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# Synchrotron microradiography study on acute lung injury of mouse caused by $PM_{2.5}$ aerosols

Yongpeng Tong [a], Guilin Zhang [a,*], Yan Li [a], Mingguan Tan [a], Wei Wang [a], Jianmin Chen [a], Yeukuang Hwu [b], Pei-Chebg Hsu [b], Jung Ho Je [c], Giorgio Margaritondo [d], Weiming Song [e], Rongfang Jiang [e], Zhihai Jiang [e]

[a] *Shanghai Institute of Applied Physics, Chinese Academy of Sciences, Shanghai 201800, China*
[b] *Institute of Physics, Academia Sinica, Nankang, Taipei*
[c] *Department of Material Science and Engineering, Pohang University of Science and Technology, Pohang, Korea*
[d] *Faculté des sciences de base, CH-1015 Lausanne, Ecole Polytechnique Fédérale de Lausanne (EPFL), Switzerland*
[e] *School of Public Health, Fudan University, Shanghai 200032, China*

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**Abstract**

In order to investigate $FeSO_4$, $ZnSO_4$ (the two of main metal compositions of Shanghai $PM_{2.5}$ (particle matter with those aerodynamical diameter <2.5 μm)) effects on acute lung injury, six solutions contained $PM_{2.5}$ aerosol particles, $FeSO_4$, $ZnSO_4$ and their mixtures were instilled intratracheally into mouse lungs for experiment. By 2 days after instillation, the live mice were checked in vivo by synchrotron refractive index microradiography. In addition after extracted and examined by dissection, the right lobes of lung were fixed by formalin, then imaged by synchrotron microradiography again. Corresponding parts of those lung tissues were embedded in paraffin for histopathologic study. The synchrotron X-ray microradiographs of live mouse lung showed different lung texture changes after instilled with different toxic solutions. Hemorrhage points in lung were observed more from those mice instilled by $FeSO_4$ contained toxin solutions groups. Bronchial epithelial hyperplasia can be observed in $ZnSO_4$ contained solution-instilled groups from histopathologic analysis. It was found that the acute lung injury of mice caused by solution of $PM_{2.5} + FeSO_4 + ZnSO_4$ was more serious than other toxin solutions. Results suggested that $FeSO_4$ mainly induced hemorrhage and $ZnSO_4$ mainly induced inflammation and bronchiolar epithelial hyperplasia in the early toxicological effects of $PM_{2.5}$.


*Keywords:* Synchrotron microradiography; Lung injury; $FeSO_4$; $ZnSO_4$; $PM_{2.5}$

## 1. Introduction

The increasing mortality and morbidity due to cardiopulmonary complications are attributed to elevated concentration levels of ambient particulate matters, in particular, of small inhalable particles [1]. Therefore, it is essential to understand in detail the mortality mechanism induced by such fine particles. Many reports suggest that $PM_{2.5}$ induce reactive oxygen species (ROS) and inflammatory mediators, resulting in vascular permeability changes, airway constriction and tissue injury [2,3]. The transition metal ions and peroxides in aerosols can induce free radicals and cause both cytotoxicity and a strong oxidation response [4]. Based on the previous report [5], it is found that the main acute effects of PM are due to soluble ions. Shanghai as one of most quickly developing cities in the world in economics, its energy exhausts are also raised quickly. The Chinese biggest iron plant—Baoshan steel plant and electronic power plants make two of the main contributions to $PM_{2.5}$ in Shanghai. Fe and Zn are the two main transition elements in $PM_{2.5}$ and high $SO_2$ (from coal burning) even make those aerosols more toxic to our health [3]. Those soluble metal constituents of residual oil fly ash (ROFA) particles can enhance the sensitization

* Corresponding author. Tel.: +86 21 59553998; fax: +86 21 59553021.
*E-mail address:* glzhang@sinap.ac.cn (G. Zhang).

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of lung injury [6]. As $PM_{2.5}$ collected from the industrial city—Shanghai contains relatively higher transition elements for instance, Fe, Zn, etc. [7], and higher sulfates [8], it is important to study the effects of the main transition compositions $FeSO_4$ and $ZnSO_4$ in $PM_{2.5}$ induced acute pneumonia process.

Many studies related to pneumonia and cytotoxicity were carried out on the histopathological examination of lung section [9–11]. Usually, in those previous work of toxicological studies on tissues, performed by optical microscopy and the scanning proton microprobe [7], only thin tissue samples (<50 μm) excised from killed rats [10,12] were used on some respiratory function tests [13] and on analyzing the structural changes [10]. Our present work proves that such critical limitation can be lifted by using edge-enhanced microradiographs with high energy of X-rays which can penetrate a mouse. The high intensity of X-rays makes it possible to achieve high resolution in a short time, for instance, 3 ms or less, as what required for imaging a live mouse without observed damage [14,15]. Here, it is important to use this method in vivo to monitor acute pulmonary toxicity after the mouse intratracheally instilled as the $PM_{2.5}$ toxic effect is a developing process [16]. Combining those studied results of the transition element Fe, Zn effects on lung epithelial cultured cells [17] with this studying result on lung tissue structure, it may understand more about Fe, Zn toxic effects in the industrial city $PM_{2.5}$.

## 2. Experimental methods

### 2.1. Aerosols sampling

$PM_{2.5}$ samples were collected by a stacked filter air sampler at the Baoshan area which is one of the industrial districts in Shanghai. The $PM_{2.5}$ aerosols were collected on Teflon filters at 6.5 m above ground at a flow rate of 78 l/min. Each sample required ∼360 h by a middle flux air sampler and all aerosol samples were collected during the period of September–November 2003.

### 2.2. Elemental analysis

The elemental analysis for $PM_{2.5}$ was carried out by a VG X7 ICP-MS instrument (Thermo electron corporation) and at least 16 elements were found.

### 2.3. Toxin sampling

Several films contained $PM_{2.5}$ (total 200 mg) were first immersed into physiological saline, then the particles were disinfected by ultrasound for 1 h at about 50 °C. During this process, it was found that most of bacteria detected by standard bacteria culture method were killed (less than 3 $ml^{-1}$). This solution was kept at low temperature (0 °C). Six solutions (pH ∼ 5.3), i.e. $PM_{2.5}$ aerosol solution 25 mg/ml, $FeSO_4$ solution 15 mg/ml, $ZnSO_4$ solution 15 mg/ml and mixed solutions of $PM_{2.5}$ 25 mg/ml + $FeSO_4$ 15 mg/ml and $PM_{2.5}$ 25 mg/ml + $FeSO_4$ 15 mg/ml + $ZnSO_4$ 15 mg/ml and saline were prepared for mice instillation.

Animal grouping and instillation: male KP600 CD-1 mice, weighing 22–26 g, were obtained from the Experimental Animal Center of Pohang University of Science and Technology, Pohang, Korea. Total of 36 mice were grouped randomly into 6 groups on an average and each group (6 mice) was, respectively, instilled intratracheally with each test materials: saline, Fe $SO_4$, $ZnSO_4$, $PM_{2.5}$, $PM_{2.5}$ + $FeSO_4$, $PM_{2.5}$ + $FeSO_4$ + $ZnSO_4$ solutions, respectively, 0.04 ml solution per mouse was instilled every day. The dose and time point used here were selected based on pre-test by histopathological examination of the lung tissues excised from the killed mouse. By 48 h after twice instillations (0 and 24 h), the right lung of live mice were observed by synchrotron X-ray imaging. Then, the mice were anesthetized with an intraperitoneal injection of 10 mg (450 mg/kg) chloral hydrate (0.2 ml of 5% Sigma chemical). After they were anesthetized, the mice were killed by cutting neck, then dissected and observed by eyes immediately. This animal experimental process is permitted by Law and Ethics Committee. Finally, the right lung tissue was fixed by formalin for further histopathological analysis.

### 2.4. Study of the irradiation influence on lung tissue during synchrotron X-ray imaging

Ten mice were taken images at their chest position for a different radiation time (3–15 ms). After dissecting those mice it was found that there were no significant changes for the lung tissue compared with those control mice without synchrotron X-ray irradiation by histopathological analysis. In addition action and behavior of mice subjected to irradiation also did not show any difference with those of the control mice. Results showed that the exposure by irradiation during the imaging within a time range of 3–15 ms did not induce significant extra effects to the mice. Finally, 3 ms were chosen for performing synchrotron X-ray imaging.

### 2.5. Lung tissue sampling and analysis

Part of the right lobe of the lung was fixed in formalin, processed and embedded in paraffin. Lung pathological sections of right lobe with a thickness of 5 μm were observed by optical microscope in order to compare with the corresponding the imaging of live mice taken by synchrotron X-rays. More than 10 lung pathological sections of each mouse lung tissue have been analyzed by optical microscope to outline toxic effects. Although it is difficult to get the section to be analyzed by optical microscope in the same area where the microradiography was imaged, some typical poisoning characters of lung tissue can be compared. The right lobe tissue was selected for experiment mainly due to the imaging process of right lobe is less affected by heart beating.