The Case Study *In Vivo* to Evaluate the Health Risks of Copying/printing Service Employees

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10 th International Particle Toxicology Conference
Düsseldorf,, Germany, 4-7 June, 2013

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"Promotion of International Cooperation Activities of Riga Stradiņš University in Science and Technologies", agreement No. 2010/0200/2DP/2.1.1.2.0/10/APIA/VIAA/006

Introduction

- The ***in vivo*** **experiments** in mostly cases **are done in** scientific **laboratories under the strongest control (**laboratories chambers, boxes, etc.): with constant air pollution, temperature, air velocity.
- It is acceptable and correct approach, but **it is far away from real situation at the workplaces form occupational medicine point of view**.
- The **increased number of the complaints about health disorders** (upper airway irritation, headaches, nausea, irritation of eyes and skin etc.**) among office workers** (especially – printing and copying processes) raises the question about indoor air pollution caused by office equipment and assessment of their impact to health.

The Objective

- The objectives of case study were **to create** the model for **case study type of *in vivo* experiment** and **to evaluate the health effects of indoor air pollution** in workplaces of **printing and copying processes** on experimental animals.

Methodes and materials (1)

- The experiment was realized using the white ***Wistar* rats**.
- The **experimental animals were exposed to passive inhalation exposure** method. The cages were placed **close to copying, printing equipment 8 hours per day 5 days a week** (modelling the environmental/occupational exposure); during weekends, animals were moved to an area with low background levels.
- **Control group** of animals **was hold in a separate room** without office equipment and with background indoor air pollution level.

Methodes and materials (2)

- Every day the **copy number of pages was listed** (average: **3000 to 5000 pages per day**). There was lack of ventilation system in premises – ventilation was done – opening windows and doors.
- Duration of experiment - **28 days**.
- Every day **was evaluated animal behaviour, appearance, and food and water consumption**.
- Animal **body weight was determined before the experiment** and **after every 7 days**.

Methodes and materials (4)

Particles were tested by:

- **number** (P-Track Ultrafine Particle Counter)

  (size range: 20 – 1000 nm)

- **surface area** (AeroTrack9000).

  »**A-alveolar fraction**

  (size range: 10 - 250 nm)

  »**TB-traheobronhial fraction**

  (size range: 250 - 1000 nm)

Methodes and materials (5)

- The air quality was tested by modern equipment, it depends on the aim of measures:
  - **VOC's and aldehydes** were tested by Gas chromatography (Varian 3800) and High-Performance Liquid Chromatography (Waters Alliance 2695);
  - **non-organic gases** (NO_2, SO_2) also O_3 were tested by spectrophotometry (Varian Cary 50);
- The **indoor climate** was characterized by parameters
  - air temperature, humidity and air flow and also carbon dioxide.

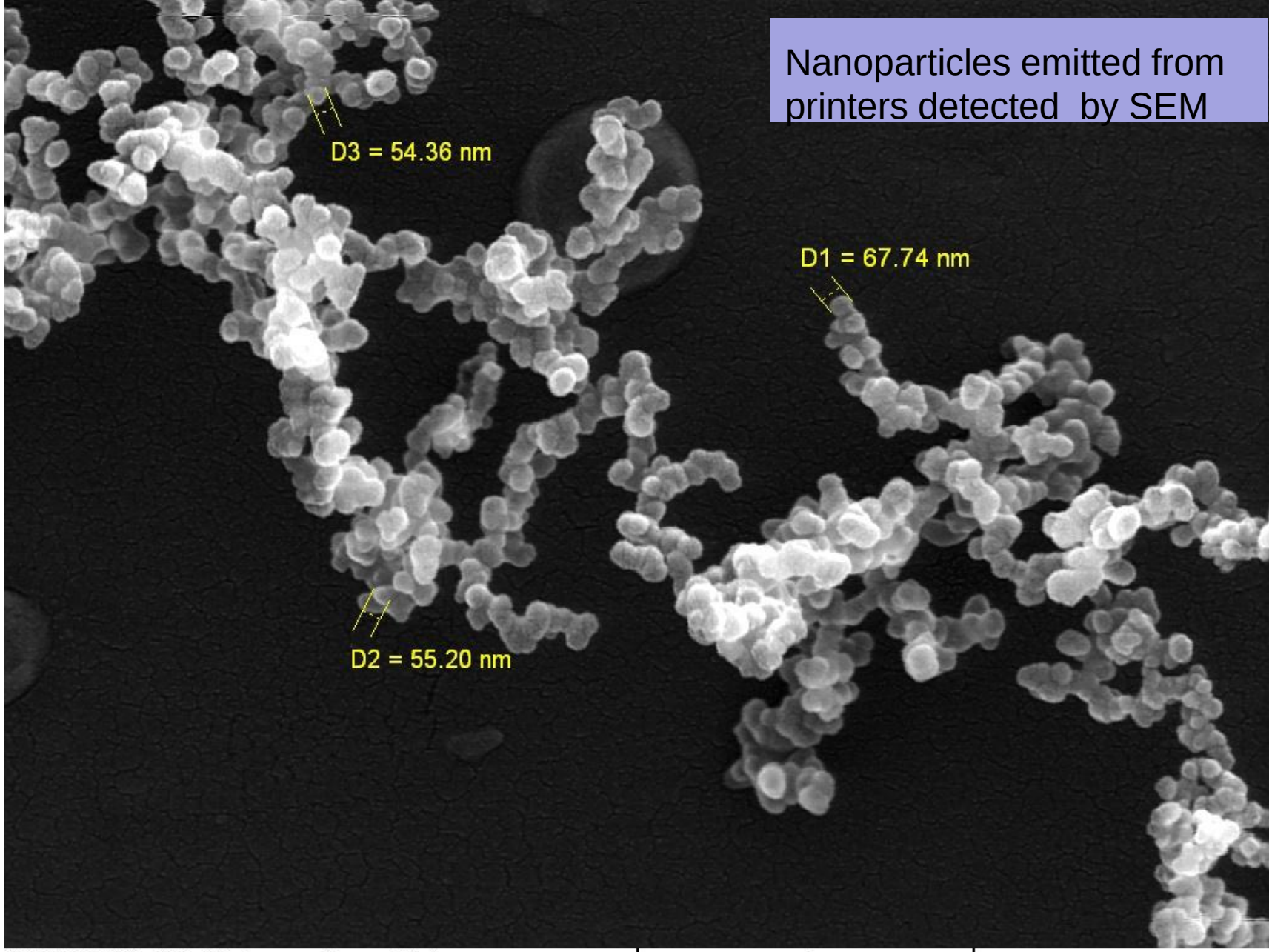
Methodes and materials (3)

- The analyses of **nasal** and **bronchoalveolar lavage**, analysis of blood, **biochemical parameters**: C-reactive protein, cytokines IL-1, IL-6, TNF-α factor, **oxidative stress factors** (superoxiddismutase (SOD), malondialdehyde (MDA), glutathione (GSH), lipid hydroperoxide (LOOH)) and **histopathological examination of tracheal and lung tissues** was done at the end of the case study experiment.

RESULTS

Results – **Indoor air exposure levels** experiment and control animals (rats)

Detected air quality indicators	Experiment (average±SDEV)	Control (average±SDEV)
Particles number, particles/cm^3	**9700 ± 1940**	3150 ± 630
• **Alveolar** fraction surface area, μm^2/cm^3	**55.0 ± 11.0**	12.5 ± 2.5
•**Tracheobronhial** fraction surface area, μm^2/cm^3	15.2 ± 3.0	9.6 ± 1.9
Volatile organic compounds, mg/m^3	**0.9 ± 0.14**	0.1 ± 0.02
Formaldehyde, mg/m^3	**0.5 ± 0.08**	0.05 ± 0.008
Ozone, mg/m^3	**0.93 ± 0.14**	0.06 ± 0.009
NOx, mg/m^3	0.12 ± 0.02	0.04 ± 0.006
SO_2, mg/m^3	0.24 ± 0.04	0.08 ± 0.012


Nanoparticles emitted from printers detected by SEM
D3 = 54.36 nm
D1 = 67.74 nm
D2 = 55.20 nm
SEM MAG: 80.00 kx
SEM HV: 25.00 kV
Date(m/d/y): 07/14/10
Vac: HiVac
WD: 5.9510 mm
Det: SE Detector
1 µm
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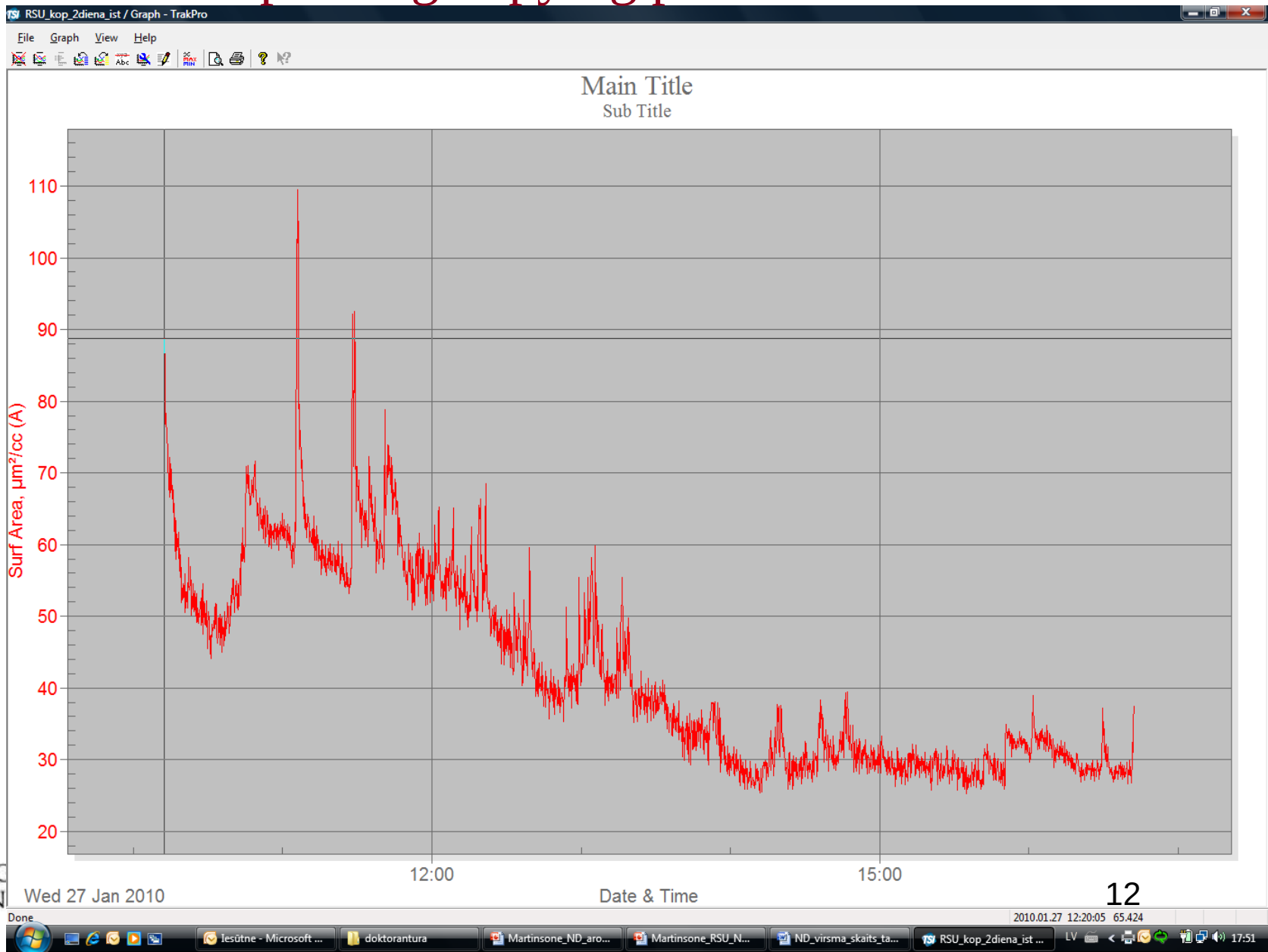
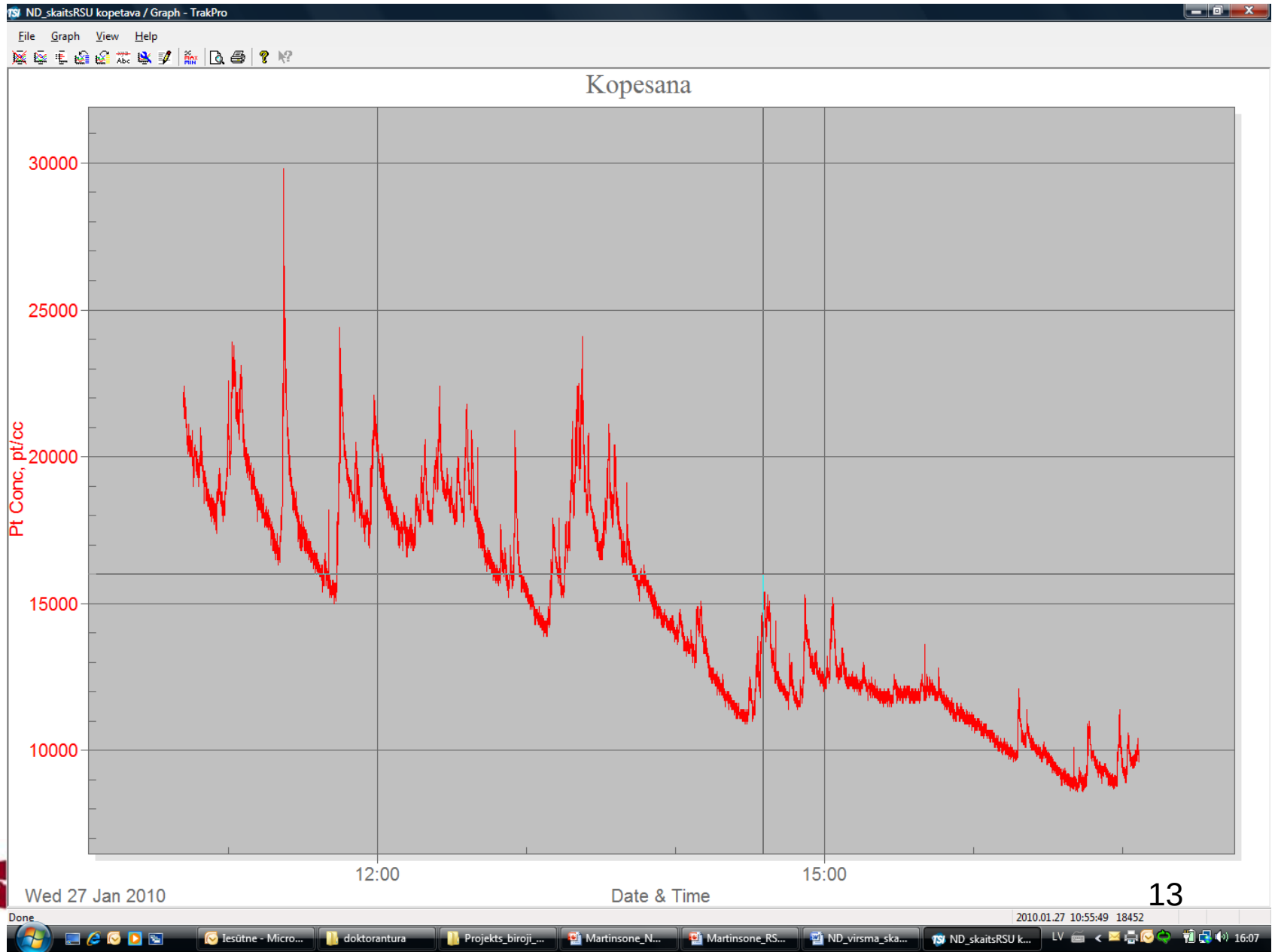
Results: **particles surface area of Alveolar fraction** during printing/copying process

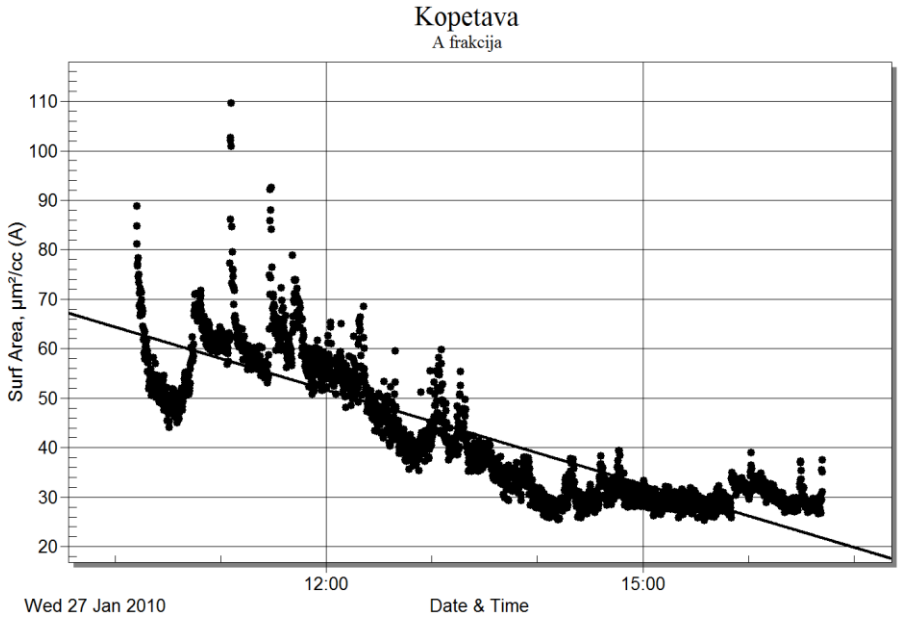


Results: **particles number** during printing/copying process

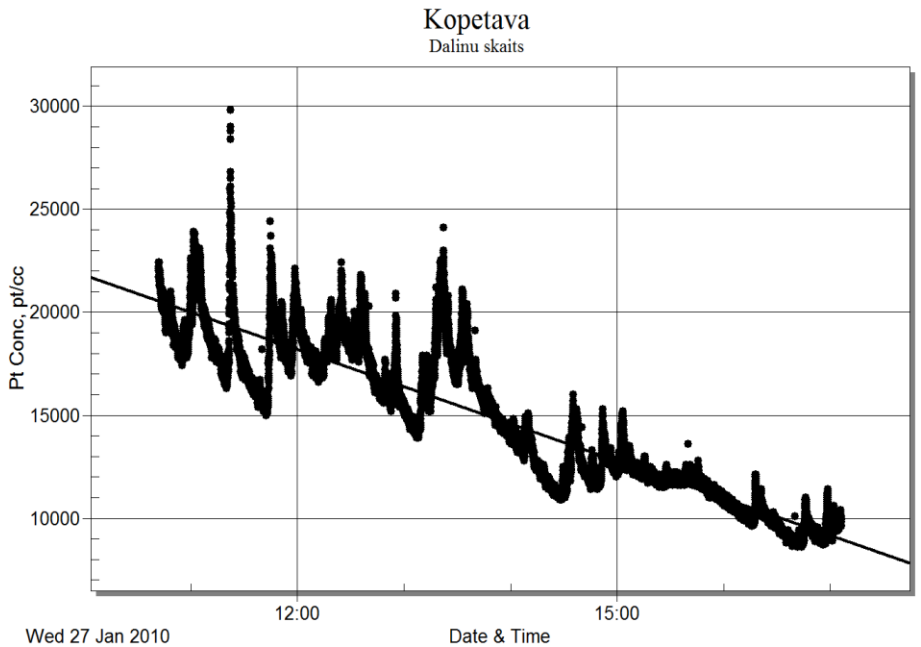


Results– **measures of particles**

Surface area measurements of **alveolar fraction**



Measurements of **particle number**



The peak concentrations of particles surface area and number have similar trend in the same time!

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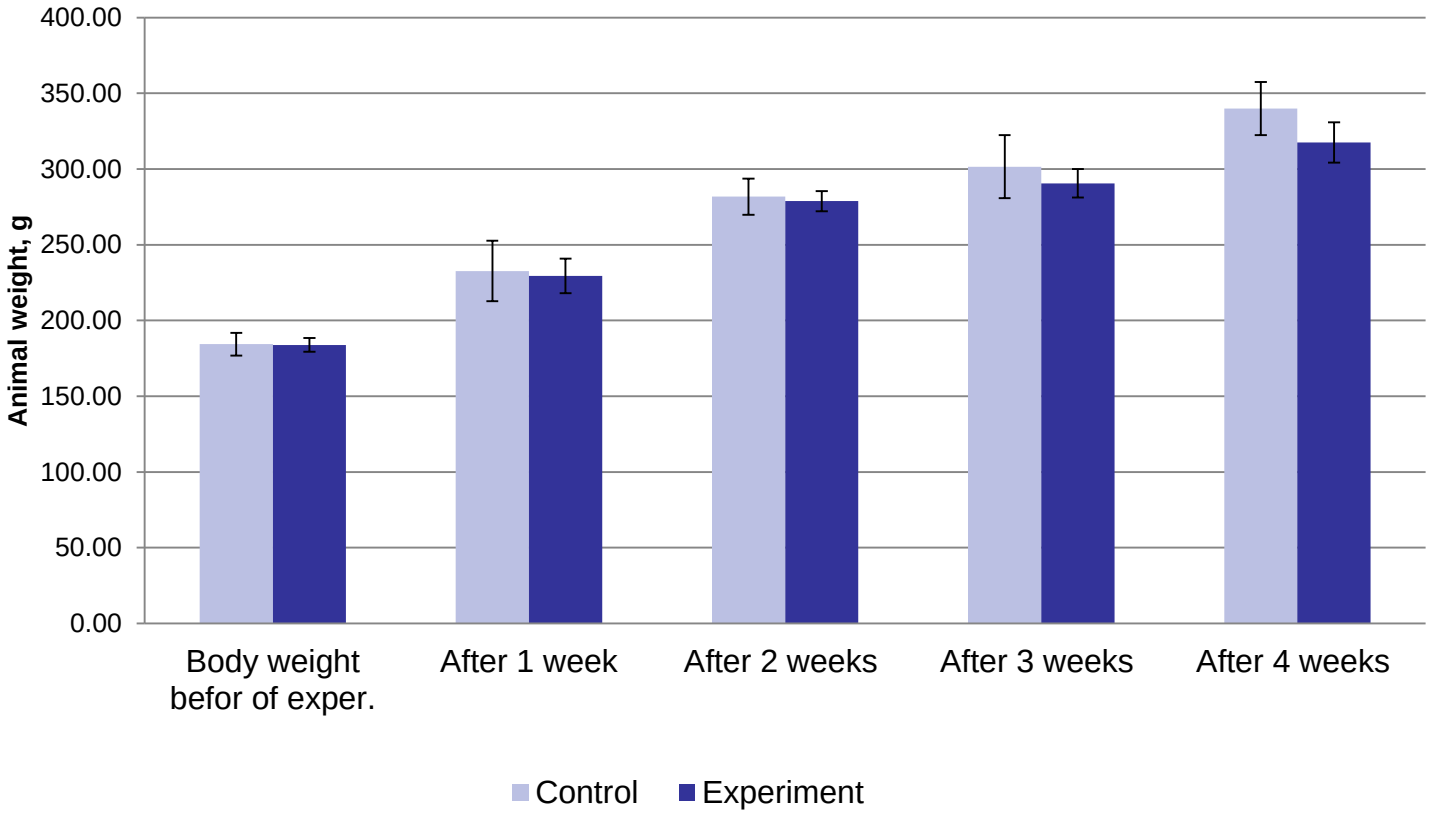
Results – **animal body weight**

- In the study was found the **decreased body weight in the experimental group animals** compared with controls at the end of the experiment.



Results - **C-reactive protein**

- The **increasing of C-reactive protein and TNF-α in the blood** indicates the evolution of inflammatory process.

Animal groups	**C – reactive** protein, μg/L		**TNF-α**, pg/mL	
	avarege	*SDEV*	average	*SDEV*
Control	**0,16**	0,03	**4,5**	0,7
Experimental	**0,22***	0,07	**11,7***	1.4

* statistical significant $p < 0.05$, compared with the control groups

Results – **nasal and bronhoalveolar lavage**

- In the **nasal lavage** were observed **increasing the total number of epithelial cells and neutrophils** (granular leukocytes), also **lymphocytes**.

Groups	Total number of cells	Macrophag.	Neutrophils	Lymphocytes	Epithelial cells
Nasal lavage					
Control	**27,4±3,2**	1,2±0,8	**11,4,0±2,3**	**8,0±1,9**	6,8±2,3
Experiment	**65,0±22,0***	0	**43,0±18,8***	**14,0±5,3***	8,0±1,2
Bronhioalveolar lavage					
Control	118,4±43,9	1,6±1,2	45,4±15,1	66,2±25,6	5,2±1,8
Experiment	107,2±52,2	0,8±0	24,2±14,6*	74,2±48,5	8,0±4,2

* statistical significant $p < 0.05$, compared with the control groups

Results – **oxidative stress**

- The **increasing of superoxiddismutase (SOD)** and **glutathione levels (GSH)** may be considered as an indication of oxidative stress increase.

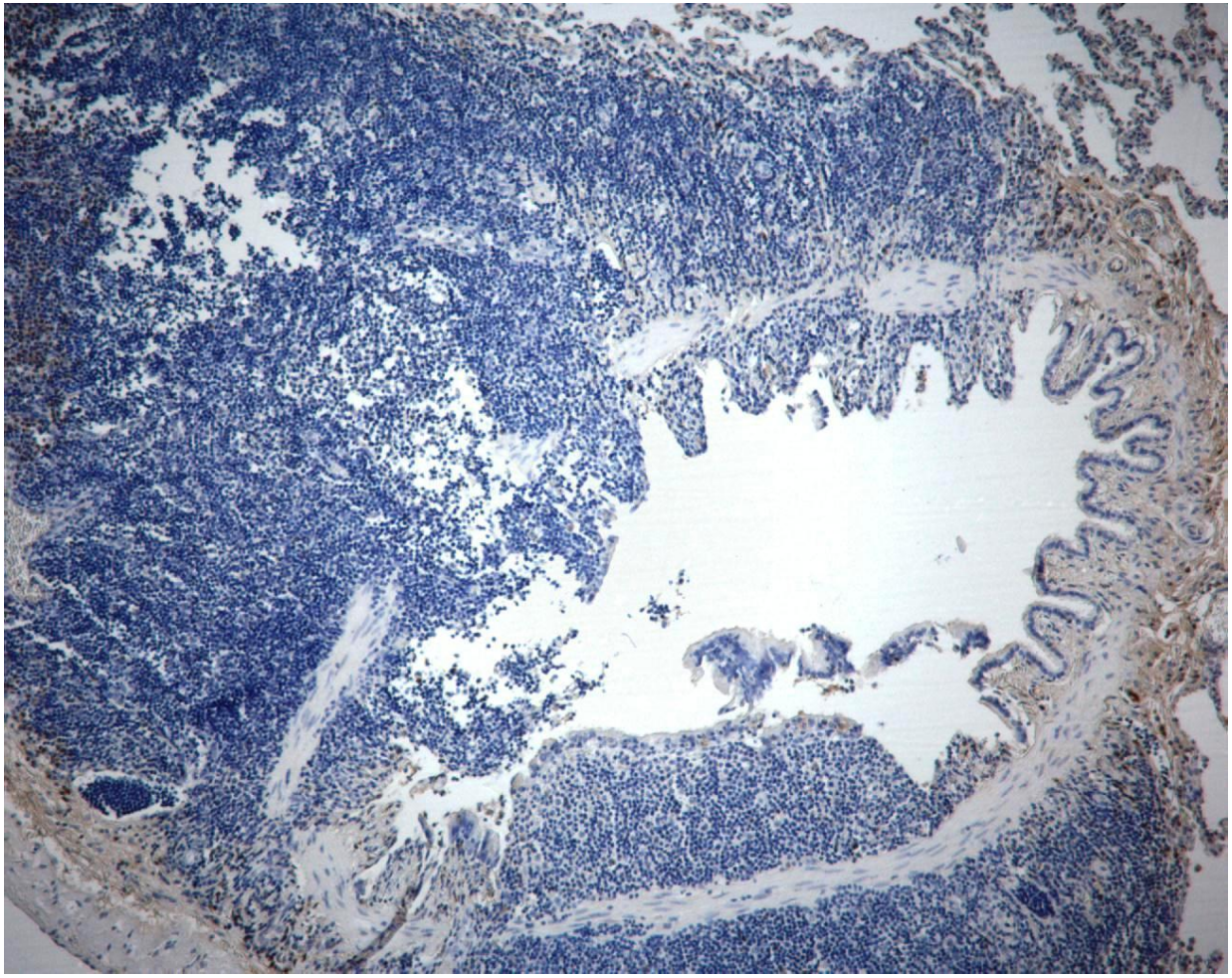
Animal groups	SOD , U/g Hb		GSH, mg/dL	
	average	*SDEV*	average	*SDEV*
Control	2424,6	*179,2*	66,51	*3,7*
Experimental	2769,8*	*249,3*	71,63*	*4,43*

* statistical significant $p < 0.05$, compared with the control groups

Results - **histopathological analyse** (1)

- The **hyperplasia of tracheal epithelial basal cell, inflammatory cell infiltration and vascular plethora in lungs indicated severe inflammation-induced changes in the lungs**.
- The **tracheal tissue** of rats for histological analysis show **reduced number of lymphoid nodules (folliculus)** ($p<0.05$) and **decreased IL-6 expression** ($p<0.05$) in the experimental group of animals compared to control animals.

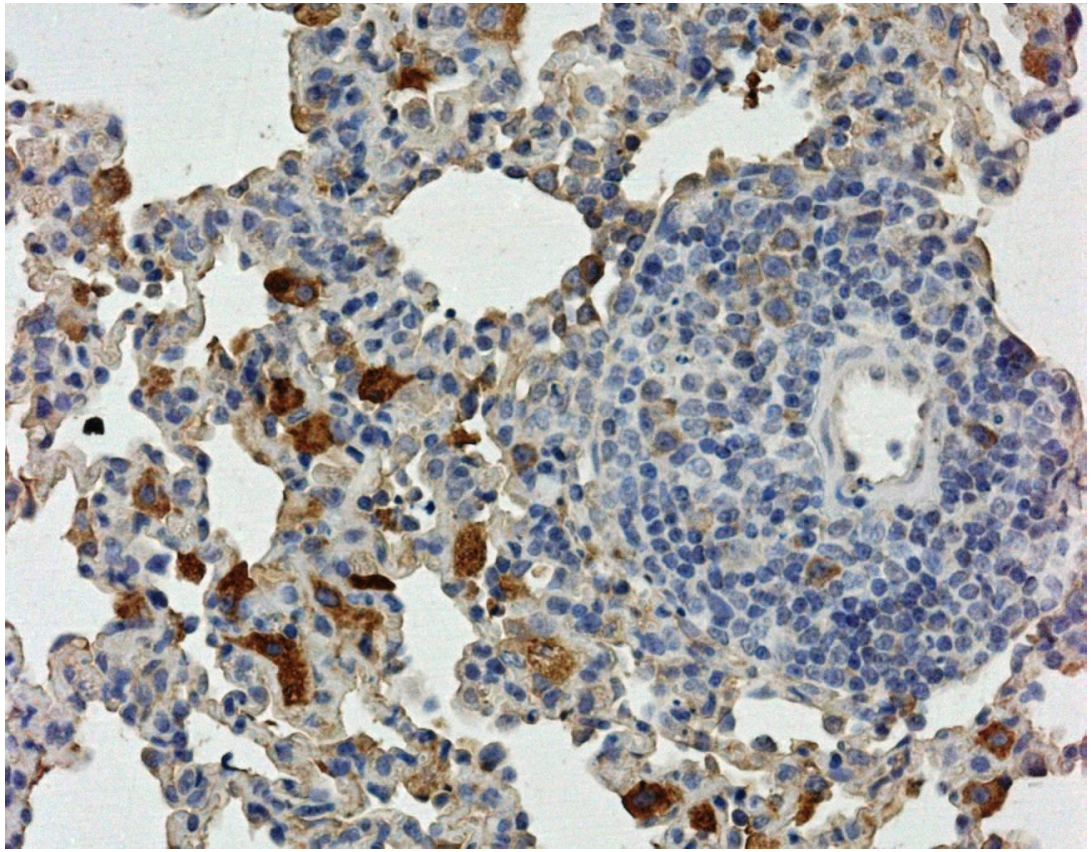
Severe **lymphocytic infiltration** due to the inflammation **in the experimental rat lung**. X100. IHC IL 6.



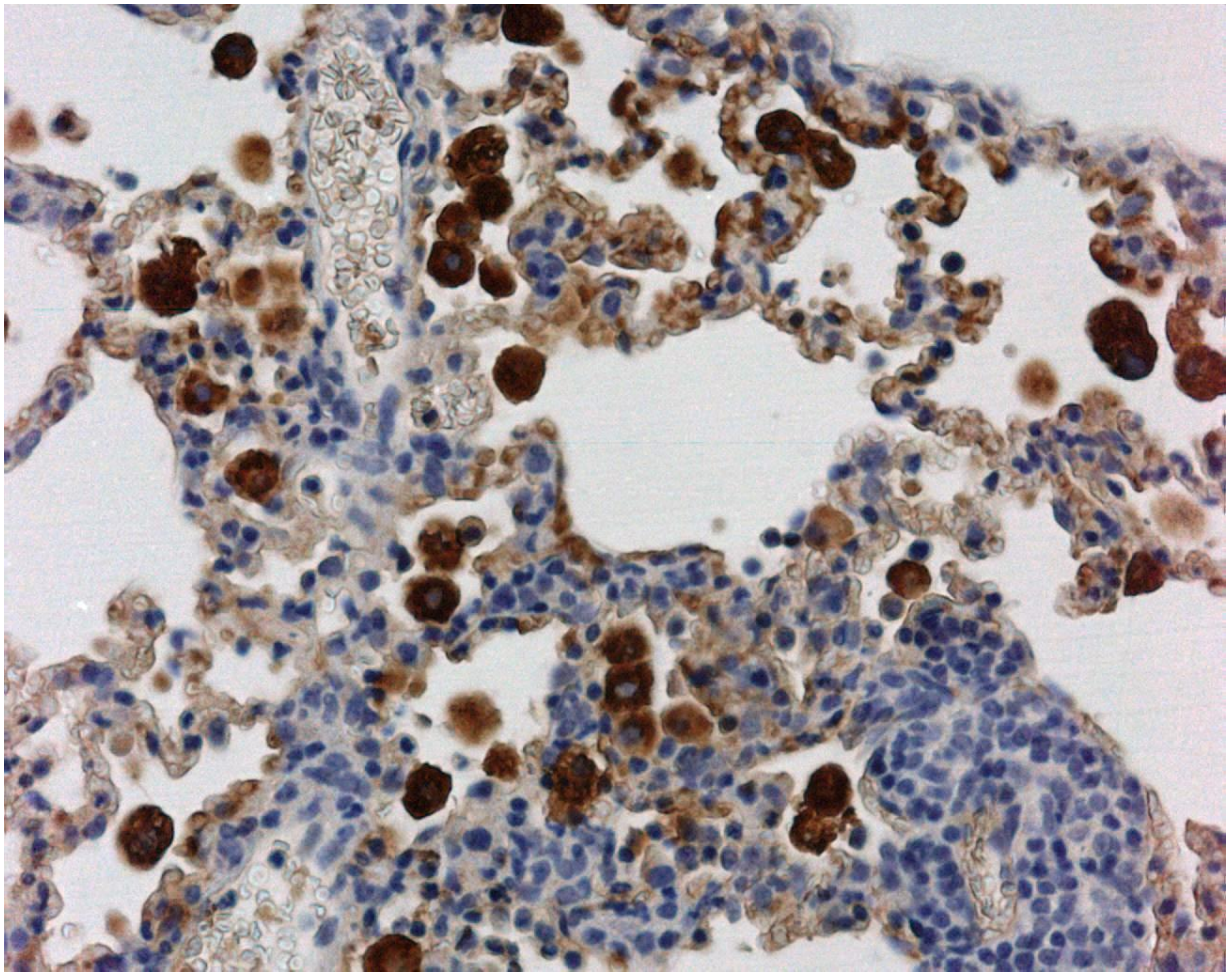
Results - **histopathological analyse** (2)

- The **decreasing of lymphatic nodules** (folliculus) in the trachea and lungs were evaluated as immune exhaustion/decompensation.
- **IL1 increased** in the trachea and the lungs after the exposure.
- **IL6 and TNF-α decreased steadily** in the tracheal tissues, but varied in the lungs.

Production of **IL 6 in the macrophages around lyphatic folliculus** and **low expresson of IL 6 in the macrophages in the folliculus** in the experimental rat lung. X400. IHC IL 6.



Conglomerates of **IL 1 expressing alveolar macrophages** in the experimental rat lung. X400. IHC IL 1



Conclutions (1)

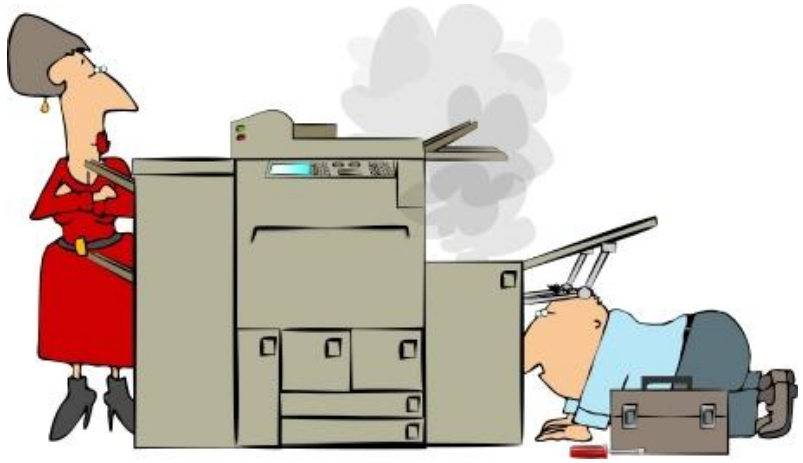
- The **measurements of indoor air quality indicates the high pollutants levels** in the premises, where was placed the animals of experimental group, and that may **lead the negative impact to animals health**, including adverse effects of particles on experimental animal respiratory tissue.
- The **analyses of nasal and bronchoalveolar lavague**, **cytokines** IL-1, IL-6, TNF-a factor, **oxidative stress factors** and **histopathological examination** of tracheal and lung tissues **indicated development of inflammatory process**.

Conclutions (2)

- Experimental ***in vivo*** **case study model should be developed for evaluation of health effects** caused by occupational environment.

Main Benefits

- Confirmation of **work – related complaints/diseases**.
- Exposure level description (especially, for particle number and surface area), what can lead the **inflammatory process** *in vivo.*
- Detection of main **indoor air quality** (**particles surface area and number**) indicators and **material** for workers health examination.



Acknowledge

Project Nr. EEA09AP-22 “Determination of indoor air pollution caused by offices equipment and estimation of possible impact on organism”

RSU Institute of Anatomy and Anthropology

RSU Laboratory of Biochemistry

Thank You for Your Attention!

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