CHAPTER 4

DISCUSSION

Airborne chemicals are exogenous substances, including both particulates and gaseous compounds that may cause adverse health effects following their interaction with biological systems. Various physical, chemical and dynamic processes may generate airborne chemicals, leading to emissions of gases and particulates into the atmosphere. While attempts have been made to reduce emissions from stationary and mobile sources, millions of people today face the adverse health effects of excessive air pollution [(85)]. Based on their physical properties, airborne chemicals can be classified into two main types. The first category includes gases and vapors, existing as distinct molecules which dissolve and form true solutions in air and follow the fundamental gas laws. Gases and vapors are treated similarly, as there is no difference in their molecular behavior. The second category is aerosols or suspended air pollutants and refers to both solid particles and liquid droplets that may vary in size, composition and origin such as dust, metal fumes, fiber, smoke, mist and fog [(86)]. Aerosols may result from different mechanical or chemical processes in both solid or liquid forms and may have spherical or nonspherical shapes with a wide range of size distributions from less than 100 nm or nanoscale particles, to fine and coarse microscale particles over 100 μm. As well as olfactory, gas exchange and blood oxygenation functions the human respiratory system has evolved to deal with xenobiotics and airborne materials that usually occur in the atmosphere. However, the respiratory system cannot always deal adequately with the wide range of airborne pollutants occurring in urban or occupational atmospheres. According to their

physicochemical characteristics, airborne chemicals may enter different regions of the respiratory tract. An understanding of airflow structures of the human respiratory tract is essential for analyzing transportation and deposition of airborne materials[87] . Insoluble gaseous compounds may cross the membrane of the pulmonary region efficiently and enter into the blood supply system. Therefore, the respiratory system is considered both as a target organ for pulmonary toxins, and also a pathway for inhaled chemicals to reach other organs distant from the lungs and elicit their effects at these extra pulmonary sites.

Traditionally, inhalation toxicology data has been generated using animal models. Inhalation toxicity tests are carried out on test animals to identify the median lethal concentration (LC50) of airborne chemicals causing death as a toxic endpoint in 50% of exposed animals. Some inhalation chambers may provide whole body exposure while many others accommodate oral–nasal exposure only. Both the concentration and fixed time period are critical parameters and approximately 30–50 animals are needed to carry out the LC50 test for a single chemical. Standard protocols have been recommended by regulatory agencies for both short-term and long-term inhalation tests.

There are some difficulties unique to inhalation studies that have been identified. Estimating the dose received by animals is one of the challenging issues of inhalation toxicology as several factors may influence the actual dose such as: airborne concentration, exposure duration and pulmonary characteristics of the test animal which modulate the deposition/absorption pattern of the airborne chemical [88]. Therefore, the selection of animal species for inhalation studies is a crucial

consideration which may influence the outcome of in vivo studies and consequently the estimated human adverse health effect.

The main technical challenge of *in vitro* testing of airborne chemicals is to mimic inhalation exposure in cultured cells or tissues. Many considerations must be investigated before adopting an *in vitro* bioassay test system for toxicity screening of airborne materials including [89]:

1. Generation and monitoring of known concentrations of test atmospheres via static or dynamic methods to investigate the dose dependent effects.

2. Using optimal exposure chambers that provide a close contact between target cells and test atmospheres to mimic *in vivo* conditions and prevent potential interactions.

3. Allowing the experiment to be carried out under the significant time course concurrently supporting the basic needs of the cellular system such as nutrition and appropriate levels of the pH, humidity and temperature.

4. Applying representative endpoints to highlight the biological pathways of human airborne chemical exposure and the potential cellular effects.

Our exposure chamber prototype provided a close contact between target cells and test atmospheres to mimic *in vivo* conditions and prevent potential interactions. We also tested for the sterility of the chamber. No contamination of bacteria was observed. This indicated that the UV lamp could destroy contaminating bacteria

Since in many studies cells are exposed to airborne chemicals ways that differ significantly from *in vivo* conditions, the necessity of direct exposure of target cells to airborne chemicals has long been recognized by researchers.

Continuous direct exposure of target cells to airborne chemicals has been developed to perform the essential requirements of toxicity testing of airborne chemicals. Technology has become available that allows cells to be cultured on permeable porous membranes in commercially available Transwell or Snapwell inserts. Once cells are established on the membrane, usually following an overnight incubation, the upper layer of the culture medium can be removed, and cells directly exposed to airborne chemicals. In fact, the liquid layer coating the cells is lowered to the same thinness as the extracellular lining layer of the conducting airways [(90)]. In a direct exposure technique at the air–liquid interface target cells can be exposed to test atmospheres continuously during the exposure time on their apical side, while being nourished from their basolateral side.

In this study, A549 cells cultured by an air/liquid interface showed more response to the vapor of benzene than that of conventional culture. Cell viability of the benzene exposed cells cultured by an air/liquid interface technique was only 30% when compared with unexposed control. Using a conventional cell culture, the viability of benzene exposed cells was about 65%, and the level of IL-6 in cells cultured by an air/liquid interface was significantly different from that of the unexposed control. These data indicated that the suitable cell culture technique for toxicity testing of inhaled substances was an air/liquid interface technique.

Dynamic delivery of test atmospheres and direct exposure of human cells to airborne chemicals have been achieved using specifically constructed exposure chambers such as the CULTEX system [(91)], or horizontal diffusion chamber systems [(92)] . These exposure chambers can accommodate cultured cells grown on microporous membranes in Transwell or Snapwell inserts respectively in physiologically relevant

conditions. Controlled test atmospheres are delivered into the exposure chamber systems using very low flow rates and target cells are exposed to airborne chemicals directly at the air–liquid interface for a significant time of exposure. Test concentrations are verified using appropriate real-time air monitoring or air sampling and analytical techniques.

Electrostatic precipitation is one of the main mechanisms to improve the deposition efficiency. Larger deposition efficiencies are not possible with typically used diffusion chargers, since bipolar diffusion charging leaves the majority of submicron-sized particles uncharged. The so-called EPDExS system selects a monodisperse subfraction of nanoparticles from a bipolarly charged polydisperse sample aerosol with a differential mobility particle sizer (DMA) and deposits the selected unipolar (positive or negative) subfraction with an efficiency of near unity onto a cell covered substrate using a constant electrical field[8].

The disadvantage of the CULTEX system and EPDExS system is that one cannot use this chamber for toxicity testing of ambient air pollution in the actual location. Our exposure chamber can be used for toxicity testing of ambient air pollution in the actual location.

Comparing the toxicity of PM measured by the exposure chamber and conventional methods, we found only PM from Warorot market induced the secretion of IL-8, but surprisingly inhibited IL 6 secretion. The particles from Saraphi District induced the secretion of both cytokines. These results are consistent with previous observations where a higher effect was reported for PM10 than PM2.5 [93], and that particles related to different sources induce effects with different intensities [94].

Nevertheless, the studies involving IL-8 evaluation have indicated that this cytokine is up regulated by PM. The possibility of an interference of PM on the measurement of IL-6, e.g. by capturing the cytokine[95] , may be the reason for IL-6 decrease in Warorot experiment, but is unlikely, considering the evidence provided by the incubation of IL-8 with PM in addition to the data reported in another study, where co-cultures of macrophages mast cells and epithelial cells exposed to the same particles resulted in increases of IL-8 [96] The fine and ultrafine fractions (mainly associated with combustion) had positive correlations with IL-8 [97]. The high proportion of PAHs in Saraphi could be explained the increase of IL-8 secretion observed in the present study. The contrasting results for IL-6 and IL-8 secretion suggest differences in the activation pathways by PM. IL-6 and IL-8 expression are controlled by different factors [98]. IL-6 expression is related to at least three signal transduction pathways; proteinkinase C, cAMP/protein kinase A and calcium ionophore [98]. IL-8 is up- regulated by the phosphorylation of Erk1/2.The phosphorylation of p38 reduces the phosphorylation of Erk1/2 and this leads to a down regulation of IL-8 secretion.