CHAPTER I
INTRODUCTION

1.1 Statement of the problem

Air pollution effects everyone everyday. Millions of people live in areas where urban smog, very small particles, and toxic pollutants posed serious health concerns. These health concerns can stem from either short-term or long-term exposure to air pollution. When people have a short-term exposure to air pollution above certain levels, they may experience temporary health problems, such as eye irritation and burning, throat irritation, and difficulty breathing. Long-term exposure to air pollution can cause chronic health concerns, such as cancer and damage to the body's immune, neurological, reproductive, and respiratory system (USEPA, 1998).

Several epidemiological studies performed in Europe and USA have documented that short-term exposure to ambient air pollution has been related to an increase in mortality and hospital admissions for illnesses of the respiratory tract. Previous researches have shown that assessment of air pollution or asbestos from a genotoxic perspective can generate DNA damage (Gluck and Gebbers, 2000 ; Trosic *et al.*,1997).

People are exposed on a daily basis to airborne particulate matter (PM). Particulate matter is regulated by the US Environmental Protection Agency (USEPA), and studies around the world have consistently demonstrated that particles with an aerodynamic diameter of less than 10 microns (PM 10), and most recently less than 2.5 micron (PM 2.5), pose a significant threat to human health. PM 2.5 particles or fine particles are most

likely to deposit in the alveoli regions of the lung, while large particles deposit in the upper respiratory, Naso-oro-pharyngeal region. Fine particles can accumulate in the respiratory system and are associated with numerous health effects. Exposure to fine particulate matter (PM 2.5) was most closely associated with increase hospital admission and emergency room visits for hearts and lung diseases (USEPA, 1998; Hong *et al.*, 1999; Hajst *et al.*, 1999). Therefore, the air pollution studies have received increased attention in recent years, recent epidemiological studies have focused on particulate air pollution.

A recent study carried out in Bangkok metropolitan area suggested that there may be as many as 4,000 to 5,500 premature deaths each year that can be attributed to short-term exposures to outdoor airborne particulate matter. In addition, hospital admissions for respiratory and cardiovascular illnesses were increased to 0.68% for each increase in the PM 10 concentrations of 10 $\mu g/m^3$ (Samet *et al.*, 2000).

Chiang Mai also has airborne particulate contamination. Chiang Mai is one of the fastest growing districts in Thailand. Its environment is unavoidably contaminated because of its economic development, population growth, and urbanization. It is reported that cancer incidence rates, especially for lung cancer in Chiang Mai, are much higher than other places in Thailand (Martin *et al.*, 1991). One study (Zhang, 1996) revealed that particulate matter in Chiang Mai varied from 17.73-211.58 $\mu g/m^3$. If these particles were mostly PM 2.5, many of these samples would be much higher than the EPA 24 hour PM 2.5 standard of 65 $\mu g/m^3$ (USEPA, 2000). These same particles expressed mutagenicity to *Salmonella typhimurium* and mutagenic expression seemed to be associated with the particulate levels of polycyclic aromatic hydrocarbons (PAHs).

Given the above studies there is ample reason to be concerned about the air quality in Chiang Mai city. However, there are very few studies that actually describe the behavior of fine particles in Chiang Mai air. This study, therefore, measured 24 hour PM (PM 10 and PM 2.5) levels over extended periods. In addition, day-time and night-time of PM samples were taken to see if there is a time difference of PM exposures. Also some of the particle samples were evaluated for genotoxicity by the reverse bacterial mutation *Salmonella* assays, and with alkaline microgel electrophoresis assay to see if there was any association between PM levels and air-borned genotoxicity .

1.2 Ambient air pollution and their sources

The trace contaminants that contribute to what we often call air pollution, vary from location to location and even from day to day. These trace contaminants to the atmosphere that we breath are a complex mixture of solid and liquid particles in an atmospheric suspension that contains numerous chemicals that are present as gasses or vapors. These trace contaminants are all mixed with the normal constituents of air, oxygen and nitrogen. The actual concentration of each pollutant present varies tremendously depending on its proximity to its source and the amount of dilution and mixing that results from different weather conditions. Often, the following categories are used to describe different kinds of air pollutants:

(1) particulate matter

(2) volatile hydrocarbons and other volatile organic compounds

(3) carbon monoxide (CO)

(4) nitrogen oxides (NO_x)

(5) sulfur oxides (SO_x)

(6) lead and other heavy metals

(7) ozone and other photochemical oxidants

(8) acids, mainly of sulfur and nitrogen

(9) Polychlorinated dioxins and furans

(10) Polynuclear aromatic hydrocarbons (PAH); polychlorinated biphenyls (PCBs).

Depending on their vapor pressure many of the hydrocarbon compounds (PAH, PCBs, dioxins and furans, alkanes, organic acids, aldehydes, alcohols, methoxyphenols, etc) can exist both in the particle and gas phases (Pankow,1994; Kamens *et al.*, 2001).

Airborne contaminates are normally retained in the lower stratosphere and eventually interact with terrestrial organisms or are dissolved in water. In large measure, the above pollutants are direct or indirect products of combustion (Frank and Perry,1980). Therefore, emission may occur wherever fuel or other materials are burned. In other words, most of pollutants entering the atmosphere come from fuel combustion, industrial process and soil waste disposal. Additional miscellaneous sources, such as atomic explosions, forest fire, soil dust, volcanoes, natural gaseous emission, and agriculture burning, contribute to the level of atmospheric pollution.

1.3 Airborne particulate matter

In order for pollution to affect terrestrial animals and plants, pollutants must be in a size range that allows it to get into a body and remain there. In other words, the pollutants must be in an aerosol, which can be defined as an airborne suspension of fluid and droplets or solid particle small enough to

posses a low settling velocity. Air contaminants constitute a poorly defined group of particles, ranging from soots and fly ashes to mineral particles (gravels, dust, cement, sand, etc.), that are 0.1-100 μm in diameter. Airborne particulate matters are a complex mixture of organic and inorganic substances and defined according to size and composition. Large particles those above 100 μm in diameter, tend to settle out and produce dust but particles smaller than 100 μm remain suspended in air, giving rise to the fraction of total suspended particulate (TSP). Particulate smaller than 10 μm in diameter are known as respirable suspended particulate (RSP) and these are further divided into; coarse particulate (2.5-10 μm in diameter), and fine particulate (<2.5 μm in diameter). Coarse particles (PM 10) come from sources such as windblown dust from the desert or agricultural fields and dust kicked up on unpaved roads by vehicle traffic. Fine particulate (PM 2.5) which contain secondarily formed aerosols, combustion particles, recondensed organic and metallic vapors are generally emitted from activities such as industrial and residential combustion and from vehicle exhaust. Fine particles are also formed in the atmosphere when gasses such as sulfur dioxide, nitrogen oxides, and volatile organic compounds, emitted by combustion activities, are transformed by chemical reactions in the air. Particulate 0.1-1 μm in diameter are responsible for visibility-reducing hazes.

The US Environmental Protection Agency regulated PM 10 an annual average of 50 $\mu g/m^3$ and a maximum of 150 $\mu g/m^3$ in a 24-hour period. PM 2.5 particles allow no more than a yearly average of 15 $\mu g/m^3$ with a maximum 24-hour average of 65 $\mu g/m^3$. Thailand standard of PM 10 allows an annual average of 50 $\mu g/m^3$ with a maximum 24-hour average of 120 $\mu g/m^3$ (Notification of National Environmental Board No.10, 1992) (Table 1). PM 2.5 particles are the most likely to lodge deeply into alveoli of the lung

while PM 10 particles are thoracic health effects. Exposure to fine particulate matter (PM 2.5) was most closely associated with increase hospital admissions and emergency room visits for heart and lung diseases (USEPA, 1998; Wordley *et al.*, 1997; Schwartz and Neas *et al.*, 2000; Hong *et al.*, 1999; Atkinson *et al.*,1999).

At least an adult human inhales 6-8 liters air/min (1 liter = 0.001 m^3) and during one day, one can inhale anywhere around 15 m^3 depending on the level of physical activity. The air quality is very important in the human life. The optimum size range for aerosol particles to get into the lung and remain there is 0.05-5 μm in diameter. Thus air samplers have been designed to collect the particulate matter in the size range most determine to human health (Frank e*t al.*,1980). An inlet sampler generally consists of an inlet to direct air to a collector, a filter to screen out larger particles that might interfere with an analysis, the collector where the sample is deposited, a flow meter and valve to calibrate the airflow, and a pump to pull air through the system (Figure.1.1). In recent years, samplers have been well developed and miniaturized so that they can be connected to individuals and used in a given field, thus allowing an estimation of exposure of the individual and pollutants level in the air of certain area.

1.4 The toxicity and genotoxicity of airborne particulate matter.

Concern over level of pollutants arose because of the adverse health effects demonstrated both on the airways and indirectly, by their presence in ambient air. Most individuals will react with significant symptoms to the challenge of large amounts of dust. The symptoms produced include nasal irritation, sneezing, moderate discharge and some mucosal congestion

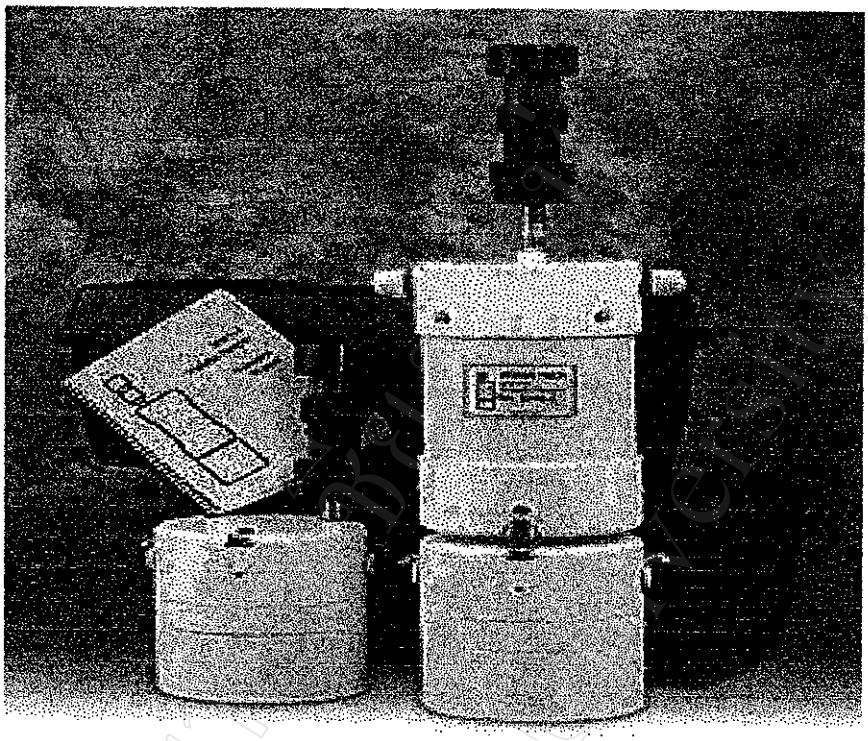
Table 1. Ambient Air Standards of Thailand (1995) (Notification of National Environmental Board No. 10, 1992)

Pollutants	1- hr average		8 - hr average		24 - hr average		1- month average		1 - year average**	
	mg/m^3	ppm	mg/m^3	ppm	mg/m^3	ppm	mg/m^3	ppm	mg/m^3	ppm
1. Carbonmonoxide (CO)	34.2	30	10.26	9	-	-	-	-	-	-
2. Nitrogen Dioxide (NO_2)	0.32	0.17	-	-	-	-	-	-	-	-
3. Sulfur Dioxide/[a] (SO_2)	0.78	0.30	-	-	0.30	0.12	-	-	0.10	0.04
4. Total Suspended Solid (TSP)	-	-	-	-	0.33	-	-	-	0.10	-
5. Particulate Matter (< 10 μ)(PM - 10)	-	-	-	-	0.12	-	-	-	0.05	-
6. Ozone (O^3)	0.20	0.10	-	-	-	-	-	-	-	-
7. Lead (Pb)	-	-	-	-	-	-	1.5	-	-	-

Remark: *At 1 standard pressurre and 25 °C

** geometric mean

/a 1-hr So_2 standard



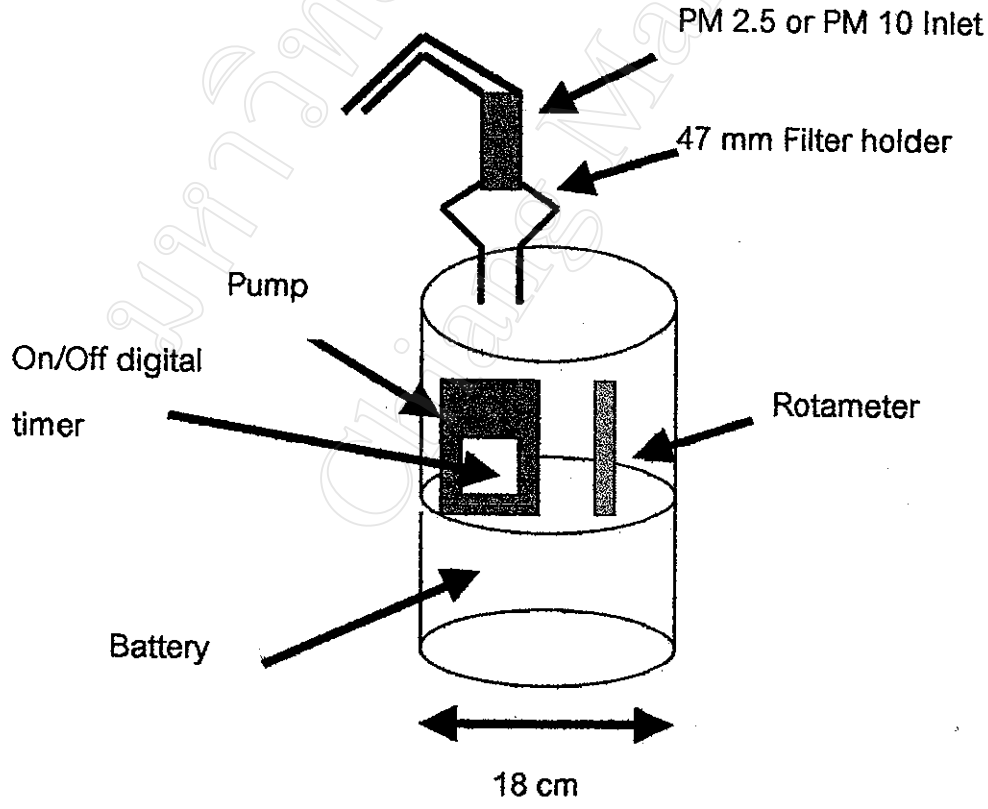


Figure 1.1 Airmetrics Minivol portable sampler (AIRmetric, Oregon, USA)

(Meltzer, 1991). All of these air pollutants not only constitute independent risks, they also act synergistically with other factors. For example, gaseous acid pollutants can be absorbed onto the surface of particulates and carried into the lungs. In all of air pollutants, the particulates are more concern. Particulates are inhaled into the respiratory system and, depending upon size, penetrate to a greater or lesser extents as far as the lower respiratory tract (TSP) and into the alveoli (RSP). In normal breathing, particulates larger than 10 μm in diameter are deposited in the proximity of the glottis, 2-10 μm in diameter in bronchioles, and smaller than 2 μm in diameter in the alveoli, although mouth breathing, as in exercise or when speaking, produces greater deposition of large particulates in the bronchioles. Material that dissolve the mucous of the airways or the surfactant layer in the alveolar region diffuse into the underlying epithelia and blood stream, thereby gaining access to other body tissues where they may cause chronic damage (Figure1.2). In addition, some specific particulates are probably or possibly carcinogenic, i.e., lung cancer as a result of exposure to diesel and petroleum exhausts (Garshick,1988; Boffetta *et al.*, 2001).

Particulate matter increase symptom severity in allergic asthmatics, in association with nitrogen dioxide, carbon monoxide, black carbon, and fine particle mass leading to therapeutic interactions by an implanted cardioverter defibillator (Peters *et al.*, 2000). One research showed that exposure to emission source particulate containing relatively high levels of transition metals, residual oil fly ash, can interact to increase Th2 cytokine production, eosinophil recruitment, and airway hyperresponsiveness in previously sensitised mice (Gavett *et al.*, 1999).

Allergic diseases of the upper and lower airways represent a major worldwide health issue. These diseases have been receiving in incidence

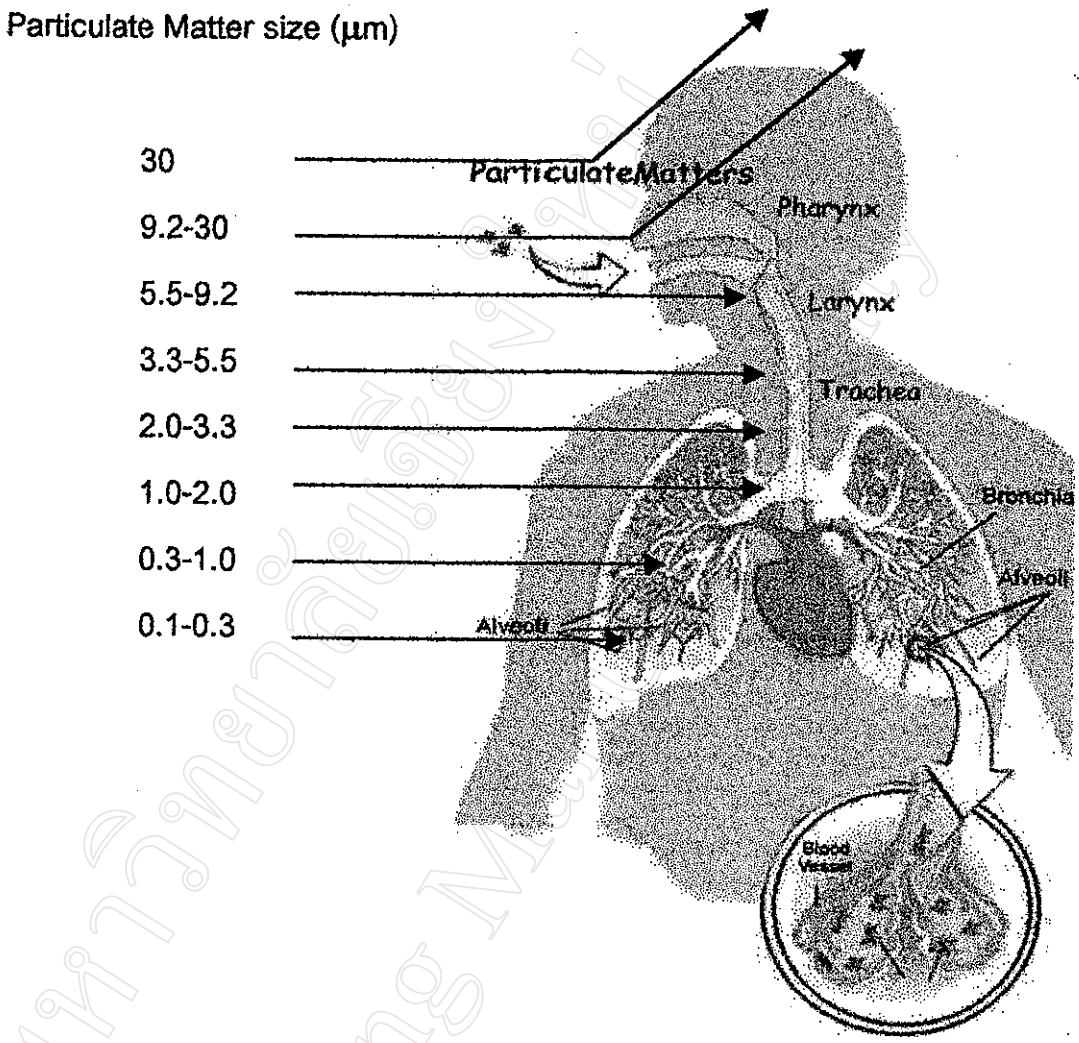


Figure1.2 Particulate matter size and its ability to penetrate into the respiratory tract

and severity throughout this century and undergoing a more recently publicize upturn. The hallmark of these disorders is increased IgE production. Epidemiologic and experimental studies suggest that air pollution, and particularly diesel exhaust particles (DEPs) may play a role in the increasing prevalence and severity of airway allergic disease (Turner, 1989; Takafuji *et al.*, 1989).

PAHs in the ambient air are more directly in contact with human beings from inhalation. They are usually adsorbed on the air particulate. Some of these compounds have been demonstrated carcinogenic. This is why the airborne particulate PAHs was focused in the air pollution research in recent years. The fate of PAHs in the air depends on the molecular size. Many lower molecular weights of PAHs are volatile. Adsorption onto the airborne particulate may be inhaled by organism including human beings. At the same time, the important health effect of particulate is that they may act as a vector to carry the pollutant to deep lung recesses. The majority of these particulate causes irritation, but those products resulting from incomplete combustion often contain PAHs, one of which is the carcinogen BaP. The concentration of BaP is quite different with different situations (Frank *et al.*, 1980) and with different seasons and weather conditions (Wei *et al.*, 1991).

Nitro-polycyclic aromatic hydrocarbons (nitro-PAH) are well known, highly mutagenic compounds present in primary combustion emissions and in airborne particulate matter (Gibson, 1983). Nitro-PAH can be formed during combustion processes or by either a photoreaction or dark reaction of polycyclic aromatic hydrocarbons (PAHs) in a polluted atmosphere (Nielson *et al.*, 1983; Pitts, 1983). 1-Nitropyrene and 2-nitrofluoranthene have been reported to be abundant particle-associated nitro-PAHs in many ambient atmospheres (Yamazaki *et al.*, 1995; Marino *et al.*, 2000) 1-Nitropyrene has

been reported many times as a direct emission product, but 2-nitrofluoranthene is believed to be formed from the reaction of gaseous fluoranthene with OH radicals under photochemical conditions (Fan *et al.*, 1995), and with the NO_3 radical and N_2H_5 in the dark (Atkinson *et al.*, 1990).

1- and 2-Nitrotriphenylenes were present at high levels both in the daytime and nighttime samples of airborne particulate matter collected in Tokyo during December 1998 (Ishii, *et al.*,2001). In addition, nitrotriphenylenes, especially 2-nitrotriphenylene, were expected to be mutagens in the airborne particulate extracts.

In addition, Claxton *et al.*(2000) documented that wintertime ambient air particulate samples collected from Boise, Idaho, USA, were shown to contain extractable organic matter that is mutagenic in *Salmonella typhimurium* microsuspension and plate-incorporation assays with TA98 and TA100. Takeneka *et al.* (1995) found that the organic extract of PAHs from DEPs (PAH-DEP), as well as the prototype AH molecule TCDD, enhance human IgE production by interleukin (IL)-4 plus CD-40 stimulated in purified human B cells and peripheral blood mononuclear cells (PBMCs). Enhanced IgE production in the human airway, resulting from exposure to PAH-DEP, may be important factor in the increase in airway allergic disease.

Evidence shows that fetuses and infants are more affected than adults by a variety of environmental toxicants because of differential exposure, physiologic immaturity, and a longer lifetime over which disease initiated in early life can develop. Studies document that there is significant transplacental transfer of PAH and environmental tobacco smoke constituents from mother to fetus. PAH-DNA adduct levels in maternal and newborn white blood cell were increased with environmental exposure to PAH from ambient pollution. Fetus is more sensitive to genetic damage than

the mother which is correlated with the lower intelligence quotient as well as poorer cognitive functioning and school performance in childhood (Perera *et al.*,1999). It is documented that an increase in ambient sulfur dioxide has had an adverse effect on neonatal mortality (Shinkura *et al.*,1999).

Benzene is volatile organic compound, one of particulate matter components, which is widely used in industry and continues to be a component of gasoline, low concentrations of benzene are present in cigarette smoke and cooking fumes (Lofroth *et al.*, 1991). Exposure to benzene has been shown to lead to aplastic anemia and acute myelogenous leukemia in human and multiple forms of cancer in rodents (Aksoy, 1989; Huff *et. al.*, 1989). Benzene can induce genotoxicity in mice detected by the alkaline comet assay (Tuo *et al.*, 1996).

In the PAHs, the B(a)P has been well documented. Much evidence has accumulated for a role of covalent binding of reactive electrophillic carcinogens to DNA in chemical carcinogens. It has been know that chemical mutagens and carcinogens can produce point mutations, frameshift mutations and chromosome translocations in mammalian cells. The electrophilic theory, metabolic activation is also used to explain the carcinogenicity of B(a)P. The metabolism of B(a)P has been studied extensively and at least 15 major phase I metabolites have been identified. Many of these metabolites are further metabolited by phase II enzymes to a bewildering number of metabolites. It has elucidated which of these metabolites and pathways are important in the carcinogenic process. B(a)P is metabolized by P450 to benzo(a)pyrene-7,8 epoxide which is hydrated by epoxide hydrase to form benzo(a)pyrene-7,8 diol. Benzo(a)pyrene-7.8-diol is considered proximate carcinogen since it must be further metabolized by P450 to form the ultimate carcinogen, benzo(a)pyrene-7,8-diol-9,10-epoxide.

It is this reactive intermediate which binds covalently to DNA forming DNA adducts. Benzo(a)pyrene binds to several bases in DNA but binds preferentially to deoxyguanine residues. Benzo(a)pyrene-7,8-diol-9,10-epoxide is highly mutagenic in eukaryotic cells and carcinogenic in rodents.

Bartsch *et al.* (1979) found that a very close positive association between the liver microsome-mediated mutagenicities of dihydrodiols that can yield bay-region diol-epoxides and carcinogen-potencies of the parent hydrocarbon. These data are consistent with the assumption that, under the assay conditions utilized, liver microsomes *in vitro* predominantly produce simple, mutagen oxides, whereas cultured cells or cells *in vivo* can carry out a three-step activation process involving the sequential formation of epoxide, diols and diol-epoxides. The latters are known assumed the ultimate carcinogenic metabolites of polycylclic hydrocarbons.

1.5 The short-term test to detect environmental genotoxin

Many different short-term tests were developed and used to detect environmental genotoxin and potential carcinogens. Probably the major factor contributing to qualitative difference in assay responses arose from differences in metabolic competence of individual assay marker organisms to activate nonelectrophilic test chemicals to DNA-reactive species. More than thirty kinds of short-term bioassay have used for detecting and screening genotoxin (Matsushima, 1991; Yushiaki, 1991). In STT systems for detecting the mutagenicity of chemicals, microorganisms such as *Salmonella*, *Escherichia* and *Bacillus*; fungi such as *Neurospora* and *Saccharomyces*; plants such as *Vicia* and *Tradescantia*; insect such as Drosophila, *Bombyx* and *Harrba bracon*; *mbyx* and *Harrba bracon;* and

tissue cells of human, hamster and mouse were extensively used (Yushiaki, 1991).

Various damages produce in chromosome and DNA are detected by short –term test. Among these short-term tests, Ames *Salmonella* microsomal test is wildely used in many laboratories because it is simple and rapid, low-cost, reproducible and reliable for detection of genotoxicants, mutagens, and genotoxic carcinogens (Matsushima, 1991). The test has also been used to determine the mutagenicity of complex environmental and biological mixtures. Many of the mutagenic compounds of these mixtures have been chemically characterized. A considerable number of mutagens first detected by the *Salmonella* test have been shown subsequently to be carcinogenic in animal tests. Therefore, this test plays important roles for screening and detection of environmental genotoxins and classification of genotoxic and non-genotoxic carcinogens.

The Salmonella microsomal assay is based on the detection of mutated histidine dependent strain (His^-) of *Salmonella typhimurium* which can revert to the wild-type (His+) if they are exposed to the mutagens. Most mutagens are not direct acting compounds and need metabolic activation to be active. Bacteria do not contain the spectrum of monooxygenase as found in higher animals, Ames (Ames *et al.*,1975) incorporated in his assays a crude fraction of rat liver obtained by centrifugation at 9,000 g, consisting mainly of microsomes as an activating system. The use of *in vitro* systems to assess potential biological activities of chemicals is limited by the absence of metabolic processes present in whole animals. Accordingly, adjuncts to *in vitro* assay frequently include the mixed function oxidase activities of the liver as a means of providing metabolic capacity (Ames *et al.*, 1975; Amacher and Turner,1982; Clive and Spector, 1975; Clive *et al.*,1979). Although the

source of the liver, the use of enzyme inducers, and the method of preparation may vary, the basic biochemistry of these enzymes are the same: using Glucose-6-phosphate or isocitrate and appropriate dehydrogenases, NADPH is generated from NADP+ and is utilized as a cofactor by the enzymes in the liver fraction (typically a 9,000g supernatant, S9). This makes the *Salmonella* mutation test available and widely using for screening and detecting mutagens and predictive carcinogens which can induce the gene mutations. By applying an operational definition of mutagen to substance that possesses a structural alerts and induces mutations in at least one of *Salmonella* strains, it has been possible to recognize some interesting and potentially important characteristics distinguishing mutagenic and non mutagenic carcinogens. The microbial systems are used for detecting gene mutations and DNA repairs, but not for detecting chromosome aberrations. The most widely used system of microbial gene mutation is *Salmonella* mutation test, in which reverse mutation in histidine requirement using *S. typhimurium* TA 98 for framshift type mutation and *S. typhimurium* TA100 for base-pair change type mutation (Yaikiaki, 1991).

1.6 The alkaline Comet assay

The Comet assay, also called the single cell gel assay (SCG) and microgel electrophoresis (MGE) was first introduced by Ostling and Johanson in 1984 as a microelectrophoretic technique for the direct visualization of DNA damage in individual cells (Ostling and Johanson, 1984). It facilitates the detection of both single and double stranded DNA breaks. The obvious distinction in protocols for single or double stranded breaks is the employment of neutral rather than alkaline lysis and

electrophoresis conditions for the double strand break detection assay. As the Comet assay is designed to evaluate DNA damage in individual cells, clearly the cells or tissues to be evaluated need to be assayed in a manner that allows distinction between the cells. Virtually any eukaryotic cell can be processed for analysis of DNA damage using the Comet assay.

In order to execute the assay, a suspension of individual cells must be prepared. The obvious concern with measuring DNA damage and strand break rejoining in tissues from animal or clinical samples is that the samples be isolated and processed without allowing additional repair or creating additional strand breaks. The most commonly examined human cells are lymphocyte populations. However, many parameters can affect the response of lymphocytes to the assay in terms of the ability to detect damage. De Meo *et al.* (1991) reported considerable intra-individual variability of comet formation using the single cell gel assay. Previous and subsequent reports have pointed to a variety of possible factors that many be responsible for differences in cell response including the age of the blood donor (Singh *et al.*, 1990, 1991), the physical activity of the donor (Hartmann *et al.*, 1994), and whether or not the donor smokes (Betti *et al.*, 1994). Moreover, cell cycle status likely causes an addition level of complexity to the problem at hand, since chromatin structure affects the role of DNA during comet formation in both the alkaline and neutral assay systems (Olive and Banath ,1993) and chromatin structure is fundamental to replication and transcriptional activity (Felsenfeld, 1992; Paranjape *et al.*, 1994). Cells are generally suspended in low melting point agarose at a final concentration of 0.5-1.0% at 35-34 ^{o}C and cast on a fully frosted microscope slide. The gels are allowed to solidify briefly. Following slide preparation, the embedded cells are lysed by gently immersing the slides in a lysis buffer. Neutral and alkaline lysis solutions are

used for double and single strand break detection assays respectively. Alkaline lysis, which appears more frequently in the literature, consists of immersing the cells in a high salt solution with detergents at a pH of 10 to > 12 for at least 1 h. Prior to electrophoresis, the slides are equilibrated in alkaline electrophoresis solution, which contains low salt, no detergents, and a generally higher pH (> 12.3). The reported time difference during both the pre-electrophoresis wash and electrophoresis steps can be attributed largely to the extent of damage and desired detectability.

Much of the variation in the reported Comet assay protocols is found during electrophoresis. The desired voltage and time of electrophoresis will obviously be related to the levels of DNA damage expressed in the cells and the salt concentration of the running buffer. Since DNA is required to migrate only a fraction of a millimeter for microscopic observation, significant DNA migration which leads to comet formation is possible with very short electrophoresis runs (20 min) and low voltages (17 Volt). Following electrophoresis, slides are washed and stained with a fluorescent DNA binding stain for image analysis. A variety of stains have been used effectively; propidium iodine is a popular choice especially for image analysis. Interestingly, damaged DNA which is denatured and subsequently renatured in the Comet assay shows less affinity for propidium iodine and other DNA stains, a property which can be used to advantage in separating damaged from undamaged cells (Olive *et al.*, 1994).

The methods of comet image analysis reported are as varied as the applications for which the Comet assay has been used. Since comets are formed upon the principle of releasing damaged DNA from the core of the nucleus with electrophoresis, several different attempts have been made to evaluate and quantify comet formation patterns. The simplest of the

techniques is to score the comets empirically on the basis of damage extent. Because the assay produces a visual endpoint, it is entirely possible to score the comets based on their appearance as either damaged, undamaged, or even with gradations, provided adequate precautions are used to protect against observer bias. This method of evaluation, although it lacks the sophistication of image analysis, has been used with some success. More commonly, the distance of DNA migration from the body of the nuclear core is used to evaluate the extent of DNA damage.

1.7 Objectives

1) To investigate the 24-hour and 8-hour levels of particulate matters, PM 10 and PM 2.5, during day-time and night-time in the air around Chiang Mai city.
2) To study the genotoxicity of extractable organic matter from particulate matter, PM 10 and PM 2.5.