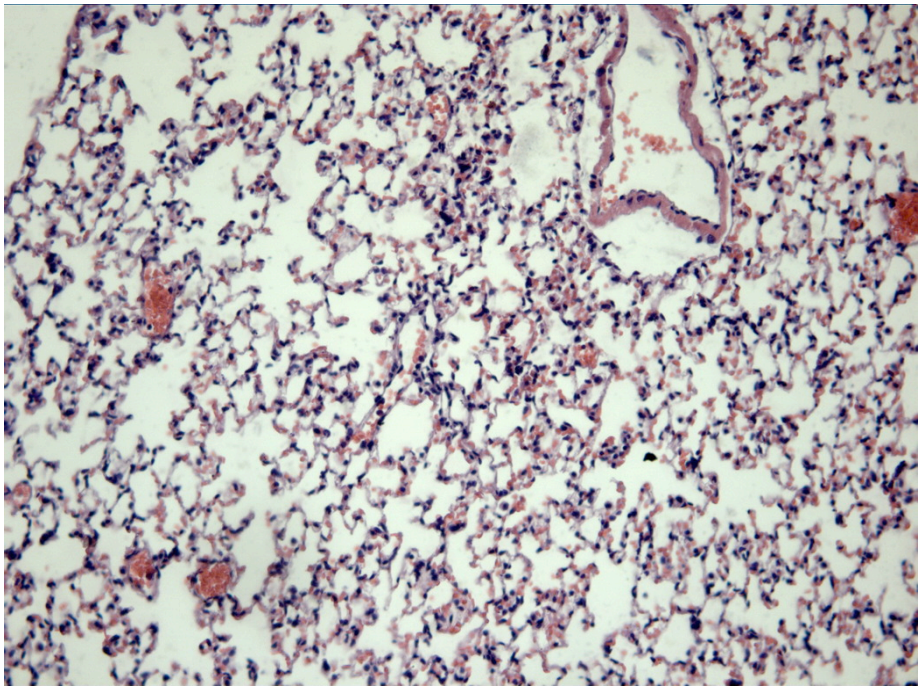

Fig.3. Lung cross-section (direct MAEAS exposed, Group 3), cut at 5 μm, stained with H/E, magnification 160x

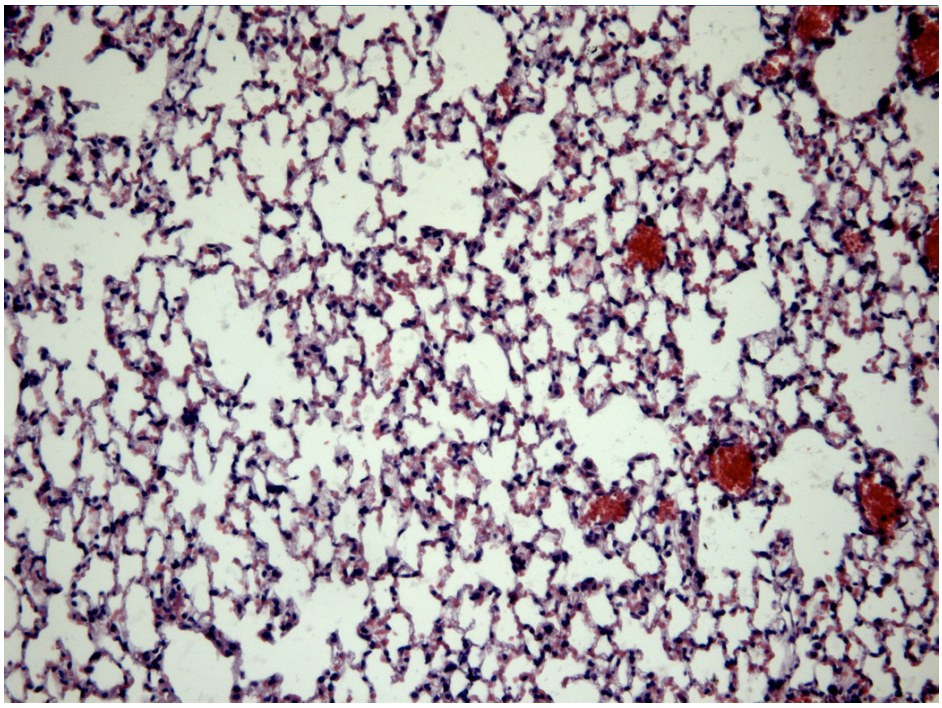

Fig. 4. Lung cross-section (direct MAEAS exposed, Group 4), cut at 5 μm, stained with H/E, magnification 160x

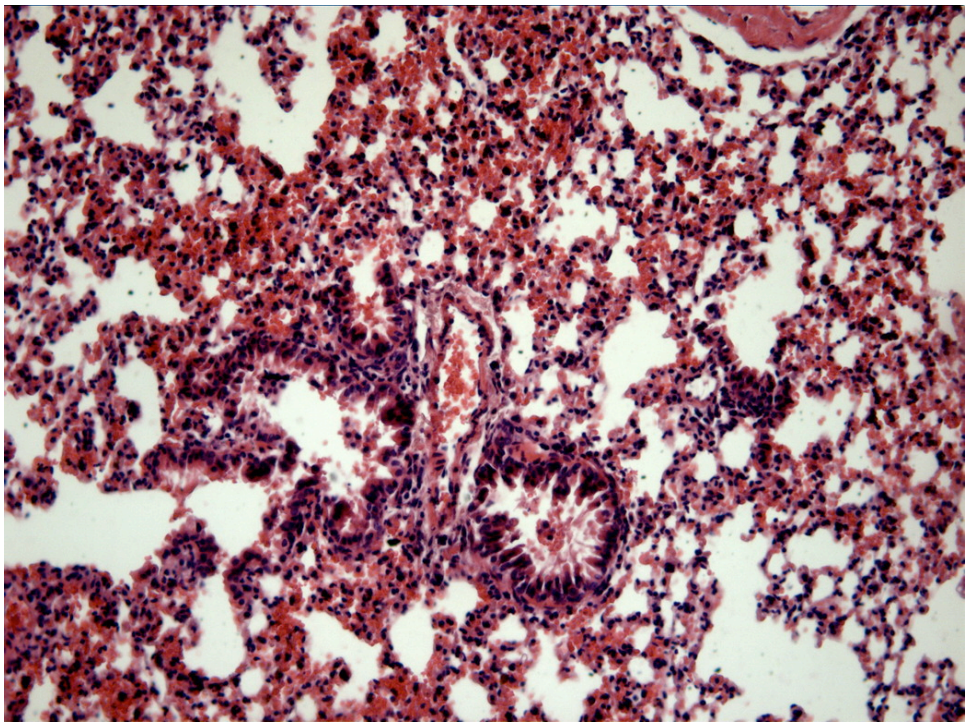

Fig. 4a. Lung cross-section (direct MAEAS-exposed, Group 4, out of overall pattern), cut at 5 μm, stained with H/E, magnification 160x

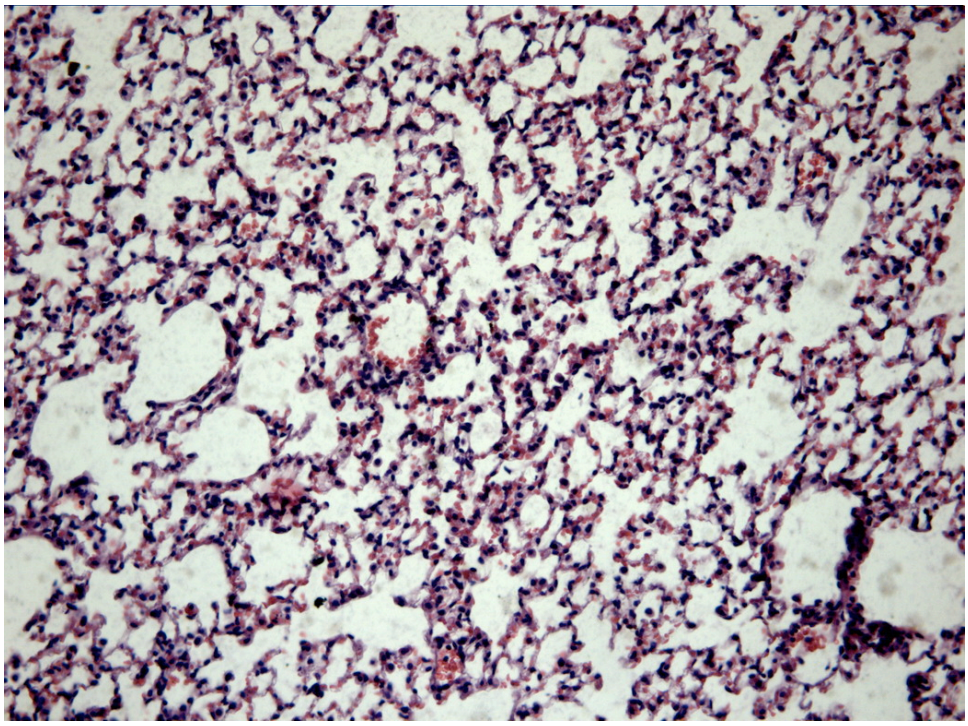

Fig. 5. Lung cross-section (direct MAEAS-exposed, Group 5), cut at 5 μm, stained with H/E, magnification 160x

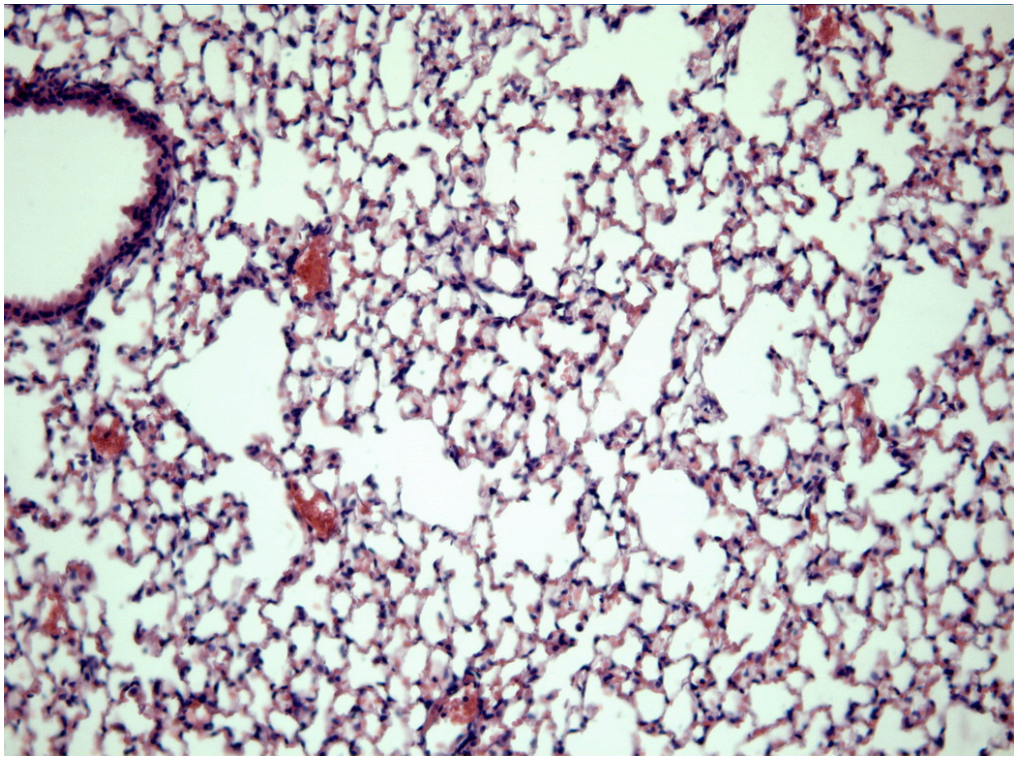

Fig. 6. Lung cross-section (indirect MAEAS-exposed, Group 6), cut at 5 µm, stained with H/E, magnification 160x

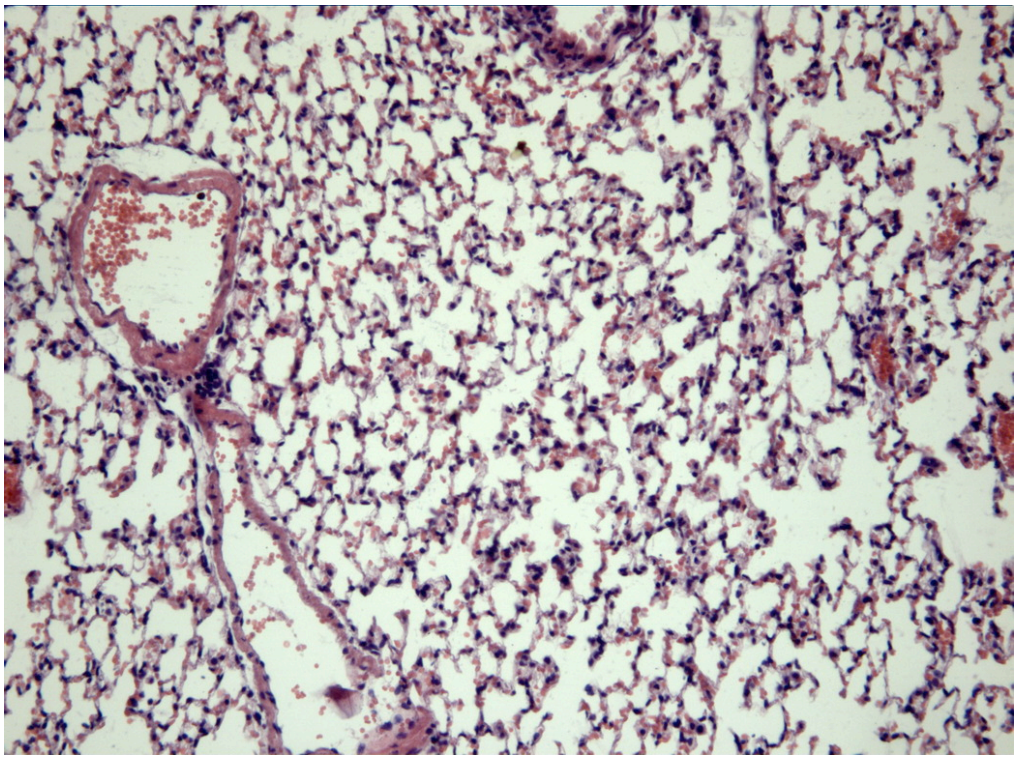

Fig 7. Lung cross-section (indirect MAEAS-exposed, Group 7), cut at 5 µm, stained with H/E, magnification 160x