| | |
|---|---|
| **From:** | [redacted] |
| **To:** | Riverside Energy Park |
| **Subject:** | Additional documentation to examiner |
| **Date:** | 07 June 2019 22:45:37 |
| **Attachments:** | Moss study airborne metals and particulates.pdf<br>Daily telegraph.pdf<br>Journal of clinical Toxicology.pdf<br>kc3-particulate-emissions-and-health-statement-of-evidence-to-ringaskiddy-inquiry.pdf<br>Scientific world journal PAH"s.pdf<br>Speech preable environmt -Cory hearing.pdf<br>Ultr fines and PAH"s.pdf<br>Ultrafines from 2003.pdf<br>YIndia - Untrfine particles and PAH.pdf |

---

Sirs,

I spoke at the Howbury Grange Community Centre on 5th June 2019 to the examiner and the public investigation into the examination of the Cory Riverside Energy Park application. I spoke on resident concerns on Air Quality and health implications.

I provided several quotes from documents regarding the issues referenced. I also stated that I would further submit, as pdf documents, the said documents quoted from and I attach them herewith as promised.

The Ultrafine Particle Toxicity document also has appended at the bottom of the page the website source address rather than copying the entire document.

Yours sincerely,

Cllr. Dave Putson
Belvedere ward
LB Bexley

[redacted]

**Groundbreaking 'moss' study reveals new airborne metals death link** Air Quality News Website extract of 4/6/19 A groundbreaking study that used wild moss to estimate human exposure to air pollution has revealed, for the first time, that airborne metals caused by human activities such as transport and industry, and not those naturally occurring in the atmosphere, increase the risk of death for humans.

Although it is well-established that airborne particulate matter kills, it is still not known which specific particles are responsible for a higher risk of mortality – whether that be particles that are considered natural in the atmosphere or those that result from human activity.

For the study, which was published in the journal Environment International, researchers from the Barcelona Institute for Global Health (ISGlobal) analysed moss samples from all over France to measure the presence of 13 elements: aluminium, arsenic, calcium, cadmium, chromium, copper, iron, mercury, sodium, nickel, lead, vanadium and zinc. The metals were then classified into two groups – those considered natural and from human activity.

They then mapped the exposure of 11,000 people living in rural France to the metals under study. The people are part of the Gazel cohort, who have been followed by scientists for various studies since the 1990s.

Bénédicte Jacquemin, IS Global and INSERM researcher and last author of the study said: 'There have been very few studies on the health effects of airborne metal pollutants, partly because of technical limitations, such as the lack of stations measuring air pollution. 'We thought that moss, because of its capacity to retain these metals, would be a useful tool for estimating the atmospheric metal exposure of people living in rural areas.'

The metals deemed to be of human activity origin were cadmium, copper, mercury, lead and zinc. While all of these metals are naturally present in the earth's crust, their presence in the atmosphere is due to human activities, such as industry, transport and heating. Between 1996 and 2017, there were 1313 deaths in the cohort, including 181 cardiovascular and 33 respiratory. Researchers believe that exposure to the metals caused by human activity was associated with an increased risk of natural-cause mortality death, while metals from natural sources were not. 'There have been very few studies on the health effects of airborne metal pollutants, partly because of technical limitations, such as the lack of stations measuring air pollution. We thought that moss, because of its capacity to retain these metals, would be a useful tool for estimating the atmospheric metal exposure of people living in rural areas,' added Bénédicte Jacquemin.

'Our results indicate that the metals present in the airborne particulate matter could be a key component in the effects of air pollution on mortality.'It is important to bear in mind that the people we included in this study live in rural areas far from major urban and industrial Centre's and road networks. This means that they are very likely to be exposed to lower levels of air pollution than people living in urban environments, which gives us an idea of the seriousness of the health effects of air pollution, even at relatively low levels of exposure. 'These findings support our hypothesis that moss bio-monitoring can be a good complementary technique for identifying the toxic components in suspended particulate matter.'

**"Chronic ozone exposure linked to heart disease, study finds"** (separate statistical modelling report.)

**EASTERN EUROPE** (p7)

Studies in the heavily polluted regions of Eastern Europe have found that air pollution can stunt the growth of foetuses [i]. At a conference in Budapest in 1996, organised by the American Cancer Association, Frederica Perera, of Columbia Univ. NY, revealed that Polish babies exposed to high levels of PM10 were on average 160g lighter and 1.04cm shorter at birth. The circumference of the head was almost a centimetre less. (This stunting is about half as severe as that seen in "crack" babies, born to mothers abusing cocaine). It is suspected that this will affect their subsequent ability to learn. The Polish research focused on **polycyclic aromatic hydrocarbons** (PAHs) which are produced by combustion and attach to PM10. This can cause mutations and increase the probability of developing cancer. Although levels of PAHs are generally lower in the West, Perera doubts that there is a threshold concentration below which air pollution does not harm foetuses. Similar findings come from the Prague Institute for Advanced Studies, in association with the EPA

[i] Edwards, Rob "Smog blights babies in the womb" New Scientist 19.10.96

**VECTOR EFFECT** (p9)

The significance of $PM_{10}$ is not limited to the presence of the particle itself. A study for the British Lung Foundation, carried out by Joy Conway, Dr.John Fleming and Professor Stephen Holgate at Southampton University, [i] used a scanning technique to study the penetration of the lung by particles of different sizes. This study suggests two possible mechanisms which could result in death; the particle penetrates deeply enough to cause inflammation in its own right or the particle acts as a vector which carries chemical pollutants deep into the lung. (More than eighty different molecules have been found attached to

particulates in urban air).[19] At a more accessible level, Friends of the Earth warned as early as 1989 that *"cancer-causing compounds called* ***polyaromatic hydrocarbons*** *cling to particulates and are carried deep into the lung"* . [ii]

[i] "Smog deaths study" Daily Telegraph, 5.7.95, p16
Download from website www.telegraph.co.uk

[ii] Friends of the Earth, "Particulate pollution from diesel vehicles". Briefing Sheet
1989

Journal of Clinical and Experimental Toxicology

# Cardiovascular health risk posed by Polycyclic Aromatic Hydrocarbon and Ultrafine Particles.

**Collins Otieno Asweto***
School of Public Health, Maseno University, Kenya
***Corresponding Author:**
Collins Otieno Asweto
School of Public Health, Maseno University, Kenya
**Tel:** [redacted]
**E-mail:** [redacted]
**Accepted date:** January 04, 2018
**Visit for more related articles at** Journal of Clinical and Experimental Toxicology

## Abstract

There is increased cardiovascular disease incidence attributed to ambient air pollution. It is estimated that ischaemic heart disease and stroke account for 80% of ambient air pollution-related early deaths. The most deleterious are the ultrafine particles (UFPs) which have more prominent potential health threat, since they can act as carriers of other strong air pollutant such as polycyclic aromatic hydrocarbons (PAHs). UFPs are small in size, hence can easily penetrate and reach cardiovascular tissues and organs. To understand their mechanisms, studies on UFPs and PAHs induced cardiovascular diseases were reviewed. The studies showed that UFPs and PAHs initiate events in atherogenesis including oxidative stress, expression of cell adhesion molecules on the surface of the endothelium, inflammation, and endothelial dysfunction. Endothelial dysfunction is associated with cardiovascular diseases such as hypertension, coronary artery disease, chronic heart failure, and peripheral vascular disease. The current review provides useful information on deleterious effect of UFP and PAH exposure on cardiovascular system. Thus advancing knowledge on UFP and PAH toxicity underscores the importance of the cardiovascular effects of air pollutants.

## Keywords

Ultrafine particles, cardiovascular, endothelial dysfunction.

# Introduction

Escalating air pollution is contributing to increased cardiovascular health problems currently witnessed in the world. Cardiac diseases such as ischaemic heart disease and strokes account for 80% of ambient air pollution-related early deaths [1]. Additionally, it is estimated that about 3 million deaths and 85 million disability adjusted live years (DALYs) resulted from particulate matter of ambient air pollution in 2012 [1]. Ambient air pollution has been on the rapid increase in most part of the world due to industrialization, urbanization, and motorization [1].

Ambient air pollutants comprise of particulate matter (PM), gases, organic compounds, and toxic metals [2,3]. Particulate matter (PM) is a blend of liquid droplets (aerosols) and solid particles like dust, soot, smoke, and dirt. PM is found in smoke, diesel exhaust, and haze that either come specifically from combustion or is a result of response to gases and sunlight or air [4]. In any case, the inhalable particles are of 10 μm in aerodynamic diameter (PM10), fine particles 2.5 μm (PM2.5), and ultra-fine particles <0.1 μm (UFP). PM2.5, most generally utilized as a representative marker of exposure to air pollution, is progressively measured and checked by national air quality monitoring networks [5]. However, experimental evidence suggests that UFP may be more dangerous than PM10 and PM2.5 due to their chemical composition, small size, large surface area/mass ratio, capability of generating reactive oxygen species (ROS), high retention rate, and deep penetration in the respiratory system [6]. Thus the World Health Organization (WHO) and the Organization for Economic Co-operation and Development (OECD) have called for a critical and comprehensive assessment of UFP safety [7]. Moreover, research substantiations demonstrate that exposure to UFPs adds to the advancement of cardiovascular disease; along these lines, triggering acute cardiac events such as altered heart rate, heart rate variability, changes in microvascular function, and systemic inflammation [8,9]. This is due to their unique physicochemical properties which have unpredicted biological effects [10-12].

Polycyclic aromatic hydrocarbons (PAHs) result from incomplete combustion of organic materials and are ever present in the environment. Human exposure to PAHs can occur via ingestion of PAHs contaminated soil, food, and water, inhalation of PAHs contaminated soil dust and air [13], and dermal contact with PAHs contaminated soil [14,15]. After PAHs entering human body via various exposure pathways, complex metabolism and unknown factors would also make PAHs reach cardiovascular tissues and organs. PAH has been previously link to development of cardiovascular

disease (CVD) such as coronary heart disease, peripheral arterial disease, stroke, and myocardial infarction [16-20].

Due to widespread sources and persistent UFPs and PAHs in the environment. Human beings are exposed to UFP and PAH mixtures in particulate phases in ambient air. Long-term exposure to high concentrations of the mixture is associated with adverse health problems. Thus, studies on PAHs in particulate matter (PM), such as UFP in ambient air, have become attention greater focus of research in recent years.

# Ultra-fine Particles and Polycyclic Aromatic Hydrocarbons

Ultra-fine Particles (UFPs) have been found to have an atmospheric concentration ten times higher in the urban air than in rural air and are considered the most detrimental of all PM fractions [21]. This is on the account of UFP size empowers them to infiltrate through the lung to the circulatory system and reach other organs [22]. In addition, UFPs act as carriers of other strong air pollutant such as Polycyclic Aromatic Hydrocarbons (PAHs) [23] because of their adsorption capabilities. UFP are incidentally generated in the environment, often as by-products of fossil fuel combustion, condensation of semi-volatile substances or industrial emissions [24,25]. In addition, the speedy growth of nanotechnology is increasing the environmental exposure to nano-size particles. This exposure may be especially chronic for those employed in research laboratories and in high tech industry where workers handle, manufacture, use and dispose of nanoparticles.

On the other hand, PAHs are ubiquitous environmental pollutants generated primarily during the incomplete combustion of organic materials such as coal, oil, petrol, and wood. Exposure to PAH has been previously link to development of cardiovascular disease (CVD) such as coronary heart disease, peripheral arterial disease, stroke, and myocardial infarction [16-20]. Besides, elevated PAH metabolites in urine has been associated with increased CVD events [18]. Moreover, a previous study reported an increased risk for fatal ischemic heart disease in relation to occupational exposure to benzo(a)pyrene [16]. The major route of PAH exposure include in halation, ingestion, and dermal contact in both occupational and non-occupational setting [26]. Many PAHs are toxic and rapidly distributed in a wide variety of tissues with a marked tendency to localize in body fat [26]. Polycyclic aromatic hydrocarbons (PAHs) are organic compound pollutants, which are ubiquitous in ambient air and exist as gases or joined to the Particulate Matter (PM) [27]. The existing interaction between UFPs and PAH from trafficrelated air pollutants [28] might be the reason for

progression of atherosclerosis observed in low level pollution below the existing regulatory standards [29,30].

# Mechanisms of Ultra-fine Particles and Polycyclic Aromatic Hydrocarbons Action

Upon entering the bloodstream, the UFPs bound with PAH get into direct contact with blood vessels and the heart endothelial cells lining. Endothelial cells are the biological barriers which mediate clearance of nanoparticles, maintain vascular function and homeostasis [31]. Moreover, in vivo and human studies have indicated damage to endothelial cells as an important mechanistic event by which inhalation of particles is associated with cardiovascular diseases [32]. The initial events in atherogenesis include the expression of cell adhesion molecules on the surface of the endothelium, inflammation, and endothelial dysfunction [33]. Both UFPs and PAH have also been shown to individually induce endothelial cell toxicity resulting in endothelial dysfunction [34-36]. Long term exposure to high levels of environmental UFPs have been shown to increase risk of arteriosclerosis, whereas short-term exposure can cause changes in heartrate variability [37]. UFP exposure depresses myocardial contractile response and coronary flow in both spontaneously hypertensive and wild-type rats [38,39].

Tithof et al. (2002) investigate the effects of polycyclic aromatic hydrocarbons contained in cigarette smoke on phospholipase A2 (PLA2) activity and apoptosis of human coronary artery endothelial cells. They found that B(a)P induce apoptosis of endothelial cells by a mechanism that involves activation of phospholipase A2 (PLA2) [40], leading to endothelium dysfunction. Endothelial dysfunction has been recognized as the source of multiple cardiovascular events that causes damage to the vascular wall, forms atherosclerotic plaque and consequently promotes vascular injury [41,42]. Besides, cardiovascular diseases such as hypertension, coronary artery disease, chronic heart failure, and peripheral vascular disease have been associated with endothelial dysfunction [43]. The underlying mechanisms are not understood, but oxidative stress and systemic inflammation have been suggested to play a role in PAH-induced CVD [44,45].

# Ultra-fine Particles and Polycyclic Aromatic Hydrocarbons Joint Effects

Ultra-fine particles have been shown to have a greater content of redox active compounds, such as prooxidative polycyclic aromatic hydrocarbons (PAHs) that could provide them with a greater prooxidative potential [46]. In addition, their smaller size and greater surface-to-mass ratio may enable

them to have greater bioavailability for the PAHs on their large surface area, making them more accessible to the contact sites of cells [46]. PAHs adsorb onto particles play a toxicological role in generating ROS, oxidative stress, and inflammation once inhaled [47]. Our previous study reported unanticipated toxicity induced by the co-exposure of UFPs and PAH, which was beyond the well-known toxicities of the individual compounds [48]. The SiNPs and B[a]P coexposure of induced excessive oxidative stress, subsequently resulting to DNA damage, cell cycle arrest, and apoptosis of endothelial cells [48]. Moreover, enhanced expression of proinflammatory and procoagulant genes have also been previously observed in SiNPs and B[a]P co-exposure [49], which is an indication of inflammation-coagulation cascade involvement in the co-exposure toxicity mechanism. Furthermore, oxidant injury plays an important role in UFPinduced adverse health effects including exacerbation and promotion of atherosclerosis [50].

In summary, the current review provides useful information on deleterious effect of UFP and PAH exposure on cardiovascular system. Thus advancing knowledge that underscores the importance of the cardiovascular effects of air pollutants. Further research is required to better understand the specific mechanisms by which PAH bounded UFP can lead to various cardiovascular effects. In addition, better parameters need to be developed to improve the assessment of PAH bounded UFP toxicity (**Figure 1**).

**An Bord Pleanála**

# Statement of Evidence

# Particulate Emissions and Health

## Proposed Ringaskiddy Waste-to-Energy Facility

**Professor C. Vyvyan Howard MB. ChB. PhD. FRCPath.**

**June 2009**

## Professor C. Vyvyan Howard MB. ChB. PhD. FRCPath.

Vyvyan Howard is a medically qualified toxico-pathologist specialising in the problems associated with the action of toxic substances on the fetus and the infant. He is Professor of Bioimaging at the University of Ulster and has written a number of papers and book chapters and spoken in a variety of forums to draw attention to the threat posed by environmental pollutants to the developing fetus.

He is a Fellow of the Royal College of Pathologists, Past President of the Royal Microscopical Society, Member of the British Society of Toxico-Pathologists, Immediate Past President of the International Society of Doctors for the Environment and Member of the European Teratology Society. He has just completed 6 years as a toxicologist on the UK Government DEFRA Advisory Committee on Pesticides.

A large part of Professor Howard's current research is the investigation of the fate toxicology of nanoparticles. His research team is in receipt of two large EU grants; 'NanoInteract and 'NeuroNano'. He has co-edited a book entitled 'Particulate Matter: Properties and Effects upon Health' published in September 1999 [1].

Vyvyan Howard has sat on two EU expert groups considering the threats and benefits posed by nanotechnology and recently addressed the House of Lords Select Committee on Science and Technology investigating the use of nanotechnology in food.

# 1 Summary:

## 1.1 Incineration and Health:

Scientific knowledge regarding the effects of solid waste incineration facilities on the health of a population living nearby is constantly being updated.

Adverse health impacts arising from both inhalation of combustion products and from contaminated food from older incineration plants, generally those operating during the 1970's through to the 1990's, are reasonably well described in the epidemiological literature. The main health endpoints studied have tended to relate to

1. respiratory symptoms and illness
2. reproductive effects, especially congenital anomalies
3. cancer.

A practical issue, and one of significant policy importance, is that the majority of published epidemiological studies relate to these older plants. With the more recent European Union regulations [2] many older plants have closed, or been fitted with more stringent emission controls. While this is obviously desirable from a public health perspective, it does raise issues of the relevance of studies around older plants, to populations affected by more modern facilities. Proponents of new facilities tend to dismiss the older research as irrelevant. Opponents take a contrary view arguing, not unreasonably, that similar claims of safety were made in relation to those older facilities when they were operating; that the risk assessments relied upon to show new incinerators are safe would not, if applied to the older plants, reveal the levels of impacts reported in the literature thus indicating that the risk assessments do not validate in real-world situations; and that epidemiology, by it's nature, involves retrospective studies. Furthermore the modern incinerators tend to be much larger than those operated historically so that although the emissions concentrations have reduced the total mass of pollutant emissions may even increase.

The comprehensive review by the Health Research Board [3], commissioned by Department of Environment and Local Government, was obviously aware of these arguments and concluded that "*there is some evidence that incinerator emissions may be associated with respiratory morbidity*" and that "*acute and chronic respiratory symptoms are associated with incinerator emissions*".

The review also confirmed that "a *number of well-designed studies have reported associations between developing certain cancers and living close to incinerator sites. Specific cancers identified include primary liver cancer, laryngeal cancer, soft-tissue sarcoma and lung cancer*".

The Health Research Board recognised the problems of isolating causation in real world epidemiology and commented that "*it is hard to separate the influences of other sources of pollutants, and other causes of cancer and, as a result, the evidence for a link between cancer and proximity to an incinerator is not conclusive*". They suggested that this could be addressed by "*further research, using reliable estimates of exposure, over long periods of time, is required to determine whether living near landfill sites or incinerators increases the risk of developing cancer. Studies of specific environmental agents and specific cancers may prove more definitive in the future*".

A more recent World Health Organisation ('WHO') report [4] similarly concludes by suggesting that "*Further insights on health effects of landfills and incinerators are likely to be gained only from studies that consider exposure pathways and biomarkers of exposure and effect, and compare waste–related exposures with those due to other sources of pollution*."

In that context this evidence reviews the possible health impacts associated with emissions from incinerators and a specifically the concerns associated with ultrafine particulates.

### 1.2 Air Pollution and Health:

The relationship between air pollution and mortality has been well known for many years. Two of the most notable pollution incidents confirming the effects of air pollution were firstly the tragic events of the Meuse Valley, Belgium, where in December 1930, in the small town of Engis 60 people died in the space of three days [5]. This disaster provided incontrovertible evidence that air pollution could kill and therefore it attracted considerable attention from the scientific community.

In a contemporary editorial in the British Medical Journal, Haldane [6] stated that "the possibility of a similar disaster happening in this country [the UK] is a matter of great public health interest". He thought that disaster had been avoided so far in London because the city emitted a lot of heat, which produced convection currents. He warned – though to no avail, against plans to build big electricity generating stations. The subsequent London pollution incident in December 1952 resulted in an increase in deaths that has been estimated to be of approximately 4,000 by Logan (1953) or 12,000 in a more recent retrospective study [7].

Despite these huge impacts, it has not been until the last decade did the scientific community focus in earnest on the potential health hazard of PM exposure [8].

### 1.3 Particulates and Health:

Epidemiological studies worldwide have consistently demonstrated links between ambient particulate matter exposure and adverse health outcomes, including increased rates of respiratory and cardiovascular illness, hospitalizations, and pre-mature mortality [9, 10]. Particles are usually defined by their size, e.g., PM10 and PM2.5, as the mass of particles with aerodynamic diameters less than 10 to 2.5 $\mu$m, respectively. Recently, however, interest has also focused on the fraction of ultrafine particles (UFP) with a diameter less than 0.1 $\mu$m, which are abundant in number but contribute little to the mass [11, 12]. The UFPs are only usually measured for research purposes and are effectively outside regulatory control. It is these emissions that are the main theme of this evidence.

Studies have shown that ultrafine particles are more toxic than larger particles [13-15]. Furthermore, individual particles have been shown to be capable of inducing inflammation and oxidative stress [15], suggesting that particle number concentrations, which are dominated by ultrafine particles, may be more indicative of some potential health impacts than particle mass concentrations. UFP are also important because of their high alveolar deposition fraction, large surface area, ability to induce inflammation, and potential to translocate into the blood circulation system. At a given mass, ultrafine particles (diameter $< 0.1$ $\mu$m) have $10^2$ to $10^3$ times more surface area than particles with diameters in the 0.1–2.5 $\mu$m range and approximately $10^5$ times more surface area than coarse particles (2.5 $\mu$m < diameter $< 10$ $\mu$m) [16]. This surface area-to-mass effect may affect the relative toxicity of particles to respiratory systems, in combination with a higher deposition efficiency of ultra fines in the alveolar region (Hughes et al., 1998).

Estimates of the number of excess deaths on a global scale due to particle inhalation have been made, and they amount to about 2 million/year of which c.370,000 per year are within the EU. The health effects are not limited to lung injuries. They deaths also include

cardiovascular diseases and cancers [17]. It is interesting in the light of these impacts to consider that as recently as 1992 the Lancet editorial was claiming that "*environmental pollution is unlikely to result in gross excess mortality*" [18].

### 1.4 Ultrafine Particles and Incineration:

Although not such a high contributor to national PM inventories incinerators appear to be very important local sources of particulate contamination. Aboh [17] assessed the contribution of a modern incinerator in Sweden to local PM2.5 levels and concluded that between 17 % and 32% of the particulates arose from the incinerator. This contribution may seem to be large compared with the relatively small increased modelled by Indaver of 0.5 $\mu g/m^3$ compared with an assessed background level of c 7 $\mu g/m^{3.}$ Indaver appears to ignore, however, the very significant contribution made to particulate burdens by SOx and, especially, NOx emissions.

### 1.5 The Precautionary Principle:

There remains significant uncertainty about the level of health impacts associated with ultrafine particulates and other emissions from incinerators.

The WHO [4] emphasises that "*priority needs for research include development and application of biomonitoring, both in human observational studies and in toxicological research, the use of pharmacokinetic models to assess the influence of factors such as metabolism and timing of exposures, and the analysis of all relevant environmental matrices, in order to evaluate chemical exposure pathways and to assess the exposure for specific subsets of the population*".

I consider that the evidence of risk of harm to human health and the environment is sufficiently high that a precautionary approach should be taken towards the permitting of new incineration capacity at least until there is much better information from the biomarker studies recommended by the WHO [4] and the Health Research Board [3].

Whilst I believe that it is sufficiently compelling in itself the uncertainties associated with the health evidence are supported by strong policy arguments in areas beyond the scope of this evidence. The 2007 WHO report [4] says "*the evidence of adverse health effects related to landfills and incinerators, although not conclusive, adds to other environmental concerns in directing waste management strategic choices towards reduction of waste production, re-use and recycling schemes, as prescribed by EU Directives*". I note that the Health Research Board review [3] includes similar commentary and says that one submission "*included a letter from the EU Environment Commissioner, which stressed that 'incinerators are not the answer to waste management .... Incinerators only reduce the volume of waste but the environmental impact of incineration is significant.'*"

The same contributor quoted the Head of EU Waste Management, who stated that incinerators need enormous input in order to be economic and that in many countries they are now considered similar to nuclear power stations and should be avoided:

*'The Commission does not support incineration. We do not consider this technique is favourable to the environment or that it is necessary to ensure a stable supply of waste for promoting combustion over the long term. Such a strategy would only slow innovation. We should be promoting prevention and recycling above all. Those countries who are in the process of drafting their planning should not base it upon incineration.'*

# 2 Properties of particulates

## 2.1 Particle Size

In 1979, the U.S. National Research Council said [19] that measuring particles by weight, without regard to particle size, has "*little utility for judging effects*". Particle size is therefore a vital consideration when it comes to air pollution and health. The respirable fraction of particles found in air are classified into size bands which are generally defined as:

| | | |
|---|---|---|
| Coarse + fine | $PM_{10}$ | The mass of particles per cubic metre which pass through a size-selective inlet with a 50% efficiency cut-off at 10 $\mu$m aerodynamic diameter |
| Fine | $PM_{2.5}$ | As for $PM_{10}$ but with a 2.5 $\mu$m cut-off. |
| Ultrafine = UFP or 'nanoparticles' | $PM_{0.1}$ | As for $PM_{10}$ but with a 100 nm cut-off, i.e. up to 0.1 $\mu$m diameter |

It is helpful to compare the size of the particles with common material like fine beach sand and human hair [20]:

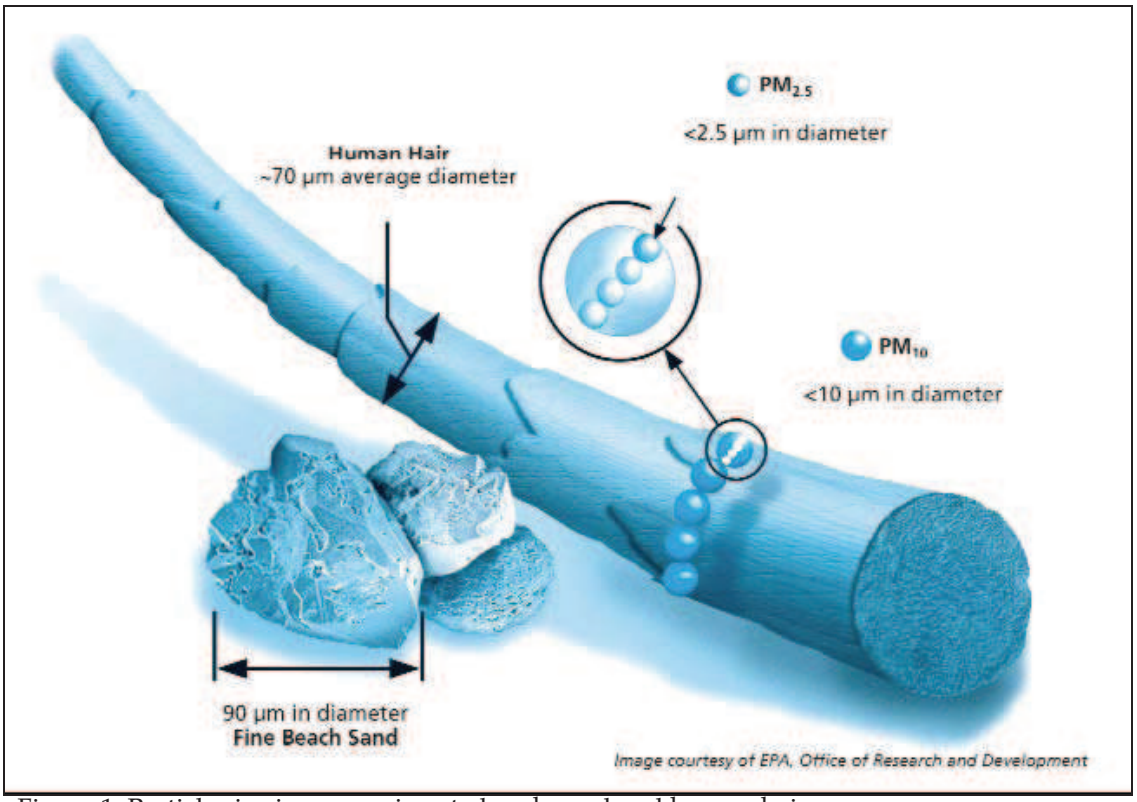


Figure 1: Particle size in comparison to beach sand and human hair

This relative size can also be illustrated by comparison to biological phenomena as per Brook et al. [21]:

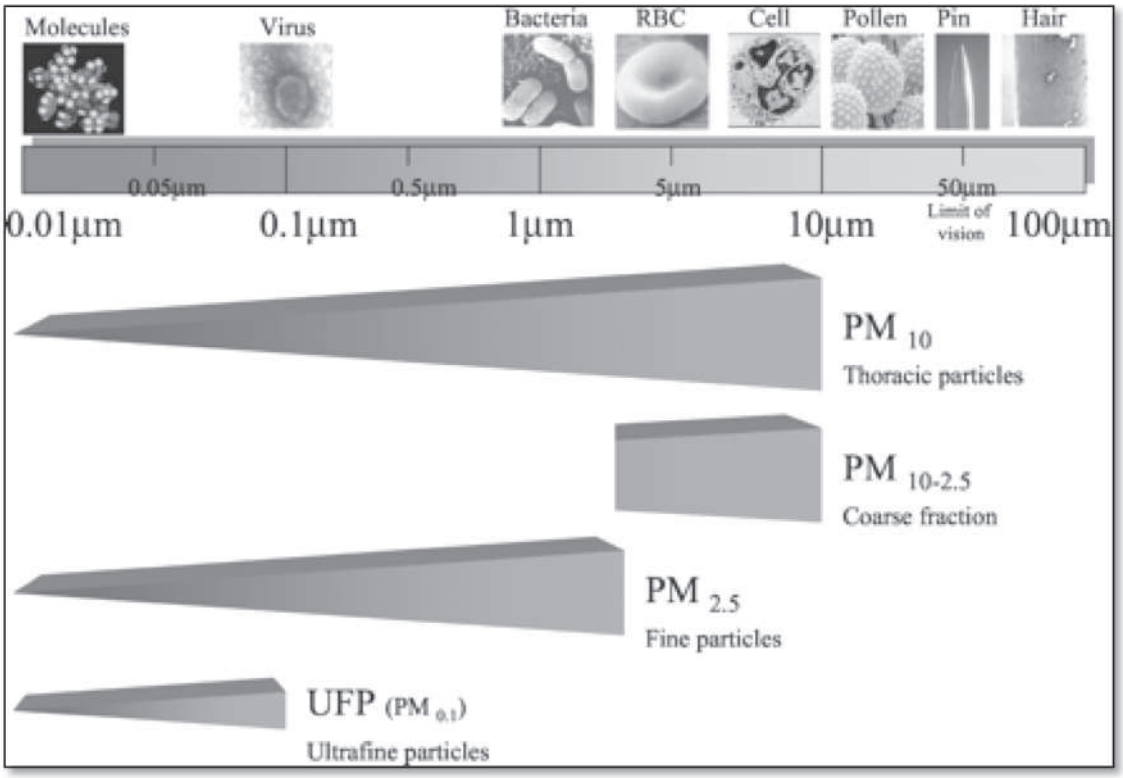

Figure 2: Particle size in comparison to common natural phenomena

The "coarse" particle mode is the difference between $PM_{10}$ and $PM_{2.5}$. It is variable because it includes wind-blown dust and some contribution from building operations; as a 'rule of thumb' $PM_{2.5}$ is normally between 50% and 80% of $PM_{10}$. [22]

The figure below summarizes what is known about particle size distribution and how size distribution is connected to more common measures of particle number and mass. The percentage values were based on 1995–1998 data from Erfurt [23] and it can be seen that whilst c 97% of the particle mass is found in the components > $PM_{0.1}$ this constitutes only 12% of the particle numbers (note that this is based on total $PM_{2.5}$ levels being 100% of the mass).

| | Contribution[a] | |
|---|---|---|
| Size (µm) | Number | Mass |
| **Ultrafine particles** | | |
| $NC_{0.01-0.03}$<br>$NC_{0.03-0.05}$<br>$NC_{0.05-0.1}$ | 88% | 3% |
| **Fine particles** | | |
| $MC_{0.1-0.5}$<br>$MC_{0.5-1.0}$<br>$MC_{1.0-2.5}$ | 12% | 97% |
| **Total ultrafine and fine particles** | | |
| 0.01–2.5 | 100% | 100% |
| **Coarse particles** | | |
| $PM_{10-2.5}$ | — | 20% |
| $TSP-PM_{10}$ | — | 30% |

[a] Based on the data from Erfurt 1995 to 1998: contribution of ultrafine and fine particles to number and mass in the size range of 0.01–2.5 µm and contribution of coarse particles to mass of total aerosol size distribution.

Size Ranges and Contribution to Number and Mass Concentration [23]

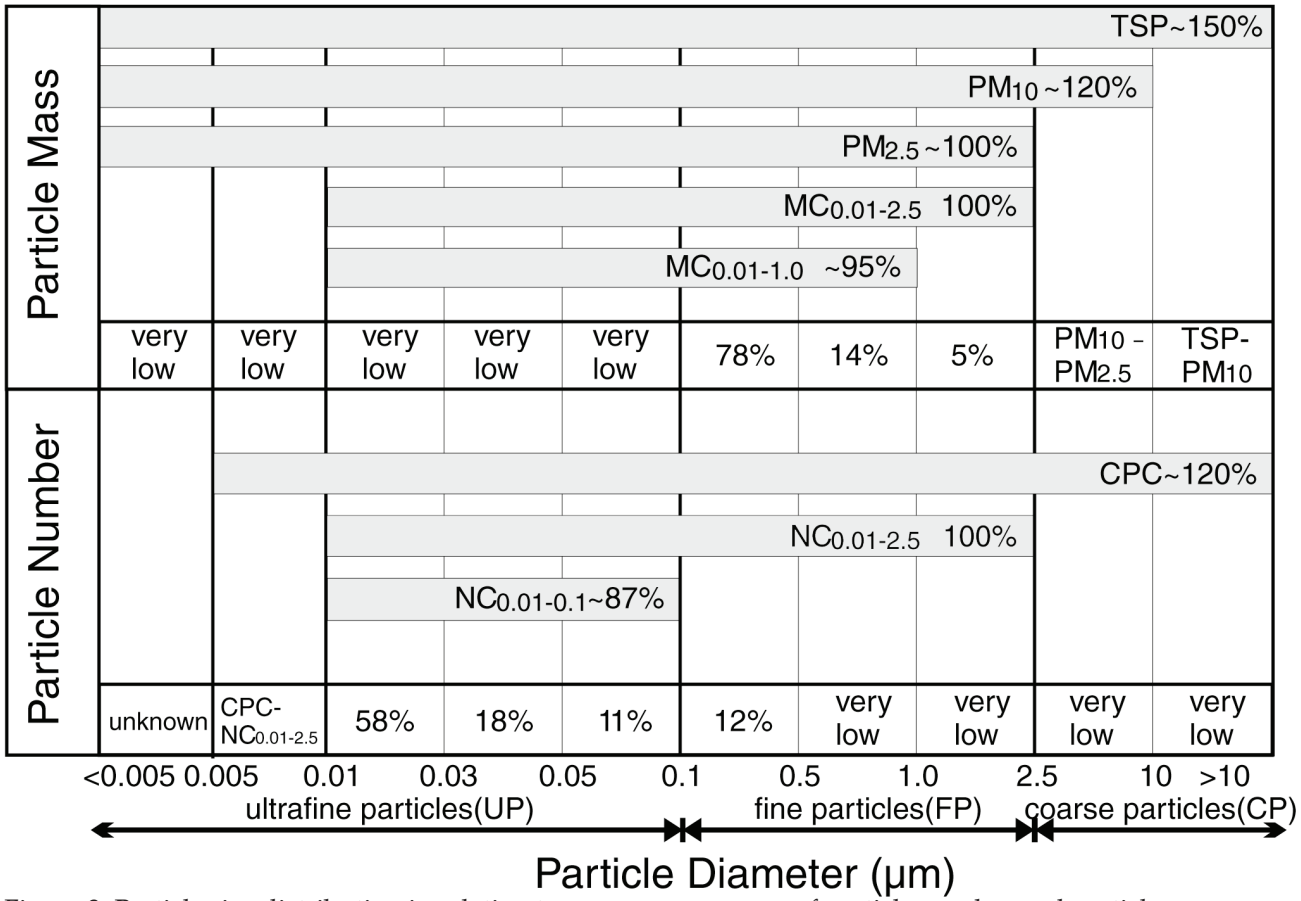


Figure 3: Particle size distribution in relation to common measures of particle number and particle mass

It is clear, therefore, that depending on their sizes, quite substantial differences in numbers or surfaces might constitute the same mass. Just one particle per $cm^3$ with a diameter of 2.5 $\mu$m is sufficient to result in a mass concentration of 10 $\mu g/m^3$ whilst more than two million particles of a diameter of 0.02 $\mu$m are needed to obtain the same mass concentration.

During the past 20 years, studies have largely been able to rule out sulphur dioxide and ozone pollution as the cause of the observed deaths although ozone is associated with increased mortality in daily time series studies (0.3–6.7% increase per 20 $\mu g/m^3$) and there is a weak association between SO2 and mortality (about 1% increase per 50 $\mu g/m^3$) which can be difficult to separate from particulate co-pollutants [24].

## 2.2 Ultrafine particles

Ultrafine particles (UFP) or nanoparticles[1], are very small pieces of matter defined as having dimensions less than $10^{-7}$ m. They constitute a small proportion of the mass of almost all types of particulate material. They also constitute the majority of the number of particles found in aerosols produced as a result of combustion processes. Their importance in the field of catalyst manufacturing, where their high surface area has a very great influence on reactivity, is widely known [25]. However, at present we know relatively little about their detailed structure, or their chemical and physical properties.

[1] Nanoparticles are smaller than 100nm, but in this evidence I take the terms to be interchangeable.

## 2.3 History and Regulation:

Regulation in Ireland of particulates as an air pollutant has been based on $PM_{10}$ (particles of <10 μm) and, more recently on $PM_{2.5}$ – although not, so far as I am aware for setting emission standards from processes like incinerators.

In common with many leading researchers in this developing field of nano-toxicology such as Donaldson's [26] and Oberdörster's [27] groups, I have long considered ultrafine particles to be the main contributor to its adverse effects. Though UFP is only a small fraction of $PM_{10}$ , Seaton et al. in 1995 [28] hypothesised biochemical processes whereby it might be the cause of acute cardiovascular effects. The 1999 Royal Society conference "Ultrafine particles in the atmosphere" and proceedings, published in 2000, consolidated the new thinking.

Urban air will often contain 100 billion ($10^{11}$) one-nanometre-diameter particles in each cubic meter of air, all of them invisible. By weight, these 100 billion particles will only amount to 0.00005 micrograms yet they may be responsible for much of the health damage created by fine-particle pollution. It is clear, therefore, that achievement of a regulatory standard does not ensure protection of health.

## 2.4 Lack of Standards and Monitoring for UFPs

Standards and monitoring are now being introduced for $PM_{2.5}$ particles – termed 'fine particles' and mostly 1,000 to 2,500nm in size – but there is nothing yet to cover the much smaller ones. The current standards are in terms of total mass, yet UFPs are generally around only one percent of the total mass but present the majority of the surface area that is reactive to human tissues. If the mass of a single inhaled 2.5 μm particle is divided into typical nanoparticles ~80nm, they would have 1000 times more surface area. For that reason alone, the mass-based PM standards are far from appropriate for UFPs.

Wichmann [23] reported some of the earliest epidemiology relating to UFPs and they showed a full distribution over particle sizes in urban air:

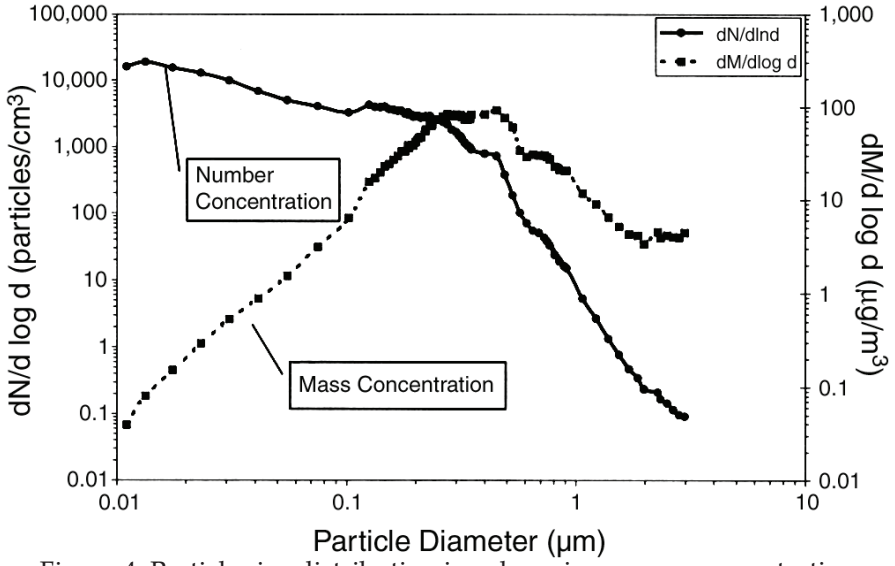

Figure 4: Particle size distribution in urban air mass vs. concentration

This does not show PM10 (cuts off at 3μm) but does indicate that most of the mass is in 0.2 to 0.5 μm particles, yet most of the particles ('number concentration') are under 0.2 μm (i.e. 200 nm).

## 2.5 Atomic Structure of Nanoparticles

It is only in the last twenty-five years, with the advent of high-resolution electron microscopy (HREM) at 0.1 nm (nanometre) levels, and the consequent ability to resolve inter-atomic spacings at this level, that any real attempt has been made to determine the atomic structure of ***individual*** particles. What has been learned is that these minute particles have an increasing proportion of surface atoms as the particle size decreases. Novel configurations of atoms have been demonstrated in nanoparticles, which cannot exist in the bulk material (Jefferson & Tilley, 1999). The imbalances between the number of atoms and number of electrons means the particles can be electrically charged and have raised chemical reactivity.

# 3 Damage to Health from Particulates

## 3.1 Fine Particles Linked to Human Deaths

US studies from the 90s first established that urban particulates in modern times were causing people to die. The 6-cities study of 1993 (Dockery et al.) was followed by the ACS study of half a million adult Americans in 151 metropolitan areas, which clearly established the relationship between fine-particle air pollution and human deaths, ruling out smoking as a cause of the observed deaths (Pope *et al.* 1995, Villeneuve *et al.* 2002, Pope *et al* 2002). This study is particularly important because it didn't simply match death certificates with pollution levels; it actually examined the characteristics (race, gender, weight and height) and lifestyle habits of all 552,138 people. Thus the study was able to rule out confounding factors of tobacco smoking (cigarettes, pipe and cigar); exposure to passive smoke; occupational exposure to fine particles; body mass index (relating to a person's weight and height); and alcohol use.

This study also controlled for changes in outdoor temperature. It found that fine-particle pollution was related to a 15% to 17% difference in death rates between the least polluted cities and the most-polluted cities. This research was vehemently attacked from a number of quarters, particularly those industries potentially most affected by the findings, which labelled it 'junk science'. However, an independent scientific panel conducted a thorough 're-analysis' and confirmed that tiny soot particles can shorten lives (HEI 2000). This basic finding was supported by a European study that found 6% of all deaths correlate with urban concentrations of fine particles, mainly from traffic [29].

The review of air pollution under the European Commission (Clean Air for Europe: CAFÉ) assisted by the WHO led to the Commission declaring in the *Thematic Strategy on Air Quality* that "*serious air pollution impacts persist*" [30].

The Commission also said "*currently in the EU there is a loss in statistical life expectancy of over 8 months due to $PM_{2.5}$ in air, equivalent to 3.6 million life years lost annually*". The thematic strategy shows that even with effective implementation of current policies this will reduce only to around 5.5 months (equivalent to 2.5 million life years lost or 272,000 premature deaths).

## 3.2 Effects of Particle Types and Mixtures

The effect of mixtures of particles of differing chemical composition entering the blood stream via the lungs in large numbers on a daily basis is beginning to be understood. There is no doubt that some particulate aerosols are indeed hazardous. However the degree of hazard associated with specific types of particle and the precise mechanisms by which exposure leads to pathology are as yet poorly understood and currently the subject of increasingly intense research.

Boekelheide [31] reported that pregnant rat dams were exposed to mixtures of phthalates (suppressors of testosterone synthesis within the fetal testis) and androgen receptor antagonists (acting at the end organs of this signalling pathway). The exposures were orchestrated so that any agent alone had very limited effects while the collective exposure robustly induced hypospadias and epididymal agenesis in the developing males. Overall, the chemicals clearly acted with dose additivity, not response additivity. These effects were induced by chemicals acting by different molecular mechanisms within different organ

systems with different absorption, distribution, metabolism, excretion patterns, and differently shaped dose response curves. By all of our familiar criteria, these chemicals are not toxicologically similar and do not share a mode of action as defined by the USEPA; and yet they can act together to inhibit this developmentally sensitive signalling pathway.

### 3.3 Threshold Levels

Successive studies have concluded there is no threshold, i.e. no level of fine-particle pollution below which no deaths occur. The ACS researchers have found that even air pollution levels that are well within legal limits are killing people, especially older people and those with chronic heart and lung ailments.

### 3.4 Respiration of particulates:

The average human lung contains about 2,300 km of airways and 480 million alveoli [32, 33]. On a daily basis, humans inhale around 10,000 litres of ambient air, which comes in close contact with a lung surface area of between 75 and 140 $m^2$. From this, 350 litres of oxygen diffuses across the alveolar capillary basement membrane into the 10,000 litres of blood flowing through the lungs daily [34]. The respiratory tract, therefore, comes into close contact with a large volume of ambient air and its components on a daily basis – the potential for uptake of contamination contained within that air is obvious.

Whilst US researchers switched to correlating $PM_{2.5}$ with health indicators authorities in Europe have tended to remained entrenched with the concept of $PM_{10.}$ There is, however, no longer and serious doubt that the size of the particles is the most important issue from a public health viewpoint and the reasons are obvious when the respiration of particles is considered in more detail.

- Particles larger than 10 $\mu$m (10 millionths of a metre) generally get caught in the nose and throat, never entering the lungs.
- Particles smaller than 10 $\mu$m ($PM_{10}$) can get into the large upper branches just below the throat where they are caught and removed (by coughing and spitting or by swallowing).
- Particles smaller than 5 $\mu$m ($PM_5$) can get into the bronchial tubes, at the top of the lungs.

Only particles smaller than 2.5$\mu$m ($PM_{2.5}$) in diameter can get down to the deepest (alveolar) portions of the lungs where gas exchange occurs between the air and the blood stream, oxygen moving in and carbon dioxide moving out [35]. The figure below shows whilst that PM ≥ 10μm in diameter enter the nose and mouth only the thoracic fraction, $PM_{10}$, passes the larynx and penetrates the trachea and bronchial regions of the lung, distributing mainly at pulmonary bifurcations. The respirable fraction, $PM_{2.5}$, and ultrafine PM, $PM_{0.1,}$ enter the nonciliated alveolar regions and deposit deep within the lungs.

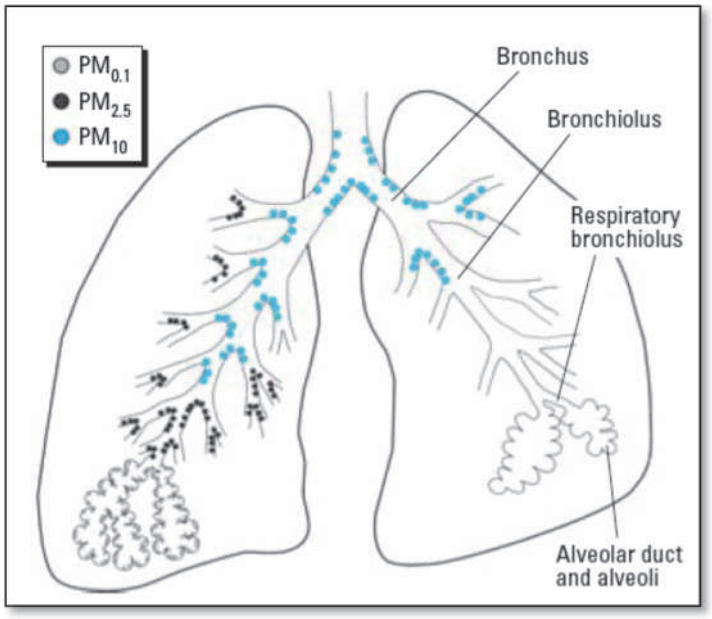


Figure 5: PM in the lungs (from [35])

Not all particles are retained. Larger particles deposit in the airways or mouth and throat, whereas smaller particles deposit in the alveolar region. A higher proportion of particles <1 $\mu$m that than those of $PM_{1.0}$ can be exhaled, thereby reducing deep lung deposition:

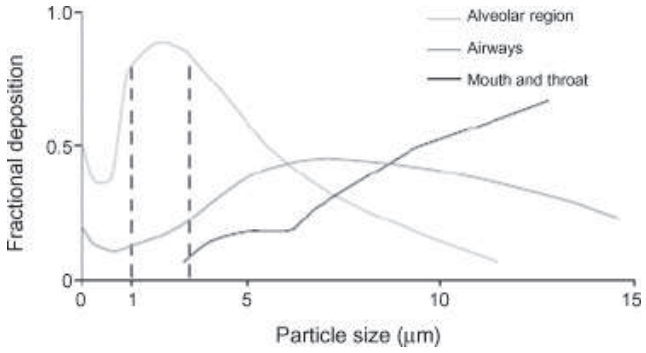


Figure 6: The effect of particle size on the deposition of aerosol particles in the human respiratory tract following a slow inhalation and a 5 s breath hold (from [33] )

### 3.5 Fate of particulates deposited in the lung

Removal of the smaller particles (<2.5 $\mu$m) deposited in the alveoli is difficult. If soluble in water, they pass directly into the blood stream within minutes. If insoluble, they are collected by scavenging cells called macrophages, which transport them to lymph nodes where they are retained for months or years (NRC, 1979). However, lung macrophage cells seem to have difficulty in recognising the smaller UFPs (those <65 nm; Donaldson et al. 1999), so may let some of them through the lung epithelium, especially during episodes of high numbers. Once they penetrate the epithelium and enter the blood stream, UFPs may be transported around the body and potentially be absorbed into cells – a process called endocytosis. Gumbleton [36], and more recently, Yang [33] have reviewed nanoparticle mobility and removal mechanisms including endocytosis. UFPs can cross biological membranes, in common with many viruses, and their mobility within the body is thought to be high.

### 3.6 The mechanism of toxic action

I have summarised and discussed a number of mechanisms by which UFPs can induce cell damage in my 2009 nanoparticle review for the WHO. Unfortunately this is not yet in the public domain and cannot yet be supplied to this inquiry. I will, however, briefly review some of the key developments here.

In recent years it has been established that Ultrafine particles:

- have a high specific surface area, which can catalyse reactions and adsorb high amounts of toxic substances (like PAH), providing a carrier deep into the lung during inhalation [28];

- have a higher deposition probability particularly in small airways and the alveolar region of the lungs than fine particles [11];

- respond differently in men and women - Women receive a greater dose than men in the head and tracheobronchial regions, for example [37];

- are less well phagocytized by alveolar macrophages than larger particles and inhibit their phagocytic ability [38];

- are taken up by other cells of the respiratory epithelium, such as epithelial cells, dendritic cells [39, 40];

- may form complexes with proteins and biomolecules which may result in functional changes of the latter [41];

- have greater access to interstitial spaces than larger particles [42, 43]);

- have access to the blood circulation [43-45];

- induce more oxidative stress than fine particles [15, 46] ;

- cause more pro-inflammatory responses than larger particles [47] ;

- have greatly enhanced toxic potential due to their free location and movement within cells, which promote interactions with intracellular proteins and organelles and even the nuclear DNA [48] ;

- adversely affect cardiac functions and vascular homeostasis [49];

- affect the immune system [27].

For all of these hypotheses there exists a growing body of studies on a mechanistic level providing plausibility or evidence, however, on different levels of causality. From many of these studies it became also clear that the hypotheses listed above may only be applicable to susceptible organisms and individuals predisposed either by disease, genetics or age while the healthy organism does not show any such sensitive reactions.

A large number of studies confirm that fine-particle pollution is responsible for, or exacerbating, a wide range of human health problems, including:

- initiating and worsening asthma, especially in children;
- increasing hospital admissions for bronchitis, asthma and other respiratory diseases;

- increasing emergency hospital visits for respiratory diseases;
- reducing lung function (though modestly) in healthy people as well as (more seriously) in those with chronic diseases;
- increasing upper respiratory symptoms (runny or stuffy nose; sinusitis; sore throat; wet cough; head colds; hay fever; and burning or red eyes);
- increasing lower respiratory symptoms (wheezing; dry cough; phlegm; shortness of breath; and chest discomfort or pain); and
- increasing heart disease.

The 1995 hypothesis of Seaton *et al.* [28] suggested that the particles retained in the deep lung cause inflammation which, in turn, releases natural chemicals into the blood stream causing coagulation of the blood. This was to explain epidemiological findings of increased cardiovascular disease in populations exposed to higher than average $PM_{10}$ exposure [50]. There may be a low exposure threshold, above which these effects will occur, but it appears the classical toxicological dose-response curve is not appropriate. The main end point under investigation is arterial damage, which is consistent with the 1965 findings of Aurerbach that smokers, who voluntarily inhale particulate aerosols, almost all sustain arterial damage themselves.

*In vivo* studies performed on laboratory animals have looked at the ability of UFPs to produce inflammation in lungs after exposure to UFP aerosols [26, 47, 51, 52] . The degree to which UFPs appear to be able to produce inflammation is related to the smallness of the particles, the 'age' of the aerosol and the level of previous exposure. It has been hypothesised [28] that the chronic inhalation of particles can set up a low grade inflammatory process that can damage the lining of the blood vessels, leading to arterial disease.

Most health studies are now using $PM_{2.5}$, though as runs of data in Europe tend to be of $PM_{10}$, uncertain corrections are often made. There are few data runs for ultrafine particles ($PM_{0.1}$), despite the finding [53] that they were on an increasing trend (while $PM_{10}$ was decreasing) and probably more hazardous.

### 3.7 UFPs penetrating into the human body

There is considerable evidence to show that inhaled UFPs can gain access to the blood stream and are then distributed to other organs in the body [54].They can even cross the placental barrier.

One needs also to compare the particle sizes with biology, as in figure two above from Brook et al. [21]. UFPs are much smaller than bacteria, against which cells can defend themselves, and of similar size or smaller than viruses, which can relatively easily penetrate between cells.

The 'passageways' for nanoparticles into and then subsequently around the body are the 'caveolar' openings in the natural membranes which separate body compartments. These openings are between 40 and 100 nm in size and are thought to be involved in the transport of 'macromolecules' such as proteins, including on occasion viruses. They also happen to be about the right size for transporting UFPs. Most of the research on that, to date, has been performed by the pharmaceutical industry, which is interested in finding

ways of improving drug delivery to target organs. This is particularly so for the brain, which is protected by the 'blood brain barrier' which can be very restrictive. This has been reviewed by Gumbleton [36].

Although there are clear advantages to the intentional and controlled targeting of 'difficult' organs, such as the brain, with nanoparticles to increase drug delivery, the obverse of this particular coin needs to be considered. When environmental UFPs (such as from traffic pollution or incineration) gain unintentional entry to the body, it appears that there is a pre-existing mechanism which can deliver them to vital organs [36]. The body is then 'wide open' to any toxic effects that they can exert. The probable reason that we have not built up any defences is that any such environmental toxic UFPs were not part of the prehistoric environment in which we evolved and therefore there was no requirement to develop defensive mechanisms.

Peters et al. [55] having established the vulnerability of remote organs – and particularly the brain - wrote "*The results indicating that particles may contribute to the overall oxidative stress burden of the brain is particularly troublesome, as these long-term health effects may accumulate over decades*". They stressed the need for increased efforts to quantify the relative risks for long-term particle exposure on the onset of Parkinson's and Alzheimer's disease adding "*both Parkinson's and Alzheimer's disease are only diagnosed once manifest clinical signs and symptoms are evident and impact the diseased persons by long years of disabilities and diminished quality of life*". The exposure of the brain to UFPs is a matter of great concern - if our limited capacity to deal with misfolded protein is exceeded then the likely sequelae would be an increase in the incidence of protein misfolding disease in the general population and a tendency to an earlier average age onset.

### 3.8 Quantifying the Established Health Impacts

A range of impacts have been reported by different researchers for different outcomes. Kunzli [56], for example, reported elevations of 10 $\mu g/m^3$ and 20 $\mu g/m^3$ in $PM_{2.5}$ were associated with 5.9% and 12.1% increases in the development of atherosclerosis in "*healthy*" people who had no previous signs of acute coronary syndromes, but had small elevation of low-density lipoprotein.

Miller et al. reported an increased relative risk of 1.76 for death from cardiovascular disease for every increase of 10 μg per cubic meter in the mean concentration of $PM_{2.5}$ [57].

By comparison, a study by the American Cancer Society showed that each increase of 10 μg per cubic meter in the mean $PM_{2.5}$ concentration was associated with an increased relative risk of 1.12 for death from cardiovascular disease, 1.18 for death from ischemic heart disease (the largest proportion of deaths), and 1.13 for death from arrhythmia, heart failure, or cardiac arrest [58].

Commenting on these data in an editorial of the New England Journal of Medicine Dockery [59] wrote:

*"A multifaceted approach that encompasses both public health and medical interventions is needed to reduce the burden of cardiovascular disease attributable to air pollution. Comprehensive management of the harmful effects of fine particles must start with intensive efforts to reduce this destructive form of air pollution. Fine particulate air pollution results not only from the combustion of carbonaceous fuels in our vehicles, power plants, and factories but also from secondary particles produced by oxidation of gaseous pollutants emitted by these same sources".*

I note that these secondary particles have not been considered in the application at all and have not been incorporated in the (very limited) assessment of risks. It is clear however that even without the consideration of secondary particulates it is not reasonable to describe the particulate emissions from the proposed incinerators as having no impacts.

### 3.9 Children as vulnerable and sensitive sub-population:

The WHO and European Commission have recognised that children are specially affected by PM pollution. The WHO *Monograph: the Effects of Air Pollution on Children's health and development: a review of the evidence* [60] reviewed factors affecting children's susceptibility, effects on pregnancy outcomes, infant and childhood mortality, lung function development, asthma and allergies, neurobehavioural development and childhood cancer. It declared that "*the amount of ill-health attributable to air pollution among European children is high*".

The *Children's Environment and Health Action Plan for Europe* (CEHAPE), adopted at the *Budapest Ministerial conference* in June 2004 [61], included air pollution in increasing concern about environmental effects on children's health. It agreed that developing organisms, especially during embryonic and foetal periods and early years of life, are often particularly susceptible. It's now recognised that the inhibition of children's lung development can be very serious, potentially meaning long term harm to their respiratory health. Evidently air pollutants, most probably including particulates, cause harm to children differently to adults.

The expert science view, summarised by Joel Schwartz [62] is that children's exposure to air pollution is of special concern because their immune system and lungs are not fully developed, so many of the epidemiological associations are likely to be causal. The review by Heinrich and Slama [63] found that ambient fine PM is associated with intra-uterine growth retardation, infant mortality; impaired lung function and postneonatal respiratory mortality, but less consistently with sudden infant death syndrome. Hertz-Picciotto et al. [64] found bronchitis in early childhood correlates with $PM_{2.5}$ and PAH levels (UFPs may be a carrier for PAH – see above). While these findings may not all be conclusive, there can be no doubt that children and even the fetus are particularly vulnerable to particulate air pollutants – while this has largely been overlooked in setting current standards and controls.

A review of health effects of poor air quality on children's health [65] emphasised the hazards associated with the siting of major particle-emitting plants and roads in the vicinity of schools or communities containing children.

### 3.10 Prenatal Exposure:

A 2007 Editorial [66] in the Journal "Reproductive Toxicology" summed up the increasing concerns associated with prenatal exposure admirably:

*"There is a major paradigm shift taking place in science that while simple is profound. It states that the root of many diseases, including reproductive diseases and dysfunctions, will not be found by examination of disease onset or etiology hours, days, weeks, or even years prior to disease onset. The new paradigm suggests that susceptibility to disease is set in utero or neonatally as a result of the influences of nutrition and exposures to environmental stressors/toxicants. In utero nutrition and/or in utero or neonatal exposures to environmental toxicants alters susceptibility to disease later*

*in life as a result of their ability to affect the programming of tissue function that occurs during development. This concept, that is still a hypothesis undergoing scientific testing and scrutiny, is called the developmental basis of health and disease".*

There is a growing recognition of the importance of the prenatal period as a ''window of exposure'' for the development of childhood, and possibly adulthood, disease [67]. Henderson et al. [68] have investigated the effects of mothers' exposure to household chemicals during pregnancy, but they acknowledged the difficulty in determining whether the reported health effects could be attributed to pre- or postnatal exposure, or even both. They observed that chemical use in the home before and after birth was highly correlated, making it difficult to separate potential effects of exposure during these periods.

Jedrychowski et al. [69] reported that prenatal exposure to $PM_{2.5}$ particulate matter had a moderate but significant impact on severity of respiratory illness in postnatal early life. The biological mechanisms whereby prenatal $PM_{2.5}$ exposure might cause adverse health outcomes in children are yet unclear. $PM_{2.5}$ is a proxy measure of a whole complex of toxic agents present in the environment – including PAHs – that could adversely affect growth and maturation of lung in early childhood.

Fine particles are usually a product of combustion processes that generate other toxic agents which may interact at the molecular level with DNA as described by Perera et al. [70]. Prenatal exposure to immunotoxic fine particles may impair the immune function of the fetus and subsequently may be responsible for an increased susceptibility of newborns and young infants to respiratory infections.

The synergism of recently proposed role of sulphur dioxide metabolites as inhibitors of enzymes and antioxidants and the adverse effects of nitrogen oxide metabolites in the early embryonic development may lead to symmetric intrauterine growth restriction and premature delivery or low birthweight. The research is directed to point out the toxics from coal combustion products as neglected causes of oxidative stress on human embryogenesis, prematurity, and low birthweight. [71]

### 3.11 Future Research:

Cormier et al [35] have reviewed the evidence for potential health impacts of particulate emissions from combustion processes. They posed a series of questions that require addressing:

- How are combustion-generated fine PM and ultrafine PM formed?
- How do their chemical properties differ from larger PM?
- What is the nature of association of chemicals with these particles?
- How is the chemical and biological reactivity of these chemicals changed by association with the particles?
- What is the role of PM-associated persistent free radicals in the environmental impacts of fine and ultrafine PM?
- What is the role of PM on cell/organ functioning at initial sites of exposure?
- What is the bioavailability of these particles to other tissues?
- How are these particles translocated to these secondary sites, and do their chemical properties change en route?
- How does acute/chronic exposure lead to adverse organ pathophysiology? Is developmental timing of exposure important?
- What effect does exposure have on predisposing to disease states or on disease progression?

- Most important, what are the specific cellular and molecular mechanisms associated with airborne exposures?

Medical science has been rather slow to fully recognize and explore the serious problems that particulate emissions cause. In spite of the thousands of papers that have been published over the past decade on the issue of UFPs it will inevitably be many years before the answers to all the questions posed are available. Meanwhile it is sensible that particulate emissions, especially those produced in conjunction with toxic chemicals, are reduced so far as possible and that new sources are avoided.

# 4 Particulate Releases from Incinerators

Modern incinerators are a major source of fine particulate emissions. In 2007, for example, Widory et al. [72] found:

*"The main sources of atmospheric particle pollution in Paris are vehicles, central heating and waste incinerators".*

It is important to bear in mind that the contribution is not just direct PM emissions, which are now relatively low in terms of total mass and emission concentrations (though not in terms of numbers). Particulate emissions and impacts also include secondary inorganic compounds which can account for a major fraction of $PM_{10}$, and especially of the $PM_{2.5}$ mass [73]. Almeida [74] found lower but still significant contributions from these secondary particles.

As $NO_x$ emissions from modern incinerators are still rather high (I understand that they normally operate close to the 200 $mg/m^3$ emission limit) then because of the increased size of modern plants compared with those operated in the early 1990's total levels are of the same order as historically – and the $NO_x$ emissions can form nitrates with metals in the incinerator plume and thus increase the toxicity and availability of the emissions as described by Moffet [75]:

*"The frequent observation of these metal-rich particles in an urban area with a high population density also has important implications for health effects. The largest fraction of the Pb-containing particles is less than 2.5 μm, meaning that these particles may be efficiently inhaled. Also, there may be important health ramifications if salts such as $Pb(NO_3)_2$ are formed because lead nitrate is soluble, and therefore more mobile within the human body".*

Indaver appear to have completely omitted any consideration of secondary particulates and their impacts from their assessment.

Table 9.2 of the application shows that the proposed Ringaskiddy incinerators would produce 125,486 $Nm^3/hr$ from the grate incinerator and 116,995 $Nm^3/hr$ from the Fluidised bed incinerator i.e a total emission of 242,481 $Nm^3/hr$. The permitted particulate emission standard, subject to statistical limits, would be 10 $mg/m^3$ and for oxides of nitrogen 200 $mg/m^3$. Daily emissions could therefore total 5,819,544 $m^3$ containing 58.2 kg of particulates and 1,164 kg of $NO_x$ .

These are large emissions in any terms – without any consideration of secondary particulates the authorised incinerator emissions would have the potential to daily fill a space 11km x 11km by 50 m deep to the WHO annual guideline of 10 $\mu g/m^3$ for $PM_{2.5}$.

Secondary particles should, of course, be considered in any case. The formation mechanism of nitrates as secondary particles is illustrated below [76]:

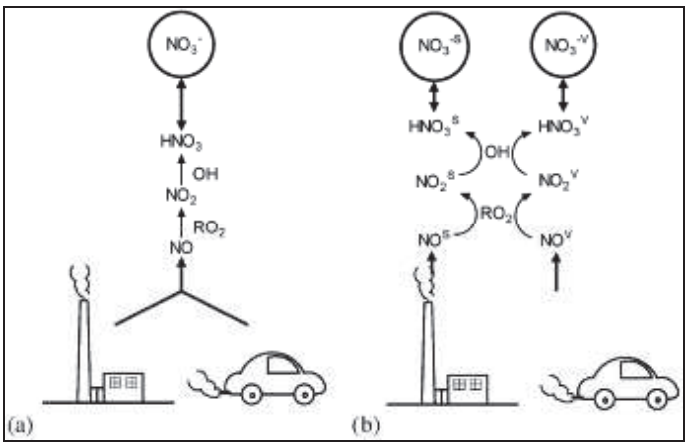


Figure 7: Illustration of source apportionment for secondary PM2.5 nitrate from two sources. (a) Formation of secondary PM2.5 nitrate in traditional air quality model using lumped NO emissions. (b) Formation of secondary PM2.5 nitrate from NO emitted from two sources tracked separately in the source-oriented air quality model used by Ying (from [76]). $RO_2$ represents a peroxy-type radical, and OH represents hydroxyl radical.

Furthermore emissions from an incinerator installed with a selective non-catalytic reduction (SNCR) $NO_x$ control system as proposed here may actually increase direct emissions of ammonium nitrate which is an important component of $PM_{2.5}$

The efficiency of the filter is therefore not the most significant aspect of the total particulate emission and control of NOx (and to a lesser extent SOx is actually more significant in terms of the contribution to ground level concentrations although neither appear to have been modelled in this application.

### 4.1 Filter Efficiency:

The proposed incinerator would use a bag filter as the main primary particulate abatement technology. For a given fibrous filter, there is a particle size, usually between 0.05 and 0.5 μm that has the minimum collection efficiency [77] ; that is, all particles, larger or smaller than this size, are collected with greater efficiency. For a given size particle, there is also a velocity for minimum collection efficiency. It is important to establish where this minimum efficiency lies, what the particle density of the emissions at that point are and what the speciation of contaminants (both metals and products of incomplete combustion) carried by those particulates is.

Waste incinerators with the most modern bag filter technology for clean-up of flue gases still emit an aerosol of ultrafine particles, unlimited by legislation [78-81].

Collection efficiencies for particles < 2.5 μm are between 5 and 30% before the filters become coated with lime and activated carbon.

| Particle size | Collection efficiency |
|---|---|
| PM10's | between 95% and 98% |
| PM 2.5's | between 65% and 70% |
| PM below 2.5 | between 5% and 30% |

Efficiency of baghouse filters for particles of differing sizes as claimed by operators. (Onyx 1999)

Though there have been improvements since 1999, the bag filter technology generally used on municipal waste incinerators is not efficient at filtering very fine particles. For particles of less than 1 μm down to about 0.2 μm the abatement efficiency is low. Although very high capture rates, based on gravimetric indices, are generally claimed, the majority by number of ultrafine particles will pass through and current standards do not take into consideration the sizes of the particles emitted by an incinerator. Thus modern plants with their very high gas fluxes are guaranteed to produce an ultrafine particulate aerosol.

Aboh [17] concluded that depending on the number of variables considered, waste incineration and local sources contributed between 17 and 32 percent of $PM_{2.5}$. Whilst the quantitative contribution from the different sources may be treated as indicative since the number of observations were small compared to the number of variables relative strength of the identified sources was seen to change when the variables included in the analysis were varied in number and character, although the same sources remained:

| | Waste incineration and local sources | Oil incineration | Biomass burning | Long distance transport (LDT) | Traffic emissions |
|---|---|---|---|---|---|
| 19 variables | 32 | 33 | 18 | 16 | 1 |
| 14 variables | 28 | 29 | 9 | 23 | 12 |
| 8 variables | 17 | 21 | 7 | 41 | 14 |
| 6 variables | 24 | 11 | 8 | 51 | 6 |

Ogulei [82] used applied multivariate data analysis methods to a combination of particle size and composition measurements in Baltimore to apportion particulate sources and found that the majority of all the observed Lead (63.4%) and most of the Zn (32.6%) could be attributed to a waste incinerator source. The closest major municipal incinerator to the monitoring site was c. 5 miles away in a direction corresponding to the direction suggested by their analysis. The contribution from this incinerator was about 7.9% which was comparable to the 9.3% contribution that was obtained in their earlier study [83]. The size distribution for this source indicated two modes at 0.02 and 0.15 mm. Whilst the incinerator made approximately the same contribution as both local petrol traffic (8.11%) and coal fired power station (10.34%) the particulate peak was smaller than each of the others and the concentration of heavy metals was much greater in the incinerator particulates.

Ultrafine particle concentrations have been shown to be raised in the plume of a hospital incinerator[3] 350 metres downwind of the plant [84].

### 4.2 Bimodal Size distribution

It has been known for many years that Aerosol emissions from combustion processes including waste incineration tend to show a bimodal mass distribution with a peak of coarse particles and another of ultrafines *[85, 86].*

Friedlander [87] wrote:

*The coarse mode consists of particles with diameters in the range between 1μm and about 100μm. In pulverized coal combustion they are formed from the nonburnable mineral inclusions within the fuel particles (Flagan and Friedlander, 1978). In addition to the large fly ash particles there often exists a*

[3] The ratio of $SO_2/NOx$ is greater than from vehicle emissions suggesting a fuel of higher sulphur content and discounting a gas fired boiler as an alternative source.

*mode of small submicron sized particles which pose a health risk because they are inhalable and may be enriched in toxic metal compounds.*

Friedlander pointed out, as we return to below, that the submicron particles are usually less efficiently captured by filter devices and hardly fall under gravity so remain longer in the air .

Ruokojarvi [88] found that half the particle mass in incinerator emissions was under 1.6*μm*, the remainder in a broad distribution up to 14.5*μm.*

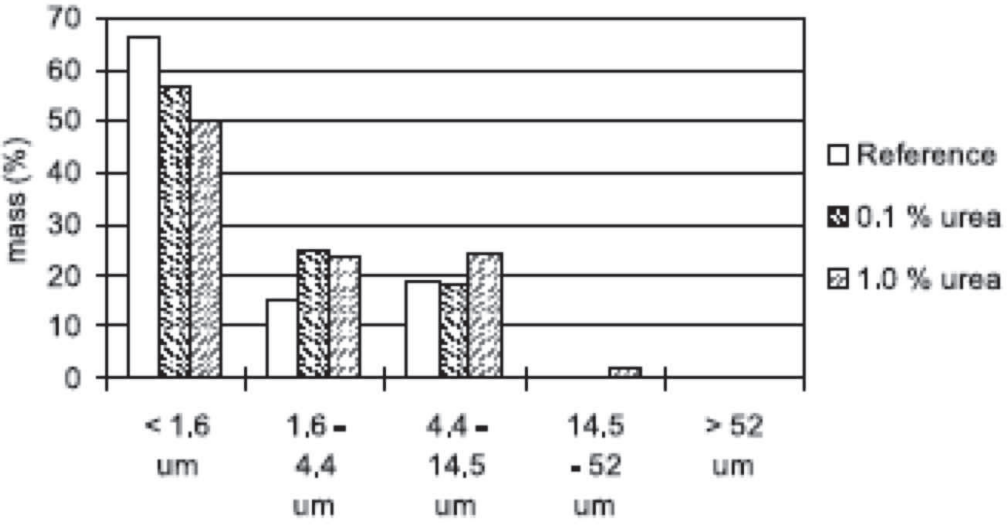


Fig. 4. Mass distribution of particles collected in the cascade centripeter samples.

This figure shows that half the mass is below 1.6μm, somewhat less than in the urban air of Wichmann [23] but it doesn't show the UFPs. Little information has been provided on particles under 1 μm size as the industry is uncomfortable over the issue. Some other data is given below.

### 4.3 Surface Area of incinerator particles:

The US EPA [89] characterisation of incinerator particulate emissions in the Table below showed that particles <0.7 μm have half the total surface area. Insofar as surface area in contact with lung's surface (epithelium cells) is relevant to exposure/dose effects, the smallest particles carry high weighting, unlike where the total mass (PM index) is considered.

| Particle Diameter (μm)[a] | Particle Radius (μm) | Surface Area/ Volume | Fraction of Total Weight | Proportion Available Surface Area | Fraction of Total Surface Area |
|---|---|---|---|---|---|
| >15.0 | 7.50 | 0.400 | 0.128 | 0.0512 | 0.0149 |
| 12.5 | 6.25 | 0.480 | 0.105 | 0.0504 | 0.0146 |
| 8.1 | 4.05 | 0.741 | 0.104 | 0.0771 | 0.0224 |
| 5.5 | 2.75 | 1.091 | 0.073 | 0.0796 | 0.0231 |
| 3.6 | 1.80 | 1.667 | 0.103 | 0.1717 | 0.0499 |
| 2.0 | 1.00 | 3.000 | 0.105 | 0.3150 | 0.0915 |
| 1.1 | 0.55 | 5.455 | 0.082 | 0.4473 | 0.1290 |
| 0.7 | 0.40 | 7.500 | 0.076 | 0.5700 | 0.1656 |
| <0.7 | 0.40 | 7.500 | 0.224 | 1.6800 | 0.4880 |

Total surface area: 3.4423 $\mu m^2$

Notes: a. Geometric mean diameter in a distribution. Distribution from EPA (1980).

Research has shown that even normally harmless bulk materials tend to become toxic when divided into ultrafine particles. Generally, the smaller the particles, the more reactive and toxic their effect [51, 52]. This is no surprise, because catalysts to enhance industrial chemical reactions are commonly made this way. Making surfaces that are irregular on the scale of just a few hundred atoms creates an enormous area of reactive surface. It is on this surface that catalytic reactions, such as the formation of halogenated organic molecules, can occur. Indeed, because of surface roughness, ash particles can have surface areas 20-30 times the surface area of equivalent spheres [90]. Some of the most reactive nanoparticles to have been studied to date are metals and spinel metal oxides [25]. The upper size limit for such enhanced toxicity of UFPs is not well defined but is generally given between 65 and 200 nm.

### 4.4 Speciation – inorganic components

Although the particles emitted from large-scale industrial combustion sources are all predominantly in the fine-particle range, their chemical compositions varies substantially depending largely upon fuel types and boiler or furnace operating conditions. This can be illustrated using the fractional abundances of the elements and chemical compounds in the particulate emissions[91].

Typical chemical abundances in source emissions

| Source | Dominant particle size | Chemical abundance (mass fractions) | | | |
|---|---|---|---|---|---|
| | | >10% | 1–10% | 0.1–1% | <0.1% |
| Coal-fired boiler | Fine | Si | $SO_4^{2-}$, OC, EC, S, Ca, Fe, Al | $NH_4^+$, P, K, Ti, V Ni, Zn, Sr, Ba, Pb | Cl, Cr, Mn, Ga, As, Se, Br, Rb, Zr |
| Incinerator | Fine | $NH_4^+$, Cl, $SO_4^{2-}$, OC | $NO_3^-$, Na, EC, Si, S, Ca, Fe, Br, Pb | K, Al, Ti, Zn, Hg | V, Mn, Cu, Ag, Sn |
| Residual oil boiler | Fine | S, $SO_4^{2-}$ | Ni, OC, EC, V | $NH_4^+$, Na, Zn, Fe, Si | K, OC, Cl, Ti, Cr, Co, Ga, Se |
| Wood waste boiler | Fine | K | Na, Fe, Mn | Zn, Br, Cl, Rb | Cr, Cu, Co, Ni, Se, Cd, Ar, Cr, Pb |

Key: OC = organic carbon, EC = elemental carbon.

This indicates incinerators are special for Pb, Hg and Br emissions (none of which come in particulates from vehicle emissions).

### 4.5 Particle Speciation:

Metal emissions from incineration of solid wastes are impacted by compositions of feedstocks and the chemical form of the metals depends on the operating conditions of the incinerator (Wey et al. [92]). A number of studies have identified the 'signature' of incinerators from the metal species. Harrison et al. reported on Birmingham air sampling in 1997 [93], finding zinc and copper to indicate an incineration source. They saw this as the large municipal refuse incinerator within the city (Tyseley), which at the time of sampling was not subject to the tighter Waste Incineration Directive limits.

In the city of Seoul, Mishra et al. [94] found via principal components analysis suggest incineration and the iron and steel industry as possibly significant sources of Pb in particulate matter. Doucet and Carignan [95] examined lead isotopes in French lichens and flyash from different municipal solid waste combustors in the Rhine valley and in other areas of France, concluding that "*these plants* (ie the incinerators) *might be an important source of industrial Pb in the atmosphere*".

Pancras reported [96] "*Large but brief 1.5-h excursions in Zn, Cd, and Pb were found to correlate with winds from the direction of an incinerator in Florida at 17km distance*".

### 4.6 Speciation – volatile and organic components

Out of over 11 million known chemicals, about 100,000 are being produced on industrial scale and about 1,000-2,000 new chemical entities are being introduced each year [97]. Any of these industrial chemicals may be disposed of by incineration and there is a near infinite number of possible combustion and incomplete combustion products that may be emitted either as particulate matter or by adsorbtion onto or reaction on the surface of particulates. Even if these emissions were monitored, and the vast majority are not, then little or nothing is known about the possible health impacts of the bulk of these emissions.

Volatile chemicals condense on particle surfaces as the incinerator exhaust gases cool. Their concentration on smaller particles is higher, being related to surface area rather than particle mass. This has been subject to particular studies for dioxin and dioxin-like chemicals, but is likely to be similar for many others e.g. [98]. It also holds for volatile chemicals that incinerator UFPs pick up from urban air, specifically the PAHs from vehicle emissions. These cannot penetrate into the body as gases, but if attached firmly to UFPs can be carried through the lung epithelium.

### 4.7 Range of chemicals coating the particles

There are thousands of chemicals emitted by incinerators. Jay and Stieglitz [99] identified 227 individual organic compounds[4] corresponding to ca. 42% of the total organic carbon

[4] Including: acetic acid, acetone, acetonitrile, aliphatic alcohol, aliphatic amide, aliphatic carbonyl, anthraquinone, benzaldehyde, benzene, benzoic acid, benzoic acid methyl ester, benzoic acid phenyl ester, benzonitrile, benzophenone, benzothiazole, benzyl alcohol, benzyl alcohol, benzylbutylphthalate, bibenzyl, bromochlorobenzene, bromochlorophenol, 2-bromo-4-chlorophenol, bromodichlorophenol, 4-bromo-2,5-dichlorophenol, butanoic acid ethyl ester, 2-butoxyethanol, butyl acetate, C10H20 HC, C10H22 HC (1), C10H22 HC (2), C11H15O2N aromatic, C12H26 HC, C12H26O alcohol, C13H28 HC, C15 acid phthalic ester, C4 alkylbenzene, C5 alkylbenzene, C6H10O2 aliphatic carbonyl, C6H12O, C8H14O cyclohexanone, derivative, C8H5BrCl3 aromatic, MW, 284, C8H5O2N, C9H18O3 aliphatic, C9H8O aromatic, caffeine,

(TOC) in flue gas from an incineration facility of MSW. The identifications exceeded ~50 ng/$m^3$, 500x higher than the dioxin emission limit set in the Waste Incineration Directive. About 3% of the TOC consisted of halogenated compounds, almost all of which were volatile compounds, while all of the identified semi- and nonvolatile halogenated compounds were aromatic compounds. Besides, 7% of the TOC was aromatic hydrocarbons and 3% of the TOC was phenols [100]. Highly carcinogenic compounds such as dibenzopyrene isomers have been identified and determined in Swedish incinerator emissions by other researchers [101] and it is likely that due to the very heterogeneous nature of the waste emissions will constantly vary with consequences for the speciation of ultrafine particulate emissions.

Similarly Leach [102] found a wide range of VOCs in ground level monitoring around the Marchwood incinerator pre and post shutdowns in November 1996. Although that incinerator has since been replaced the results are indicative of the range of post combustion VOCs that are likely to be found in more modern facilities.

---

chlorobenzene, chlorobenzoic acid, 4-chlorobenzoic acid, chloroform, 2-chloro-6-methylphenol, 4-(chloromethyl)toluene, 2-chlorophenol, 4-chlorophenol, cholesterol., cyclohexane, cyclopentasiloxanedecamet, hyl, cyclotetrasiloxaneoctamethy, l, decane, decanecarboxylic acid, dibenzothiophene, dibutylphthalate, 1,2-dichlorobenzene, 1,3-dichlorobenzene, 1,4-dichlorobenzene, 2,4-dichloro-6-cresol, dichloromethane, 2,6-dichloro-4-nitrophenol, 2,4-dichlorophenol, dichloromethylphenol, 1,3-diethylbenzene, diisooctylphthalate, 2,2'-dimethylbiphenyl, 2,3'-dimethylbiphenyl, 2,4'-dimethylbiphenyl, 3,3'-dimethylbiphenyl, 3,4'-dimethylbiphenyl, 1,2-dimethylcyclohexane, 1,2-dimethylcyclopentane, 1,3-dimethylcyclopentane, dimethyldioxane, dimethyloctane, 2,2-dimethyl-3-pentanol, dimethylphthalate, 2,6-di-t-butyl-*p*benzoquinone, 2,4-di-t-butylphenol, docosane, dodecane, dodecanecarboxylic acid, eicosane, ethanol-1-(2-butoxyethoxy), ethyl acetate, 4-ethylacetophenone, ethyl benzaldehyde, ethylbenzene, ethylbenzoic acid, 2-ethylbiphenyl, ethylcyclohexane, ethylcyclopentane, ethyldimethylbenzene, ethylhexanoic acid, 1-ethyl-2-methylbenzene, 1-ethyl-4-methylbenzene, ethylmethylcyclohexane, 2-ethylnaphthalene-1,2,3,4-, tetrahydro, 1-ethyl-3,5-xylene, 2-ethyl-1,4-xylene, fluorene, fluorenone, fluoroanthene, formic acid, 2-furanecarboxaldehyde, heneicosane, heptadecane, heptadecanecarboxylic acid, heptane, 20, heptanecarboxylic acid, 2-heptanone, hexachlorobenzene, hexachlorobiphenyl, hexadecane, hexadecane amide, hexadecanoic acid, hexadecanoic acid, hexadecyl ester, 9-hexadecene carboxylic, acid, hexanecarboxylic acid, 2-hexanone, hydroxybenzonitrile, hydroxychloroacetophenone, 2-hydroxy-3,5-, dichlorobenzaldehyde, hydroxymethoxybenzaldehy, de, 2-(hydroxymethyl) benzoic, acid, iodomethane, 1(3H)-isobenzofuranone-5-, methyl, isopropylbenzene, methyl acetophenone, 2-methylbenzaldehyde, 4-methylbenzaldehyde, methylbenzoic acid, 4-methylbenzyl alcohol, 2-methylbiphenyl, methylcyclohexane, methyldecane, 3-methyleneheptane, 5-methyl-2-furane, carboxaldehyde, methylhexadecanoic acid, 2-methylhexane, 3-methylhexane, methyl hexanol, 2-methylisopropylbenzene, 2-methyloctane, 2-methylpentane, methylphenanthrene, nonedecane, 4-methylphenol, 1-methyl-2-, phenylmethylbenzene, 2-methyl-2-propanol, 1-methyl-(1-, propenyl)benzene, 2-methylpropyl acetate, 1-methyl-2-propylbenzene, 1-methyl-3-propylbenzene, methylpropylcyclohexane, 12-, methyltetradecanecarboxyli, c acid, naphthalene, N-bearing aromatic, MW, 405, nitrogen compd, MW 269, 2-nitrostyrene, nonane, octadecadienal, octadecadienecarboxylic, acid, octadecane, octadecanecarboxylic acid, octane, octanoic acid, paraldehyde, pentachlorobenzene, pentachlorobiphenyl, pentachlorobiphenyl, pentachlorophenol, pentadecacarboxylic acid, pentane, pentanecarboxylic acid, phenanthrene, phenol, phthalic ester, phthalic ester, propylbenzene, propylcyclohexane, pyrene, Si organic compd, sulphonic acid m.w. 192, sulphonic acid m.w. 224, 2-t-butyl-4-methoxyphenol, tetrachlorobenzene, 1,2,3,5-tetrachlorobenzene, tetrachlorobenzofuran, tetrachloroethylene, 2,3,4,6-tetrachlorophenol, tetradecanecarboxylic acid, tetradecanoic acid isopropyl, ester, toluene, 1,2,3-trichlorobenzene, 1,2,4-trichlorobenzene, 1,2,4-trimethylbenzene, 1,2,5-trichlorobenzene, trichloroethene, trichlorofluoromethane, 3,4,6-trichloro-1-methylphenol, 2,3,4-trichlorophenol, 2,3,5-trichlorophenol, 2,4,6-trichlorophenol, 3,4,5-trichlorophenol, tridecanoic acid, 1,3,5-trimethylbenzene, trimethylcyclohexane, undecane, xylene

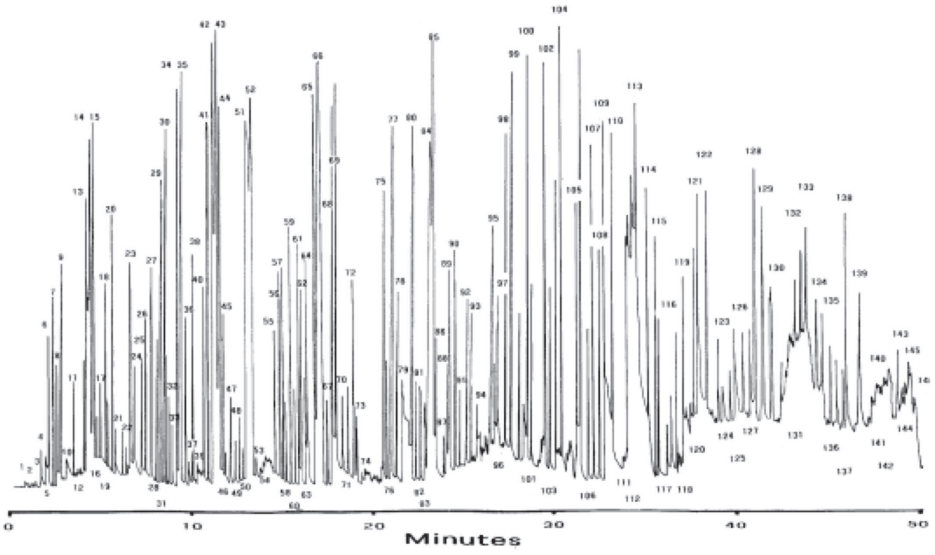


Fig. 4. Representative cGC-FID chromatogram of VOC identified at Sample Station 4, located 100 m south of Marchwood municipal incinerator (September 1996). Peak identifications are given in Table 2.

The toxicity of chemically-coated particles can be enhanced over expectations for single chemicals, because of synergies (coalitive effect, cosynergism and potentiation).

### 4.8 Dioxins and PCBs on Small Particles:

Fängmark et al. [13] concluded from analyzing incinerator flyash that chlorinated organics tend to be concentrated on the smaller particles. A similar result by Ruokojärvi et al. [9] found the < 1.6-$\mu$m fraction was disproportionately loaded. The distribution of PCDD/F with particle size in atmospheric dust collected at four Japanese sites was examined by Kurokawa et al. [11]. The maximum size collected was 30 $\mu$m in aerodynamic diameter, and the smallest 0.1 $\mu$m. Particles less than 1.1 $\mu$m contributed 50% of the total PCDD/F, with an almost equivalent I-TEQ proportion. The distribution of homologues changed with size, with the fraction of less chlorinated congeners in the homologue groups increasing with increasing particle size.

Chang [5] sampled air around a 1995 incinerator in Taiwan that had been fitted with activated carbon filtration to reduce the dioxin emissions to the EU standard of 0.1 ng/m3 and still found PCDD/F concentrations downwind of the MWI to be the highest and upwind to be the lowest among all sampling sites, concluding the MWI is noticeably contributing to dioxin levels in the ambient atmosphere.

Similarly Chao [103] sampled sites 1.1 and 2.1 km downwind from a municipal incinerator in central Taiwan and showed that PCDD/Fs were associated with the full size range of atmospheric particles.

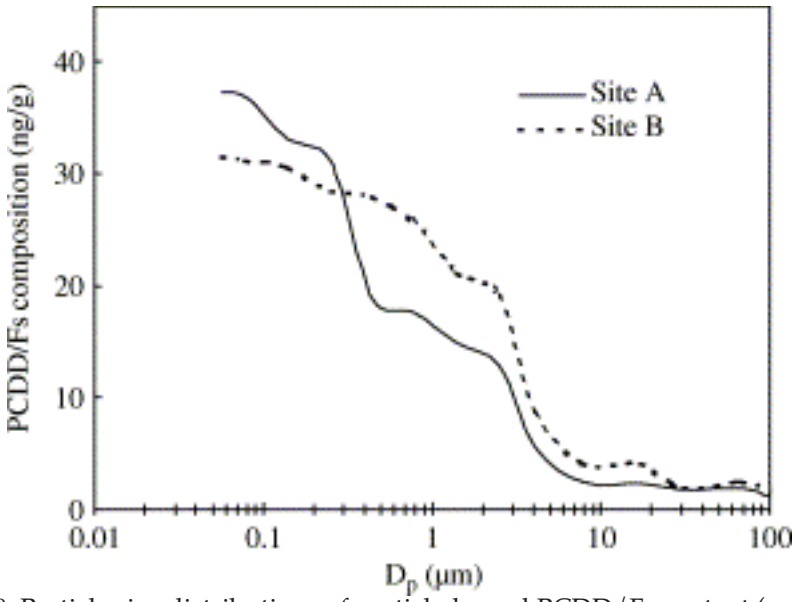

Fig8: Particle size distributions of particle-bound PCDD/Fs content (ng $g^{-1}$)

More than 80% of the PCDD/Fs and toxic equivalents (TEQs) were found to be associated with fine particles of aerodynamic diameter 2.0 $\mu$m. Generally a smaller particle had a higher PCDD/Fs content and the dioxin concentration can be seen to increase to the very finest particles. The particle size distributions of PCDD/Fs and TEQs were shifted to larger particles with increasing time and distance.

Professor Sakai [104] analysed the mass balance of total and dioxin-like (co-planar) PCBs across a municipal waste incinerator and found that whereas the input of Co-PCBs into the MSW incineration facilities was 0.13–0.29 $\mu$g-TEQ per ton waste, the total output of Co-PCBs (the sum of Co-PCBs released from emission gas, fly ash, and bottom ash) was 4.9$\mu$g-TEQ per ton waste. Whilst over 90% of the total PCBs were destroyed in the incineration process the toxicity of the output was found to be higher than that of the input. This emphasizes the importance of assessing PCB emissions as well as those of dioxins and as the indications are that PCB synthesis was taking place post-combustion it is likely that the contaminants on the smallest particles would include PCBs as well as dioxins.

### 4.9 Halogenated Dioxins

It should be noted that whilst currently 17 dioxins and furans are measured there are actually many more – and this has been recognised for more than 20 years. In 1987, for example, Schechter [105] wrote:

*"We are faced with the problem that animal data, upon which risk assessment and standard setting is based, is very incomplete. Also, as noted by Buser, in addition to the 200 plus chlorinated dibenzodioxins and dibenzofurans which may exist, there may be 5,000 chlorinated, brominated or bromochlorodioxins and dibenzofurans which may exist from incineration sources and which may be of potential concern".*

Since 1987 it has been demonstrated beyond doubt that brominated and mixed halogenated dioxins are produced by incinerators and that their toxicity is similar to - and sometime greater – than the chlorinated dioxins. In spite of this these dioxins are still not incorporated into incinerator risk assessments.

### 4.10 Combined Particle Size Distribution and Speciation:

Unfortunately few researchers have combined data on particle size distribution and speciation. Greenberg [106] tested emissions from the Nicosia incinerator and found 70-90% of the Zn, Cu, Cd and Pb to reside in the smallest particles (< 0.8*μm)*. However, that facility had only an electrostatic precipitator at the time, so the results are not directly transferrable to a more modern plant with a bag filter. Nonetheless it is clear that the majority of the metals exposure should be anticipated to arise from the ultrafine fraction of the emissions.

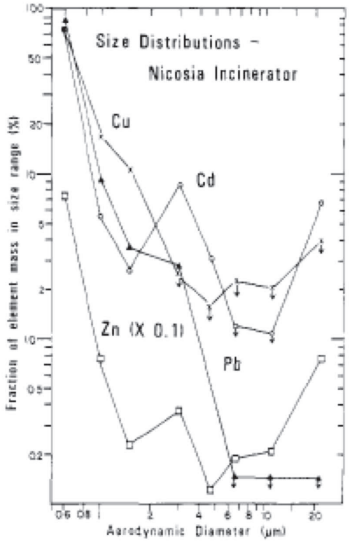


**Figure 2.** Size distributions of particles bearing indicated elements in terms of stack-gas mass concentration of the element (i.e., total mass per unit volume times elemental concentration in mass) vs. particle diameter, normalized to 100% for each element
Data obtained from analyses of material collected with cascade impactor. First stage collected all particles with $D > 15$ μm; last stage (back-up filter) collected particles <0.8 μm

### 4.11 Future Risks – Disposal of Nanotechnology wastes:

Nanomaterials are already reportedly used in over 800 products and the sales of which were valued at $147 billion in 2007 and are expected to soar over the coming years with a predicted value of $3.1 trillion by 2015 [107]. Inevitably the quantities of waste containing nanoparticles will increase rapidly but little thought has yet been given to the consequences of this. When products are incinerated, the thermal properties of nanoparticles determine their fate. There is evidence that at least some nanoparticles will pass through incinerators and be dispersed into the environment..

Franco [108] writes: " *whereas the onset temperature reaction for C60 is very low (315 °C), carbon nanotubes display very low reactivity under combustion conditions (onset temperature = 820 °C) and hence may not breakdown in an incinerator [109]. In theory, this means that they could end up in the gaseous effluent and released into the atmosphere*".

This is a significant concern given the inability to filter ultra-fine particles even with modern bag filters [78-81]. Any nanoparticles released from an incinerator increase the risk described above and incineration may increasingly play a role as a very effective delivery mechanism directly into the alveoli for a wide range of products of waste nanotechnology products.

### 4.12 Risk Assessment:

The risk assessment in relation to particulates that has been undertaken by the Indaver is rather simplistic. The principle assumption, and the basis for the conclusion, it that if air quality standards are not exceeded by the combination of existing ambient concentrations and the marginal increase from the incinerator then no harm is assumed to occur.

This approach is, of course, fundamentally flawed for those emissions, like particulates for which no safe level can be demonstrated.

Kunzli [110] wrote "*In many countries, policy makers currently face the problem that air quality criteria regulations are intended to "protect health", including the health of the most vulnerable people; to date, research has failed to obtain any evidence for a no-effect threshold. Thus, similar to carcinogens, the natural "threshold" might be zero exposure. Therefore, non-zero target values of clean air acts, inherently assume that some health impact of air pollution may be accepted. Impact assessors must choose a level below which they explicitly want to ignore the impact on air pollution*".

Chao [103] comments that even though a large number of atmospheric dispersion models exist and are readily available for use, the risk assessor is generally faced with little or no data on the atmospheric particle size distribution of PCDD/Fs. Lohman and Seigneur [111] conclude that "*it is essential to obtain accurate characterizations of the particle size distribution of particulate PCDD/F because the dry deposition flux is very sensitive to the particle size distribution*". Without such data accurate risk assessment is not possible and yet there is no evidence that it has been collected or used in relation to this application.

### 4.13 Conclusions on UFPs from Incinerators:

Not only do a high proportion of the UFPs escape the filters, but they are chemically reactive and carry a wide range of products of incomplete combustion and adsorbed metals with them. The subsequent direct uptake of these respirable particles and the ready transfer from the lungs into the blood stream may be part of the reason that traditional toxicology is at a loss to explain the level of impacts for such apparently low exposures.

Aerosols in the ultra-fine size range have much higher mobility in the air and can more effectively deposit in the respiratory system.

Ultrafine particles have been found to be chemically highly reactive, even when originating from a relatively unreactive bulk material [25]. The massive surface area associated with a small mass of nanometre-sized particles can act as a catalytic surface for the secondary formation of organic compounds such as the *de novo* synthesis of dioxins.

The relative toxicity of ultrafine particles arising from different processes remains un-researched. The levels of heavy and transition metal inputs in municipal solid waste are very much higher than with conventional fuels. Such increases must inevitably be associated with an increase in toxicity and consequently the likelihood of adverse health effects among the local receptors.

In my opinion, there is also a need to determine the relative toxicity of the particulate aerosols in the gases emitted by different waste disposal routes, to facilitate rational decisions as to the best disposal method, particularly with respect to public health. This should be addressed urgently but, in the meantime with the significant prospects of serious harm to health, high weight must be given to the precautionary principle.

## 5 The Precautionary Principle

The Twenty-fourth Report of the Royal Commission on Environmental Pollution, *Chemicals in Products: Safeguarding the Environment and Human Health*, [112]pointed out that the historical record is replete with unexpected toxicological impacts arising following the use of anthropogenic chemicals.

The Royal Commission emphasized that whilst we have learnt a great deal from some of the early episodes we may still be caught unawares, as witnessed with the emergence of a large number of different endocrine disrupting chemicals during the 1980s and 1990s.

"*It was not foreseen that low concentrations of chemicals used as antifouling agents (tributyltin), surfactants (nonyl phenol), flame retardants (polybrominated diphenylethers) and plasticisers (phthalates) would bind to hormone receptors or disrupt hormone metabolism in birds, reptiles, fish and invertebrates and influence sperm counts and the development of testicular malignancy in humans* [113, 114]."

These examples refer to chemicals whose reactivity it was felt was reasonably well understood. This is not the case with the UFPs with their wide range of chemical loading that are released in large quantities from modern incinerators. Apart from the fact that we know they are likely to be harmful at concentrations well below current air quality standards little is known of about the likely extent of environmental effects or their likelihood of causing unintended harm. Furthermore as nanotechnology expands there are even greater future risks from relying on technologies which, in at least some cases, are more likely to disperse them into the atmosphere than to destroy them as described above.

Having reviewed the science and the hazards of ultrafine particles I agree with Kunzli [110] who wrote "*In the light of all the uncertainties and limitations, researchers should not lose sight of the general patterns and perspectives. Given the current level of evidence of the association between air pollution and health, the precautionary principle may provide excellent guide to rigorously implement clean air strategies*".

The precautionary principle is part of the framework for sustainable development and I consider that the principle should be regarded more seriously when considering incineration processes, where there is significant scientific uncertainty and serious risks of harm.

The precautionary principle in its modern formulation is a means to safeguard public health. The European Commission advised the inclusion of public health in 2000 (European Commission Communication on Precautionary Principle, 2 February 2000), saying that the precautionary principle should be applied where "*there are reasonable grounds for concern that potential hazards may affect the environment or human, animal or plant health, and when at the same time the lack of scientific information precludes a detailed scientific evaluation*".

The EU Treaty Article 174(2) as amended at Nice 2004 recognized that scientific evaluation can be inconclusive and accorded priority to public health:

> a precautionary approach must be paramount, as opposed to acting only where proof or very strong suspicion of harm can be demonstrated. The Precautionary Principle should be applied where the possibility of harmful effects on health or the environment has been identified and preliminary scientific evaluation proves inconclusive for assessing the level of risk. Account should be taken of social and environmental costs in examining the level of risk, but the protection of public

health, including the effects of the environment on public health, must be given priority.

I would therefore recommend that this application should not be approved in the light of the likely risks to public health and the Environment detailed in this evidence.

# 6 EndNotes:


1. Maynard, R. and C. Howard, Eds, *Particulate Matter: Properties and Effects upon Health*. 1999, Oxford: BIOS Scientific Publishers.
2. European Commission, *DIRECTIVE 2000/76/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 4 December 2000 on the incineration of waste.* Official Journal of the European Communities, 2000. **L 332**.
3. Health Research Board, et al., *Health and Environmental Effects of Landfilling and Incineration of Waste – A Literature Review*. 2003, Dublin: Health Research Board. viii, 284 p.
4. World Health Organisation, *Population health and waste management: scientific data and policy options Report of a WHO workshop Rome, Italy, 29-30 March 2007*. 2007.
5. Nemery, B., P.H.M. Hoet, and A. Nemmar, *The Meuse Valley fog of 1930: an air pollution disaster.* The Lancet, 2001. **357**(9257): p. 704-708.
6. Haldane, J.S., *Atmospheric Pollution and Fogs.* Br Med J, 1931. **1**(3660): p. 366-367.
7. Bell, M.L. and D.L. Davis, *Reassessment of the lethal London fog of 1952: novel indicators of acute and chronic consequences of acute exposure to air pollution.* Environ Health Perspect, 2001. **109 Suppl 3**: p. 389-94.
8. Polichetti, G., et al., *Effects of particulate matter (PM10, PM2.5 and PM1) on the cardiovascular system.* Toxicology. **In Press, Corrected Proof**.
9. Pope, A.C., 3rd and D.W. Dockery, *Health Effects of Fine Particulate Air Pollution: Lines that Connect.* Journal of the Air & Waste Management Association, 2006. **56**: p. 709-742.
10. Nawrot, T.S., et al., *Stronger associations between daily mortality and fine particulate air pollution in summer than in winter: evidence from a heavily polluted region in western Europe.* J Epidemiol Community Health, 2007. **61**(2): p. 146-149.
11. Donaldson, K., X.Y. Li, and W. MacNee, *Ultrafine (nanometre) particle mediated lung injury.* Journal of Aerosol Science, 1998. **29**(5-6): p. 553-560.
12. Penttinen, P., et al., *Number concentration and size of particles in urban air: effects on spirometric lung function in adult asthmatic subjects.* Environ Health Perspect, 2001. **109**(4): p. 319-23.
13. Wahlin, P., et al., *Pronounced decrease of ambient particle number emissions from diesel traffic in Denmark after reduction of the sulphur content in diesel fuel.* Atmospheric Environment, 2001. **35**(21): p. 3549-3552.
14. Donaldson, K., et al., *Combustion-derived nanoparticles: A review of their toxicology following inhalation exposure.* Particle and Fibre Toxicology, 2005. **2**(1): p. 10.
15. Li, N., et al., *Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage.* Environ Health Perspect, 2003. **111**(4): p. 455-60.
16. Harrison, R., et al., *Measurement of number, mass and size distribution of particles in the atmosphere.* Philos Trans R Soc A, 2000. **358**: p. 2567–2579.
17. Aboh, I.J.K., et al., *EDXRF characterisation of elemental contents in PM2.5 in a medium-sized Swedish city dominated by a modern waste incineration plant.* X-Ray Spectrometry, 2007. **36**(2): p. 104-110.
18. Bencko, V., *Use of human hair as a biomarker in the assessment of exposure to pollutants in occupational and environmental settings.* Toxicology, 1995. **101**(1-2): p. 29-39.
19. National Research Council, N.A.o.S., *"Airborne Particles" quoted by RACHEL'S ENVIRONMENT & HEALTH WEEKLY #440 4th May1995 The Holy Grail of Scientific Certainty*. 1979, Baltimore, Md: University Park Press.
20. Bai, N., et al., *The pharmacology of particulate matter air pollution-induced cardiovascular dysfunction.* Pharmacology & Therapeutics, 2007. **113**(1): p. 16-29.
21. Brook, R., et al., *Air pollution and cardiovascular disease: a statement for healthcare professionals from the Expert Panel on Population and Prevention Science of the American Heart Association.* Circulation, 2004. **109**: p. 2655 - 2671.

22. Ballester, F., et al., *Reducing ambient levels of fine particulates could substantially improve health: a mortality impact assessment for 26 European cities.* J Epidemiol Community Health, 2008. **62**(2): p. 98-105.
23. Wichmann, H.-E., et al., *Daily Mortality and Fine and Ultrafine Particles in Erfut, Germany. Part I: Role of Particle Number and Particle Mass. Research Report 98*. 2000, Health Effects Institute: Cambridge MA.
24. Sawyer, R.F., *Science based policy for addressing energy and environmental problems.* Proceedings of the Combustion Institute, 2009. **32**(1): p. 45-56.
25. Jefferson, D.A. and E.E.M. Tilley, *The structural and physical chemistry of nanoparticles*, in *Particulate matter: properties and effects upon health*, R.L. Maynard and C.V. Howard, Editors. 1999, BIOS Scientific Publishers Ltd: Oxford. p. 63-84.
26. Donaldson, J., V. Stone, and W. MacNee, *The toxicology of ultrafine particles*, in *Particulate matter: properties and effects upon health*, R.L. Maynard and C.V. Howard, Editors. 1999, BIOS Scientific Publishers Ltd: Oxford. p. 115-129.
27. Oberdorster, G., E. Oberdorster, and J. Oberdorster, *Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles.* Environ Health Perspect, 2005. **113**: p. 823 - 839.
28. Seaton, A., et al., *Particulate air pollution and acute health effects.* The Lancet, 1995. **345**(8943): p. 176-178.
29. Kunzli, N., et al., *Public-health impact of outdoor and traffic-related air pollution: a European assessment.* The Lancet, 2000. **356**(9232): p. 795-801.
30. Commision of the European Communities, *COM(2005) 446 final COMMUNICATION FROM THE COMMISSION TO THE COUNCIL AND THE EUROPEAN PARLIAMENT Thematic Strategy on air pollution {SEC(2005) 1132}*. 2005.
31. Boekelheide, K., *Mixed Messages.* Toxicol. Sci., 2007. **99**(1): p. 1-2.
32. Ochs, M., et al., *The Number of Alveoli in the Human Lung.* Am. J. Respir. Crit. Care Med., 2004. **169**(1): p. 120-124.
33. Yang, W., J.I. Peters, and R.O. Williams Iii, *Inhaled nanoparticles--A current review.* International Journal of Pharmaceutics, 2008. **356**(1-2): p. 239-247.
34. Salvi, S., *Health effects of ambient air pollution in children.* Paediatric Respiratory Reviews, 2007. **8**(4): p. 275-280.
35. Cormier, S.A., et al., *Origin and health impacts of emissions of toxic by-products and fine particles from combustion and thermal treatment of hazardous wastes and materials.* Environ Health Perspect, 2006. **114**(6): p. 810-7.
36. Gumbleton, M., *Caveolae as potential macromolecule trafficking compartments within alveolar epithelium.* Advanced Drug Delivery Reviews, 2001. **49**(3): p. 281-300.
37. Kim, C.S. and P.A. Jaques, *Respiratory dose of inhaled ultrafine particles in healthy adults.* Philosophical Transactions of the Royal Society of London. Series A: Mathematical, Physical and Engineering Sciences, 2000. **358**(1775): p. 2693-2705.
38. Lundborg, M., et al., *Human Alveolar Macrophage Phagocytic Function is Impaired by Aggregates of Ultrafine Carbon Particles.* Environmental Research, 2001. **86**(3): p. 244-253.
39. Muller, A.K., et al., *DNA damage in lung after oral exposure to diesel exhaust particles in Big BlueÆ rats.* Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 2004. **550**(1-2): p. 123-132.
40. Bermudez, E., et al., *Pulmonary Responses of Mice, Rats, and Hamsters to Subchronic Inhalation of Ultrafine Titanium Dioxide Particles.* Toxicol. Sci., 2004. **77**(2): p. 347-357.
41. Borm, P.J. and W. Kreyling, *Toxicological hazards of inhaled nanoparticles--potential implications for drug delivery.* J Nanosci Nanotechnol, 2004. **4**(5): p. 521-31.
42. Stearns, R.C., J.D. Paulauskis, and J.J. Godleski, *Endocytosis of Ultrafine Particles by A549 Cells.* Am. J. Respir. Cell Mol. Biol., 2001. **24**(2): p. 108-115.
43. Oberdorster, G., et al., *Extrapulmonary translocation of ultrafine carbon particles following whole-body inhalation exposure of rats.* J Toxicol Environ Health A, 2002. **65**(20): p. 1531-43.

44. Nemmar, A., et al., *Passage of Inhaled Particles Into the Blood Circulation in Humans.* Circulation, 2002. **105**(4): p. 411-414.
45. Kreyling, W.G., et al., *Translocation of ultrafine insoluble iridium particles from lung epithelium to extrapulmonary organs is size dependent but very low.* J Toxicol Environ Health A, 2002. **65**(20): p. 1513-30.
46. Stone, V., et al., *Increased calcium influx in a monocytic cell line on exposure to ultrafine carbon black.* Eur Respir J, 2000. **15**: p. 297 - 303.
47. Donaldson, K., et al., *Ultrafine particles.* Occup Environ Med, 2001. **58**(3): p. 211-216.
48. Geiser, M., et al., *Ultrafine particles cross cellular membranes by nonphagocytic mechanisms in lungs and in cultured cells.* Environ Health Perspect, 2005. **113**(11): p. 1555-60.
49. Stone, P.H. and J.J. Godleski, *First steps toward understanding the pathophysiologic link between air pollution and cardiac mortality.* American Heart Journal, 1999. **138**(5): p. 804-807.
50. Dockery, D.W., et al., *An association between air pollution and mortality in six U.S. cities.* N Engl J Med, 1993. **329**(24): p. 1753-9.
51. Donaldson, K., et al., *Ultrafine particles: mechanisms of lung injury.* Philosophical Transactions of the Royal Society of London. Series A: Mathematical, Physical and Engineering Sciences, 2000. **358**(1775): p. 2741-2749.
52. Oberdorster, G., *Toxicology of ultrafine particles: In vivo studies.* Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences, 2000. **358**(1775): p. 2719-2740.
53. Wichmann, H.E. and A. Peters, *Epidemiological evidence of the effects of ultrafine particle exposure.* Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences, 2000. **358**(1775): p. 2751-2769.
54. Howard, C.V., *Nano-particles and Toxicity - Annex (P15-20) in No Small Matter II: The Case for a Global Moratorium Size Matters! April 2003*, in *Occasional Paper Series 7:1* 2003, ETC Group
55. Peters, A., et al., *Translocation and potential neurological effects of fine and ultrafine particles a critical update.* Part Fibre Toxicol, 2006. **3**: p. 13.
56. Kunzli, N., et al., *Ambient air pollution and atherosclerosis in Los Angeles.* Environ Health Perspect, 2005. **113**(2): p. 201-6.
57. Miller, K.A., et al., *Long-Term Exposure to Air Pollution and Incidence of Cardiovascular Events in Women.* N Engl J Med, 2007. **356**(5): p. 447-458.
58. Pope, C.A., III, et al., *Cardiovascular Mortality and Long-Term Exposure to Particulate Air Pollution: Epidemiological Evidence of General Pathophysiological Pathways of Disease.* Circulation, 2004. **109**(1): p. 71-77.
59. Dockery, D.W. and P.H. Stone, *Cardiovascular Risks from Fine Particulate Air Pollution.* N Engl J Med, 2007. **356**(5): p. 511-513.
60. World Health Organisation, *Effects of air pollution on children's health and development - a review of the evidence*. 2005, Special Programme on Health and Environment, European Centre For Environment and Health: Bonn.
61. World Health Organisation Europe. *Children's Environment and Health Action Plan for Europe*. in *Fourth Ministerial Conference on Environment and Health*. 2005. Budapest, Hungary.
62. Schwartz, J., *Air pollution and children's health.* Pediatrics, 2004. **113**(4 Suppl): p. 1037-43.
63. Heinrich, J. and R. Slama, *Fine particles, a major threat to children.* International Journal of Hygiene and Environmental Health, 2007. **210**(5): p. 617-622.
64. Hertz-Picciotto, I., et al., *Early childhood lower respiratory illness and air pollution.* Environ Health Perspect, 2007. **115**(10): p. 1510-8.
65. Kunzli, N., et al., *Breathless in Los Angeles: The Exhausting Search for Clean Air.* Am J Public Health, 2003. **93**(9): p. 1494-1499.

66. Heindel, J.J., *Role of exposure to environmental chemicals in the developmental basis of disease and dysfunction.* Reproductive Toxicology, 2007. **23**(3): p. 257-259.
67. Yeatts, K., et al., *A brief targeted review of susceptibility factors, environmental exposures, asthma incidence, and recommendations for future asthma incidence research.* Environ Health Perspect, 2006. **114**(4): p. 634-40.
68. Henderson, J., et al., *Household chemicals, persistent wheezing and lung function: effect modification by atopy?* Eur Respir J, 2008. **31**(3): p. 547-554.
69. Jedrychowski, W., et al., *Early wheezing phenotypes and severity of respiratory illness in very early childhood: Study on intrauterine exposure to fine particle matter.* Environment International, 2009. **35**(6): p. 877-884.
70. Perera, F.P., et al., *Molecular evidence of an interaction between prenatal environmental exposures and birth outcomes in a multiethnic population.* Environ Health Perspect, 2004. **112**(5): p. 626-30.
71. Mohorovic, L., *First two months of pregnancy--critical time for preterm delivery and low birthweight caused by adverse effects of coal combustion toxics.* Early Human Development, 2004. **80**(2): p. 115-123.
72. Widory, D., *Nitrogen isotopes: Tracers of origin and processes affecting PM10 in the atmosphere of Paris.* Atmospheric Environment, 2007. **41**(11): p. 2382-2390.
73. Querol, X., et al., *Speciation and origin of PM10 and PM2.5 in selected European cities.* Atmospheric Environment, 2004. **38**(38): p. 6547-6555.
74. Almeida, S.M., et al., *Approaching PM2.5 and PM2.5†-†10 source apportionment by mass balance analysis, principal component analysis and particle size distribution.* Science of The Total Environment, 2006. **368**(2-3): p. 663-674.
75. Moffet, R., et al., *Characterization of Aerosols Containing Zn, Pb, and Cl from an Industrial Region of Mexico City.* Environmental Science & Technology, 2008. **42**(19): p. 7091–7097.
76. Ying, Q. and M.J. Kleeman, *Source contributions to the regional distribution of secondary particulate matter in California.* Atmospheric Environment, 2006. **40**(4): p. 736-752.
77. Hinds, W.C., *Filtration*, in *Aerosol Technology Properties, Behavior, and Measurement of Airborne Particles*. 1999, Wiley: New York. p. 182-205.
78. Lighty, J.S., J.M. Veranth, and A.F. Sarofim, *Combustion aerosols: factors governing their size and composition and implications to human health.* J Air Waste Manag Assoc, 2000. **50**(9): p. 1565-618; discussion 1619-22.
79. Buonanno, G., G. Ficco, and L. Stabile, *Size distribution and number concentration of particles at the stack of a municipal waste incinerator.* Waste Management, 2009. **29**(2): p. 749-755.
80. Maguhn, J., et al., *On-line analysis of the size distribution of fine and ultrafine aerosol particles in flue and stack gas of a municipal waste incineration plant: effects of dynamic process control measures and emission reduction devices.* Environ Sci Technol, 2003. **37**(20): p. 4761-70.
81. Yuan, C.-S., et al., *Partition and size distribution of heavy metals in the flue gas from municipal solid waste incinerators in Taiwan.* Chemosphere, 2005. **59**(1): p. 135-145.
82. Ogulei, D., et al., *Source apportionment of Baltimore aerosol from combined size distribution and chemical composition data.* Atmospheric Environment, 2006. **40**(Supplement 2): p. 396-410.
83. Ogulei, D., et al., *Receptor modeling for multiple time resolved species: The Baltimore supersite.* Atmospheric Environment, 2005. **39**(20): p. 3751-3762.
84. Shi, J.P., et al., *Sources and concentration of nanoparticles (<10 nm diameter) in the urban atmosphere.* Atmospheric Environment, 2001. **35**(7): p. 1193-1202.
85. Bennett, R.L. and K.T. Knapp, *Characterization of particulate emissions from municipal wastewater sludge incinerators.* Environ Sci Technol, 1982. **16**(12): p. 831-836.
86. Markowski, G.R., et al., *A submicron aerosol mode in flue gas from a pulverized coal utility boiler.* Environ Sci Technol, 1980. **14**(11): p. 1400-1402.

87. Friedlander, S.K., W. Koch, and H.H. Main, *Scavenging of a coagulating fine aerosol by a coarse particle mode.* Journal of Aerosol Science, 1991. **22**(1): p. 1-8.
88. Ruokojarvi, P., et al., *Effect of urea on fly ash PCDD/F concentrations in different particle sizes.* Chemosphere, 2001. **43**(4-7): p. 757-762.
89. United States Environmenal Protection Agency, *Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds Part I: Estimating Exposure to Dioxin-Like Compounds Volume 4: Site-Specific Assessment Procedures p 3-73 Draft Final EPA/600/P-00/001Ad March 2000*. 2000.
90. Rose, W.I., C.M. Riley, and S. Dartevelle, *Sizes and Shapes of 10-Ma Distal Fall Pyroclasts in the Ogallala Group, Nebraska.* The Journal of Geology, 2003. **111**(1): p. 115-124.
91. Morawska, L. and J. Zhang, *Combustion sources of particles. 1. Health relevance and source signatures.* Chemosphere, 2002. **49**(9): p. 1045-1058.
92. Wey, M.Y., J.L. Su, and J.C. Chen, *Influence of operating conditions on the formation of heavy metal compounds during incineration.* Journal of the Air & Waste Management Association, 1999. **49**(4): p. 444-453.
93. Harrison, R.M., et al., *Comparative receptor modelling study of airborne particulate pollutants in Birmingham (United Kingdom), Coimbra (Portugal) and Lahore (Pakistan).* Atmospheric Environment, 1997. **31**(20): p. 3309-3321.
94. Mishra, V.K., et al., *Wintertime sources and distribution of airborne lead in Korea.* Atmospheric Environment, 2004. **38**(17): p. 2653-2664.
95. Doucet, F.J. and J. Carignan, *Atmospheric Pb isotopic composition and trace metal concentration as revealed by epiphytic lichens: : an investigation related to two altitudinal sections in Eastern France.* Atmospheric Environment, 2001. **35**(21): p. 3681-3690.
96. Pancras, J.P., et al., *Identification of sources and estimation of emission profiles from highly time-resolved pollutant measurements in Tampa, FL.* Atmospheric Environment, 2006. **40**(Supplement 2): p. 467-481.
97. Younes, M., *Specific issues in health risk assessment of endocrine disrupting chemicals and international activities.* Chemosphere, 1999. **39**(8): p. 1253-1257.
98. Kurokawa, Y., et al., *Distribution of polychlorinated dibenzo-p-dioxins and dibenzofurans in various sizes of airborne particles.* Chemosphere, 1998. **37**(9-12): p. 2161-2171.
99. Jay, K. and L. Stieglitz, *Identification and quantification of volatile organic components in emissions of waste incineration plants.* Chemosphere, 1995. **30**(7): p. 1249-1260.
100. Kato, M., K. Urano, and T. Tasaki, *Development of Semi- and Nonvolatile Organic Halogen as a New Hazardous Index of Flue Gas.* Environ Sci Technol, 2000. **34**(19): p. 4071-4075.
101. Bergvall, C. and R. Westerholm, *Identification and Determination of Highly Carcinogenic Dibenzopyrene Isomers in Air Particulate Samples from a Street Canyon, a Rooftop, and a Subway Station in Stockholm.* Environ Sci Technol, 2007. **41**(3): p. 731-737.
102. Leach, J., A. Blanch, and A.C. Bianchi, *Volatile organic compounds in an urban airborne environment adjacent to a municipal incinerator, waste collection centre and sewage treatment plant.* Atmospheric Environment, 1999. **33**(26): p. 4309-4325.
103. Chao, M.R., et al., *Size distribution of particle-bound polychlorinated dibenzo-p-dioxins and dibenzofurans in the ambient air of a municipal incinerator.* Atmospheric Environment, 2003. **37**(35): p. 4945-4954.
104. Sakai, S.-i., et al., *Substance flow analysis of coplanar PCBs released from waste incineration processes.* Journal of Material Cycles and Waste Management, 1999. **1**(1): p. 62-74.
105. Schecter, A., *Risk assessment and standard setting: Rapporteur's summary and discussion.* Chemosphere, 1987. **16**(8-9): p. 2205-2210.
106. Greenberg, R.R., et al., *Composition of particles emitted from the Nicosia municipal incinerator.* Environ Sci Technol, 1978. **12**(12): p. 1329-1332.
107. Singh, N., et al., *NanoGenotoxicology: The DNA damaging potential of engineered nanomaterials.* Biomaterials, 2009. **30**(23-24): p. 3891-3914.

108. Franco, A., et al., *Limits and prospects of the ‘‘incremental approach’’ and the European legislation on the management of risks related to nanomaterials.* Regulatory Toxicology and Pharmacology, 2007. **48**: p. 171–183.
109. Cataldo, F., *A study on the thermal stability to 1000 C of various carbon allotropes and carbonaceous matter both under nitrogen and in air.* Fullerenes Nanotubes and Carbon Nanostructures, 2002. **10**(4): p. 293–311.
110. Kunzli, N., *The public health relevance of air pollution abatement.* Eur Respir J, 2002. **20**(1): p. 198-209.
111. Lohman, K. and C. Seigneur, *Atmospheric fate and transport of dioxins: local impacts.* Chemosphere, 2001. **45**(2): p. 161-171.
112. Royal Commission on Environmental Pollution (RCEP), *24th report on Chemicals in Products - Safeguarding the Environment and Human Health Cm 5827 June 2003* 2003.
113. Sharpe, R.M., *Male Reproductive Health Disorders and the Potential Role of Exposure to Environmental Chemicals*. 2009, Commissioned by CHEM Trust.
114. ENDS, *Chemical threat to men’s sexual health.* Environmental Data Services (ENDS), 2009. **412**: p. 6.

ScientificWorldJournal. 2012; 2012: 980843.
Published online 2012 Apr 29. doi: 10.1100/2012/980843
PMCID: PMC3350969
PMID: 22649329

# Polycyclic Aromatic Hydrocarbons Bound to PM 2.5 in Urban Coimbatore, India with Emphasis on Source Apportionment

R. Mohanraj,* S. Dhanakumar, and G. Solaraj
Author information Article notes Copyright and License information Disclaimer


Go to:

## Abstract

Coimbatore is one of the fast growing industrial cities of Southern India with an urban population of 1.9 million. This study attempts to evaluate the trends of airborne fine particulates (PM 2.5) and polyaromatic hydrocarbons (PAH) on them. The PM 2.5 mass was collected in polytetra fluoroethylene filters using fine particulate sampler at monthly intervals during March 2009 to February 2010. PAHs were extracted from PM 2.5 and estimated by high-performance liquid chromatography. It is alarming to note that PM 2.5 values ranged between 27.85 and 165.75 $\mu$g/m3 and exceeded the air quality standards in many sampling events. The sum of 9 PAHs bound to PM 2.5 in a single sampling event ranged from 4.1 to 1632.3 ng/m3. PAH diagnostic ratios and principal component analysis results revealed vehicular emissions and diesel-powered generators as predominant sources of PAH in Coimbatore.

Go to:

## 1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) among the urban air toxics are of global concern due to their multiple effects on human population. Attention is more importantly on PAHs bound to PM 2.5 and ultrafine fraction of the airborne particulates that are reportedly known for their higher health risk [1, 2]. About 80% of particulate matter in urban environment belongs to the size class of fine (PM 2.5) and ultrafine (PM 0.1) particles [3]. PAHs with two or three benzene rings existed in the vapour phase, whereas PAHs with more than five rings were observed mainly in the particulate phase [4]. PAH size distributions in the atmosphere are influenced by the growth of combustion-generated particles and the variation of PAH adsorption and absorption affinity based on physical characteristics and chemical composition [5, 6]. Higher-

molecular-weight (HMW) PAHs, due to their lower vapor pressures, continue to remain glued with fine particles in urban areas where emission sources are high. The PAH "mix" is expected to vary seasonally and geographically as a consequence of changes in emission sources.
Major anthropogenic emission sources of PAHs include biomass burning, coal and petroleum combustion, and coke and metal production [7, 8]. Open burning of biomass, agricultural waste, and municipal solid waste also contribute to atmospheric PAHs. However, vehicular and domestic emissions mainly influence the variation in PAH concentration across the urban to rural gradient [9]. Reportedly higher concentrations of PAHs in urban air have become a major health issue mainly due to their well-known carcinogenic and mutagenic properties [10, 11].
Links between PAH exposure and elevated levels of DNA adducts, mutations, and reproductive defects have strengthened the notorious impact of PAHs [12, 13]. Studies also hint that PAHs can inhibit growth of diatoms and development effects in wildlife. PAHs are also likely to cause transboundary effects and ultimately interfere with the global carbon cycle [14].
In many Asian regions where rapid economic drive is noticed, studies have shown that motor vehicles (especially diesel-engine vehicles), factories, and home heating are the principal sources of atmospheric PAHs and NPAHs [15]. Two wheelers, a popular means of transport in Asian region, are believed to emit particulate PAHs even higher than gasoline- and diesel-powered passenger cars and light- and heavy-duty vehicles [16].
In many Indian cities, rapid urbanization mushrooming of industrialization have triggered the growth of transportation by all means including two wheeler sector. Consequently, these cities suffer from serious air quality problems including rise in PAHs [17, 18]. Annual PAH emissions of India are estimated to be 90 Gg $y^{-1}$ by Zhang and Tao [14]. Reports on fine-particulate-bound PAH are unavailable in many cities. In this context, the present work was aimed to explore the PM 2.5-bound PAH concentration in Coimbatore city and source apportionment. Another objective was to assess the influence of environmental parameters such as temperature, rain, wind speed, and relative humidity on PAH concentration.

Go to:

## 2. Materials and Methods

### 2.1. Study Area Description

Coimbatore city and its environs with a population of 1,990,000 occupies the 225th position among the principal urban agglomerations of world as in 2011 (http://www.citypopulation.de/world/Agglomerations.html).

Coimbatore city is one of the top ten fastest growing cities of India and one among the top 100 in the world. There are about 25.000 small-scale industries functioning in and around Coimbatore. The number of medium- and large-scale textile mills present in Coimbatore is 312. In the wake of urbanization and industrialization, the number of automobiles is also rising at a similar pace. Among the sources of air pollution, vehicular emissions, industrial emissions, and smoke arising from the garbage dump are prominent in the city. For the current study, five locations namely C1-Small Industries Developmental Corporation (SIDCO), C2-Kuniyamuthur, C3-Kavundampalayam, C4-100 ft road, and C5-Lakshmi mills were selected to represent industrial, suburban residential, mixed commercial and residential, urban commercial, and urban highway, respectively (Figure 1).

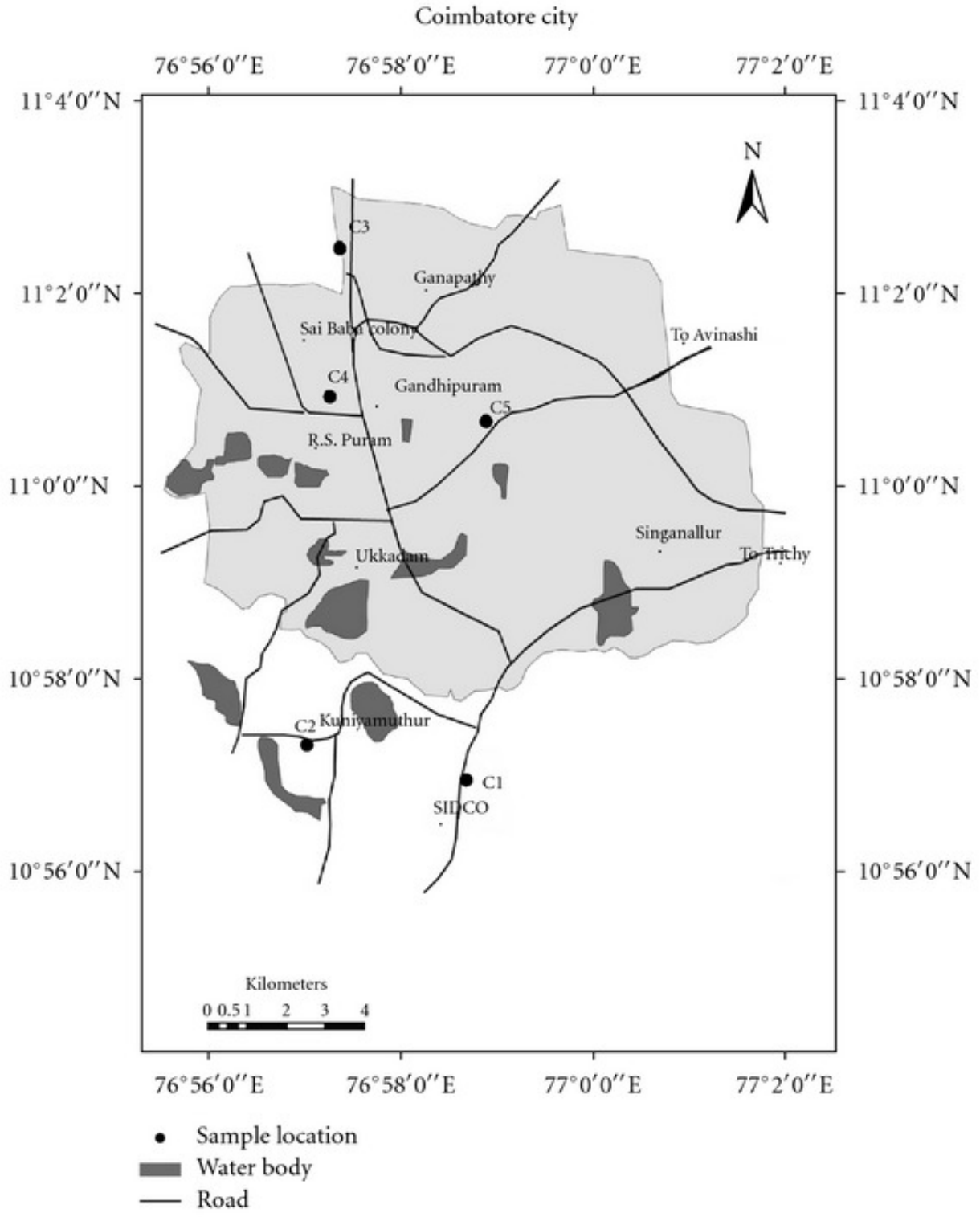


Figure 1

Map showing study sites in Coimbatore.

The total number of vehicles registered in Coimbatore city as in 2010 had exceeded 1 million. Over the quinquennium ending in 2006, 23 metros posted a compound annual growth rate of 8.3% in the number of total vehicle registrations. Significantly, among the second-tier cities

Coimbatore with 12.9% growth rates stood first in Tamilnadu state, India, followed by Madurai city (10.9%) [19]. Coimbatore holds a share of 0.84% and 7.46% of total registered motor vehicles in India and Tamilnadu, respectively. Slower growth in goods vehicle category hints an economic shift from commodity-producing sector (agriculture and industry) towards service sector. High growth in personalized motor vehicles reflects rising per capita income of middle class market competition in automobile sector due globalization coupled with convenient financing options. This proliferation in the personalized mode of transport has evidently increased traffic congestion and particulate air pollution.

## 2.2. Fine Particulate Matter Sample Collection

Fine particulate matter (PM 2.5) sample collection was carried out by Fine Particulate Sampler (Model: APM 550, Manufacturer: Envirotech, New Delhi, India) during March 2009 to February 2010 at five locations once a month in each location for a period of 24 h. The sampling days were different for each location and not simultaneous. Sampler's omnidirectional inlet design prevents the entry of particles greater than 10 microns from the ambient air by a clean aerodynamic cut-point. Further, particles finer than 10 microns in the air stream pass to a second impactor that has an aerodynamic cut-point at 2.5 microns. PM 2.5 particles are finally retained in 47 mm diameter polytetrafluoroethylene (PTFE) filters. The sampler was operated in each station at a constant flow rate of 1 $m^3$/hr. After 24 hours of operation, PM 2.5 deposited on the preweighed PTFE filters is estimated gravimetrically and stored in desiccators for PAH investigations.

## 2.3. PAH Analysis

It is assumed that fine particulate matter is uniformly distributed in the PTFE membrane filters. For more precise extraction of PAH from the PTFE filters, several solvents (cyclohexane, benzene, acetonitrile, methanol, dichloromethane) in various combinations were attempted and eventually dichloromethane:methanol (60 : 40) was chosen. A small piece of particulate laden PTFE filter measuring an area of 3.14 $cm^2$ was taken for extraction in a dark bottle by adding one mL of dichloromethane:methanol (60 : 40) mixture. Then the filter and solvent mixture was allowed to incubate for half an hour and then ultrasonically extracted for another half an hour [20]. The process was repeated 2-3 times, and final volume was made up to 2 mL. The extracted solution was filtered through 0.2 $\mu$m PTFE filter and then redissolved in acetonitrile for HPLC analysis.

HPLC (Manufacturer: Waters, USA) with PAH C18 column (5 $\mu$m 4.6 × 250 mm) and fluorescence detector (model: 2475) was used for detection of PAH compounds. Calibrations were performed using 9 individual PAH standards obtained from Supelco, USA, and subsequently samples were analyzed. Chromatographic peaks were identified on the basis of retention time. Fluorescence detector was set at 250 and 425 nm wavelengths, for excitation and emission, respectively. The gradient elution started with 50% water and 50% acetonitrile (up to 5 min), then acetonitrile was increased with a linear gradient to 100% and maintained up to 28 min. The last 12 min elution (28 to 40 min) was performed in 50 : 50 gradients of water and acetonitrile. A total of 9 PAHs were analyzed for this study, namely, phenanthrene (PHENAN), anthracene (ANTH), benzo(a)anthracene (BaA), chrysene (CHRY), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo(a)pyrene (BaP), indeno-1, 2, 3-cd pyrene (IcdP), and benzo(ghi)perylene (BghiP). Results of the experiment were subjected to statistics using Statistical Package for Social Scientists (SPSS 11.0). Meteorological data (wind speed, temperature, humidity, and rainfall) obtained from Indian Meteorological Department (IMD) used for further statistical analysis (Table 1). A three-day average inclusive of sampling days and a day prior to it was considered for each meteorological variable.

Table 1

Meteorological conditions in Coimbatore during the study period1.

| | |
|---|---|
| | [1]Source: India Meteorological Department (IMD), New Delhi.<br>In order to ensure precision, blank PTFE filters were analyzed for PAH contamination prior to outdoor sample collection. The concentrations of PAH in blank filters were below detection limits for all 9 PAHs investigated. Recovery efficiency was estimated by spike method. Briefly, a half portion of the PTFE filters were spiked with a pre-determined amount of standard PAH solutions. |

| Month | Temp. (°C) | Humidity (%) | Wind speed (km/h) | Rainfall (cm) |
|---|---|---|---|---|
| March | 28 ± 1 | 52 ± 8 | 7 ± 2 | 0 |
| April | 29 ± 1 | 57 ± 10 | 9 ± 2 | 0 |
| May | 28 ± 1 | 69 ± 6 | 11 ± 3 | 3 ± 8 |
| June | 27 ± 1 | 70 ± 7 | 15 ± 1 | 0 |
| July | 26 ± 1 | 77 ± 6 | 16 ± 4 | 1 ± 2 |
| August | 26 ± 1 | 74 ± 5 | 14 ± 4 | 2 ± 7 |
| September | 26 ± 1 | 75 ± 5 | 13 ± 3 | 2 ± 7 |
| October | 27 ± 1 | 67 ± 10 | 8 ± 5 | 2 ± 6 |
| November | 25 ± 1 | 78 ± 8 | 6 ± 2 | 8 ± 17 |
| December | 25 ± 1 | 70 ± 6 | 7 ± 2 | 0 |
| January | 25 ± 1 | 59 ± 6 | 7 ± 2 | 0 |
| February | 27 ± 2 | 51 ± 6 | 6 ± 1 | 0 |

Figure 2
PM 2.5 trends in Coimbatore.

| | |
|---|---|
| | *BDL below detectable level. In the urban sampling station at Lakshmi mills, CHRY, BbF, and BghiP were observed as predominant PAHs with annual average of 103.4, 33.2, and 26.6 ng/m3, respectively. BghiP has been identified as the tracer of gasoline emissions [36]. Earlier studies had confirmed substantial contribution of BghiP and IcdP from sparking ignition engines [31]. Maximum mean level of BaP, a potent carcinogen, was recorded to be the highest at |

| Descriptive statistics | Concentration of PAHs in ng/m3 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | PHENAN | ANTH | BaA | CHRY | BbF | BkFi | BaP | BghiP | IcdP | TPAHs |
| *SIDCO* | | | | | | | | | | |
| Minimum | BDL | BDL | BDL | BDL | BDL | BDL | 0.1 | BDL | BDL | 4.2 |
| Maximum | 17.4 | 88.3 | 292.7 | 113.4 | 75.5 | 10.4 | 18.7 | 73.3 | 18.6 | 619.1 |
| Mean | 5.5 | 25.4 | 40.1 | 27.2 | 15.2 | 2.1 | 6.1 | 17.4 | 4.5 | 143.5 |
| *Lakshmi mills* | | | | | | | | | | |
| Minimum | 15.9 | BDL | BDL | BDL | 3.6 | BDL | BDL | 4.9 | 10.9 | 119.4 |
| Maximum | 81.9 | 40.2 | 177 | 195.2 | 48.4 | 14.4 | 21.2 | 59.1 | 30 | 419.2 |
| Mean | 38.8 | 4.5 | 30.8 | 103.4 | 33.2 | 6.5 | 11.8 | 26.6 | 20.4 | 276.0 |
| *Kuniamuthur* | | | | | | | | | | |
| Minimum | BDL | BDL | BDL | 0.2 | 0.2 | 0.1 | BDL | 0.1 | 0.1 | 4.1 |
| Maximum | 157.7 | 85.0 | 142.2 | 312.0 | 157.7 | 40.2 | 20.2 | 56.5 | 84.2 | 744.3 |
| Mean | 27.3 | 12.9 | 40.3 | 73.0 | 30.0 | 7.7 | 5.7 | 16.1 | 15.2 | 233.2 |
| *Kavundampalayam* | | | | | | | | | | |
| Minimum | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | 23.7 |
| Maximum | 80.4 | 278.7 | 225.0 | 805.1 | 43.5 | 66.3 | 40.7 | 98.8 | 218.8 | 1632.3 |
| Mean | 17.4 | 43.5 | 41.9 | 277.5 | 16.3 | 12.5 | 10.4 | 30.5 | 36.4 | 486.4 |
| *100 feet road* | | | | | | | | | | |
| Minimum | BDL | BDL | BDL | BDL | BDL | BDL | BDL | 6.7 | BDL | 13.7 |
| Maximum | 35.5 | 55.6 | 28.5 | 196.8 | 18.4 | 8.0 | 16.3 | 37.1 | 41.5 | 325.6 |
| Mean | 12.1 | 20.7 | 8.0 | 81.4 | 3.9 | 3.7 | 9.1 | 21.0 | 15.2 | 175.0 |
| *Limit of detection (LOD) of PAHs (μg/mL)* | | | | | | | | | | |
| LOD (μg/mL) | 0.0042 | 0.001 | 0.006 | 0.006 | 0.005 | 0.007 | 0.009 | 0.008 | 0.006 | — |

| S. No | Total PAHs | Range | City | Reference |
|---|---|---|---|---|
| 1 | ∑ 8 PAHs | 150–1800 | Delhi | [21] |
| 2 | ∑ 5 PAHs | 244–1481 | Chennai | [22] |
| 3 | ∑ 5 PAHs | 197–2397 | Kanpur | [22] |
| 4 | ∑ 5 PAHs | 284–2114 | Kolkata | [22] |

| | | | | |
|---|---|---|---|---|
| 5 | ∑12 PAHs | 177–1201 | Delhi | [23] |
| 5 | ∑ 11 PAHs | 326–791 | Chennai | [24] |
| 6 | ∑ 9 PAHs | 4.1–1632 | Coimbatore | The present study |

| | **PM 2.5** | **Temp** | **Humidity** | **Wind speed** | **Rainfall** | **PHENAN** | **ANTH** | **BaA** | **CHRY** | **BbF** | **BkF** | **BaP** | **BghiP** | **IcdP** | **TPAHs** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PM 2.5 | 1.0 | | | | | | | | | | | | | | |
| Temp | -0.5 | 1.0 | | | | | | | | | | | | | |
| Humidity | -0.1 | 0.35 | 1.0 | | | | | | | | | | | | |
| Windspeed | −.69* | 0.46 | 0.53 | 1.0 | | | | | | | | | | | |
| Rainfall | 0.25 | 0.47 | 0.25 | -0.3 | 1.0 | | | | | | | | | | |
| PHENAN | -0.1 | 0.06 | -0.24 | -0.1 | -0.03 | 1.0 | | | | | | | | | |
| ANTH | 0.39 | -0.4 | −.70* | -0.5 | -0.26 | 0.15 | 1.0 | | | | | | | | |
| BaA | 0.37 | -0.6 | -0.43 | -0.1 | -0.57 | 0.17 | .71* | 1.0 | | | | | | | |
| CHRY | 0.13 | 0.14 | -0.44 | -0.2 | 0.13 | .78* | 0.13 | 0.2 | 1.0 | | | | | | |
| BbF | 0.48 | -0.5 | -0.14 | -0.4 | -0.16 | .68* | 0.23 | 0.5 | 0.43 | 1.0 | | | | | |
| BkF | 0.17 | -0.1 | -0.37 | -0.3 | -0.09 | .93** | 0.09 | 0.2 | .83** | .77* | 1.0 | | | | |
| BaP | .72* | -0.4 | -0.53 | −.72* | 0.16 | 0.40 | 0.46 | 0.5 | 0.58 | .67* | 0.57 | 1.0 | | | |
| BghiP | 0.63 | −.72* | -0.17 | -0.3 | -0.27 | -0.03 | 0.14 | 0.2 | 0.07 | 0.43 | 0.27 | 0.3 | 1.0 | | |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| IcdP | 0.3[illegible] | -0.3 | -0.26 | -0.3 | -0.14 | .83** | 0.13 | 0.4 | .75* | .90** | .93** | .67* | 0.43 | 1.0 |
| TPAHs | 0.2[illegible] | -0.3 | -0.46 | -0.2 | -0.27 | .82** | 0.35 | 0.5 | .80** | .77* | .87** | 0.6 | 0.30 | .92** | 1.0 |

| **Sampling stations** | **BaP/ BaP+ Chr** | **BbF/ BkF** | **BaP / BghiP** | **Ind/ BghiP** | **Ind/ (Ind+ BghiP)** | **BaA/ (Chr+ BaA)** | **BghiP/ BaP** |
|---|---|---|---|---|---|---|---|
| SIDCO | 0.52 | 2.98 | 0.24 | 0.20 | 0.26 | 0.53 | 2.99 |
| Lakshmi mills | 0.09 | 3.85 | 0.58 | 1.15 | 0.48 | 0.13 | 2.11 |
| Kuniyamuthur | 0.07 | 5.49 | 0.74 | 1.12 | 0.44 | 0.34 | 2.81 |
| Kaundampalayam | 0.05 | 1.67 | 1.60 | 1.99 | 0.66 | 0.24 | 1.16 |
| 100 feet road | 0.18 | 0.89 | 0.53 | 0.73 | 0.36 | 0.12 | 2.01 |
| | | | | | | | |
| Diesel vehicles | >0.73 [25, 26] | >0.5 [27] | | ~1.0 [28] | 0.35–0.70 [29] | 0.38–0.64 [30] | 1.2–2.2 [31] |
| Industrial furnaces | | | | | 0.36–0.57 [32] | 0.23–0.89 [32] | 0.02–0.06 [32] |
| Catalyst-equipped cars | | | | | 0.21–0.22 [30] | 0.22–0.55 [33] | 2.5–3.3 [31] |

Figure 3

Factor analysis for PAH concentration in Coimbatore city.

I was invited to speak to groups of residents, and informally to individual residents who wanted to express their concerns about declining air quality and health hazards as a consequence of the building of the R E P.

As a consequence of the “evidence” provided by the first speaker at the Open Floor hearing on 4th June 2019 in which he referenced NOx emissions I felt that I should speak today and provide further written material (which will be provided to the Examiner under separate cover) which covers in detail NOx emissions and the probable linkage between Ultra Fine Particulates (UFP’s) and Polycyclic Aromatic Hydrocarbons (PAH’s) and the negative health implications that these linkages generate.

Cory literature :

The generalised statistic on the Cory website offers 100,000 truck journeys removed from London’s roads every year thanks to use of our infrastructure and lighterage fleet on the River Thames

Using the fleet of tugs and barges in this way removes c.100,000 truck movements from London’s congested streets every year and reduces the company’s carbon footprint, saving around 13,500 tonnes of $CO_2$ annually.

———————————————————————————

75% of transported waste to be conducted by tugs on the river.
The Cory statement above offers this as a saving of 100,000 truck movements per year.
However, this infers that the remaining 25% of waste transport will be by truck. If 100,000 truck movements saved per year equates to 75% of the waste movement that then provides anecdotal evidence of 33,333 actual ADDITIONAL yearly truck movements.

| | | | | |
|---|---|---|---|---|
| Yearly additional truck movements. | | | | 33,333 |
| Thus | | | | |
| Daily. | “ | “ | “ | 91.32 |
| Hourly | | | | 3.8 |

Effectively One additional truck every 15 minutes to the Cory plant , for every hour, every day, every year.

Trucks burn diesel fuel.
Tugboats burn diesel fuel.

Neither, transport method will have 100% efficient burning of the fuel.
A new incinerator plant may be highly efficient, but will it be 100% efficient ? As an incinerator plant ages it is a reasonable assumption that the efficiency levels will drop.

Polycyclic Aromatic Hydrocarbons (PAH) are created as a consequence of inefficient burning, fuel I.e petrol, diesel, biomass, and other industrial processes.

PAH’s have negative health consequences as they offer three chemical changes if absorbed into the body, they are :

Carcinogenic - cancer forming

Mutragenic - permanent cell changes, mutations (as in cancers)

Tetarogenic - causing the deformation within foetuses

I was able to identify documents that suggest there is a probable link between an incinerator plant that, even with the best filter and scrubbing technology, releases Ultra Fine particulates into the atmosphere and the Polycyclic Aromatic Hydrocarbons that are generated from burning such as lorry burning diesel engines.

It is my intention to quote from a full document, extracts of documents and then hand that additional information to the Examiner today.

As of today the Cory representative has stated that this is a Low Carbon footprint project. I had spoken to consultants at the public consultations where I got 3 different answers, Low Carbon, Carbon Neutral, Carbon Negative. With this clarification we know at least know that with this project we will still be adding carbon to the atmosphere despite having an acknowledged Climate Emergency.

# Ultrafine Particle Toxicity

Tian Xia , Paavo Korge , James N. Weiss , Ning Li , M. Indira Venkatesen , Constantinos Sioutas , and Andre Nel
**Published:**1 October 2004https://doi.org/10.1289/ehp.7167**Cited by:**231

## *Abstract*

Particulate pollutants cause adverse health effects through the generation of oxidative stress. A key question is whether these effects are mediated by the particles or their chemical compounds. In this article we show that aliphatic, aromatic, and polar organic compounds, fractionated from diesel exhaust particles (DEPs), exert differential toxic effects in RAW 264.7 cells. Cellular analyses showed that the quinone-enriched polar fraction was more potent than the polycyclic aromatic hydrocarbon (PAH)–enriched aromatic fraction in $O_2^{\bullet -}$ generation, decrease of membrane potential ($\Delta\Psi m$), loss of mitochondrial membrane mass, and induction of apoptosis. A major effect of the polar fraction was to promote cyclosporin A (CsA)–sensitive permeability transition pore (PTP) opening in isolated liver mitochondria. This opening effect is dependent on a direct effect on the PTP at low doses as well as on an effect on $\Delta\Psi m$ at high doses in calcium ($Ca^{2+}$)-loaded mitochondria. The direct PTP effect was mimicked by redox-cycling DEP quinones. Although the aliphatic fraction failed to perturb mitochondrial function, the aromatic fraction increased the $Ca^{2+}$ retention capacity at low doses and induced mitochondrial swelling and a decrease in $\Delta\Psi m$ at high doses. This swelling effect was mostly CsA insensitive and could be reproduced by a mixture of PAHs present in DEPs. These chemical effects on isolated mitochondria could be reproduced by intact DEPs as well as ambient ultrafine particles (UFPs). In contrast, commercial polystyrene nanoparticles failed to exert mitochondrial effects. These results suggest that DEP and UFP effects on the PTP and $\Delta\Psi m$ are mediated by adsorbed chemicals rather than the particles themselves.

https://ehp.niehs.nih.gov/doi/full/10.1289/ehp.7167

**EXTRACT FROM EVIDENCE GIVEN TO INQUIRY 2003**

Primary particles are those emitted directly into the atmosphere from traffic, combustion sources and windblown dust. Secondary particles are formed in the atmosphere by chemical reactions, mainly through the oxidation of sulphur dioxide and nitrogen dioxide, to sulphate and nitrate particles. Clearly, a waste incinerator would be producing both types.

**To appreciate more fully the nature of the particulates being discussed, 1600 $PM_{10}$ particles would fit on the dot of the letter i in normal print. For $PM_{2.5}$ the figure would be 25,600 particles. In the case of ultra-fine particles, 0.001 micrometers or one nanometer, it takes 160 billion to cover the dot of the i.**[1]

The process of incineration turns solids and liquids partly into gases and partly into tiny particles of soot or ash. The resulting particles are exceedingly small when they are emitted to the environment. Scientists who study particles make a distinction between coarse and fine particles. Fine particles behave entirely differently from coarse particles and are much more dangerous to humans. Fine particles are much more difficult and expensive to control and are also invisible. Incinerators emit large numbers of particles, despite the best control technology. Half of all the particles emitted will have a diameter of less than 2 micrometers and the majority of those will have a diameter of 0.3 micrometers. **Each pound of fine particles emitted from an incinerator will consist of 140 quadrillion individual particles.** (A quadrillion is 1000 trillion). Over a year, an incinerator meeting US federal standards will legally emit anywhere from ten to one thousand tons of fine particles depending on the size of the incinerator.[2]

The human body has evolved to cope with its environment and has mechanisms capable of dealing with coarse particles. The membranes of the nose, throat and lungs help to trap dust. The deepest region of the lungs, the alveoli, where oxygen passes into the blood, should be protected from airborne particles. But we have not evolved sufficiently to deal with fine particles and once these reach the alveoli there is no clearance mechnism to remove them. Once lodged in the lung, fine particles, with their enormous surface area enriched with toxics, provide an efficient means of delivering metals and organic pollutants directly into the bloodstream. Those which are not soluble are retained in the deep lung for long periods (months or years).

Recent research suggests that certain spots in the lungs may accumulate far more cancer-causing airborne particles than was previously thought. A new computer model suggests that cells on the spurs between airways in the lungs build up inhaled carcinogens to concentrations at least a hundred times higher than elsewhere in the lungs, according to Thomas Heistracher of the Polytechnical University of Salzburg in Austria.[3] Current pollution regulations ignore such buildups but it means that allowable concentrations should be reduced.. Further experiments are to be carried out.

[1] Montague, Peter, "The Holy Grail of scientific certainty"
Rachel's Environment & Health Weekly No.440 4.5.95

2 Montague, Peter "Fine particles; the danger of incineration"
Rachel's Hazardous Waste News No 131 30.5.89
Download from website www.enviroweb.org/publications/rachel on 25.03.99

3 "Hot spots in lungs collect pollution" The Guardian, 24.4.03, Life Section, p7

The Scientific World Journal
Volume 2012, Article ID 980843, 8 pages
http://dx.doi.org/10.1100/2012/980843

**Research Article**

# Polycyclic Aromatic Hydrocarbons Bound to PM 2.5 in Urban Coimbatore, India with Emphasis on Source Apportionment

R. Mohanraj, S. Dhanakumar, and G. Solaraj
Department of Environmental Management, Bharathidasan University, Tamilnadu Tiruchirappalli 620024, India

Received 13 October 2011; Accepted 21 November 2011
Academic Editor: Wan Kuen Jo



## Abstract

Coimbatore is one of the fast growing industrial cities of Southern India with an urban population of 1.9 million. This study attempts to evaluate the trends of airborne fine particulates (PM 2.5) and polyaromatic hydrocarbons (PAH) on them. The PM 2.5 mass was collected in polytetra fluoroethylene filters using fine particulate sampler at monthly intervals during March 2009 to February 2010. PAHs were extracted from PM 2.5 and estimated by high-performance liquid chromatography. It is alarming to note that PM 2.5 values ranged between 27.85 and 165.75 $\mu$g/m3 and exceeded the air quality standards in many sampling events. The sum of 9 PAHs bound to PM 2.5 in a single sampling event ranged from 4.1 to 1632.3 ng/m3. PAH diagnostic ratios and principal component analysis results revealed vehicular emissions and diesel-powered generators as predominant sources of PAH in Coimbatore.

## 1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) among the urban air toxics are of global concern due to their multiple effects on human population. Attention is more importantly on PAHs bound to PM 2.5 and ultrafine fraction of the airborne particulates that are reportedly known for their higher health risk [1, 2]. About 80% of particulate matter in urban environment belongs to the size class of fine (PM 2.5) and ultrafine (PM 0.1) particles [3]. PAHs with two or three benzene rings existed in the vapour phase, whereas PAHs with more than five rings were observed mainly in the particulate phase [4]. PAH size distributions in the atmosphere are influenced by the growth of combustion-generated particles and the variation of PAH adsorption and absorption affinity based on physical characteristics and chemical composition [5, 6]. Higher-molecular-weight (HMW) PAHs, due to their lower vapor pressures, continue to remain glued with fine particles in urban areas where emission sources are high. The PAH “mix” is expected to vary seasonally and geographically as a consequence of changes in emission sources.

Major anthropogenic emission sources of PAHs include biomass burning, coal and petroleum combustion, and coke and metal production [7, 8]. Open burning of biomass, agricultural waste, and municipal solid waste also contribute to atmospheric PAHs. However, vehicular and domestic emissions mainly influence the variation in PAH concentration across the urban to rural gradient [9]. Reportedly higher concentrations of PAHs in urban air have become a major health issue mainly due to their well-known carcinogenic and mutagenic properties [10, 11].

Links between PAH exposure and elevated levels of DNA adducts, mutations, and reproductive defects have strengthened the notorious impact of PAHs [12, 13]. Studies also hint that PAHs can inhibit growth of diatoms and development effects in wildlife. PAHs are also likely to cause transboundary effects and ultimately interfere with the global carbon cycle [14].

In many Asian regions where rapid economic drive is noticed, studies have shown that motor vehicles (especially diesel-engine vehicles), factories, and home heating are the principal sources of atmospheric PAHs and NPAHs [15]. Two wheelers, a popular means of transport in Asian region, are believed to emit particulate PAHs even higher than gasoline- and diesel-powered passenger cars and light- and heavy-duty vehicles [16].

In many Indian cities, rapid urbanization mushrooming of industrialization have triggered the growth of transportation by all means including two wheeler sector. Consequently, these cities suffer from serious air quality problems including rise in PAHs [17, 18]. Annual PAH emissions of India are estimated to be 90 $Gg\,y^{-1}$ by Zhang and Tao [14]. Reports on fine-particulate-bound PAH are unavailable in many cities. In this context, the present work was aimed to explore the PM 2.5-bound PAH concentration in Coimbatore city and source apportionment. Another objective was to assess the influence of environmental parameters such as temperature, rain, wind speed, and relative humidity on PAH concentration.

## 2. Materials and Methods

### 2.1. Study Area Description

Coimbatore city and its environs with a population of 1,990,000 occupies the 225th position among the principal urban agglomerations of world as in 2011 (http://www.citypopulation.de/world/Agglomerations.html). Coimbatore city is one of the top ten fastest growing cities of India and one among the top 100 in the world. There are about 25.000 small-scale industries functioning in and around Coimbatore. The number of medium- and large-scale textile mills present in Coimbatore is 312. In the wake of urbanization and industrialization, the number of automobiles is also rising at a similar pace. Among the sources of air pollution, vehicular emissions, industrial emissions, and smoke arising from the garbage dump are prominent in the city. For the current study, five locations namely C1-Small Industries Developmental

Corporation (SIDCO), C2-Kuniyamuthur, C3-Kavundampalayam, C4-100 ft road, and C5-Lakshmi mills were selected to represent industrial, suburban residential, mixed commercial and residential, urban commercial, and urban highway, respectively (Figure 1).

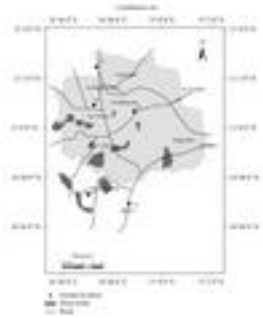

**Figure 1:** Map showing study sites in Coimbatore.

The total number of vehicles registered in Coimbatore city as in 2010 had exceeded 1 million. Over the quinquennium ending in 2006, 23 metros posted a compound annual growth rate of 8.3% in the number of total vehicle registrations. Significantly, among the second-tier cities Coimbatore with 12.9% growth rates stood first in Tamilnadu state, India, followed by Madurai city (10.9%) [19]. Coimbatore holds a share of 0.84% and 7.46% of total registered motor vehicles in India and Tamilnadu, respectively. Slower growth in goods vehicle category hints an economic shift from commodity-producing sector (agriculture and industry) towards service sector. High growth in personalized motor vehicles reflects rising per capita income of middle class market competition in automobile sector due globalization coupled with convenient financing options. This proliferation in the personalized mode of transport has evidently increased traffic congestion and particulate air pollution.

### 2.2. Fine Particulate Matter Sample Collection

Fine particulate matter (PM 2.5) sample collection was carried out by Fine Particulate Sampler (Model: APM 550, Manufacturer: Envirotech, New Delhi, India) during March 2009 to February 2010 at five locations once a month in each location for a period of 24 h. The sampling days were different for each location and not simultaneous. Sampler's omnidirectional inlet design prevents the entry of particles

greater than 10 microns from the ambient air by a clean aerodynamic cut-point. Further, particles finer than 10 microns in the air stream pass to a second impactor that has an aerodynamic cut-point at 2.5 microns. PM 2.5 particles are finally retained in 47 mm diameter polytetrafluoroethylene (PTFE) filters. The sampler was operated in each station at a constant flow rate of 1 $m^3$/hr. After 24 hours of operation, PM 2.5 deposited on the preweighed PTFE filters is estimated gravimetrically and stored in desiccators for PAH investigations.

### 2.3. PAH Analysis

It is assumed that fine particulate matter is uniformly distributed in the PTFE membrane filters. For more precise extraction of PAH from the PTFE filters, several solvents (cyclohexane, benzene, acetonitrile, methanol, dichloromethane) in various combinations were attempted and eventually dichloromethane:methanol (60 : 40) was chosen. A small piece of particulate laden PTFE filter measuring an area of 3.14 $cm^2$ was taken for extraction in a dark bottle by adding one mL of dichloromethane:methanol (60 : 40) mixture. Then the filter and solvent mixture was allowed to incubate for half an hour and then ultrasonically extracted for another half an hour [20]. The process was repeated 2-3 times, and final volume was made up to 2 mL. The extracted solution was filtered through 0.2 $\mu$m PTFE filter and then redissolved in acetonitrile for HPLC analysis.

HPLC (Manufacturer: Waters, USA) with PAH C18 column (5 $\mu$m 4.6 × 250 mm) and fluorescence detector (model: 2475) was used for detection of PAH compounds. Calibrations were performed using 9 individual PAH standards obtained from Supelco, USA, and subsequently samples were analyzed. Chromatographic peaks were identified on the basis of retention time. Fluorescence detector was set at 250 and 425 nm wavelengths, for excitation and emission, respectively. The gradient elution started with 50% water and 50% acetonitrile (up to 5 min), then acetonitrile was increased with a linear gradient to 100% and maintained up to 28 min. The last 12 min elution

(28 to 40 min) was performed in 50 : 50 gradients of water and acetonitrile. A total of 9 PAHs were analyzed for this study, namely, phenanthrene (PHENAN), anthracene (ANTH), benzo(a)anthracene (BaA), chrysene (CHRY), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo(a)pyrene (BaP), indeno-1, 2, 3-cd pyrene (IcdP), and benzo(ghi)perylene (BghiP). Results of the experiment were subjected to statistics using Statistical Package for Social Scientists (SPSS 11.0). Meteorological data (wind speed, temperature, humidity, and rainfall) obtained from Indian Meteorological Department (IMD) used for further statistical analysis (Table 1). A three-day average inclusive of sampling days and a day prior to it was considered for each meteorological variable.

**Table 1:** Meteorological conditions in Coimbatore during the study period1.

In order to ensure precision, blank PTFE filters were analyzed for PAH contamination prior to outdoor sample collection. The concentrations of PAH in blank filters were below detection limits for all 9 PAHs investigated. Recovery efficiency was estimated by spike method. Briefly, a half portion of the PTFE filters were spiked with a pre-determined amount of standard PAH solutions. Both the spiked portion of filter and another half of the original filter were subjected to the similar analytical method. In general, recoveries were obtained in the range from 72 to 98% throughout the analysis and values reported were mean of 3 replicates. Reproducibility of the PAH level was calculated based on relative standard deviations (RSDs) by 6-replicate analyses of the same aliquots.

## 3. Results and Discussion

PM 2.5 values in Coimbatore varied between 27.85 and 165.75 $\mu$g/m3 with an average of 76.28 $\mu$g/m3 (Figure 2). A maximum of 165.75 $\mu$g/m3 was recorded in the urban location C3 (Kaundampalayam) during the month of January. Since

Kaundampalayam lies at bottleneck edge of the city with roads bound to other towns and touristic locations like Ooty, traffic snarl is a common issue at this exit point. Moreover open garbage dumps present in the vicinity smoldering invariably further exacerbate air pollution levels. Industrial site C1 (SIDCO) with an annual mean of 91.69 $\mu$g/m3 of PM 2.5 was noted as the most polluted site. It is observed that during the month of June all the stations recorded lower PM values compared to other months indicating a cleansing role of southwest monsoonal rains that are prominent in Coimbatore.

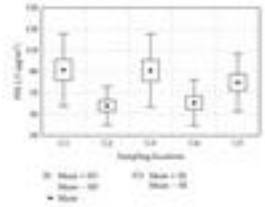

**Figure 2:** PM 2.5 trends in Coimbatore.

Lower particulate levels were recorded in suburban residential area C2 (Kuniamuthur), in the range of 36.2 to 88.40 $\mu$g/m3 with average of 57.93 $\mu$g/m3. PM 2.5 trend was observed in the following sequence: industrial (SIDCO) > mixed commercial and residential (Kaundampalayam) > urban highway (Lakshmi mills) > urban commercial (100 ft road) > suburban residential (Kuniamuthur). Levels of fine particulate matter in half the number of samples exceeded the 24-hour standard limit of National Ambient Air Quality Standards [34] (NAAQS) (65 $\mu$g/m3). Analysis of variance of fine particulate matter elucidated statistically significant difference () between sampling location and sampling events. Although no published data is available on PM 2.5 in Coimbatore for comparison, the present observation showed substantially higher concentrations compared to earlier observations of PM 10 indicating the rise in emission sources [35].

Descriptive statistics for nine PAH compounds identified in five sampling stations at Coimbatore are shown in Table 2. Annual average of nine PAHs at Kaundampalayam, Lakshmi mills, Kuniamuthur, 100 feet road, and SIDCO was 486.4, 276, 233.2, 175, and 143.48 ng/m3, respectively. As mentioned earlier, at

Kaundampalayam emissions from traffic and open garbage dump are probable sources for high levels of PAH. In SIDCO industrial area average of 9 PAHs observed during the study period was 143.48 ng/m3 without any significant monthly variation compared to other stations. However, BaA value in SIDCO recorded a onetime high value of 292.7 ng/m3 during December indicating an unusual source. In SIDCO, engineering and machining industries are mainly involved in production of various accessories and machines required for textile machines, electrical pumps, motors, and other machines, consisting of more than 4000 employees. Plastic industries are involved in manufacturing polythene bags, pipes, joints, and so forth. Main sources of air pollution in SIDCO are probably metal casting operations, cotton textiles, and diesel generators used as power backups. The majority of industries in SIDCO are engaged in metal fabrication and engineering works which obviously did not contribute to elevated levels of PAH although PM 2.5 values are high.

**Table 2:** Descriptive statistics for PAH concentrations in Coimbatore city.

In the urban sampling station at Lakshmi mills, CHRY, BbF, and BghiP were observed as predominant PAHs with annual average of 103.4, 33.2, and 26.6 ng/m3, respectively. BghiP has been identified as the tracer of gasoline emissions [36]. Earlier studies had confirmed substantial contribution of BghiP and IcdP from sparking ignition engines [31]. Maximum mean level of BaP, a potent carcinogen, was recorded to be the highest at Lakshmi mills (11.8 ng/m3) followed by Kaundampalayam (10.4 ng/m3). BaP concentration in Coimbatore city was tenfold higher than the NAAQS standard limit of 1 ng/m3. Motorcycles (both 2 strokes and 4 strokes) generally comprise 60–70% of vehicular fleet in Coimbatore city [37]. An earlier study hints that PAH emission factors were the largest for the 2-Stk/Cb

motorcycles. Moreover, the 2-Stk/Cb motorcycles had the largest total BaP equivalent emission factor of 10.8 $\mu$g/km [38]. Apart from automobile emissions, diesel generator sets used by the industries and medical waste incinerators are also suspected sources of elevated level of BaP [39]. Comparing the total PAH level in Coimbatore with other Indian cities, the present observations were higher than in Chennai and moderately lower than in Kanpur and Kolkata (Table 3).

**Table 3:** Comparison of total PAHs (ng/m3) in various cities of the India.

Domestic cooking fuels also significantly contribute to PAH emissions. A notable percent of the population depends on the following fuels: kerosene, charcoal, wood, straw/shrub/grass, agricultural crop waste, and dung cake for cooking and other domestic purposes [40]. An estimated 61.4 kg/year emission rate of total $BaP_{eq}$ from cooking sources was detected earlier [41].

To analyze the extent of seasonal variation in the PAH concentrations, the year was divided into four seasons that is, winter (December to February), summer (March to May), southwest monsoon (June to August), and northeast monsoon (September to November), as per regional meteorological considerations. The mean concentration of total PAHs in five sampling stations to recorded be 481.8, 193.7, 232.3, and 138.9 ng/m3 during winter, northeast monsoon, southwest monsoon, and summer season, respectively. Higher PAH levels in winter season suggest the influence of lower temperature and wind speed on stagnation of PAH. On the other hand, the alleviation role of monsoonal rains is also reflected clearly in those sampling months. Summer season recording the lowest levels also confirms photochemical degradation and dispersion of PAHs [42]. Elevated PAH concentration in winter is observed as a common phenomenon in many urban areas [43].

The relationship between PAHs with PM2.5 and meteorological parameters is given in Table 4. Strong positive correlation between IcdP with BbF, BkF, and PHENAN suggests similar source of origin, and negative relationship between these PAHs and temperature infers the role of photolytic degradation [25]. Moderate negative correlation observed between wind speed, rainfall, and individual PAHs suggests dilution and washout effect.

**Table 4:** Correlation between meteorological parameters and PAH.

PAH diagnostic ratio computation is one of the most abundantly used methods to identify emission sources based on the concentrations of specific PAH compounds or groups of PAHs. In the current study, site-specific PAH ratios attempted are shown in Table 5. Mean ratio of Ind/(Ind+ BghiP) ranging between 0.26 and 0.66 in all sampling stations indicates likely sources from diesel-driven trucks and catalyst cars. Higher ratio (0.5) of BaA/(CHRY+ BaA) in SIDCO industrial region suggests the use of furnace oil and foundry based furnace operations as source of PAHs. Ratio of BaA/(CHRY+ BaA) observed to be less than 0.3 in other sampling sites in the city suggests catalyst-equipped gasoline cars as a probable source.

**Table 5:** Site-specific diagnostic ratio of PAHs and their probable sources.

Principal component analysis was attempted to identify sources of particulate-bound PAHs in Coimbatore by applying varimax rotation with Kaiser Normalization. The number of significant factors within the data was established by considering only those with an Eigen value >1.0. The degree of association between each variable and each factor was given by its loading on that factor.

In PCA analysis, the total variability of data set is represented by two factors with an account of 74.8% (Figure 3). Factor 1 was loaded with ANTH, CHRY, BkF, BaP, and BghiP, which represents 47.4% of total variance. Most of the factor 1 loadings are tracers of diesel- and gasoline-powered vehicular emissions [5]. Apart from vehicular emissions, diesel generators may also contribute a notable level of these PAHs in Coimbatore city. Therefore, this factor is characteristic of vehicular and diesel generator emissions. Many industrial units in Coimbatore use diesel generators in the event of frequent electricity interruption from public distribution. According to the National Institute of Urban Affairs [44] report, totally 36.579 industrial units are in Coimbatore district, out of which 2.462 units (large industrial units: 138, Medium industrial units 1.082, and small industrial units 1.242) are located in urban limits.

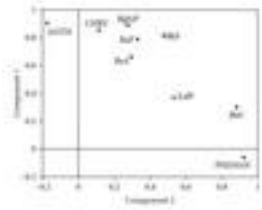

**Figure 3:** Factor analysis for PAH concentration in Coimbatore city.

Factor 2 represents 27.4% of variation with loadings of PHENAN and BbF suggesting combustion of wood materials and backyard burning of biomass as another source [45]. According to the Ministry of Health and Family Welfare [40], nearly 34% of urban and 75% of rural population use wood as a cooking fuel in the state of Tamilnadu. Earlier study also hinted that the ambient PAH concentrations increased significantly during cooking hours and incomplete burning of biomass [46].

## 4. Conclusions

Concentrations of PAHs associated with ambient PM 2.5 were determined at five sites in urban Coimbatore area, for a period of one year. It was found that the level of fine particulate matter (PM 2.5) and its associated polyaromatic hydrocarbons is almost similar to other major metropolitan cities in India. Maximum annual average of total of 9 PAHs was observed to

be the highest in urban bottleneck (Kaundampalayam: 486.4 ng/m3) than core urban areas (Lakshmi mills: 276 ng/m3, 100 feet road: 175 ng/m3). The most potent carcinogenic and genotoxic PAH, BaP, was recorded to be the maximum at Lakshmi mills (Annual mean = 11.8 ng/m3). The present observation shows that BaP concentration in Coimbatore city was tenfold higher than the NAAQS standard limit of 1 ng/m3. Seasonal variation pattern shows significant rise in PAHs concentration during winter compared to other seasons. Source apportionment, analysis reveals vehicular emissions and diesel generator as predominant emission sources of PAH in Coimbatore. Higher ratio (0.5) of BaA/(CHRY+ BaA) in SIDCO industrial region suggests the use of furnace oil and foundry-based furnace operations as source of PAHs. A need for inventory of air trajectory within urban canyon and implementation of good practices such as land use policy and vehicular emission control is imperative.

## Acknowledgments

The authors gratefully acknowledge the Defence Research and Developmental Organization (DRDO), Government of India, for financial support and UGC innovative program for instrumental facility. They are also thankful to Dr. P. A. Azeez, Salim Ali Centre, Coimbatore, for his valuable inputs and support.

## References

1. T. W. Hesterberg, W. B. Bunn, G. R. Chase et al., "A critical assessment of studies on the carcinogenic potential of diesel exhaust," *Critical Reviews in Toxicology*, vol. 36, no. 9, pp. 727–776, 2006. View at Publisher · View at Google Scholar · View at Scopus
2. D. J. Briggs, K. de Hoogh, C. Morris, and J. Gulliver, "Effects of travel mode on exposures to particulate air pollution," *Environment International*, vol. 34, no. 1, pp. 12–22, 2008. View at Publisher · View at Google Scholar · View at Scopus
3. L. Morawska, S. Thomas, N. Bofinger, D. Wainwright, and D. Neale, "Comprehensive characterization of aerosols in a

subtropical urban atmosphere: particle size distribution and correlation with gaseous pollutants," *Atmospheric Environment*, vol. 32, no. 14-15, pp. 2467–2478, 1998. View at Publisher · View at Google Scholar · View at Scopus

4. S. O. Baek, M. E. Goldstone, P. W. W. Kirk, J. N. Lester, and R. Perry, "Concentrations of particulate and gaseous polycyclic aromatic hydrocarbons in London air following a reduction in the lead content of petrol in the United Kingdom," *Science of the Total Environment*, vol. 111, no. 2-3, pp. 169–199, 1992. View at Google Scholar · View at Scopus
5. C. Venkataraman, J. M. Lyons, and S. K. Friedlander, "Size distributions of polycyclic aromatic hydrocarbons and elemental carbon. 1. Sampling, measurement methods, and source characterization," *Environmental Science Technology*, vol. 28, no. 4, pp. 555–562, 1994. View at Google Scholar · View at Scopus
6. J. O. Allen, N. M. Dookeran, K. A. Smith, A. F. Sarofim, K. Taghizadeh, and A. L. Lafleur, "Measurement of polycyclic aromatic hydrocarbons associated with size-segregated atmospheric aerosols in Massachusetts," *Environmental Science and Technology*, vol. 30, no. 3, pp. 1023–1031, 1996. View at Google Scholar · View at Scopus
7. S. O. Baek, M. E. Goldstone, P. W. W. Kirk, J. N. Lester, and R. Perry, "Phase distribution and particle size dependency of polycyclic aromatic hydrocarbons in the urban atmosphere," *Chemosphere*, vol. 22, no. 5-6, pp. 503–520, 1991. View at Google Scholar · View at Scopus
8. Y. Zhang, H. Dou, B. Chang et al., "Emission of polycyclic aromatic hydrocarbons from indoor straw burning and emission inventory updating in China," *Annals of the New York Academy of Sciences*, vol. 1140, pp. 218–227, 2008. View at Publisher · View at Google Scholar · View at Scopus

9. K. Ravindra, R. Sokhi, and R. Van Grieken, “Atmospheric polycyclic aromatic hydrocarbons: source attribution, emission factors and regulation,” *Atmospheric Environment*, vol. 42, no. 13, pp. 2895–2921, 2008. View at Publisher · View at Google Scholar · View at Scopus
10. K. De-Raat, “Polycyclic aromatic hydrocarbons and mutagens in ambient air particles,” *Toxicological and Environmental Chemistry*, vol. 16, p. 259, 1988. View at Google Scholar
11. N. T. Kim Oanh, L. H. Nghiem, and Y. L. Phyu, “Emission of polycyclic aromatic hydrocarbons, toxicity, and mutagenicity from domestic cooking using sawdust briquettes, wood, and kerosene,” *Environmental Science and Technology*, vol. 36, no. 5, pp. 833–839, 2002. View at Publisher · View at Google Scholar · View at Scopus
12. F. Perera, K. Hemminki, W. Jedrychowski et al., “In Utero DNA damage from environmental pollution is associated with somatic gene mutation in newborns,” *Cancer Epidemiology Biomarkers and Prevention*, vol. 11, no. 10 I, pp. 1134–1137, 2002. View at Google Scholar · View at Scopus
13. L. Gaspari, S. -S. Chang, R. M. Santella, S. Garte, P. Pedotti, and E. Taioli, “Polycyclic aromatic hydrocarbon-DNA adducts in human sperm as a marker of DNA damage and infertility,” *Mutation Research—Genetic Toxicology and Environmental Mutagenesis*, vol. 535, no. 2, pp. 155–160, 2003. View at Publisher · View at Google Scholar
14. Y. Zhang and S. Tao, “Global atmospheric emission inventory of polycyclic aromatic hydrocarbons (PAHs) for 2004,” *Atmospheric Environment*, vol. 43, no. 4, pp. 812–819, 2009. View at Publisher · View at Google Scholar · View at Scopus
15. K. Hayakawa, N. Tang, T. Kameda, and A. Toriba, “Atmospheric behaviors of polycyclic aromatic hydrocarbons and nitro polycyclic aromatic hydrocarbons

in East Asia," *Asian Journal of Atmospheric Environment*, vol. 1, p. 19, 2007. View at Google Scholar
16. P. Spezzano, P. Picini, D. Cataldi, F. Messale, and C. Manni, "Particle- and gas-phase emissions of polycyclic aromatic hydrocarbons from two-stroke, 50-cm3 mopeds," *Atmospheric Environment*, vol. 42, no. 18, pp. 4332–4344, 2008. View at Publisher · View at Google Scholar · View at Scopus
17. H. Sharma, V. K. Jain, and Z. H. Khan, "Atmospheric polycyclic aromatic hydrocarbons (PAHs) in the urban air of Delhi during 2003," *Environmental Monitoring and Assessment*, vol. 147, no. 1–3, pp. 43–55, 2008. View at Publisher · View at Google Scholar · View at Scopus
18. P. Kulkarni and C. Venkataraman, "Atmospheric polycyclic aromatic hydrocarbons in Mumbai, India," *Atmospheric Environment*, vol. 34, no. 17, pp. 2785–2790, 2000. View at Publisher · View at Google Scholar · View at Scopus
19. Ministry of Shipping, Road Transport & Highways, *Road Transport Year Book (2006-07)*, Transport Research Wing, Government of India, New Delhi, India, 2009.
20. M. P. Colombini, R. Fuoco, S. Giannarelli et al., "Determination of polyaromatic hydrocarbons in atmospheric particulate samples by HPLC with fluorescence detection: a field application," *Microchemical Journal*, vol. 59, no. 2, pp. 228–238, 1998. View at Google Scholar · View at Scopus
21. R. V. Shankar, *Characterization of atmospheric polynuclear aromatic hydrocarbons with special reference to automobile exhaust*, Ph.D. thesis, Jawaharlal Nehru University, New Delhi, India, 1990.
22. National Environmental Engineering Research Institute, *Ambient Air Quality Status for Ten Cities of India (1991–2005)*, Nagpur, India, 2006.
23. H. Sharma, V. K. Jain, and Z. H. Khan, "Characterization and source identification of polycyclic aromatic hydrocarbons (PAHs) in the urban environment of Delhi,"

*Chemosphere*, vol. 66, no. 2, pp. 302–310, 2007. View at Publisher · View at Google Scholar · View at Scopus
24. R. Mohanraj, G. Solaraj, and S. Dhanakumar, "Fine particulate phase PAHs in ambient atmosphere of Chennai metropolitan city, India," *Environmental Science and Pollution Research*, vol. 18, no. 5, pp. 764–771, 2011. View at Publisher · View at Google Scholar
25. T. T. Hien, P. P. Nam, S. Yasuhiro, K. Takayuki, T. Norimichi, and B. Hiroshi, "Comparison of particle-phase polycyclic aromatic hydrocarbons and their variability causes in the ambient air in Ho Chi Minh City, Vietnam and in Osaka, Japan, during 2005-2006," *Science of the Total Environment*, vol. 382, no. 1, pp. 70–81, 2007. View at Publisher · View at Google Scholar · View at Scopus
26. N. R. Khalili, P. A. Scheff, and T. M. Holsen, "PAH source fingerprints for coke ovens, diesel and gasoline engines, highway tunnels, and wood combustion emissions," *Atmospheric Environment*, vol. 29, no. 4, pp. 533–542, 1995. View at Publisher · View at Google Scholar · View at Scopus
27. P. K. Pandey, K. S. Patel, and J. Lenicek, "Polycyclic aromatic hydrocarbons: need for assessment of health risks in India? Study of an urban-industrial location in India," *Environmental Monitoring and Assessment*, vol. 59, no. 3, pp. 287–319, 1999. View at Publisher · View at Google Scholar
28. A. M. Caricchia, S. Chiavarini, and M. Pezza, "Polycyclic aromatic hydrocarbons in the urban atmospheric particulate matter in the city of Naples (Italy)," *Atmospheric Environment*, vol. 33, no. 23, pp. 3731–3738, 1999. View at Publisher · View at Google Scholar · View at Scopus
29. A. Gogou, N. Stratigakis, M. Kanakidou, and E. G. Stephanou, "Organic aerosols in Eastern Mediterranean: components source reconciliation by using molecular markers and atmospheric back trajectories," *Organic*

*Geochemistry*, vol. 25, no. 1-2, pp. 79–96, 1996. View at Publisher · View at Google Scholar · View at Scopus
30. M. A. Sicre, J. C. Marty, A. Saliot, X. Aparicio, J. Grimalt, and J. Albaiges, "Aliphatic and aromatic hydrocarbons in different sized aerosols over the Mediterranean Sea: occurrence and origin," *Atmospheric Environment*, vol. 21, no. 10, pp. 2247–2259, 1987. View at Google Scholar · View at Scopus
31. W. F. Rogge, L. M. Hildemann, M. A. Mazurek, G. R. Cass, and B. R. T. Simoneit, "Sources of fine organic aerosol. 2. Noncatalyst and catalyst-equipped automobiles and heavy-duty diesel trucks," *Environmental Science and Technology*, vol. 27, no. 4, pp. 636–651, 1993. View at Google Scholar · View at Scopus
32. H. H. Yang, W. J. Lee, S. J. Chen, and S. O. Lai, "PAH emission from various industrial stacks," *Journal of Hazardous Materials*, vol. 60, no. 2, pp. 159–174, 1998. View at Publisher · View at Google Scholar · View at Scopus
33. M. F. Simcik, S. J. Eisenreich, and P. J. Lioy, "Source apportionment and source/sink relationships of PAHs in the coastal atmosphere of Chicago and Lake Michigan," *Atmospheric Environment*, vol. 33, no. 30, pp. 5071–5079, 1999. View at Publisher · View at Google Scholar · View at Scopus
34. *National Ambient Air Quality Standards*, Ministry of Forest and Environment, Government of India, New Delhi, India, 2009.
35. R. Mohanraj, P. A. Azeez, and T. Priscilla, "Heavy metals in airborne particulate matter of urban Coimbatore," *Archives of Environmental Contamination and Toxicology*, vol. 47, no. 2, pp. 162–167, 2004. View at Google Scholar
36. R. M. Harrison, D. I. T. Smith, and L. Luhana, "Source apportionment of atmospheric polycyclic aromatic hydrocarbons collected from an urban location in Birmingham, U.K," *Environmental Science and Technology*,

vol. 30, no. 3, pp. 825–832, 1996. View at Publisher · View at Google Scholar · View at Scopus
37. R. Mohanraj and P. A. Azeez, "Automobile pollution in Urban Coimbatore, India," *Nature Environment and Pollution Technology*, vol. 4, p. 626, 2005. View at Google Scholar
38. H. H. Yang, L. T. Hsieh, H. C. Liu, and H. H. Mi, "Polycyclic aromatic hydrocarbon emissions from motorcycles," *Atmospheric Environment*, vol. 39, no. 1, pp. 17–25, 2005. View at Publisher · View at Google Scholar · View at Scopus
39. I. F. Mao, C. N. Chen, Y. C. Lin, and M. L. Chen, "Airborne particle PM2.5/PM10 mass distribution and particle-bound PAH concentrations near a medical waste incinerator," *Atmospheric Environment*, vol. 41, no. 11, pp. 2467–2475, 2007. View at Publisher · View at Google Scholar · View at Scopus
40. Ministry of Health and Family Welfare, *National Family Health 2005-06 Survey—Tamilnadu*, Ministry of Health and Family Welfare, Government of India, New Delhi, India, 2008.
41. C. T. Li, Y. C. Lin, W. J. Lee, and P. J. Tsai, "Emission of polycyclic aromatic hydrocarbons and their carcinogenic potencies from cooking sources to the urban atmosphere," *Environmental Health Perspectives*, vol. 111, no. 4, pp. 483–487, 2003. View at Google Scholar · View at Scopus
42. H. Sharma, V. K. Jain, and Z. H. Khan, "Characterization and source identification of polycyclic aromatic hydrocarbons (PAHs) in the urban environment of Delhi," *Chemosphere*, vol. 66, no. 2, pp. 302–310, 2007. View at Publisher · View at Google Scholar · View at Scopus
43. S. S. Park, Y. J. Kim, and C. H. Kang, "Atmospheric polycyclic aromatic hydrocarbons in Seoul, Korea," *Atmospheric Environment*, vol. 36, no. 17, pp. 2917–2924, 2002. View at Publisher · View at Google Scholar · View at Scopus

44. National Institute of Urban Affairs, *Appraisal of City Development Plan Coimbatore*, New Delhi, India, 2006.
45. R. K. Larsen and J. E. Baker, "Source apportionment of polycyclic aromatic hydrocarbons in the urban atmosphere: a comparison of three methods," *Environmental Science and Technology*, vol. 37, no. 9, pp. 1873–1881, 2003. View at Publisher · View at Google Scholar · View at Scopus
46. A. Bhargava, R. N. Khanna, S. K. Bhargava, and S. Kumar, "Exposure risk to carcinogenic PAHs in indoor-air during biomass combustion whilst cooking in rural India," *Atmospheric Environment*, vol. 38, no. 28, pp. 4761–4767, 2004. View at Publisher · View at Google Scholar · View at Scopus

Environmental Health Perspectives

OPEN ACCESS



**Research**
Open Access

# Quinones and Aromatic Chemical Compounds in Particulate Matter Induce Mitochondrial Dysfunction: Implications for Ultrafine Particle Toxicity

Tian Xia , Paavo Korge , James N. Weiss , Ning Li , M. Indira Venkatesen , Constantinos Sioutas , and Andre Nel

**Published:**1 October 2004https://doi.org/10.1289/ehp.7167**Cited by:**231

# *Abstract*

Particulate pollutants cause adverse health effects through the generation of oxidative stress. A key question is whether these effects are mediated by the particles or their chemical compounds. In this article we show that aliphatic, aromatic, and polar organic compounds, fractionated from diesel exhaust particles (DEPs), exert differential toxic effects in RAW 264.7 cells. Cellular analyses showed that the quinone-enriched polar fraction was more potent than the polycyclic aromatic hydrocarbon (PAH)–enriched aromatic fraction in $O_2^{\bullet -}$ generation, decrease of membrane potential ($\Delta\Psi m$), loss of mitochondrial membrane mass, and induction of apoptosis. A major effect of the polar fraction was to promote cyclosporin A (CsA)–sensitive permeability transition pore (PTP) opening in isolated liver mitochondria. This opening effect is dependent on a direct effect on the PTP at low doses as well as on an effect on $\Delta\Psi m$ at high doses in calcium ($Ca^{2+}$)-loaded mitochondria. The direct PTP effect was mimicked by redox-cycling DEP quinones. Although the aliphatic fraction failed to perturb mitochondrial function, the aromatic fraction increased the $Ca^{2+}$ retention capacity at low doses and induced mitochondrial swelling and a decrease in $\Delta\Psi m$ at high doses. This swelling effect was mostly CsA insensitive and could be reproduced by a mixture of PAHs present in DEPs. These chemical effects on isolated mitochondria could be reproduced by intact DEPs as well as ambient ultrafine particles (UFPs). In contrast, commercial polystyrene nanoparticles failed to exert mitochondrial effects. These results suggest that DEP and UFP effects on the PTP and $\Delta\Psi m$ are mediated by adsorbed chemicals rather than the particles themselves.


There is increasing evidence that particulate pollutants induce inflammatory responses in the cardiorespiratory system (**Nel et al. 1998**; **Nightingale et al. 2000**; **Saldiva et al. 2002**). These proinflammatory effects have been linked to the ability of particulate matter (PM), such as diesel exhaust particles (DEPs), to generate reactive oxygen species (ROSs) and oxidative stress in macrophages, bronchial epithelial cells, and lung microsomes (**Gurgueira et al. 2002**; **Hiura et al. 1999**; **Kumagai et al. 1997**; **Nel et al. 2001**). The pro-oxidative effects of the intact particles can be mimicked by organic chemical components extracted from these particles (**Hiura et al. 1999**; **Kumagai et al. 1997**; **Li et al. 2002**). The PM-induced oxidative stress response is a

hierarchical event, which is characterized by the induction of antioxidant and cytoprotective responses at lower tiers of oxidative stress and by pro-inflammatory and cytotoxic responses at higher levels of oxidative stress (**Li et al. 2002**; **Xiao et al. 2003**).

Mitochondrial damage is a key event in PM-induced cytotoxicity (**Hiura et al. 1999**, **2000**). The initial response to PM is a decrease in mitochondrial membrane potential ($\Delta\Psi m$) and increased $O_2^{\bullet -}$ production, followed by cytochrome *c* release and inner mitochondrial membrane damage (**Hiura et al. 1999**, **2000**; **Upadhyay et al. 2003**). It is also of interest that the smallest and potentially most toxic ambient particles, ultrafine particles (UFPs), lodge inside damaged mitochondria (**Li et al. 2003**). UFPs have a physical diameter $< 0.1$ μm, which allows them to penetrate deep into the lung as well as into systemic circulation (**Nemmar et al. 2002**). Although it is still a matter of debate whether UFPs target the mitochondrion directly or enter the organelle secondary to oxidative damage (**Li et al. 2003**), PM-induced mitochondrial perturbation has important biologic effects, which include the initiation of apoptosis and decreased ATP production (**Hiura et al. 2000**). Although the particles themselves may play a role in mitochondrial damage, it has been demonstrated that the organic chemicals adsorbed on the particle surface mimic the effects of the intact particles (**Hiura et al. 1999**). These effects can also be reproduced by functionalized aromatic and polar chemical groups fractionated from DEPs by silica gel chromatography (**Alsberg et al. 1985**; **Li et al. 2000**). These compounds are toxicologically relevant because the aromatic fraction is enriched in polycyclic aromatic hydrocarbons (PAHs), whereas the polar fraction contains several oxy-PAH compounds, including quinones (**Alsberg et al. 1985**; **Li et al. 2000**). Quinones are able to redox cycle and to produce ROSs, whereas PAHs can be converted to quinones by cytochrome P450, epoxide hydrolase, and dihydrodiol dehydrogenase (**Penning et al. 1999**).

A key mitochondrial target for oxidizing chemicals is the permeability transition pore (PTP) (**Jajte 1997**; **Susin et al. 1998**; **Zoratti and Szabo 1995**). This calcium ($Ca^{2+}$)-, voltage-, and pH-sensitive pore is permeant to molecules of $< 1.5$ kDa and opens in the mitochondrial inner membrane when matrix $Ca^{2+}$ levels are increased, especially when accompanied by oxidative stress (**Bernardi 1999**; **Kushnareva and Sokolove 2000**; **Zoratti and Szabo 1995**). PTP opening causes massive *in vitro* mitochondrial swelling, outer membrane rupture, and release of proapoptotic factors such as cytochrome *c* (**Susin et al. 1998**). In addition, mitochondria become depolarized, causing inhibition of oxidative phosphorylation and stimulation of ATP hydrolysis. PTP opening is inhibited by cyclosporin A (CsA), which inhibits the peptidyl-prolyl *cis-trans* isomerase activity of cyclophilin D (**Bernardi 1999**). This has led to the proposal that PTP transition is mediated by a $Ca^{2+}$-triggered

conformational change of inner membrane proteins (**Woodfield et al. 1998**). However, although this model may explain the action of some PTP modulators, PTP open–close transitions are also regulated by physiologic factors, drugs, and chemicals (**Jajte 1997**; **Kushnareva and Sokolove 2000**). **Walter et al. (2000)** characterized endogenous ubiquinones that stimulate or inhibit pore function by means of a putative quinone binding site in the PTP.

The goal of our study was to clarify how redox-cycling DEP chemicals affect mitochondrial function, as well as to compare ambient UFPs with commercial nanoparticle effects on mitochondria. Aromatic, polar, and aliphatic chemical fractions, prepared by silica gel chromatography, were used to study CsA-sensitive mitochondrial swelling (PTP opening), $\Delta\Psi m$, $Ca^{2+}$ loading capacity, and mitochondrial respiration. We also compared isolated mitochondrial responses with perturbation of mitochondrial function in intact RAW 264.7 cells. Our data show that mitochondrial perturbation and induction of apoptosis by polar DEP chemicals involve CsA-sensitive PTP opening that can be mimicked by representative redox-cycling quinones present in DEPs. In contrast, the aromatic chemical fraction induced mostly CsA-insensitive mitochondrial swelling, which can be mimicked by a mixture of PAHs. Ambient UFPs induced a combination of aromatic and polar effects, whereas polystyrene nanoparticles were inactive.

# Materials and Methods

## *Reagents.*

Tetramethylrhodamine methyl ester (TMRM), propidium iodide (PI), sucrose, HEPES buffer salts, EGTA, ascorbic acid, succinate, malate, glutamate, carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), alamethacin (Ala), and tetraphenylphosphonium chloride were from Sigma (St. Louis, MO). The annexin V–fluorescein isothio-cyanate (FITC) kit was obtained from Trevigen (Gaithersburg, MD). 3,3′-Dihexyl-oxabarbocyanine iodide ($DiOC_6$), 10 *N*-nonylacridine orange (NAO), Calcium Green-5N, and hydroethidine (HE) were obtained from Molecular Probes (Eugene, OR). The PAH working standard (no. 8310) was purchased from Cerilliant Corporation (Round Rock, TX). All organic solvents used were of Fisher optima grade (Fisher Scientific, Hampton, NH), and the solid chemicals were of analytical reagent grade.

## *Preparation of crude DEP extracts.*

DEPs were obtained from M. Sagai (National Institute of Environment Studies, Tsukuba, Ibaraki, Japan). These particles were collected from a 4JB1-type light-duty, 2.74-L, four-cylinder Isuzu diesel engine (Isuzu Automobile Co., Tokyo, Japan) under a load of 6 kilogram meter onto a cyclone impactor (**Kumagai et al. 1997**). The particles were scraped from the glass fiber filters and stored as a powder under nitrogen gas. The particles

consist of aggregates in which individual particles are < 1 μm in diameter. The chemical composition of these particles, including PAH and quinone analysis, has been previously described (**Li et al. 2000**). DEP methanol extracts were prepared by suspending 100 mg particles in 25 mL methanol, followed by sonication and centrifuging the suspension at 2,000 rpm for 10 min at 4°C (**Hiura et al. 1999**). The supernatant was transferred to a preweighed polypropylene tube and dried under nitrogen gas. The tube was reweighed to determine the methanol-extractable phase. The dried extract was dissolved in DMSO, and aliquots stored at –80°C in the dark.

## *DEP fractionation by silica gel chromatography.*

DEPs (1.2 g) were sonicated in 200 mL methylene chloride, and the extract was filtered with a 0.45-μm nylon filter in a Millipore filtration system (**Li et al. 2000**). The methylene chloride extract was concentrated by rotoevaporation, and asphaltenes (insoluble, aromatic chemicals with nitrogen, oxygen, and sulfur heteroatoms) were precipitated by adding 25 mL hexane and shaking. The contents were left overnight in the freezer and then centrifuged, and the supernatant was collected. The precipitate was washed twice with hexane, and the washings were combined with the first hexane extract, concentrated, and dried over anhydrous sodium sulfate. The extract thus prepared was subjected to gravity-fed silica gel column chromatography. Three columns (1.5 × 50 cm) were packed with 26 g activated silica gel between 1 cm anhydrous sodium sulfate and conditioned with hexane. The extract was split into three equal aliquots and applied to each column. Aliphatic, aromatic, and polar fractions were successively eluted at 1.5 mL/min with 70 mL hexane, 150 mL hexane:methylene chloride (3:2, vol/vol), and 90 mL methylene chloride:methanol (1:1, vol/vol), respectively. The elution of the aromatic fraction was monitored by ultraviolet light at 365 nm. The respective eluates were combined and concentrated by rotoevaporation and made up to 1 mL in a 4-mL graduated vial, the aliphatic fraction in hexane and the others in methylene chloride. The vials were tightly sealed with a silicone-lined cap and stored at –80°C until use. The weight of the fractions was determined in a microbalance after evaporating off the hexane or methylene chloride from a known sample volume. Alkanes in the aliphatic fraction were characterized by gas chromatography (Varian 3400 with an SPI injector; Varian Inc., Palo Alto, CA) equipped with a flame ionization detector and a DB-5 column (30 m, 0.25 mm inner diameter, 0.25 μm film). The fractions were dried with $N_2$ gas and redissolved in DMSO for *in vitro* biologic studies.

## *PAH and quinone analyses.*

PAH content in each fraction was determined by an HPLC-fluorescence method that detects a signature group of 16 PAHs (**Li et al. 2003**). Quinone content was analyzed as described by **Cho et al. (2004)**. Briefly, quinones in the samples were derivatized and evaporated to approximately 50 μL under

nitrogen; then, 100 mg zinc, anhydrous tetrahydrofuran, and 200 µL acetic anhydride were added to samples. After heating at 80°C for 15 min, samples were cooled to room temperature and an additional 100 mg zinc was added, followed by an additional 15 min of heating. The reaction was quenched with 0.5 mL water and 2 mL pentane. After centrifugation at 750 × *g* for 10 min, the pentane layer was evaporated to dry and the residue was reconstituted in 50–100 µL dry acetonitrile. 1,2-Naphthoquinone (NQ), 1,4-NQ, phenanthraquinone (PQ), and anthraquinone (AQ) were analyzed by the electron-impact gas chromatography/mass spectrometry technique using an HP MSD mass spectrometer (Hewlitt Packard, Palo Alto, CA) equipped with an automatic sampler (**Cho et al. 2004**).

## *Cell culture and stimulation.*

RAW 264.7 cells were cultured in a 5% carbon dioxide in Dulbecco modified Eagle medium (DMEM) containing 10% fetal calf serum, 5,000 U/mL penicillin, 500 µg/mL streptomycin, and 2 mM l-glutamine. For exposure to DEP extracts and its fractions, aliquots of $3 \times 10^6$ cells were cultured in six-well plates in 3 mL medium at 37°C for the indicated time periods.

## *Cellular staining with fluorescent probes and flow cytometry.*

Cells were stained with fluorescent dyes diluted in DMEM, except for annexin V and PI, which were prepared in a commercial binding buffer (Trevigen). The following dye combinations were added for 15–30 min at 37°C in the dark: *a*) 0.25 µg/mL annexin V plus 47.5 µg/mL PI in 500 µL binding buffer (assessment of apoptosis); *b*) 20 nM $DiOC_6$ plus 2 µM HE (assessment of $\Delta\Psi m$ and mostly $O_2^{\bullet-}$ production, respectively); *c*) 100 nM NAO plus 2 µM HE (to assess cardiolipin mass and $O_2^{\bullet-}$ production, respectively). Flow cytometry was performed using a FACScan (Becton Dickinson, Mountain View, CA) equipped with a single 488-nm argon laser. $DiOC_6$, NAO, and annexin V-FITC were analyzed using excitation and emission settings of 488 nm and 535 nm (Fl-1 channel); PI, 488 nm and 575 nm (Fl-2 channel); and HE, 518 nm and 605 nm (Fl-3 channel). Forward and side scatter were used to gate out cellular fragments.

## *Preparation of mouse liver mitochondria and experimental conditions.*

We removed livers from Balb/c mice and isolated mito-chondria by a standard differential centrifugation procedure as previously described (**Xia et al. 2002**). Briefly, liver tissue was homogenized with four strokes of a Teflon pestle in buffer A (250 mM sucrose, 1 mM EGTA, and 5 mM HEPES, pH 7.4) on ice. After centrifugation at 1,000 × *g* for 10 min at 4°C, the supernatant was removed and recentrifuged at 10,000 × *g* for 10 min. The pellet was sequentially washed with buffer A and buffer B (buffer A without EGTA). The pellet was resuspended in buffer B and used within 5 hr after isolation.

Mitochondrial protein content was determined by the Bradford method (**Xia et al. 2002**).
Most of the isolated mitochondrial experiments were conducted in a fiberoptic spectrofluorimeter (Ocean Optics, Dunedin, FL), which uses a closed, continuously stirred cuvette at room temperature (**Korge et al. 2002**). Mitochondria were added to the cuvette at 0.1 mg/mL in a standard buffer containing 250 mM sucrose and 5 mM HEPES, pH 7.4. Substrates, $Ca^{2+}$, PI, inhibitors, and fluorescent indicators were added at the indicated concentrations as shown for each experiment.

## *Mitochondrial swelling assay.*

Mitochondria (0.1 mg/mL) were incubated in swelling buffer containing 250 mM sucrose, 5 mM HEPES (pH 7.4), 2 µM rotenone, 1 mM PI, and 4.2 mM succinate at room temperature. Mitochondria were then exposed to different chemicals.
Changes in mitochondrial volume were estimated by measuring 90° light scatter with excitation and emission wavelengths set at 520 nm (**Walter et al. 2000**). Changes in matrix volume were reported as a percentage of maximum (100%) swelling induced by 10µg Ala at the end of each run.

## *Measurement of ΔΨm.*

TMRM was included at 400 nM, and ΔΨm was estimated at a wavelength of 570 nm (**Korge et al. 2002**). Decrease in ΔΨm was expressed as percentage decrease in TMRM fluorescence compared with the effect of 1 µM CCCP (100%) in fully energized mitochondria. Light scattering was recorded simultaneously with TMRM fluorescence. In some experiments, ΔΨm was estimated using an ion-selective electrode to measure tetraphenylphosphonium ion ($TPP^+$) distribution with a Flex-Ref electrode and Duo 18 recording system (World Precision Instruments, Sarasota, FL). $TPP^+$ was added to a final concentration of 3 µM, and the mitochondria were energized by adding succinate at 4.2 mM.

## *Calcium Green-5N assay to assess mitochondrial $Ca^{2+}$ retention capacity.*

Changes in extramitochondrial $Ca^{2+}$ concentration were followed by measuring Calcium Green-5N (1 µM, salt form) fluorescence at excitation and emission wavelengths of 475 and 530 nm, respectively. Individual $Ca^{2+}$ additions were calibrated by adding known quantities of $Ca^{2+}$ to the buffer in the presence of mitochondria and CCCP to block $Ca^{2+}$ uptake. Addition of chemical materials did not exhibit autofluorescence in our spectrofluorimetry assays.

## *Assessment of mitochondrial respiration.*

Mitochondrial respiration was carried out in the fiberoptic spectrofluorimeter in the presence of different substrates: succinate, 4.2 mM (complex II); malate/

pyruvate/glutamate, 5 mM each (complex I); tetramethyl-*p*-phenylenediamine (TMPD) and ascorbate, 0.2 mM and 2.5 mM, respectively (complex IV) (**Korge et al. 2002**). The addition of 2 μM CCCP was used as an inducer of maximal respiration. The partial pressure of $O_2$ in the buffer was continuously recorded by a fiber-optic oxygen sensor (Foxy Al-300; Ocean Optics, Dunedin, FL).

## *Collection of UFPs and assessment of their chemical composition.*

UFPs were collected using the Versatile Aerosol Concentration Enrichment System (VACES) in Downey, California, as previously described by **Li et al. (2003)**. Highly concentrated liquid particle suspensions were obtained by connecting the concentrated output flow from the VACES to a liquid impinger (BioSampler; SKC West Inc., Fullerton, CA). Particles were injected into the BioSampler in a swirling flow pattern so that they could be collected in a small volume of water by a combination of inertial and centrifugal forces.

For chemical analysis, we collected two reference filter samples in parallel with the VACES. The first sample was collected on a Teflon filter (47 mm, polytetrafluoroethylene, 2μm pore; Gelman Science, Ann Arbor, MI). Mass concentrations were determined by weighing the Teflon filter before and after each field test in a Mettler 5 Microbalance (Mettler-Toledo Inc., Highstown, NJ). Laboratory and field blanks were used for quality assurance. The Teflon filters were then analyzed by X-ray fluorescence for measurement of trace-element and metal concentrations. The second collection was done on two 47-mm quartz filters (Pallflex Corp., Putnam, CT). These filters were used for measurement of inorganic ions as well as for determining PAH, elemental carbon (EC), and organic carbon (OC) concentrations. A slice of approximately 0.2 $cm^2$ from each filter was placed in a platinum boat containing manganese dioxide. The sample was acidified with an aliquot of HCl and heated to 115°C to form $CO_2$ as an index of particle-associated carbon. The boat was then inserted into a dual-zone furnace, where $MnO_2$ oxidized OC at 550°C and EC at 850°C. A flame ionization detector converted the $CO_2$ combustion product to $CH_4$ for detection. The remaining filter was divided in two equal parts: one half was analyzed by means of ion chromatography to determine the concentrations of particulate sulfate and nitrate; the other half was analyzed by a HPLC-fluorescence method for detection of a group of signature PAHs as previously described (**Li et al. 2003**).

## *Statistics.*

The experiments were reproduced four times, except where otherwise stated. Results were analyzed by Student's *t*-test, and changes were considered significant at $p < 0.05$.

# Results

## *Differential toxicity and mitochondrial effects exerted by aliphatic, aromatic, and polar DEP fractions.*

Previous data from our laboratory showed that crude organic DEP extracts mimic the effects of intact particles in ROS production and cytotoxicity (**Hiura et al. 1999**). Mitochondria play a key role in DEP-induced toxicity, as shown by an early decrease in ΔΨm, loss of inner membrane mass, caspase 9 activation, and onset of apoptosis (**Hiura et al. 2000**). To clarify which organic chemicals play a role in this cytotoxicity, the crude extract was fractionated by silica gel chromatography, as previously described (**Li et al. 2000**). Elution with increasingly polar solvents resulted in the recovery of aliphatic, aromatic, and polar fractions in the amounts shown in Table 1. Although the aromatic fraction was enriched for PAHs (Table 2), the polar fraction was devoid of this chemical group but contained an abundance of quinones (Table 3). No quinones were present in the aromatic fraction (Table 3).

**Table 1** Recovery of each fraction from 1.2 g DEPs.

| Fraction | Elution solvent | Solvent | Amount (mg) | Recovery (%)*a* |
|---|---|---|---|---|
| **Aliphatic** | **Hexane** | **Hexane** | **281.4** | **23.5** |
| **Aromatic** | **Hexane:MC (3:2)*b*** | **MC** | **125.6** | **10.5** |
| **Polar** | **MC:methanol (1:1)*b*** | **MC** | **119.8** | **10.0** |
| **Total** | | | **526.8** | **44.0** |

MC, methylene chloride.

aFrom 1.2 g DEPs, 347.6 mg asphaltene was recovered; this represents 29% recovery.

bVol:vol.

**Table 2** PAH content in each DEP fraction (ng/1.2 g DEPs).

| PAH | Crude extract | Aliphatic | Aromatic | Polar |
|---|---|---|---|---|
| **NAP** | **10,149** | **25.5** | **4,420** | **0** |
| **ACE** | **7,470** | **0** | **513** | **0** |
| **FLU** | **17,483** | **0** | **7,461** | **0** |

| | | | | |
|---|---|---|---|---|
| **PHE** | **179,714** | **17.2** | **133,069** | **0** |
| **ANT** | **2,759** | **0** | **1,133** | **145** |
| **FLT** | **77,278** | **0** | **54,122** | **1,266** |
| **PYR** | **60,425** | **0** | **28,024** | **59.6** |
| **BAA** | **10,349** | **0** | **7,392** | **0** |
| **CRY** | **18,026** | **0** | **9,237** | **0** |
| **BBF** | **5,510** | **0** | **2,053** | **0** |
| **BKF** | **2,275** | **0.33** | **391** | **0** |
| **BAP** | **1,777** | **0.51** | **30.2** | **0** |
| **DBA** | **1,841** | **0.69** | **106** | **0** |
| **BGP** | **2,104** | **1.32** | **130** | **0** |
| **IND** | **2,045** | **0** | **119** | **0** |

Abbreviations: ACE, acenaphthalene; ANT, anthracene; BAA, benzo(*a*)anthracene; BAP, benzo(*a*)pyrene; BBF, benzo(*b*)fluoranthene; BGP, benzo(*g,h,i*)perylene; BKF, benzo(*k*)fluoranthene; CRY, chrysene; DBA, dibenz(*a,h*)anthracene; FLT, fluoranthene; FLU, fluorene; IND, indeno(1,2,3-*c,d*)pyrene; NAP, naphthalene; PHE, phenanthrene; PYR, pyrene.

**Table 3** Quinone content in DEP fractions (ng/mg fraction).

| Quinone | Crude extract | Aliphatic | Aromatic | Polar |
|---|---|---|---|---|
| **1,2 NQ** | **22.34** | **ND** | **ND** | **25.09** |
| **1,4 NQ** | **19.94** | **ND** | **ND** | **75.88** |
| **9,10 PQ** | **18.73** | **ND** | **ND** | **66.25** |
| **9,10 AQ** | **69.34** | **ND** | **ND** | **405.02** |

ND, none detected.

RAW 264.7 cells were treated with these chemicals and assessed for evidence of apoptosis (Figure 1). Figure 1A and 1B show representative flow cytometry panels of an experiment that was performed in triplicate. The results demonstrate the induction of annexin $V_+/PI_-$ (lower right) and annexin

$V_+/PI_+$ (upper right) cells by the crude extract. These represent early and late apoptotic events, respectively, and can be combined with live (annexin $V_-/PI_-$, lower left) and dead (annexin $V_-/PI_+$, upper left) cells to provide a graphic display of cellular viability/toxicity (Figure 1C). This presentation format demonstrates that the polar fraction is considerably more toxic than the aromatic fraction, whereas the aliphatic fraction has no effect on cell viability (Figure 1C).

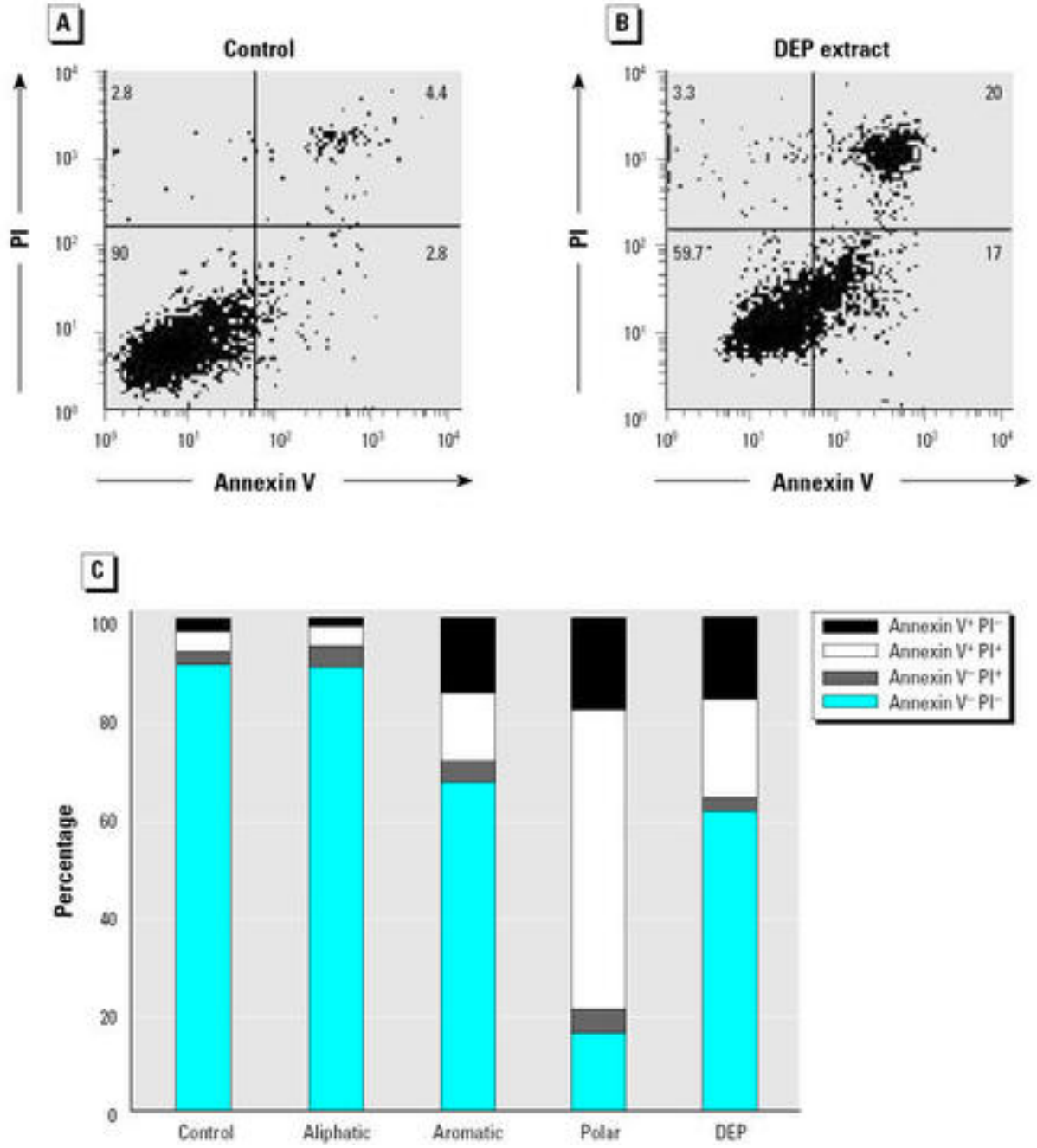


**Figure 1** Flow cytometry showing that DEP fractions induce apoptosis in RAW 264.7 cells. (*A*) Control. (*B*) DEP. Cells were treated with 25 µg/mL of the crude DEP extract for 12 hr, stained with annexin V-FITC and PI, and analyzed by flow cytometry. (*C*) Flow data expressed as a stack diagram, in which the crude extract data are compared with the effects of aliphatic, aromatic, and polar fraction, each used at 25 µg/mL; the data are

representative of three experiments in which the induction of apoptosis by the crude DEP material, as well as the aromatic and polar fractions, was statistically significant ($p < 0.05$).

To explore mitochondrial perturbation, we assessed ΔΨm and ROS production by dual-color $DiOC_6$/HE fluorescence (**Hiura et al. 1999**). $DiOC_6$ reflects ΔΨm, whereas HE measures mostly $O_2^{\bullet -}$ production. This analysis shows that although the aliphatic fraction was inactive, the aromatic and polar fractions induced the appearance of $DiOC_{6low}$/$HE_{high}$ subpopulations (Figure 2A). These effects were dose dependent (not shown), with the polar being more active than the aromatic fraction at comparable dose levels (Figure 2). To test whether $O_2^{\bullet -}$ production is related to inner membrane damage, we also performed dual-color NAO/HE fluorescence (Figure 2B). NAO binds to the inner membrane phospholipid, cardiolipin. Although NAO fluorescence is ΔΨm sensitive, a decrease in fluorescence reflects inner membrane damage. Both polar and aromatic compounds led to a decrease in inner membrane mass, whereas the aliphatic fraction was inactive (Figure 2). Cells with damaged mitochondria also showed increased HE fluorescence, which is in accordance with increased $O_2^{\bullet -}$ production by cells with reduced ΔΨm (Figure 2A). Overall, the polar fraction was more active than the aromatic fraction in its ability to induce these mitochondrial effects (Figure 2). Taken together, these results demonstrate that the aliphatic, aromatic, and polar fractions exert differential toxic effects on mitochondria and cellular viability.

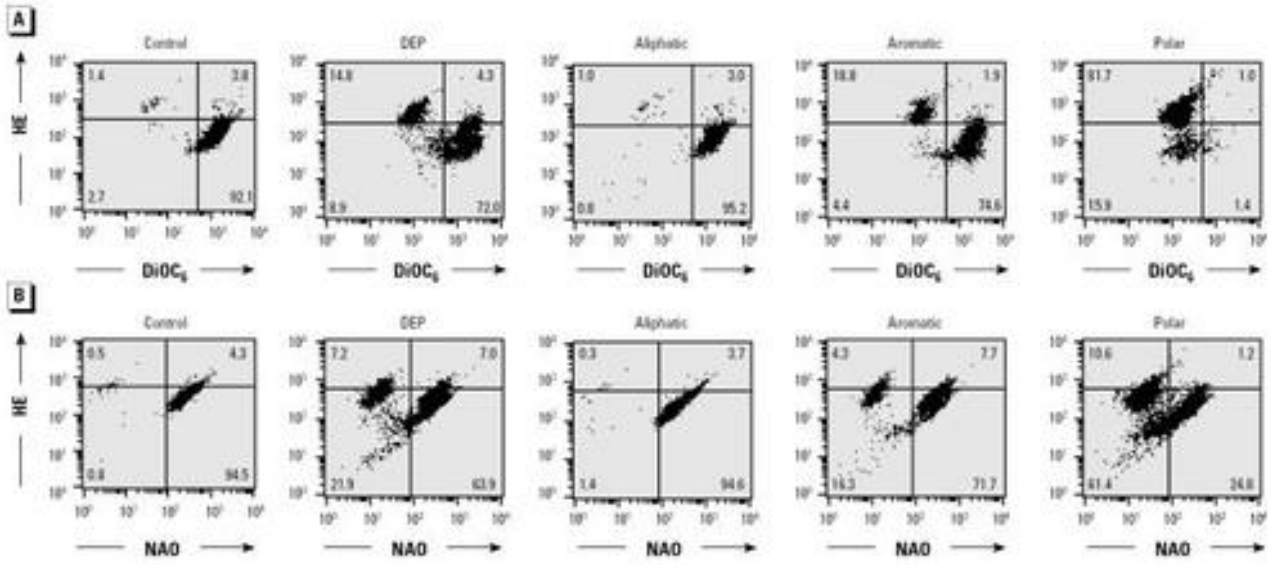


**Figure 2** Changes in ΔΨm, mitochondria mass, and ROS production induced by DEP chemicals in RAW 264.7 cells dual-color stained with either (*A*) HE (detects mostly $O_2^{\bullet -}$) plus $DiOC_6$ (ΔΨm) or (*B*) NAO (mitochondria mass) plus HE. RAW 264.7 cells were treated with 100 μg/mL DEP extract or its fractions for 5.5 hr before staining. Data are representative of two experiments.

## *Differential effects of the polar fraction on membrane depolarization and PTP opening.*

To further explore the action of functionalized DEP chemical groups on mitochondrial function, we performed a series of studies in isolated liver mitochondria. First, ΔΨm was recorded with a $TPP_+$ electrode after the addition of phosphate and succinate to the mitochondrial preparation (**Kushnareva and Sokolove 2000**). The addition of CCCP, a protonophore uncoupler, led to a quick dissipation of the ΔΨm (Figure 3A). Although the carrier (DMSO) and the aliphatic fraction were inactive (Figure 3A,B), the crude extract as well as the polar fraction induced a dose-dependent decline in ΔΨm (Figure 3C,D). The polar material was more potent and induced a faster rate of depolarization (Figure 3D).

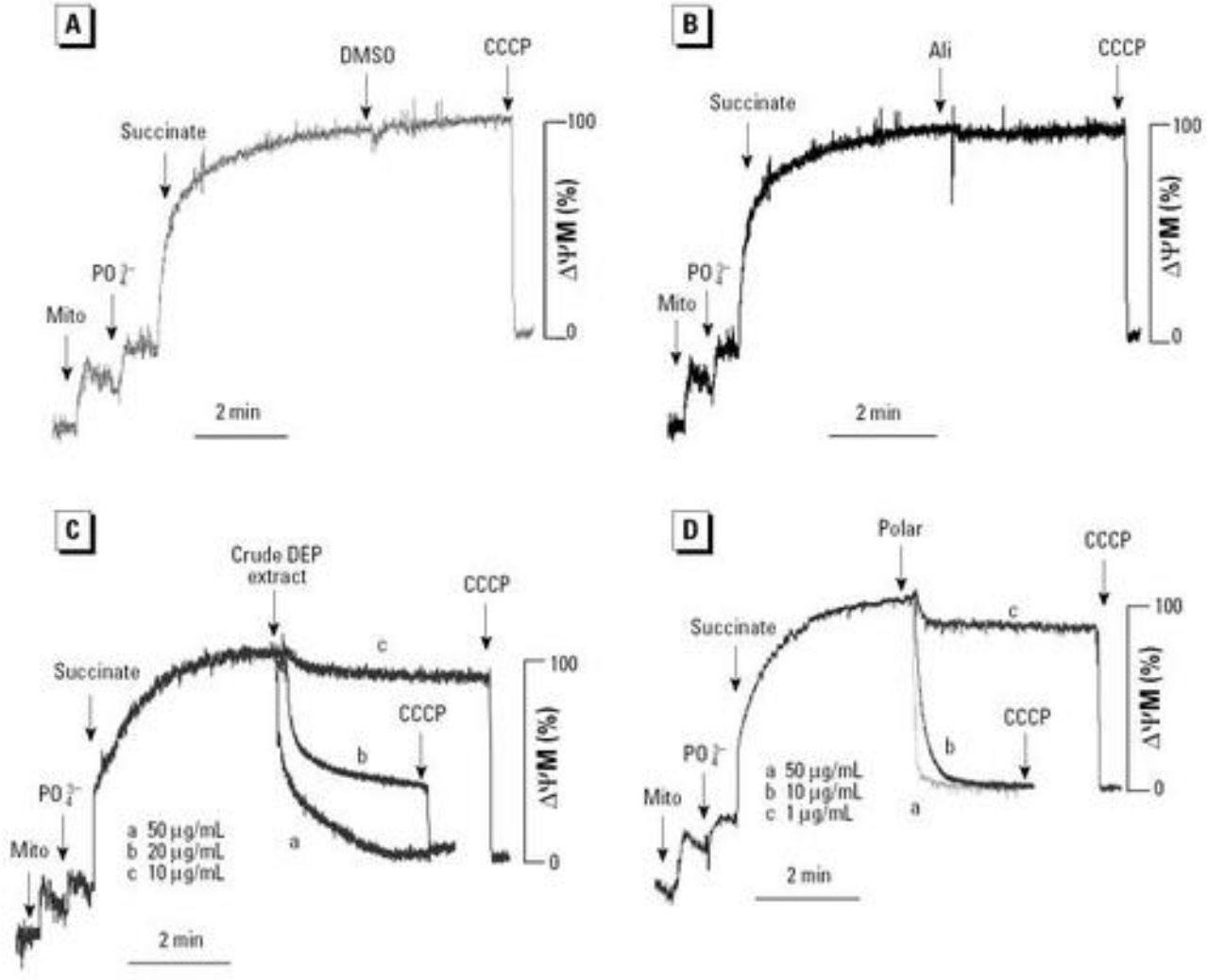


**Figure 3** Effects of organic DEP chemicals on ΔΨm in isolated mitochondria (Mito) incubated with 3 µM $TPP_+$, 1 mM phosphate ($PO_{43-}$), 4.2 mM succinate, and chemicals. (*A*) DMSO carrier. (*B*) Aliphatic fraction at 100 µg/mL. (*C*) Crude DEP extract. (*D*) Polar fraction. DEP extract and polar fraction were added as indicated by the arrows; CCCP was used to completely

depolarize the mitochondria and to serve as a quantitative control. Data are representative of four experiments.

If mitochondria are well polarized, addition of a large $Ca^{2+}$ load leads to matrix $Ca^{2+}$ uptake and PTP opening (**Korge et al. 2002**). PTP opening leads to mitochondrial swelling, which can be followed by using 90° light scatter in a spectrophotometer (Figure 4A, a). In mitochondria that had not been subjected to a $Ca^{2+}$ load, addition of a small and nondepolarizing polar dose (1–2.5 µg/mL; Figure 3) caused spontaneous induction of mitochondrial swelling (Figure 4B, c and d). Compared with the lack of response to the DMSO carrier, these results were statistically significant ($p < 0.01$). In contrast, higher doses of the polar fraction (≥5 µg/mL) caused a statistically significant ($p < 0.01$) inhibition of $Ca^{2+}$-induced mitochondrial swelling (Figure 4A). The same effect ($p < 0.01$) was seen with the crude DEP extract (not shown). This inhibition of swelling can be attributed to the ΔΨm-reducing effects of these higher concentrations. This is similar to the ΔΨm dissipation by CCCP, which prevents the rise in matrix $Ca^{2+}$ required for PTP opening. If, on the other hand, matrix $Ca^{2+}$ is already elevated, ΔΨm depolarization promotes PTP opening because the PTP open probability is voltage dependent and increases with depolarization. To test this theory, isolated mitochondria were preexposed to a small $Ca^{2+}$ load (10 µM) that is insufficient to induce PTP opening, and then exposed to a higher polar concentration range. This led to a dose-dependent induction of mitochondrial swelling at all doses tested (Figure 4C). DMSO and the aliphatic fraction had no effect on mitochondrial swelling (not shown).

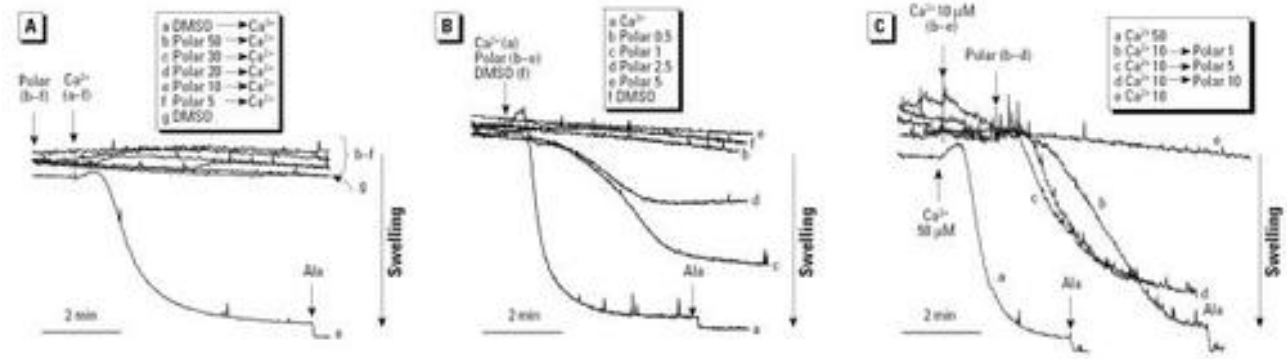


**Figure 4** Effects of DEP and the polar fraction on mitochondrial swelling. (*A*) 50 µM $Ca^{2+}$ added after DMSO and different doses of polar fraction (5, 10, 20, 30, 50 µg/mL); the control was DMSO alone. The data are representative of four experiments in which the inhibitory effect of polar concentrations ≥5 µg/mL on $Ca^{2+}$-induced swelling was statistically significant ($p < 0.01$). (*B*) 50 µM $Ca^{2+}$ introduced to induce swelling as a positive control; polar material (0.5, 1, 2.5, 5 µg/mL) was added in the absence of a $Ca^{2+}$ stimulus, and the control was DMSO alone. See “Materials and Methods” for details. (*C*) When previously loaded with a small amount of 10 µM $Ca^{2+}$, the subsequent

addition of the polar material (1, 5, 10 µg/mL) induced near-maximal mitochondrial swelling at all doses tested.

To confirm that mitochondrial swelling induced by the crude extract and polar fraction was due to PTP opening, we examined the effects of the PTP inhibitor CsA (Figure 5). Similar to its effect on $Ca^{2+}$-induced swelling, CsA added before the addition of the polar fraction (Figure 5A, a) abrogated polar-induced mitochondrial swelling in a statistically significant fashion ($p < 0.01$) (Figure 5B). $Ca^{2+}$-dependent mitochondrial swelling by the polar fraction was confirmed by prior addition of EGTA, which led to a significant reduction in the rate and magnitude of mitochondrial swelling in the presence of 1 µg/mL of the polar material (Figure 5C, b vs. c).

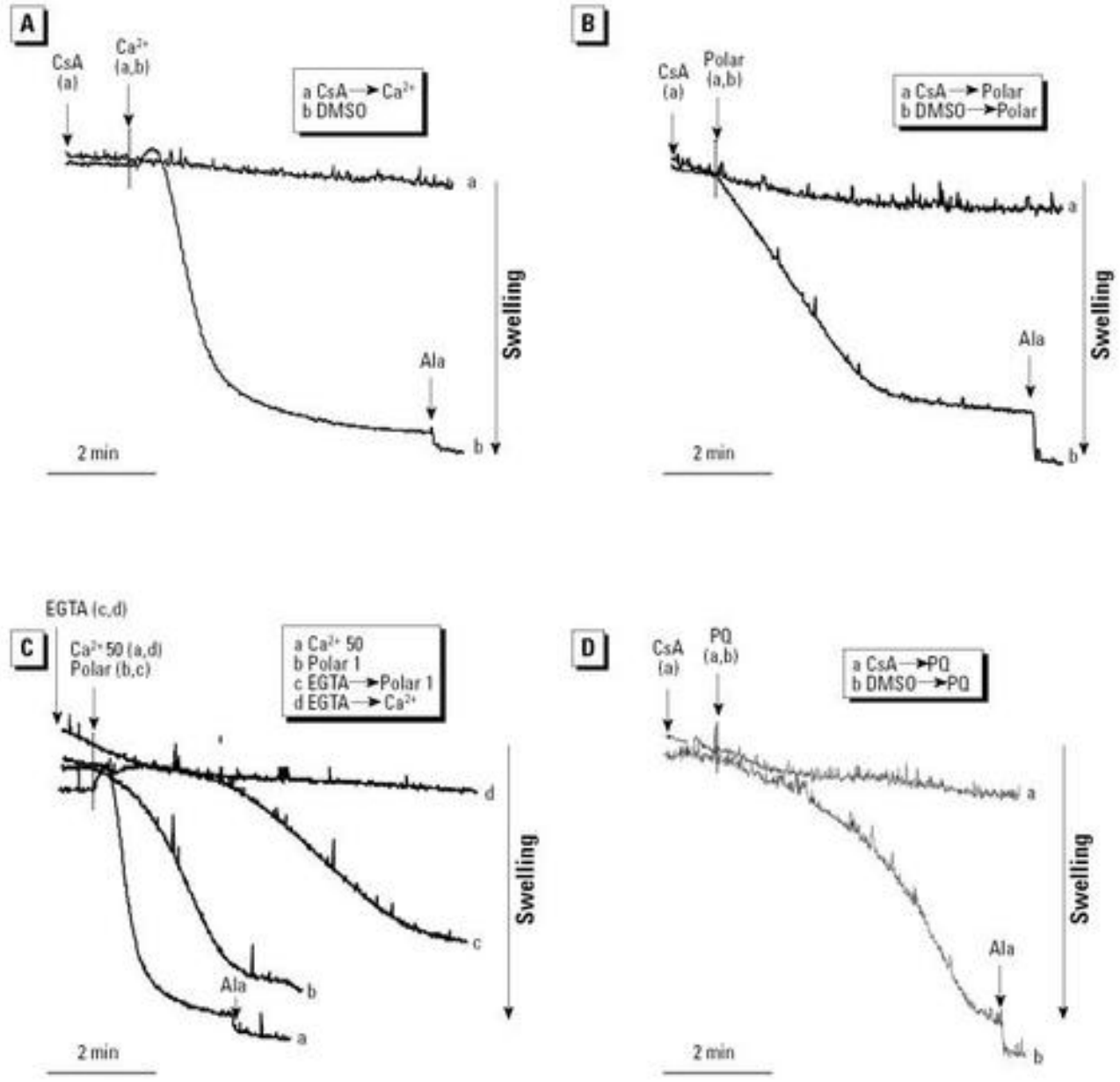


**Figure 5** Calcium-dependent PTP transition by the polar fraction and PQ in mitochondria incubated in swelling buffer. Mitochondria were then incubated with 1 µM CsA or DMSO before the addition of 50 µM $Ca^{2+}$ (*A*), 1 µg/mL polar fraction (*B*), and 5 µM PQ (*D*). (*C*) EGTA was added before the introduction of

1 µg/mL polar fraction. See “Materials and Methods” for details. The data are representative of four experiments, in which the swelling effect of the polar fraction and PQ where both statistically significant at $p < 0.01$. The inhibition by CsA was also statistically significant at $p < 0.01$.

The polar fraction contains a number of chemicals, among which the quinones participate in the generation of oxidative stress and covalent protein modification (**Penning et al. 1999**). We tested a number of DEP quinones (Table 3) for their effects on mitochondrial swelling, including PQ, 1,2-naphthaquinone, and AQ. PQ induced statistically significant ($p < 0.01$) mitochondrial swelling with slower kinetics than did the $Ca^{2+}$ load stimulus (Figure 5D). This effect was totally suppressed by CsA, indicating that quinones stimulate PTP activity in a $Ca^{2+}$-dependent fashion (Figure 5D). Similar results were obtained with 1,2-naphthaquinone, whereas a nonredox-cycling quinone, AQ, was inactive (not shown). These results suggest that redox-cycling quinones play a role in the cytotoxic effects of DEPs on the mitochondrion.

All considered, the data presented indicate that polar chemicals induce mitochondrial swelling due to PTP opening. This involves direct action on the PTP at low doses, as well as rapid-onset $\Delta\Psi m$ depolarization at higher doses, provided that the matrix $Ca^{2+}$ concentration is already elevated. In the absence of $Ca^{2+}$ loading, higher polar doses inhibit mitochondrial swelling, most likely due to interference in $Ca^{2+}$ accumulation as a result of $\Delta\Psi m$ depolarization.

## *Interference in the function of respiratory complexes by the polar fraction.*

Mitochondrial uncoupling increases mitochondrial respiration, which can be assessed by measuring oxygen consumption with an oxygen-sensing electrode (Figure 6). Although the polar fraction increased mitochondrial respiration as a consequence of its depolarizing effect (not shown), the induction of maximal respiration by CCCP in the presence of succinate showed that subsequent addition of the polar fraction caused a slowing of respiration (Figure 6A). The crude DEP extract had the same effect, whereas the aromatic or aliphatic fractions did not affect maximal mitochondrial respiration (Figure 6A). These findings indicate that the polar fraction and crude DEPs interfere in the function of complex II in the inner membrane. Similar results were obtained when using malate/glutamate/pyruvate, which are substrates for complex I (not shown). However, there was no effect when ascorbate and TMPD were used, implying that complex IV was not affected by the polar chemicals (Figure 6B). We propose that exogenous quinones present in the polar fraction might compete with the ubiquinones, which play a critical role in electron transfer in the inner membrane complexes. Transfer of those electrons to molecular dioxygen could explain $O_2^{\bullet -}$ production.

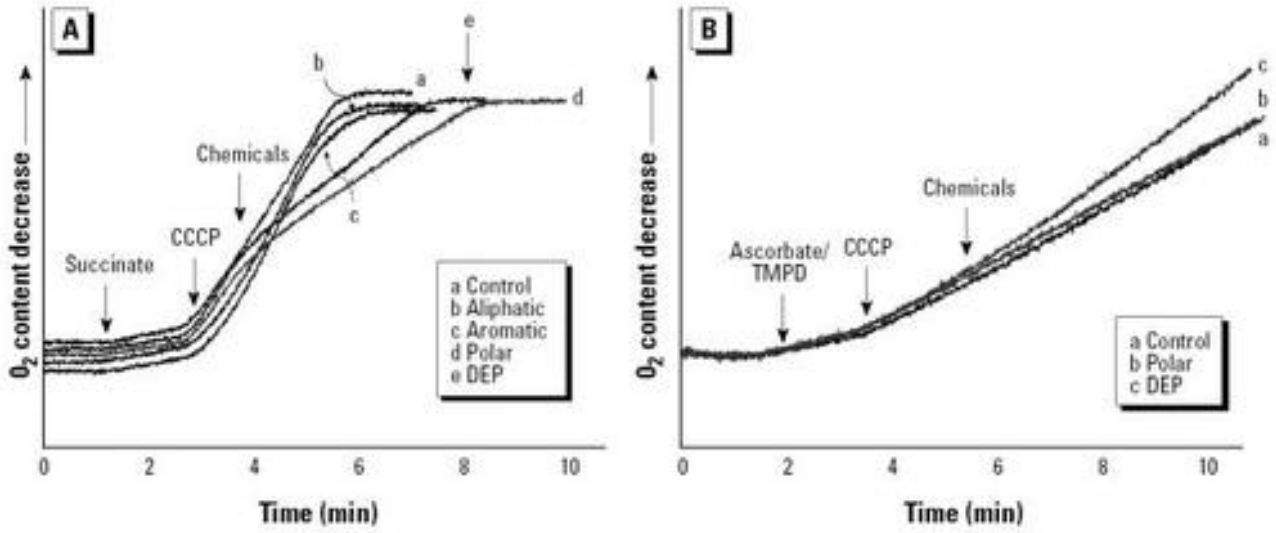


**Figure 6** Effects of organic DEP chemicals on mitochondrial respiration. (*A*) Succinate as a complex II substrate. (*B*) Ascorbic acid/TMPD as complex IV substrates. See "Materials and Methods" for details. Maximal mitochondrial respiration was initiated by 2 µM CCCP before the addition of DEP or its fractions at 50 µg/mL. Data are representative of three experiments.

## *Unique effects on ΔΨm, mitochondrial swelling, and $Ca^{2+}$ retention capacity exerted by the aromatic fraction and PAHs.*

Treatment with the aromatic fraction induced a dose-dependent ΔΨm decrease in isolated liver mitochondria at doses > 10 µg/mL (not shown). Unlike that observed with the polar fraction (Figure 3D), this depolarization was incomplete compared with CCCP (not shown). In addition, the aromatic fraction induced spontaneous mitochondrial swelling in a dose-dependent fashion (Figure 7A, b–f). In non-$Ca^{2+}$-loaded mitochondria, this effect started at aromatic doses ≥10 µg/mL (Figure 7A), whereas lower doses (e.g., 5 µg/mL) actually inhibited $Ca^{2+}$-induced swelling (Figure 7B). This is the opposite from the effect observed with the polar fraction, which interfered in mitochondrial swelling at high doses but induced spontaneous swelling at low doses (Figure 4B,C). Taken together, these data suggest that differences in the chemical composition of the aromatic and polar fractions lead to differential effects on mitochondrial function.

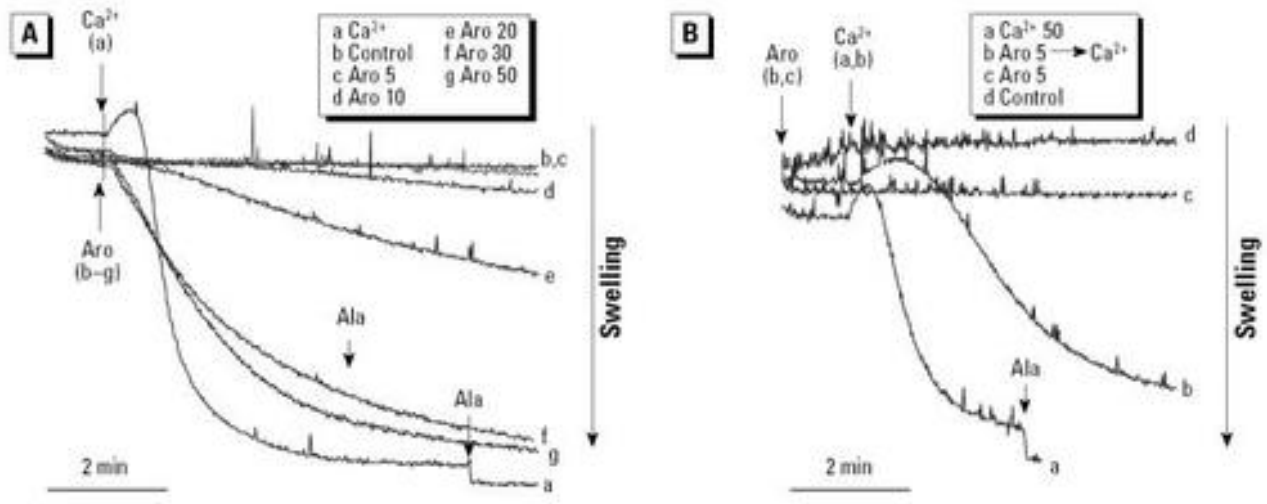


**Figure 7** Effects of the aromatic fraction on mitochondrial swelling. (*A*) 50 µM $Ca_{2+}$, DMSO alone, or different doses of aromatic (Aro) fraction (5, 10, 20, 30, 50 µg/mL); mitochondrial swelling was statistically significant ($p < 0.01$) at aromatic doses ≥20 µg/mL. (*B*) 50 µM $Ca_{2+}$, 5 µg/mL aromatic fraction (Aro) followed by 50µM $Ca_{2+}$, 5 µg/ml Aro alone, or control (DMSO alone). The data are representative of four experiments.

PAHs are the main components of the aromatic fraction and are capable of inducing apoptosis (**Li et al. 2000**). To test if PAHs exert an effect on isolated mitochondria, we used a commercial source composed of 16 DEP PAHs to conduct the swelling assay. This demonstrated that the PAH mix can induce slow-onset swelling in non-$Ca_{2+}$-loaded mitochondria, which mimics the effects of the aromatic fraction (Figure 8). This swelling effect was incomplete and was partially but statistically significantly ($p < 0.05$) inhibited by CsA (Figure 8B). CsA exerted the same effect on the induction of swelling by the aromatic fraction (Figure 8A).

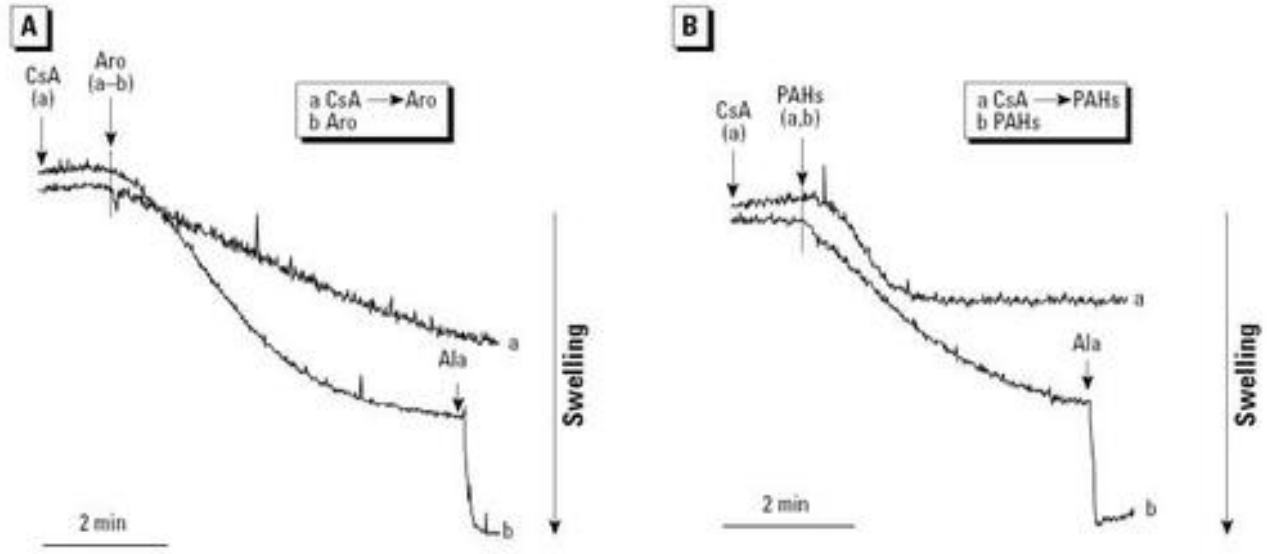


**Figure 8** Effects of the aromatic fraction and PAHs on mitochondrial swelling. (*A*) 1 µM CsA followed by the addition of 20 µg/mL aromatic fraction (Aro) or

20 µg/mL Aro alone; the experiment was reproduced four times, with statistically significant ($p < 0.05$) inhibition of mitochondrial swelling by CsA. (*B*) CsA followed by 7.8 µg/mL PAHs or PAHs alone; the experiment was reproduced four times, with statistically significant stimulation by PAHs ($p < 0.01$) and inhibition ($p < 0.01$) of the swelling effect by CsA. See “Materials and Methods” for details.

## *Use of mitochondrial calcium retention capacity to study differences between the polar and aromatic fractions on PTP opening.*

Calcium Green-5N is a fluorescent dye that can be used to assess the $Ca^{2+}$ retention capacity of isolated mitochondria. The addition of small amounts of $Ca^{2+}$ leads to a rapid matrix uptake into isolated energized mitochondria (Figure 9A). With repeated $Ca^{2+}$ pulses, matrix $Ca^{2+}$ eventually triggers PTP opening, which leads to depolarization and release of $Ca^{2+}$ from the matrix (Figure 9A). This leads to a precipitous and sustained increase in fluorescence intensity (Figure 9A). This response is statistically significantly ($p < 0.01$) inhibited by CsA, which increased the number of $Ca^{2+}$ pulses from 4 to 14 (Figure 9B). The aliphatic fraction had no effect on the number of $Ca^{2+}$ pulses (Figure 9C), whereas 1 µg/mL of the polar material reduced the number of $Ca^{2+}$ pulses required to trigger PTP transition (Figure 9D). This finding is compatible with the ability of the polar fraction to induce spontaneous mitochondrial swelling in a $Ca^{2+}$-dependent fashion (Figure 4C). Higher polar concentrations induced immediate release of ambient accumulated $Ca^{2+}$, which reflects its depolarizing effect (Figure 9C). Similar results were obtained with the crude DEP extract: a reduction in the required number of $Ca^{2+}$ pulses at low doses and precipitous $Ca^{2+}$ release at high doses (not shown).

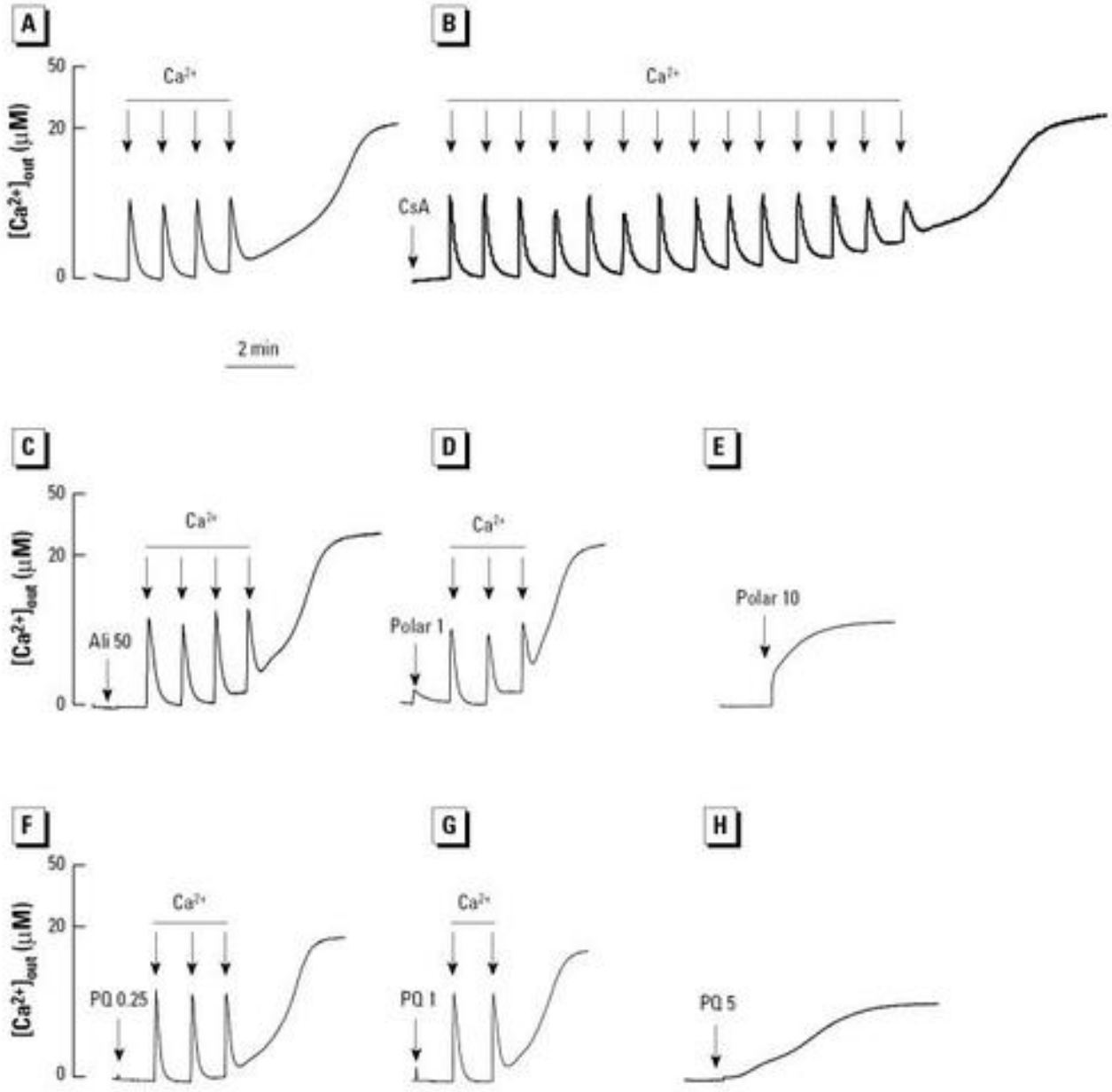


**Figure 9** Effect of the polar fraction and quinones on the $Ca^{2+}$ retention capacity of isolated mitochondria incubated with 1 μM Calcium Green-5N. After the addition of mitochondria, the following chemicals were added: (*A*) DMSO (carrier), (*B*) CsA, (*C*) aliphatic (Ali), (*D*) 1 μg/mL polar fraction, (*E*) 10 μg/mL polar fraction, (*F*) 0.25 μM PQ, (*G*) 1 μM PQ, and (*H*) 5 μM PQ. Each arrow represents one 5 μM $Ca^{2+}$ pulse. Data are representative of four experiments.

Because we have shown that DEP quinones mimic the effect of the polar fraction in spontaneous mitochondrial swelling, we also tested these quinones in the Calcium Green-5N assay. PQ reduced the required number of $Ca^{2+}$ applications to achieve PTP from 3, to 2, to 0 at PQ concentrations of 0.25, 1, and 5 μg/mL, respectively (Figure 9F–H). CsA could significantly ($p < 0.01$) increase the number of $Ca^{2+}$ pulses required for precipitous $Ca^{2+}$ release in

the presence of PQ, suggesting PTP involvement. Similar results were obtained with 1,2-NQ but not with AQ (not shown).
Examination of the aromatic fraction in the Calcium Green-5N assay showed that doses < 10 µg/mL increased the $Ca^{2+}$ retention capacity (Figure 10A,B). This is in keeping with the ability of the aromatic fraction to inhibit $Ca^{2+}$-induced PTP opening in this dose range (Figure 7B). At higher doses, the aromatic fraction induced a short $Ca^{2+}$ burst, probably related to $\Delta\Psi m$ depolarization, which is followed by a progressive decline in the ability of the matrix to accumulate $Ca^{2+}$ (Figure 10C). This depolarization was incomplete and not CsA sensitive (not shown). In order to determine whether this effect is related to the PAHs present in the aromatic fraction, the DEP PAH mixture was separately tested. PAHs mimicked the effect of the aromatic fraction in the low and high dose range (Figure 10D,E). Taken together, these results confirm that the polar and aromatic DEP compounds exert fundamentally different actions on mitochondria.

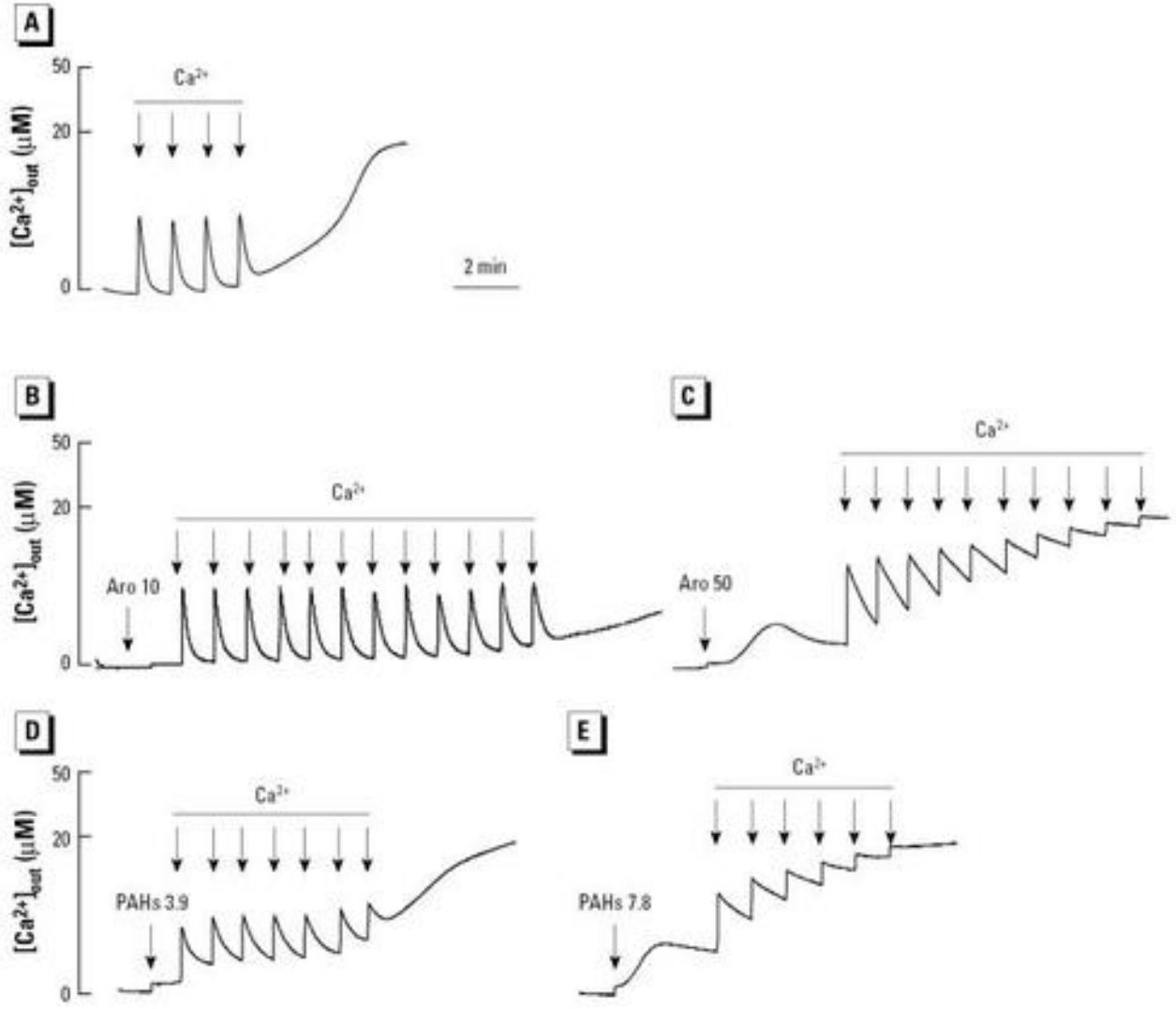


**Figure 10** Effect of the aromatic fraction and PAHs on the $Ca^{2+}$ retention capacity of isolated mitochondria incubated with 1 µM Calcium Green-5N. After the addition of mitochondria, the following chemicals were added: (*A*)

DMSO, (*B*) aromatic (Aro) 10 µg/mL, (*C*) Aro 50 µg/mL, (*D*) PAH mix 3.9 µg/mL, and (*E*) PAH mix 7.8 µg/mL. Each arrow represents one 5 µM $Ca^{2+}$ pulse. Data are representative of three experiments.

## *Effects of ambient UFPs on mitochondrial responses.*

A key question is whether the effects of the DEP chemicals can be reproduced with intact DEP and "real-life" ambient particles (**Li et al. 2003**). Intact DEPs induce apoptosis (**Hiura et al. 1999**), and ambient UFPs induce structural damage and lodge inside mitochondria in RAW 264.7 cells and epithelial cells (**Li et al. 2003**). When UFPs, collected by a particle concentrator in the Los Angeles Basin (**Kim et al. 2001**), were tested in the mitochondrial swelling assay, we observed spontaneous PTP opening at doses of 4.8 and 7.7 µg/mL in non-$Ca^{2+}$-loaded mitochondria (Figure 11, b and c). Swelling was partially reversed by CsA (Figure 11, d). At a dose of 1.9 µg/mL, UFPs did not induce spontaneous PTP opening but interfered with $Ca^{2+}$-induced swelling (not shown). This is similar to the effect of sonicated DEP, which interfered in $Ca^{2+}$-induced mitochondrial swelling in a dose-dependent fashion but failed to induce spontaneous swelling (Table 4). This could relate to differences in the particle size (the DEP powder used here contains particle aggregates) as well as differences in the bioavailability of surface chemical compounds on these particles. The chemical composition of UFPs is shown in Table 5. In contrast to the particulate pollutants, artificial polystyrene microspheres (size < 100 nm) did not exert an effect on mitochondrial swelling, and the mitochondria remained fully responsive to Ala (Figure 11, a).

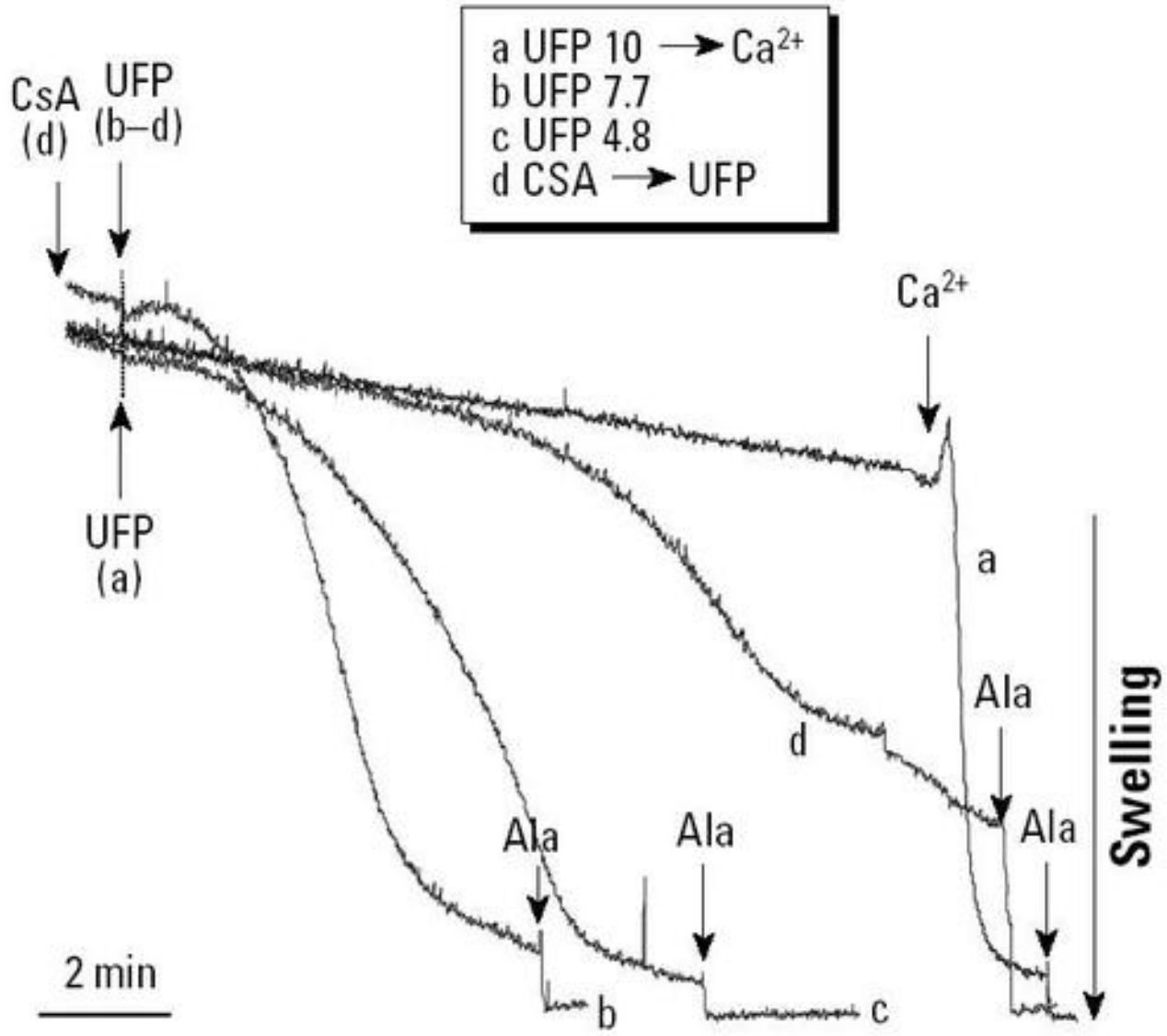


**Figure 11** Effects of UFP on mitochondrial swelling conducted in the presence of 10 µg/mL UFP followed by $Ca^{2+}$ (50 µM), 7.7 µg/mL UFP without $Ca^{2+}$ loading, 4.8 µg/mL UFP without $Ca^{2+}$ loading, or 1 µM CsA followed by 7.7 µg/mL UFP. Data are representative of three experiments.

**Table 4** Comparison of DEP and UFP effects on isolated mitochondria.

| Assay | DEP particle | Ambient UFPs |
|---|---|---|
| **ΔΨm** | **Dose-dependent delayed or rapid depolarization** | **Rapid depolarization** |
| | **CsA insensitive** | **CsA insensitive** |
| **Mitochondrial $Ca^{2+}$ retention capacity** | **Decreased retention capacity** | **Decreased retention capacity** |

| | | |
|---|---|---|
| | **CsA sensitive** | **CsA sensitive** |
| **Mitochondrial swelling** | **Dose-dependent inhibition of $Ca^{2+}$-induced swelling** | **Inhibition of $Ca^{2+}$-induced swelling at low doses (1 µg/mL)** |
| | **No spontaneous swelling effects at any dose** | **Spontaneous swelling at doses > 1.9 µg/mL** |
| | | **Partially CsA sensitive** |

All assays were performed as described in “Materials and Methods”; DEPs were sonicated and tested in the dose range 1–50 µg/mL.

**Table 5** Chemical composition of UFPs (percentage of PM mass).

| **Major elements (%)** | **Inorganic ions (%)** | **EC** | **OC** | **PAH** |
|---|---|---|---|---|
| **Na (0.84)** | **Nitrate (4.9)** | | | **PHE (1.75)** |
| **Al (8.80)** | **Sulfate (17.6)** | | | **FLT (2.72)** |
| **Si (14.19)** | | | | **PYR (2.94)** |
| **Cl (0.10)** | | | | **BAA (1.90)** |
| **K (0.67)** | | | | **CRY (2.53)** |
| **Ca (2.05)** | | | | **BBF (2.39)** |
| **Ti (0.47)** | | | | **BKF (1.04)** |
| **V (0.08)** | | | | **BAP (2.45)** |
| **Cr (0.07)** | | | | **BGP (10.38)** |

| | | | | |
|---|---|---|---|---|
| **Mn (0.09)** | | | | **IND (3.04)** |
| **Fe (3.20)** | | | | |
| **Ni (0.05)** | | | | |
| **Cu (0.19)** | | | | |
| **Zn (0.10)** | | | | |
| **Br (0.01)** | | | | |
| **Sr (0.01)** | | | | |
| **Zr (0.01)** | | | | |
| **Ba (0.10)** | | | | |
| **Pb (0.02)** | | | | |
| **Total 31%** | **23%** | **2%** | **41%** | **31.1%** |

Abbreviations: BAA, benzo(*a*)anthracene; BAP, benzo(*a*)pyrene; BBF, benzo(*b*)fluoranthene; BGP, benzo(*ghi*)perylene; BKF, benzo(*k*)fluoranthene; CRY, chrysene; FLT, fluoranthene; IND, indeno(1,2,3-*cd*)pyrene; PHE, phenanthrene; PYR, pyrene. All species are expressed as a percentage of the total PM mass except PAHs, which are expressed in nanograms per milligram of PM mass. The data show an excellent balance between the total mass and the sum of the measured chemical species, which account for 97% of the total UFP mass. OC is the most predominant species, contributing 41% of the mass. Trace elements and metals, such as Al, Si, Ca, and Fe, are also significant. BGP is the most abundant PAH in the UFP mode.

In the Calcium Green-5N assay, ambient UFPs induced instantaneous $Ca^{2+}$ release but reduced $Ca^{2+}$ retention capacity in a dose-dependent manner (Figure 12A vs. Figure 12C–F). CsA prevented this effect (Figure 12G). Sonicated DEPs had a similar effect that was also CsA sensitive (Table 4). In contrast, polystyrene microspheres (80 nm) had no effect on $Ca^{2+}$ retention capacity (Figure 12B). This suggests that the effect of the ambient UFP is dependent on their content of redox-cycling chemicals. Taken together with the data shown in Figure 11, the UFP effects appear to be a summation of the effects of polar and aromatic chemical compounds.

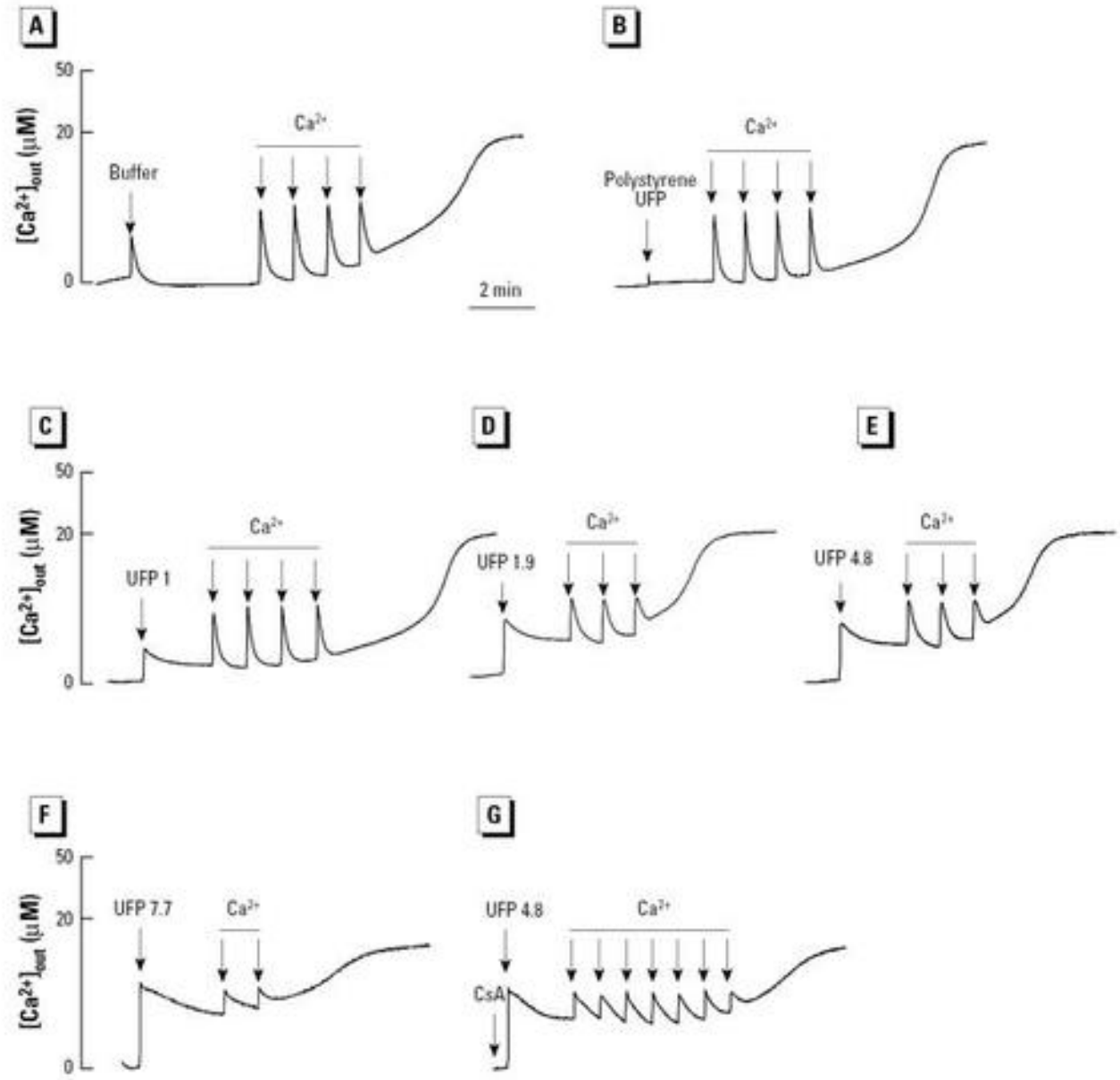


**Figure 12** Effect of UFPs on $Ca^{2+}$ retention capacity of isolated mitochondria incubated with 1 μM Calcium Green-5N. After the addition of mitochondria, the following chemicals were added: (*A*) carrier buffer, (*B*) 10 μg/mL polystyrene microspheres, (*C*) 1 μg/mL UFP, (*D*) 1.9 μg/mL UFP, (*E*) 4.8 μg/mL UFP, (*F*) 7.7 μg/mL UFP, (*G*) CsA followed by the addition of 4.8 μg/mL UFP. Each arrow represents one 5 μM $Ca^{2+}$ pulse. Data are representative of three experiments.

## Discussion

In this study we looked at the effects of distinct DEP chemical fractions on mitochondrial function. A major effect of the polar fraction was to promote mitochondrial swelling, both directly at the level of PTP opening and indirectly by promoting ΔΨm depolarization. Mitochondrial swelling by the polar fraction and the redox-cycling quinones involved the induction of $Ca^{2+}$-dependent PTP opening, as determined by the inhibitory effect of CsA and EGTA. Polar interference in inner membrane function likely targets membrane complexes

I–III, as determined using different substrates in the mitochondrial respiratory chain. The polar fraction also contains chemical substances that induce mitochondrial swelling, even at low doses that have no effect on ΔΨm. This effect could be mimicked by DEP quinones, which are enriched in the polar fraction. Although the aliphatic fraction failed to affect mitochondrial function, the aromatic fraction induced a decrease in ΔΨm that is likely secondary to PTP perturbation. This effect is mostly $Ca^{2+}$ independent and can be mimicked by PAHs. At low doses, the aromatic fraction increased the $Ca^{2+}$ retention capacity, suggesting interference in PTP function. However, at higher doses, the aromatic fraction induced partial ΔΨm depolarization, which could promote swelling if matrix $Ca^{2+}$ was already elevated. The polar and aromatic effects on isolated mitochondria could be mimicked, in part, by ambient UFPs and intact DEPs, which contain an abundance of both functionalized chemical species. In contrast, commercial polystyrene nanoparticles, which lack these chemicals, were inactive. The above effects on isolated mitochondria were accompanied by effects on apoptosis and ΔΨm in intact RAW 264.7 cells.

There is a paucity of data about the mechanisms by which ambient PM induces adverse health effects. There is also a considerable debate as to whether the particles themselves or their chemical components are responsible for injurious effects in the respiratory tract and cardiovascular system (**Brown et al. 2000**; **Oberdörster 1996**). Our view is that both the particles and the chemicals are important. First, the particles are effective carriers of chemical compounds, many of which are semi-volatile organic substances that will not otherwise gain access to the deeper regions of the lung. Second, the particle surface may act as an important catalyst for chemical reactions involved in ROS generation (**Brown et al. 2000**). Third, particles localize inside target cells, and it is possible that their subcellular localization may be determined by chemical composition. This could explain why ambient UFPs lodge inside mitochondria in epithelial cells and macrophages and why these particles are more potent than larger-sized particles in perturbing mitochondrial function (Figure 12). One possibility is that the negative charge of the mitochondrial matrix or the positive charge in the intermembrane space attracts chemical dipoles that are present in the polar material. Another possibility is that the large surface area of UFPs may promote the bioavailability of the absorbed chemicals. UFPs are known to have increased solubility, compared with larger sized particles of the same composition because of the increased surface-to-volume ratio for smaller particle sizes (**Navrotsky 2001**). This could explain why UFPs induce spontaneous swelling, whereas the major effect of DEPs is inhibition of $Ca^{2+}$-induced swelling (Table 4). PAHs and quinones are representative chemical groups that may be released in different amounts from DEPs and UFPs. The

type of PAH (e.g., 4-, 5-, or 6-ring PAHs) could also play a role in determining bioavailability.

How does mitochondrial perturbation lead to adverse PM health effects? An obvious mechanism is ROS production in mitochondria (**Hiura et al. 1999**). Although oxidative stress is increasingly recognized as a key component in tissue damage by DEPs, there is still a great deal of uncertainty about the origin of ROS. It is possible that one-electron transfers to molecular dioxygen in the mitochondrial inner membrane could contribute to $O_2^{\bullet -}$ generation. This effect is compatible with the effects of the polar fraction on inner membrane complexes I–III (Figure 6) and increased HE fluorescence in RAW 264.7 cells (Figure 2). We propose that quinones play a role in redirecting electron transfer to molecular $O_2$ in the inner membrane. This effect could be enhanced by PTP transition, which disrupts the $\Delta\Psi m$ and increases $O_2^{\bullet -}$ generation (**Zoratti and Szabo 1995**). This does not imply that $O_2^{\bullet -}$ generation by mitochondria is the only PM-induced source of ROS production. In fact, it is well known that in phagocytic cells mitochondria are a minor source for ROS production compared with NADPH oxidase and lysosomes (**Bassoe et al. 2003**).

PM contains a number of polar chemical substances, including quinones, ketones, aldehydes, sulfur compounds, and dibutyl phthalate (**Shuetzle et al. 1981**). Although much needs to be learned about the biologic effects of these substances, there is a substantive biologic literature describing tissue injury by quinones (**Penning et al. 1999**). The endogenous ubiquinones play a key role in one-electron transfers in the mitochondrial inner membrane as well as PTP transition (**Fontaine et al. 1998**; **Walter et al. 2000**). **Walter et al. (2000)** described three classes of ubiquinones that affect the PTP: group I ubiquinones (Ub0, decyl-Ub, Ub10, 2,3,5-trimethyl-6-geranyl-1,4-benzoquinone, and 2,3-dimethyl-6-decyl-1,4-benzoquinone) act as PTP inhibitory quinones that enhance the $Ca^{2+}$ load required for PTP opening; group II quinones [2,3-dimethoxy-5-methyl-6-(10-hydroxydecyl)-1,4-benzoquinone and 2,5-dihydroxy-6-undecyl-1,4-benzoquinone] act as PTP-activating quinones that dramatically decrease the $Ca^{2+}$ load required for PTP opening; group III or PTP-inactive quinones [2,3,5-trimethyl-6-(3-hydroxyisoamyl)-1,4-benzoquinone and Ub5] are neutral in their effect but have the ability to counteract the effects of group I and II quinones (**Walter et al. 2000**). Although the mechanism of PTP perturbation is unclear, it has been proposed that competition between these groups is mediated through the occupancy of a common quinone binding site in the PTP (**Walter et al. 2000**). According to this hypothesis, ligation by stimulating (group II) quinones facilitates PTP opening at a relatively small $Ca^{2+}$ load, whereas a larger $Ca^{2+}$ load would be required to access the $Ca^{2+}$ binding site when liganded with inactive (group III) quinones, and an even larger $Ca^{2+}$ load when liganded

with inhibitory (group I) quinones (**Walter et al. 2000**). If a mixture of quinones is present, they could compete in a concentration- and affinity-dependent manner for binding to the PTP site.

Although the applicability of this model to exogenous quinones is uncertain, it is interesting that redox-cycling NQs have been shown to induce $Ca^{2+}$-dependent, CsA-sensitive PTP transition (**Henry and Wallace 1995**; **Palmeira and Wallace 1997**). On the other hand, non–redox-cycling quinones with sulfhydrylarylating potential (e.g., benzoquinone) induce direct, $Ca^{2+}$-independent depolarization and mitochondrial swelling that is insensitive to CsA inhibition (**Henry and Wallace 1995**; **Palmeira and Wallace 1997**). These findings are compatible with our data that redox-cycling DEP quinones (e.g., PQ and 1,2-NQ) induce a $Ca^{2+}$-dependent, CsA-sensitive PTP transition, whereas a non–redox-cycling DEP quinone (AQ) had no effect (Figure 5D). This suggests that the redox-cycling quinones present in DEPs are responsible for PTP transition. In the absence of $Ca^{2+}$ loading, this effect disappears at higher polar concentrations that prevent $Ca^{2+}$ accumulation (Figure 4C, Figure 9D,E). The mechanism by which exogenous quinones perturb PTP activity is unknown. One possibility is binding to the putative ubiquinone binding site mentioned above. Another is the oxidative modification of thiol-dependent PTP components by redox-cycling quinones (**Henry and Wallace 1995**; **Palmeira and Wallace 1997**). Whatever the exact explanation, our data indicate that DEP quinones affect mitochondrial function independent of other biologic effects these compounds may have.

It is interesting that the aromatic fraction differs from the polar fraction in its effect on mitochondrial function. The key difference appears to be the ability of the aromatic compounds to interfere in $Ca^{2+}$-induced PTP opening at low doses (Figure 10B) while inducing mostly CsA-insensitive swelling at higher doses (Figure 7A). These effects are mimicked by the PAHs, suggesting that they play a key role in the toxic effect of the aromatic compounds (Figure 10D,E). Although we lack a definitive molecular explanation for the PAH effects, their action at lower doses resembles PTP inhibition by CsA (Figure 10D). Whether this represents occupation of an inhibitory binding site similar to group II ubiquinones or interference in cyclophylin D binding to the pore is unknown. Lemasters and colleagues have postulated that the PTP has two open conductance modes: one activated by $Ca^{2+}$ and inhibited by CsA and the other independent of $Ca^{2+}$ and CsA insensitive (**He and Lemasters 2002**; **Lemasters et al. 2002**). Induction of the $Ca^{2+}$-independent open state has been suggested to be mediated by oxidative chemicals, such as phenylarsine oxide (PAO) and $HgCl_2$, which lead to misfolding of integral membrane proteins at high doses (**He and Lemasters 2002**). It is possible that high doses of aromatic chemicals could act in similar fashion (**Lemasters et al. 2002**). According to the protein misfolding hypothesis, cyclophilin D protects

against this effect by acting as a chaperone for the damaged proteins (**Lemasters et al. 2002**). That could lead to decreased cyclophilin D binding to the PTP, which may explain why the aromatic fraction interferes in $Ca^{2+}$-induced PTP opening (Figure 7B). At a high aromatic dose, the number of misfolded protein clusters could overwhelm the ability of the chaperones to prevent nonspecific channel formation, leading to CsA-insensitive mitochondrial swelling (Figure 7A).

We have frequently referred to the role of $Ca^{2+}$ in PM-induced mitochondrial effects, including the fact that certain quinones affect mitochondrial function and PTP opening in a $Ca^{2+}$-dependent fashion (**Henry and Wallace 1995**). PAH diol epoxides have been shown to increase cytosolic $Ca^{2+}$ in airway epithelial cells (**Jyonouchi et al. 2001**), which theoretically could affect mitochondrial function, as demonstrated by the ability of some PAH species to induce apoptosis (**Solhaug et al. 2004**). In addition to the contribution of chemicals, the particles themselves play a role in intracellular $Ca^{2+}$ release, as demonstrated by treating alveolar macrophages with carbon black particles (**Brown et al. 2004**).

In addition to using a $Ca^{2+}$-dependent pathway, redox-cycling DEP chemicals may perturb the PTP in a thiol-dependent manner. In this regard, **Constantini et al. (1996)** proposed that oxidation of vicinol thiol groups in the PTP by ROS or electrophilic chemicals may lead to induction of permeability transition. Bernardi and colleagues have provided data that suggest that two distinct thiol groups are implicated in modulating PTP activity (**Chernyak and Bernardi 1996**; **Constantini et al. 1996**). One thiol group is sensitive to glutathione (GSH) oxidation, whereas the other responds to the redox state of the matrix NAD(P). The adenine nucleotide transporter (ANT) protein, a proposed structural PTP component, has three cysteine residues that show differential reactivity toward various thiol and oxidizing reagents in a conformation-dependent fashion (**Majima et al. 1993**, **1994**, **1995**). These cysteines could represent the thiol groups that regulate cyclophilin D binding as well as the effects of membrane potential on the PTP. This could explain the synergy between intracellular $Ca^{2+}$ flux and oxidative stress in PTP opening. Interestingly, ANT uses its vicinal thiols to bind to a PAO column (**Halestrap et al. 1997**). Treatment of isolated mitochondria with a crude DEP extract prevents ANT binding to PAO, suggesting that this protein is oxidatively modified at vicinal thiol groups (Xia et al., unpublished data). The thiol hypothesis also explains the prevention of mitochondrial damage by *N*-acetylcysteine, which, in addition to its effect as a radical scavenger, serves as a precursor for GSH synthesis as well as electrophilic binding to prooxidative DEP chemicals (**Xiao et al. 2003**). Under physiologic conditions, GSH may play an important role in protecting the vicinal thiols associated with

the PTP, hence the association of a drop in GSH levels with DEP-induced apoptosis.

A final point of interest is the potent effect of ambient UFPs on mitochondrial function, compared with no effect from commercial UFPs (Figure 11). This finding is of great importance to the burgeoning field of nanotechnology, which has attracted attention because of the possible interference of nanoparticles in biologic processes (**Brumfiel 2003**). Although it is possible that very small particles may exert toxic effects and induce intracellular $Ca^{2+}$ flux based on their small size and high surface area, independent of their chemical makeup (**Brown et al. 2001**, **2004**), our data indicate that the injurious effect of ambient UFP is dependent on chemical composition. In addition to the presence of organic chemicals, transition metals may contribute to particle toxicity. By using a mitochondrial end point, we have shown that it is possible to develop a mechanistic approach to particle toxicity. Similar approaches could be used to study the effects of commercial nanoparticles, which, in addition to their chemical composition, may exert mitochondrial effects based on size, surface area, and surface charge.

## Correction

The concentration of DEP extract and its fractions was incorrect in Figure 2 of the manuscript published online; it has been corrected here.


- Figures
- References
- Related
- Details



- **Cited By**

  Walker D, Pennell K and Jones D (2018) The Mitochondrial Exposome **Mitochondrial Dysfunction Caused by Drugs and Environmental Toxicants**, 10.1002/9781119329725.ch41, (613-637)

  Maurer L, Luz A and Meyer J (2018) Detection of Mitochondrial Toxicity of Environmental Pollutants Using Caenorhabditis elegans **Mitochondrial Dysfunction Caused by Drugs and Environmental Toxicants**, 10.1002/9781119329725.ch43, (655-689)

  Veranth J, Ghandehari H and Grainger D (2018) Nanoparticles in the Lung ☆ **Comprehensive Toxicology**, 10.1016/

B978-0-12-801238-3.64194-6, (322-341), .
Finch C (2018) The Toxic Nature of the Three Smokes **The Role of Global Air Pollution in Aging and Disease**, 10.1016/B978-0-12-813102-2.00002-9, (37-81), .
Erkekoglu P and Kocer-Gumusel B (2018) Toxicity assessment of nanopharmaceuticals **Inorganic Frameworks as Smart Nanomedicines**, 10.1016/B978-0-12-813661-4.00013-4, (565-603), .
Wang Y, Plewa M, Mukherjee U and Verma V (2018) Assessing the cytotoxicity of ambient particulate matter (PM) using Chinese hamster ovary (CHO) cells and its relationship with the PM chemical composition and oxidative potential, **Atmospheric Environment**, 10.1016/j.atmosenv.2018.02.025, 179, (132-141), Online publication date: 1-Apr-2018.
Matsunaga T, Kamase K, Takasawa H, Yamaji Y, Endo S, El-Kabbani O and Ikari A (2018) Facilitation of 9,10-phenanthrenequinone-elicited neuroblastoma cell apoptosis by NAD(P)H:quinone oxidoreductase 1, **Chemico-Biological Interactions**, 10.1016/j.cbi.2017.10.028, 279, (10-20), Online publication date: 1-Jan-2018.
Sánchez-Soberón F, Cuykx M, Serra N, Linares V, Bellés M, Covaci A and Schuhmacher M (2018) In-vitro metabolomics to evaluate toxicity of particulate matter under environmentally realistic conditions, **Chemosphere**, 10.1016/j.chemosphere.2018.06.065, 209, (137-146), Online publication date: 1-Oct-2018.
Wang F, Zhang H, Geng N, Ren X, Zhang B, Gong Y and Chen J (2018) A metabolomics strategy to assess the combined toxicity of polycyclic aromatic hydrocarbons (PAHs) and short-chain chlorinated paraffins (SCCPs), **Environmental Pollution**, 10.1016/j.envpol.2017.11.073, 234, (572-580), Online publication date: 1-Mar-2018.
Bliss B, Tran K, Sioutas C and Campbell A (2018) Ambient ultrafine particles activate human monocytes: Effect of dose, differentiation state and age of donors, **Environmental Research**, 10.1016/j.envres.2017.11.019, 161, (314-320), Online publication date: 1-Feb-2018.
Forman H and Finch C (2018) A critical review of assays for hazardous components of air pollution, **Free Radical Biology and Medicine**, 10.1016/j.freeradbiomed.2018.01.030, 117, (202-217), Online publication date: 1-Mar-2018.
Tositti L, Brattich E, Parmeggiani S, Bolelli L, Ferri E and Girotti S (2018) Airborne particulate matter biotoxicity estimated by

chemometric analysis on bacterial luminescence data, **Science of The Total Environment**, 10.1016/j.scitotenv.2018.06.024, 640-641, (1512-1520), Online publication date: 1-Nov-2018.
Lavrich K, Corteselli E, Wages P, Bromberg P, Simmons S, Gibbs-Flournoy E and Samet J (2018) Investigating mitochondrial dysfunction in human lung cells exposed to redox-active PM components, **Toxicology and Applied Pharmacology**, 10.1016/j.taap.2018.01.024, 342, (99-107), Online publication date: 1-Mar-2018.
Qin Y, Li G, Gao Y, Zhang L, Ok Y and An T (2018) Persistent free radicals in carbon-based materials on transformation of refractory organic contaminants (ROCs) in water: A critical review, **Water Research**, 10.1016/j.watres.2018.03.012, 137, (130-143), Online publication date: 1-Jun-2018.
Nichols C, Shepherd D, Hathaway Q, Durr A, Thapa D, Abukabda A, Yi J, Nurkiewicz T and Hollander J (2017) Reactive oxygen species damage drives cardiac and mitochondrial dysfunction following acute nano-titanium dioxide inhalation exposure, **Nanotoxicology**, 10.1080/17435390.2017.1416202, 12:1, (32-48), Online publication date: 2-Jan-2018.
Stevens J, Padilla S, DeMarini D, Hunter D, Martin W, Thompson L, Gilmour M, Hazari M and Farraj A (2017) Zebrafish Locomotor Responses Reveal Irritant Effects of Fine Particulate Matter Extracts and a Role for TRPA1, **Toxicological Sciences**, 10.1093/toxsci/kfx217, 161:2, (290-299), Online publication date: 1-Feb-2018.
Brinchmann B, Skuland T, Rambøl M, Szoke K, Brinchmann J, Gutleb A, Moschini E, Kubátová A, Kukowski K, Le Ferrec E, Lagadic-Gossmann D, Schwarze P, Låg M, Refsnes M, Øvrevik J and Holme J (2018) Lipophilic components of diesel exhaust particles induce pro-inflammatory responses in human endothelial cells through AhR dependent pathway(s), **Particle and Fibre Toxicology**, 10.1186/s12989-018-0257-1, 15:1, Online publication date: 1-Dec-2018.
Fiordelisi A, Piscitelli P, Trimarco B, Coscioni E, Iaccarino G and Sorriento D (2017) The mechanisms of air pollution and particulate matter in cardiovascular diseases, **Heart Failure Reviews**, 10.1007/s10741-017-9606-7, 22:3, (337-347), Online publication date: 1-May-2017.
Gali N, Jiang S, Yang F, Sun L and Ning Z (2017) Redox characteristics of size-segregated PM from different public transport microenvironments in Hong Kong, **Air Quality,**

**Atmosphere & Health**, 10.1007/s11869-017-0473-0, 10:7, (833-844), Online publication date: 1-Sep-2017.
Holland N, Fraiser C, Sloan R, Devlin R, Brown D and Wingard C (2017) Ultrafine Particulate Matter Increases Cardiac Ischemia/Reperfusion Injury via Mitochondrial Permeability Transition Pore, **Cardiovascular Toxicology**, 10.1007/s12012-017-9402-6, 17:4, (441-450), Online publication date: 1-Oct-2017.
Johansen J and Esbaugh A (2017) Sustained impairment of respiratory function and swim performance following acute oil exposure in a coastal marine fish, **Aquatic Toxicology**, 10.1016/j.aquatox.2017.04.002, 187, (82-89), Online publication date: 1-Jun-2017.
Raftery T, Jayasundara N and Di Giulio R (2017) A bioenergetics assay for studying the effects of environmental stressors on mitochondrial function in vivo in zebrafish larvae, **Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology**, 10.1016/j.cbpc.2016.12.001, 192, (23-32), Online publication date: 1-Feb-2017.
Samek L, Furman L, Mikrut M, Regiel-Futyra A, Macyk W, Stochel G and van Eldik R (2017) Chemical composition of submicron and fine particulate matter collected in Krakow, Poland. Consequences for the APARIC project, **Chemosphere**, 10.1016/j.chemosphere.2017.08.090, 187, (430-439), Online publication date: 1-Nov-2017.
Bae M, Schauer J, Lee T, Jeong J, Kim Y, Ro C, Song S and Shon Z (2017) Relationship between reactive oxygen species and water-soluble organic compounds: Time-resolved benzene carboxylic acids measurement in the coastal area during the KORUS-AQ campaign, **Environmental Pollution**, 10.1016/j.envpol.2017.07.100, 231, (1-12), Online publication date: 1-Dec-2017.
Niu X, Ho S, Ho K, Huang Y, Sun J, Wang Q, Zhou Y, Zhao Z and Cao J (2017) Atmospheric levels and cytotoxicity of polycyclic aromatic hydrocarbons and oxygenated-PAHs in PM 2.5 in the Beijing-Tianjin-Hebei region, **Environmental Pollution**, 10.1016/j.envpol.2017.08.099, 231, (1075-1084), Online publication date: 1-Dec-2017.
Zhang Y, Wang L, Feng H, Hu G, Wang L, Liu J, Gao X, Shang J, Zhu T, Tang S and Jia G (2017) Effects of 1,4-naphthoquinone aged carbon black particles on the cell membrane of human bronchial epithelium, **Environmental Toxicology and Pharmacology**, 10.1016/j.etap.2017.06.012, 54, (21-27), Online

publication date: 1-Sep-2017.
Soeur J, Belaïdi J, Chollet C, Denat L, Dimitrov A, Jones C, Perez P, Zanini M, Zobiri O, Mezzache S, Erdmann D, Lereaux G, Eilstein J and Marrot L (2017) Photo-pollution stress in skin: Traces of pollutants (PAH and particulate matter) impair redox homeostasis in keratinocytes exposed to UVA1, **Journal of Dermatological Science**, 10.1016/j.jdermsci.2017.01.007, 86:2, (162-169), Online publication date: 1-May-2017.
Roubicek D and Souza-Pinto N (2017) Mitochondria and mitochondrial DNA as relevant targets for environmental contaminants, **Toxicology**, 10.1016/j.tox.2017.06.012, 391, (100-108), Online publication date: 1-Nov-2017.
Ye J, Salehi S, North M, Portelli A, Chow C and Chan A (2017) Development of a Novel Simulation Reactor for Chronic Exposure to Atmospheric Particulate Matter, **Scientific Reports**, 10.1038/srep42317, 7:1, Online publication date: 1-Dec-2017.
Sheng K and Lu J (2016) Typical airborne quinones modulate oxidative stress and cytokine expression in lung epithelial A549 cells, **Journal of Environmental Science and Health, Part A**, 10.1080/10934529.2016.1237127, 52:2, (127-134), Online publication date: 28-Jan-2017.
Kraegeloh A and Unfried K (2017) The Safety of Nanomaterials on Molecular and Cellular Scale **Nanomaterials Handbook**, 10.1201/9781315371795-23, (629-662), Online publication date: 13-Jun-2017.
Nishio T, Usami M, Awaji M, Shinohara S and Sato K (2015) Dual effects of acetylsalicylic acid on ERK signaling and Mitf transcription lead to inhibition of melanogenesis, **Molecular and Cellular Biochemistry**, 10.1007/s11010-015-2613-x, 412:1-2, (101-110), Online publication date: 1-Jan-2016.
Sato K, Ando R, Kobayashi H and Nishio T (2016) 2-Ethoxybenzamide stimulates melanin synthesis in B16F1 melanoma cells via the CREB signaling pathway, **Molecular and Cellular Biochemistry**, 10.1007/s11010-016-2823-x, 423:1-2, (39-52), Online publication date: 1-Dec-2016.
Balmes J and Eisner M (2016) Indoor and Outdoor Air Pollution **Murray and Nadel's Textbook of Respiratory Medicine**, 10.1016/B978-1-4557-3383-5.00074-9, (1331-1342.e5), .
Du X, Crawford D, Nacci D and Oleksiak M (2016) Heritable oxidative phosphorylation differences in a pollutant resistant Fundulus heteroclitus population, **Aquatic Toxicology**, 10.1016/j.aquatox.2016.05.007, 177, (44-50), Online publication date: 1-

Aug-2016.
Zhou H, He J, Zhao B, Zhang L, Fan Q, Lü C, Dudagula , Liu T and Yuan Y (2016) The distribution of PM10 and PM2.5 carbonaceous aerosol in Baotou, China, **Atmospheric Research**, 10.1016/j.atmosres.2016.03.019, 178-179, (102-113), Online publication date: 1-Sep-2016.
Lee J (2016) Mitochondrial drug targets in neurodegenerative diseases, **Bioorganic & Medicinal Chemistry Letters**, 10.1016/j.bmcl.2015.11.032, 26:3, (714-720), Online publication date: 1-Feb-2016.
Kumar R and Abraham A (2016) PVP- coated naringenin nanoparticles for biomedical applications – In vivo toxicological evaluations, **Chemico-Biological Interactions**, 10.1016/j.cbi.2016.07.012, 257, (110-118), Online publication date: 1-Sep-2016.
Lui K, Bandowe B, Ho S, Chuang H, Cao J, Chuang K, Lee S, Hu D and Ho K (2016) Characterization of chemical components and bioreactivity of fine particulate matter (PM 2.5 ) during incense burning, **Environmental Pollution**, 10.1016/j.envpol.2016.02.053, 213, (524-532), Online publication date: 1-Jun-2016.
Ghadikolaei M (2016) Effect of alcohol blend and fumigation on regulated and unregulated emissions of IC engines—A review, **Renewable and Sustainable Energy Reviews**, 10.1016/j.rser.2015.12.128, 57, (1440-1495), Online publication date: 1-May-2016.
Bandowe B and Nkansah M (2016) Occurrence, distribution and health risk from polycyclic aromatic compounds (PAHs, oxygenated-PAHs and azaarenes) in street dust from a major West African Metropolis, **Science of The Total Environment**, 10.1016/j.scitotenv.2016.02.142, 553, (439-449), Online publication date: 1-May-2016.
Chen I, Huang H, Chen P and Chiang H (2016) Sirtuin 3 Protects against Urban Particulate Matter-Induced Autophagy in Human Bronchial Epithelial Cells, **Toxicological Sciences**, 10.1093/toxsci/kfw073, 152:1, (113-127), Online publication date: 1-Jul-2016.
Mukherjee B, Maji R, Roychowdhury S and Ghosh S (2016) Toxicological Concerns of Engineered Nanosize Drug Delivery Systems, **American Journal of Therapeutics**, 10.1097/01.mjt.0000433947.16654.75, 23:1, (e139-e150), .
Huang J, Lim M, Zhao B, Shao L and Lao L (2015) PM 2.5 and

ash residue from combustion of moxa floss , **Acupuncture in Medicine**, 10.1136/acupmed-2015-010914, 34:2, (101-106), Online publication date: 1-Apr-2016.
Kumagai Y, Abiko Y and Cong N (2016) Chemical toxicology of reactive species in the atmosphere: two decades of progress in an electron acceptor and an electrophile, **The Journal of Toxicological Sciences**, 10.2131/jts.41.SP37, 41:Special, (SP37-SP47), .
Sha B, Gao W, Cui X, Wang L and Xu F (2015) The potential health challenges of TiO 2 nanomaterials , **Journal of Applied Toxicology**, 10.1002/jat.3193, 35:10, (1086-1101), Online publication date: 1-Oct-2015.
Seriani R, Junqueira M, de Toledo A, Martins M, Seckler M, Alencar A, Negri E, Silva L, Mauad T, Saldiva P and Macchione M (2014) Diesel exhaust particulates affect cell signaling, mucin profiles, and apoptosis in trachea explants of Balb/C mice, **Environmental Toxicology**, 10.1002/tox.22000, 30:11, (1297-1308), Online publication date: 1-Nov-2015.
Zhou X, Wang B, Jiang P, Chen Y, Mao Z and Gao C (2015) Uptake of cerium oxide nanoparticles and its influence on functions of mouse leukemic monocyte macrophages, **Journal of Nanoparticle Research**, 10.1007/s11051-014-2815-2, 17:1, Online publication date: 1-Jan-2015.
Du X, Crawford D and Oleksiak M (2015) Effects of Anthropogenic Pollution on the Oxidative Phosphorylation Pathway of Hepatocytes from Natural Populations of Fundulus heteroclitus, **Aquatic Toxicology**, 10.1016/j.aquatox.2015.06.009, 165, (231-240), Online publication date: 1-Aug-2015.
Lee K, Cao J, Lee C, Hsiao T, Yeh C, Huynh T, Han Y, Li X, Chuang K, Tian L, Ho K and Chuang H (2015) Inhibition of the WNT/β-catenin pathway by fine particulate matter in haze: Roles of metals and polycyclic aromatic hydrocarbons, **Atmospheric Environment**, 10.1016/j.atmosenv.2015.03.017, 109, (118-129), Online publication date: 1-May-2015.
Hamad S, Schauer J, Heo J and Kadhim A (2015) Source apportionment of PM2.5 carbonaceous aerosol in Baghdad, Iraq, **Atmospheric Research**, 10.1016/j.atmosres.2014.12.017, 156, (80-90), Online publication date: 1-Apr-2015.
Lovera-Leroux M, Crobeddu B, Kassis N, Petit P, Janel N, Baeza-Squiban A and Andreau K (2015) The iron component of particulate matter is antiapoptotic: A clue to the development of lung cancer after exposure to atmospheric pollutants?,

**Biochimie**, 10.1016/j.biochi.2015.09.030, 118, (195-206), Online publication date: 1-Nov-2015.
Tissier F, Dussauze M, Lefloch N, Theron M, Lemaire P, Le Floch S and Pichavant-Rafini K (2015) Effect of dispersed crude oil on cardiac function in seabass Dicentrarchus labrax, **Chemosphere**, 10.1016/j.chemosphere.2015.04.026, 134, (192-198), Online publication date: 1-Sep-2015.
Elie M, Choi J, Nkrumah-Elie Y, Gonnerman G, Stevens J and Tanguay R (2015) Metabolomic analysis to define and compare the effects of PAHs and oxygenated PAHs in developing zebrafish, **Environmental Research**, 10.1016/j.envres.2015.05.009, 140, (502-510), Online publication date: 1-Jul-2015.
Xia M, Viera-Hutchins L, Garcia-Lloret M, Noval Rivas M, Wise P, McGhee S, Chatila Z, Daher N, Sioutas C and Chatila T (2015) Vehicular exhaust particles promote allergic airway inflammation through an aryl hydrocarbon receptor–notch signaling cascade, **Journal of Allergy and Clinical Immunology**, 10.1016/j.jaci.2015.02.014, 136:2, (441-453), Online publication date: 1-Aug-2015.
Lim J, Lim C, Kim S and Hong J (2015) Characterizations of organic compounds in diesel exhaust particulates, **Journal of Environmental Sciences**, 10.1016/j.jes.2015.03.013, 34, (171-183), Online publication date: 1-Aug-2015.
Li H, Fang C, Shi W, Gurusamy S, Li S, Krishnan M and George S (2015) Size and site dependent biological hazard potential of particulate matters collected from different heights at the vicinity of a building construction, **Toxicology Letters**, 10.1016/j.toxlet.2015.08.002, 238:3, (20-29), Online publication date: 1-Nov-2015.
Antiñolo M, Willis M, Zhou S and Abbatt J (2015) Connecting the oxidation of soot to its redox cycling abilities, **Nature Communications**, 10.1038/ncomms7812, 6:1, Online publication date: 1-Dec-2015.
Vejerano E, Ma Y, Holder A, Pruden A, Elankumaran S and Marr L (2015) Toxicity of particulate matter from incineration of nanowaste, **Environmental Science: Nano**, 10.1039/C4EN00182F, 2:2, (143-154)
Langie S, Koppen G, Desaulniers D, Al-Mulla F, Al-Temaimi R, Amedei A, Azqueta A, Bisson W, Brown D, Brunborg G, Charles A, Chen T, Colacci A, Darroudi F, Forte S, Gonzalez L, Hamid R, Knudsen L, Leyns L, Lopez de Cerain Salsamendi A, Memeo L, Mondello C, Mothersill C, Olsen A, Pavanello S, Raju J, Rojas E, Roy R, Ryan E, Ostrosky-Wegman P, Salem H, Scovassi A, Singh

N, Vaccari M, Van Schooten F, Valverde M, Woodrick J, Zhang L, van Larebeke N, Kirsch-Volders M and Collins A (2015) Causes of genome instability: the effect of low dose chemical exposures in modern society, **Carcinogenesis**, 10.1093/carcin/bgv031, 36:Suppl 1, (S61-S88), Online publication date: 1-Jun-2015.
Maynard R (2015) Air pollution, **Human & Experimental Toxicology**, 10.1177/0960327115603585, 34:12, (1253-1257), Online publication date: 1-Dec-2015.
SAGAI M and WIN-SHWE T (2015) Oxidative Stress Derived from Airborne Fine and Ultrafine Particles and the Effects on Brain-Nervous System: Part 1, **Nippon Eiseigaku Zasshi (Japanese Journal of Hygiene)Nippon Eiseigaku Zasshi (Japanese Journal of Hygiene)**, 10.1265/jjh.70.127, 70:2, (127-133), .
He X, Lin M, Li F, Sha B, Xu F, Qu Z and Wang L (2015) Advances in studies of nanoparticle–biomembrane interactions, **Nanomedicine**, 10.2217/nnm.14.167, 10:1, (121-141), Online publication date: 1-Jan-2015.
Filippi C, Pryde A, Cowan P, Lee T, Hayes P, Donaldson K, Plevris J and Stone V (2014) Toxicology of ZnO and TiO 2 nanoparticles on hepatocytes: Impact on metabolism and bioenergetics , **Nanotoxicology**, 10.3109/17435390.2014.895437, 9:1, (126-134), Online publication date: 2-Jan-2015.
Chen G, Shim S and Wen J (2014) Potential Toxicity of Food Ingredients Loaded in Nano- and Microparticles **Nano- and Microencapsulation for Foods**, 10.1002/9781118292327.ch15, (363-381)
O'Hagan H (2013) Chromatin modifications during repair of environmental exposure-induced DNA damage: A potential mechanism for stable epigenetic alterations, **Environmental and Molecular Mutagenesis**, 10.1002/em.21830, 55:3, (278-291), Online publication date: 1-Apr-2014.
Varela-Valencia R, Gómez-Ortiz N, Oskam G, de Coss R, Rubio-Piña J, del Río-García M, Albores-Medina A and Zapata-Perez O (2014) The effect of titanium dioxide nanoparticles on antioxidant gene expression in tilapia (Oreochromis niloticus), **Journal of Nanoparticle Research**, 10.1007/s11051-014-2369-3, 16:4, Online publication date: 1-Apr-2014.
Westman O, Larsson M, Venizelos N, Hollert H and Engwall M (2014) An oxygenated metabolite of benzo[a]pyrene increases hepatic β-oxidation of fatty acids in chick embryos, **Environmental Science and Pollution Research**, 10.1007/s11356-013-2471-6, 21:9, (6243-6251), Online publication date:

1-May-2014.
Dussauze M, Camus L, Le Floch S, Pichavant-Rafini K, Geraudie P, Coquillé N, Amérand A, Lemaire P and Theron M (2014) Impact of dispersed fuel oil on cardiac mitochondrial function in polar cod Boreogadus saida, **Environmental Science and Pollution Research**, 10.1007/s11356-014-2618-0, 21:24, (13779-13788), Online publication date: 1-Dec-2014.
Donateo A, Gregoris E, Gambaro A, Merico E, Giua R, Nocioni A and Contini D (2014) Contribution of harbour activities and ship traffic to PM2.5, particle number concentrations and PAHs in a port city of the Mediterranean Sea (Italy), **Environmental Science and Pollution Research**, 10.1007/s11356-014-2849-0, 21:15, (9415-9429), Online publication date: 1-Aug-2014.
Cheng W, Rong Y, Shi T, Zhou T, Liu Y and Chen W (2014) Size-dependent biological effects on vascular endothelial cells induced by different particulate matters, **Journal of Huazhong University of Science and Technology [Medical Sciences]**, 10.1007/s11596-014-1276-2, 34:3, (314-321), Online publication date: 1-Jun-2014.
Zimmerman N, Godri Pollitt K, Jeong C, Wang J, Jung T, Cooper J, Wallace J and Evans G (2014) Comparison of three nanoparticle sizing instruments: The influence of particle morphology, **Atmospheric Environment**, 10.1016/j.atmosenv.2013.12.023, 86, (140-147), Online publication date: 1-Apr-2014.
Elie M, Williamson R, Clausen C and Yestrebsky C (2014) Application of a magnesium/co-solvent system for the degradation of polycyclic aromatic hydrocarbons and their oxygenated derivatives in a spiked soil, **Chemosphere**, 10.1016/j.chemosphere.2014.10.042, 117, (793-800), Online publication date: 1-Dec-2014.
Vattanasit U, Navasumrit P, Khadka M, Kanitwithayanun J, Promvijit J, Autrup H and Ruchirawat M (2014) Oxidative DNA damage and inflammatory responses in cultured human cells and in humans exposed to traffic-related particles, **International Journal of Hygiene and Environmental Health**, 10.1016/j.ijheh.2013.03.002, 217:1, (23-33), Online publication date: 1-Jan-2014.
Lee K, Wong C, Chuang K, Bien M, Cao J, Han Y, Tian L, Chang C, Feng P, Ho K and Chuang H (2014) Methionine oxidation in albumin by fine haze particulate matter: An in vitro and in vivo study, **Journal of Hazardous Materials**, 10.1016/j.jhazmat.2014.04.029, 274, (384-391), Online publication date: 1-Jun-2014.

Anderson A, Phair J, Benson J, Meenan B and Davis J (2014) Investigating the use of endogenous quinoid moieties on carbon fibre as means of developing micro pH sensors, **Materials Science and Engineering: C**, 10.1016/j.msec.2014.07.038, 43, (533-537), Online publication date: 1-Oct-2014.
Wang D, Shafer M, Schauer J and Sioutas C (2014) Development of a Technology for Online Measurement of Total and Water-Soluble Copper (Cu) in PM 2.5 , **Aerosol Science and Technology**, 10.1080/02786826.2014.937478, 48:8, (864-874), Online publication date: 3-Aug-2014.
Totlandsdal A, Øvrevik J, Cochran R, Herseth J, Bølling A, Låg M, Schwarze P, Lilleaas E, Holme J and Kubátová A (2013) The occurrence of polycyclic aromatic hydrocarbons and their derivatives and the proinflammatory potential of fractionated extracts of diesel exhaust and wood smoke particles, **Journal of Environmental Science and Health, Part A**, 10.1080/10934529.2014.854586, 49:4, (383-396), Online publication date: 21-Mar-2014.
Carlsten C, MacNutt M, Zhang Z, Sava F and Pui M (2014) Anti-Oxidant N-Acetylcysteine Diminishes Diesel Exhaust-Induced Increased Airway Responsiveness in Person with Airway Hyper-Reactivity, **Toxicological Sciences**, 10.1093/toxsci/kfu040, 139:2, (479-487), Online publication date: 1-Jun-2014.
Laumbach R, Kipen H, Ko S, Kelly-McNeil K, Cepeda C, Pettit A, Ohman-Strickland P, Zhang L, Zhang J, Gong J, Veleeparambil M and Gow A (2014) A controlled trial of acute effects of human exposure to traffic particles on pulmonary oxidative stress and heart rate variability, **Particle and Fibre Toxicology**, 10.1186/s12989-014-0045-5, 11:1, Online publication date: 1-Dec-2014.
Pierdominici M, Maselli A, Cecchetti S, Tinari A, Mastrofrancesco A, Alfè M, Gargiulo V, Beatrice C, Di Blasio G, Carpinelli G, Ortona E, Giovannetti A and Fiorito S (2014) Diesel exhaust particle exposure in vitro impacts T lymphocyte phenotype and function, **Particle and Fibre Toxicology**, 10.1186/s12989-014-0074-0, 11:1, Online publication date: 1-Dec-2014.
Sato K, Minai Y and Watanabe H (2013) Effect of monochromatic visible light on intracellular superoxide anion production and mitochondrial membrane potential of B16F1 and B16F10 murine melanoma cells, **Cell Biology International**, 10.1002/cbin.10069, 37:6, (633-637), Online publication date: 1-Jun-2013.
Gini M, Lianou M, Chalbot M, Kotronarou A, Kavouras I and Helmis C (2012) Quantification of Environmental Tobacco Smoke

Contribution on Outdoor Particulate Aliphatic and Polycyclic Aromatic Hydrocarbons, **Archives of Environmental Contamination and Toxicology**, 10.1007/s00244-012-9844-6, 64:3, (347-356), Online publication date: 1-Apr-2013.
Wang D, Pakbin P, Shafer M, Antkiewicz D, Schauer J and Sioutas C (2013) Macrophage reactive oxygen species activity of water-soluble and water-insoluble fractions of ambient coarse, PM2.5 and ultrafine particulate matter (PM) in Los Angeles, **Atmospheric Environment**, 10.1016/j.atmosenv.2013.05.031, 77, (301-310), Online publication date: 1-Oct-2013.
Lin L, Liu I, Chuang H, Lin H and Chuang K (2013) Size and composition effects of household particles on inflammation and endothelial dysfunction of human coronary artery endothelial cells, **Atmospheric Environment**, 10.1016/j.atmosenv.2013.05.045, 77, (490-495), Online publication date: 1-Oct-2013.
Chiang L, Chen H, Lee C, Chuang K, Chen T, Yeh C, Wang L, Lee W, Lin L, Tseng H and Chuang H (2013) Serum protein oxidation by diesel exhaust particles: Effects on oxidative stress and inflammatory response in vitro, **Chemico-Biological Interactions**, 10.1016/j.cbi.2013.10.013, 206:2, (385-393), Online publication date: 1-Nov-2013.
Betha R and Balasubramanian R (2013) Emissions of particulate-bound elements from biodiesel and ultra low sulfur diesel: Size distribution and risk assessment, **Chemosphere**, 10.1016/j.chemosphere.2012.07.052, 90:3, (1005-1015), Online publication date: 1-Jan-2013.
Elie M, Clausen C and Yestrebsky C (2013) Reductive degradation of oxygenated polycyclic aromatic hydrocarbons using an activated magnesium/co-solvent system, **Chemosphere**, 10.1016/j.chemosphere.2013.02.031, 91:9, (1273-1280), Online publication date: 1-May-2013.
Kanaly R and Hamamura N (2013) 9,10-Phenanthrenedione biodegradation by a soil bacterium and identification of transformation products by LC/ESI-MS/MS, **Chemosphere**, 10.1016/j.chemosphere.2013.03.054, 92:11, (1442-1449), Online publication date: 1-Sep-2013.
Gillespie P, Tajuba J, Lippmann M, Chen L and Veronesi B (2013) Particulate matter neurotoxicity in culture is size-dependent, **NeuroToxicology**, 10.1016/j.neuro.2011.10.006, 36, (112-117), Online publication date: 1-May-2013.
Ekstrand-Hammarström B, Magnusson R, Österlund C, Andersson B, Bucht A and Wingfors H (2013) Oxidative stress

and cytokine expression in respiratory epithelial cells exposed to well-characterized aerosols from Kabul, Afghanistan, **Toxicology in Vitro**, 10.1016/j.tiv.2012.12.022, 27:2, (825-833), Online publication date: 1-Mar-2013.
Yang M and Zhang X (2013) Comparative Developmental Toxicity of New Aromatic Halogenated DBPs in a Chlorinated Saline Sewage Effluent to the Marine Polychaete Platynereis dumerilii , **Environmental Science & Technology**, 10.1021/es401841t, 47:19, (10868-10876), Online publication date: 1-Oct-2013.
Mao Z, Zhou X and Gao C (2013) Influence of structure and properties of colloidal biomaterials on cellular uptake and cell functions, **Biomaterials Science**, 10.1039/c3bm00137g, 1:9, (896), .
Wang D, Kam W, Cheung K, Pakbin P and Sioutas C (2013) Development of a Two-Stage Virtual Impactor System for High Concentration Enrichment of Ultrafine, PM 2.5 , and Coarse Particulate Matter , **Aerosol Science and Technology**, 10.1080/02786826.2012.744446, 47:3, (231-238), Online publication date: 1-Mar-2013.
Meyer J, Leung M, Rooney J, Sendoel A, Hengartner M, Kisby G and Bess A (2013) Mitochondria as a Target of Environmental Toxicants, **Toxicological Sciences**, 10.1093/toxsci/kft102, 134:1, (1-17), Online publication date: 1-Jul-2013., Online publication date: 1-Jul-2013.
Mohamed S and Sayed H (2013) Hazards of nanotechnology, **The Egyptian Journal of Histology**, 10.1097/01.EHX.0000428980.13426.2d, 36:2, (389-399), Online publication date: 1-Jun-2013.
Chu S, Zhang H, Maher C, McDonald J, Zhang X, Ho S, Yan B, Chillrud S, Perera F, Factor P and Miller R (2013) Prenatal and Postnatal Polycyclic Aromatic Hydrocarbon Exposure, Airway Hyperreactivity, and Beta-2 Adrenergic Receptor Function in Sensitized Mouse Offspring, **Journal of Toxicology**, 10.1155/2013/603581, 2013, (1-9), .
Kioumourtzoglou M, Zanobetti A, Schwartz J, Coull B, Dominici F and Suh H (2013) The effect of primary organic particles on emergency hospital admissions among the elderly in 3 US cities, **Environmental Health**, 10.1186/1476-069X-12-68, 12:1, Online publication date: 1-Dec-2013.
Chan J, Charrier J, Kodani S, Vogel C, Kado S, Anderson D, Anastasio C and Van Winkle L (2013) Combustion-derived flame generated ultrafine soot generates reactive oxygen species and

activates Nrf2 antioxidants differently in neonatal and adult rat lungs, **Particle and Fibre Toxicology**, 10.1186/1743-8977-10-34, 10:1, (34), .
Kazlauskienė A, Valentukevičienė M and Ignatavičius G (2013) Sustainability assessment of heavy metals and road maintenance salts in sweep sand from roadside environment, **Technological and Economic Development of Economy**, 10.3846/20294913.2013.796500, 19:2, (224-236), Online publication date: 1-Jun-2013.
Wong J, Chan H and Kwok P (2013) Electrostatics in pharmaceutical aerosols for inhalation, **Therapeutic Delivery**, 10.4155/tde.13.70, 4:8, (981-1002), Online publication date: 1-Aug-2013.
Kudo S, Sekiguchi K, Kim K, Kinoshita M, Möller D, Wang Q, Yoshikado H and Sakamoto K (2012) Differences of chemical species and their ratios between fine and ultrafine particles in the roadside environment, **Atmospheric Environment**, 10.1016/j.atmosenv.2012.08.039, 62, (172-179), Online publication date: 1-Dec-2012.
Totlandsdal A, Herseth J, Bølling A, Kubátová A, Braun A, Cochran R, Refsnes M, Øvrevik J and Låg M (2012) Differential effects of the particle core and organic extract of diesel exhaust particles, **Toxicology Letters**, 10.1016/j.toxlet.2011.10.025, 208:3, (262-268), Online publication date: 1-Feb-2012.
Nelin T, Joseph A, Gorr M and Wold L (2012) Direct and indirect effects of particulate matter on the cardiovascular system, **Toxicology Letters**, 10.1016/j.toxlet.2011.11.008, 208:3, (293-299), Online publication date: 1-Feb-2012.
Chuang H, Fan C, Chen K, Chang-Chien G and Chan C (2012) Vasoactive alteration and inflammation induced by polycyclic aromatic hydrocarbons and trace metals of vehicle exhaust particles, **Toxicology Letters**, 10.1016/j.toxlet.2012.08.012, 214:2, (131-136), Online publication date: 1-Oct-2012.
Jaiswal P, Srivastava S, Gupta J and Thakur I (2012) Dibenzofuran induces oxidative stress, disruption of trans-mitochondrial membrane potential (ΔΨm) and G1 arrest in human hepatoma cell line, **Toxicology Letters**, 10.1016/j.toxlet.2012.08.014, 214:2, (137-144), Online publication date: 1-Oct-2012.
Lackey L and Paulson S (2011) Influence of Feedstock: Air Pollution and Climate-Related Emissions from a Diesel Generator Operating on Soybean, Canola, and Yellow Grease Biodiesel,

**Energy & Fuels**, 10.1021/ef2011904, 26:1, (686-700), Online publication date: 19-Jan-2012.
Shang Y, Chen C, Li Y, Zhao J and Zhu T (2012) Hydroxyl Radical Generation Mechanism During the Redox Cycling Process of 1,4-Naphthoquinone, **Environmental Science & Technology**, 10.1021/es203032v, 46:5, (2935-2942), Online publication date: 6-Mar-2012.
Li Y, Zhu T, Zhao J and Xu B (2012) Interactive Enhancements of Ascorbic Acid and Iron in Hydroxyl Radical Generation in Quinone Redox Cycling, **Environmental Science & Technology**, 10.1021/es301834r, 46:18, (10302-10309), Online publication date: 18-Sep-2012.
Ghio A, Carraway M and Madden M (2012) Composition of Air Pollution Particles and Oxidative Stress in Cells, Tissues, and Living Systems, **Journal of Toxicology and Environmental Health, Part B**, 10.1080/10937404.2012.632359, 15:1, (1-21), Online publication date: 1-Jan-2012.
Kao Y, Chen Y, Cheng T, Chiung Y and Liu P (2011) Zinc Oxide Nanoparticles Interfere With Zinc Ion Homeostasis to Cause Cytotoxicity, **Toxicological Sciences**, 10.1093/toxsci/kfr319, 125:2, (462-472), Online publication date: 1-Feb-2012., Online publication date: 1-Feb-2012.
RISTOVSKI Z, MILJEVIC B, SURAWSKI N, MORAWSKA L, FONG K, GOH F and YANG I (2012) Respiratory health effects of diesel particulate matter, **Respirology**, 10.1111/j.1440-1843.2011.02109.x, 17:2, (201-212), Online publication date: 1-Feb-2012.
Andreau K, Leroux M and Bouharrour A (2012) Health and Cellular Impacts of Air Pollutants: From Cytoprotection to Cytotoxicity, **Biochemistry Research International**, 10.1155/2012/493894, 2012, (1-18), .
Hoogendoorn B, Berube K, Gregory C, Jones T, Sexton K, Brennan P, Brewis I, Murison A, Arthur R, Price H, Morgan H and Matthews I (2012) Gene and protein responses of human lung tissue explants exposed to ambient particulate matter of different sizes, **Inhalation Toxicology**, 10.3109/08958378.2012.742600, 24:14, (966-975), Online publication date: 1-Dec-2012.
Maynard R (2017) Nano-technology and nano-toxicology, **Emerging Health Threats Journal**, 10.3402/ehtj.v5i0.17508, 5:1, (17508), Online publication date: 1-Jan-2012.
Holder A, Carter B, Goth–Goldstein R, Lucas D and Koshland C (2012) Increased cytotoxicity of oxidized flame soot,

**Atmospheric Pollution Research**, 10.5094/APR.2012.001, 3:1, (25-31), Online publication date: 1-Jan-2012.
Cheng W, Currier J, Bromberg P, Silbajoris R, Simmons S and Samet J (2011) Linking Oxidative Events to Inflammatory and Adaptive Gene Expression Induced by Exposure to an Organic Particulate Matter Component, **Environmental Health Perspectives**, 120:2, (267-274), Online publication date: 1-Feb-2012.
Lupo P, Symanski E, Waller D, Chan W, Langlois P, Canfield M and Mitchell L (2010) Maternal Exposure to Ambient Levels of Benzene and Neural Tube Defects among Offspring: Texas, 1999–2004, **Environmental Health Perspectives**, 119:3, (397-402), Online publication date: 1-Mar-2011.
Chuang H, Jones T, Chen Y, Bell J, Wenger J and BéruBé K (2011) Characterisation of airborne particles and associated organic components produced from incense burning, **Analytical and Bioanalytical Chemistry**, 10.1007/s00216-011-5209-7, 401:10, (3095-3102), Online publication date: 1-Dec-2011.
Sha B, Gao W, Wang S, Xu F and Lu T (2011) Cytotoxicity of titanium dioxide nanoparticles differs in four liver cells from human and rat, **Composites Part B: Engineering**, 10.1016/j.compositesb.2011.05.009, 42:8, (2136-2144), Online publication date: 1-Dec-2011.
Leme D, Grummt T, Heinze R, Sehr A, Skerswetat M, de Marchi M, Machado M, de Oliveira D and Marin-Morales M (2011) Cytotoxicity of water-soluble fraction from biodiesel and its diesel blends to human cell lines, **Ecotoxicology and Environmental Safety**, 10.1016/j.ecoenv.2011.08.012, 74:8, (2148-2155), Online publication date: 1-Nov-2011.
Musa Bandowe B, Sobocka J and Wilcke W (2011) Oxygen-containing polycyclic aromatic hydrocarbons (OPAHs) in urban soils of Bratislava, Slovakia: Patterns, relation to PAHs and vertical distribution, **Environmental Pollution**, 10.1016/j.envpol.2010.10.011, 159:2, (539-549), Online publication date: 1-Feb-2011.
Justino C, Rocha-Santos T and Duarte A (2011) Sampling and characterization of nanoaerosols in different environments, **TrAC Trends in Analytical Chemistry**, 10.1016/j.trac.2010.12.002, 30:3, (554-567), Online publication date: 1-Mar-2011.
Faiola C, Johansen A, Rybka S, Nieber A, Thomas C, Bryner S, Johnston J, Engelhard M, Nachimuthu P and Owens K (2011) Ultrafine Particulate Ferrous Iron and Anthracene Associations

with Mitochondrial Dysfunction, **Aerosol Science and Technology**, 10.1080/02786826.2011.581255, 45:9, (1109-1122), Online publication date: 1-Sep-2011.
Zhigacheva I, Evseenko L, Burlakova E, Voronkov M and Krivosheeva L (2011) A new organosilicon adsorbent for air-gas mixture purification, **Catalysis in Industry**, 10.1134/S2070050411020176, 3:2, (170-175), Online publication date: 1-Jun-2011.
Tashakkor A, Chow K and Carlsten C (2011) Modification by antioxidant supplementation of changes in human lung function associated with air pollutant exposure: A systematic review, **BMC Public Health**, 10.1186/1471-2458-11-532, 11:1, Online publication date: 1-Dec-2011.
Mutlu E, Engen P, Soberanes S, Urich D, Forsyth C, Nigdelioglu R, Chiarella S, Radigan K, Gonzalez A, Jakate S, Keshavarzian A, Budinger G and Mutlu G (2011) Particulate matter air pollution causes oxidant-mediated increase in gut permeability in mice, **Particle and Fibre Toxicology**, 10.1186/1743-8977-8-19, 8:1, (19), .
Layshock J, Wilson G and Anderson K (2010) Ketone and quinone-substituted polycyclic aromatic hydrocarbons in mussel tissue, sediment, urban dust, and diesel particulate matrices, **Environmental Toxicology and Chemistry**, 10.1002/etc.301, 29:11, (2450-2460), Online publication date: 1-Nov-2010.
Teles A, Kumagai Y, Brain S, Teixeira S, Varriano A, Barreto M, de Lima W, Antunes E, Muscará M and Costa S (2009) Involvement of sensory nerves and TRPV1 receptors in the rat airway inflammatory response to two environment pollutants: diesel exhaust particles (DEP) and 1,2-naphthoquinone (1,2-NQ), **Archives of Toxicology**, 10.1007/s00204-009-0427-x, 84:2, (109-117), Online publication date: 1-Feb-2010.
Veranth J, Ghandehari H and Grainger D (2010) Nanoparticles in the Lung **Comprehensive Toxicology**, 10.1016/B978-0-08-046884-6.00928-3, (453-475), .
Eisner M and Balmes J (2010) Indoor and Outdoor Air Pollution **Murray and Nadel's Textbook of Respiratory Medicine**, 10.1016/B978-1-4160-4710-0.00067-5, (1601-1618), .
Walgraeve C, Demeestere K, Dewulf J, Zimmermann R and Van Langenhove H (2010) Oxygenated polycyclic aromatic hydrocarbons in atmospheric particulate matter: Molecular characterization and occurrence, **Atmospheric Environment**, 10.1016/j.atmosenv.2009.12.004, 44:15, (1831-1846), Online

publication date: 1-May-2010.
Lee J and Lane D (2010) Formation of oxidized products from the reaction of gaseous phenanthrene with the OH radical in a reaction chamber, **Atmospheric Environment**, 10.1016/j.atmosenv.2010.03.008, 44:20, (2469-2477), Online publication date: 1-Jun-2010.
Jung D and Di Giulio R (2010) Identification of mitochondrial cytochrome P450 induced in response to polycyclic aromatic hydrocarbons in the mummichog (Fundulus heteroclitus), **Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology**, 10.1016/j.cbpc.2009.09.003, 151:1, (107-112), Online publication date: 1-Jan-2010.
Wei Y, Han I, Hu M, Shao M, Zhang J and Tang X (2010) Personal exposure to particulate PAHs and anthraquinone and oxidative DNA damages in humans, **Chemosphere**, 10.1016/j.chemosphere.2010.08.055, 81:10, (1280-1285), Online publication date: 1-Nov-2010.
Eiguren-Fernandez A, Shinyashiki M, Schmitz D, DiStefano E, Hinds W, Kumagai Y, Cho A and Froines J (2010) Redox and electrophilic properties of vapor- and particle-phase components of ambient aerosols, **Environmental Research**, 10.1016/j.envres.2010.01.009, 110:3, (207-212), Online publication date: 1-Apr-2010.
Ruipérez V, Darios F and Davletov B (2010) Alpha-synuclein, lipids and Parkinson's disease, **Progress in Lipid Research**, 10.1016/j.plipres.2010.05.004, 49:4, (420-428), Online publication date: 1-Oct-2010.
George S, Pokhrel S, Xia T, Gilbert B, Ji Z, Schowalter M, Rosenauer A, Damoiseaux R, Bradley K, Mädler L and Nel A (2009) Use of a Rapid Cytotoxicity Screening Approach To Engineer a Safer Zinc Oxide Nanoparticle through Iron Doping, **ACS Nano**, 10.1021/nn901503q, 4:1, (15-29), Online publication date: 26-Jan-2010.
Polidori A, Kwon J, Turpin B and Weisel C (2009) Source proximity and residential outdoor concentrations of PM2.5, OC, EC and PAHs, **Journal of Exposure Science & Environmental Epidemiology**, 10.1038/jes.2009.39, 20:5, (457-468), Online publication date: 1-Jul-2010.
Pakbin P, Hudda N, Cheung K, Moore K and Sioutas C (2010) Spatial and Temporal Variability of Coarse (PM 10–2.5 ) Particulate Matter Concentrations in the Los Angeles Area , **Aerosol Science and Technology**,

10.1080/02786821003749509, 44:7, (514-525), Online publication date: 4-Jun-2010.
Laumbach R and Kipen H (2010) Acute effects of motor vehicle traffic-related air pollution exposures on measures of oxidative stress in human airways, **Annals of the New York Academy of Sciences**, 10.1111/j.1749-6632.2010.05604.x, 1203:1, (107-112), Online publication date: 1-Aug-2010.
Samet J and Tal T (2010) Toxicological Disruption of Signaling Homeostasis: Tyrosine Phosphatases as Targets, **Annual Review of Pharmacology and Toxicology**, 10.1146/annurev.pharmtox.010909.105841, 50:1, (215-235), Online publication date: 1-Feb-2010.
Hou L, Zhu Z, Zhang X, Nordio F, Bonzini M, Schwartz J, Hoxha M, Dioni L, Marinelli B, Pegoraro V, Apostoli P, Bertazzi P and Baccarelli A (2010) Airborne particulate matter and mitochondrial damage: a cross-sectional study, **Environmental Health**, 10.1186/1476-069X-9-48, 9:1, Online publication date: 1-Dec-2010.
Yoshizaki K, Brito J, Toledo A, Nakagawa N, Piccin V, Junqueira M, Negri E, Carvalho A, Ligeiro de Oliveira A, Tavares de Lima W, Saldiva P, Mauad T and Macchione M (2010) Subchronic effects of nasally instilled diesel exhaust particulates on the nasal and airway epithelia in mice, **Inhalation Toxicology**, 10.3109/08958371003621633, 22:7, (610-617), Online publication date: 1-Jun-2010.
Sehlstedt M, Behndig A, Boman C, Blomberg A, Sandström T and Pourazar J (2010) Airway inflammatory response to diesel exhaust generated at urban cycle running conditions, **Inhalation Toxicology**, 10.3109/08958378.2010.529181, 22:14, (1144-1150), Online publication date: 1-Dec-2010.
Møller P, Jacobsen N, Folkmann J, Danielsen P, Mikkelsen L, Hemmingsen J, Vesterdal L, Forchhammer L, Wallin H and Loft S (2009) Role of oxidative damage in toxicity of particulates, **Free Radical Research**, 10.3109/10715760903300691, 44:1, (1-46), Online publication date: 1-Jan-2010.
Li N, Wang M, Bramble L, Schmitz D, Schauer J, Sioutas C, Harkema J and Nel A (2009) The Adjuvant Effect of Ambient Particulate Matter Is Closely Reflected by the Particulate Oxidant Potential, **Environmental Health Perspectives**, 117:7, (1116-1123), Online publication date: 1-Jul-2009.
Cheng X, Qin Y and Ivessa A (2009) Loss of mitochondrial DNA under genotoxic stress conditions in the absence of the yeast

DNA helicase Pif1p occurs independently of the DNA helicase Rrm3p, **Molecular Genetics and Genomics**, 10.1007/s00438-009-0438-6, 281:6, (635-645), Online publication date: 1-Jun-2009.
Verma V, Ning Z, Cho A, Schauer J, Shafer M and Sioutas C (2009) Redox activity of urban quasi-ultrafine particles from primary and secondary sources, **Atmospheric Environment**, 10.1016/j.atmosenv.2009.09.019, 43:40, (6360-6368), Online publication date: 1-Dec-2009.
Vondráček J, Krčmář P, Procházková J, Trilecová L, Gavelová M, Skálová L, Szotáková B, Bunček M, Radilová H, Kozubík A and Machala M (2009) The role of aryl hydrocarbon receptor in regulation of enzymes involved in metabolic activation of polycyclic aromatic hydrocarbons in a model of rat liver progenitor cells, **Chemico-Biological Interactions**, 10.1016/j.cbi.2009.03.011, 180:2, (226-237), Online publication date: 1-Jul-2009.
Matsunaga T, Arakaki M, Kamiya T, Endo S, El-Kabbani O and Hara A (2009) Involvement of an aldo-keto reductase (AKR1C3) in redox cycling of 9,10-phenanthrenequinone leading to apoptosis in human endothelial cells, **Chemico-Biological Interactions**, 10.1016/j.cbi.2009.05.005, 181:1, (52-60), Online publication date: 1-Sep-2009.
Kasumba J, Hopke P, Chalupa D and Utell M (2009) Comparison of sources of submicron particle number concentrations measured at two sites in Rochester, NY, **Science of The Total Environment**, 10.1016/j.scitotenv.2009.05.040, 407:18, (5071-5084), Online publication date: 1-Sep-2009.
Mo Y, Wan R, Chien S, Tollerud D and Zhang Q (2009) Activation of endothelial cells after exposure to ambient ultrafine particles: The role of NADPH oxidase, **Toxicology and Applied Pharmacology**, 10.1016/j.taap.2009.01.017, 236:2, (183-193), Online publication date: 1-Apr-2009.
Eom H and Choi J (2009) Oxidative stress of silica nanoparticles in human bronchial epithelial cell, Beas-2B, **Toxicology in Vitro**, 10.1016/j.tiv.2009.07.010, 23:7, (1326-1332), Online publication date: 1-Oct-2009.
Eom H and Choi J (2009) Oxidative stress of CeO2 nanoparticles via p38-Nrf-2 signaling pathway in human bronchial epithelial cell, Beas-2B, **Toxicology Letters**, 10.1016/j.toxlet.2009.01.028, 187:2, (77-83), Online publication date: 1-Jun-2009.
Meng H, Xia T, George S and Nel A (2009) A Predictive

Toxicological Paradigm for the Safety Assessment of Nanomaterials, **ACS Nano**, 10.1021/nn9005973, 3:7, (1620-1627), Online publication date: 28-Jul-2009.
Hu L, Mao Z and Gao C (2009) Colloidal particles for cellular uptake and delivery, **Journal of Materials Chemistry**, 10.1039/b815958k, 19:20, (3108), .
Soberanes S, Urich D, Baker C, Burgess Z, Chiarella S, Bell E, Ghio A, De Vizcaya-Ruiz A, Liu J, Ridge K, Kamp D, Chandel N, Schumacker P, Mutlu G and Budinger G (2008) Mitochondrial Complex III-generated Oxidants Activate ASK1 and JNK to Induce Alveolar Epithelial Cell Death following Exposure to Particulate Matter Air Pollution, **Journal of Biological Chemistry**, 10.1074/jbc.M808844200, 284:4, (2176-2186), Online publication date: 23-Jan-2009.
Arhami M, Sillanpää M, Hu S, Olson M, Schauer J and Sioutas C (2009) Size-Segregated Inorganic and Organic Components of PM in the Communities of the Los Angeles Harbor, **Aerosol Science and Technology**, 10.1080/02786820802534757, 43:2, (145-160), Online publication date: 2-Jan-2009.
Gong L, Xu B and Zhu Y (2009) Ultrafine Particles Deposition Inside Passenger Vehicles, **Aerosol Science and Technology**, 10.1080/02786820902791901, 43:6, (544-553), Online publication date: 15-May-2009.
Moore K, Krudysz M, Pakbin P, Hudda N and Sioutas C (2009) Intra-Community Variability in Total Particle Number Concentrations in the San Pedro Harbor Area (Los Angeles, California), **Aerosol Science and Technology**, 10.1080/02786820902800900, 43:6, (587-603), Online publication date: 15-May-2009.
Cattaneo A, Garramone G, Taronna M, Peruzzo C and Cavallo D (2009) Personal exposure to airborne ultrafine particles in the urban area of Milan, **Journal of Physics: Conference Series**, 10.1088/1742-6596/151/1/012039, 151, (012039), Online publication date: 1-Feb-2009.
Stevens T, Cho S, Linak W and Gilmour M (2008) Differential Potentiation of Allergic Lung Disease in Mice Exposed to Chemically Distinct Diesel Samples, **Toxicological Sciences**, 10.1093/toxsci/kfn248, 107:2, (522-534), Online publication date: 1-Feb-2009., Online publication date: 1-Feb-2009.
Andreev G, Minashkin V, Nevskii I and Putilov A (2009) Nanotechnology-Derived materials: Potential risk in preparation and use, **Russian Journal of General Chemistry**, 10.1134/

S107036320909028X, 79:9, (1974-1981), Online publication date: 1-Sep-2009.
Xia T, Li N and Nel A (2009) Potential Health Impact of Nanoparticles, **Annual Review of Public Health**, 10.1146/annurev.publhealth.031308.100155, 30:1, (137-150), Online publication date: 1-Apr-2009.
Zhao Y, Usatyuk P, Gorshkova I, He D, Wang T, Moreno-Vinasco L, Geyh A, Breysse P, Samet J, Spannhake E, Garcia J and Natarajan V (2009) Regulation of COX-2 Expression and IL-6 Release by Particulate Matter in Airway Epithelial Cells, **American Journal of Respiratory Cell and Molecular Biology**, 10.1165/rcmb.2008-0105OC, 40:1, (19-30), Online publication date: 1-Jan-2009.
Park E, Park Y and Park K (2009) Acute Toxicity and Tissue Distribution of Cerium Oxide Nanoparticles by a Single Oral Administration in Rats, **Toxicological Research**, 10.5487/TR.2009.25.2.079, 25:2, (79-84), Online publication date: 1-Jun-2009.
Lee J, Lane D, Huh J, Yi S and Kim Y (2009) Analysis of Organic Compounds in Ambient PM 2.5 over Seoul using Thermal Desorption-comprehensive Two Dimensional Gas Chromatography-time of Flight Mass Spectrometry (TD-GCxGC-TOFMS) , **Journal of Korean Society for Atmospheric Environment**, 10.5572/KOSAE.2009.25.5.420, 25:5, (420-431), Online publication date: 31-Oct-2009.
Wegesser T, Pinkerton K and Last J (2009) California Wildfires of 2008: Coarse and Fine Particulate Matter Toxicity, **Environmental Health Perspectives**, 117:6, (893-897), Online publication date: 1-Jun-2009.
Fruin S, Westerdahl D, Sax T, Sioutas C and Fine P (2008) Measurements and predictors of on-road ultrafine particle concentrations and associated pollutants in Los Angeles, **Atmospheric Environment**, 10.1016/j.atmosenv.2007.09.057, 42:2, (207-219), Online publication date: 1-Jan-2008.
Matsunaga T, Kamiya T, Sumi D, Kumagai Y, Kalyanaraman B and Hara A (2008) L-Xylulose reductase is involved in 9,10-phenanthrenequinone-induced apoptosis in human T lymphoma cells, **Free Radical Biology and Medicine**, 10.1016/j.freeradbiomed.2007.12.024, 44:6, (1191-1202), Online publication date: 1-Mar-2008.
Sillanpää M, Geller M, Phuleria H and Sioutas C (2008) High collection efficiency electrostatic precipitator for in vitro cell

exposure to concentrated ambient particulate matter (PM), **Journal of Aerosol Science**, 10.1016/j.jaerosci.2007.12.001, 39:4, (335-347), Online publication date: 1-Apr-2008.
Kocbach A, Herseth J, Låg M, Refsnes M and Schwarze P (2008) Particles from wood smoke and traffic induce differential pro-inflammatory response patterns in co-cultures, **Toxicology and Applied Pharmacology**, 10.1016/j.taap.2008.07.002, 232:2, (317-326), Online publication date: 1-Oct-2008.
Tal T, Bromberg P, Kim Y and Samet J (2008) Epidermal growth factor receptor activation by diesel particles is mediated by tyrosine phosphatase inhibition, **Toxicology and Applied Pharmacology**, 10.1016/j.taap.2008.09.013, 233:3, (382-388), Online publication date: 1-Dec-2008.
Stevens J, Zahardis J, MacPherson M, Mossman B and Petrucci G (2008) A new method for quantifiable and controlled dosage of particulate matter for in vitro studies: The electrostatic particulate dosage and exposure system (EPDExS), **Toxicology in Vitro**, 10.1016/j.tiv.2008.05.013, 22:7, (1768-1774), Online publication date: 1-Oct-2008.
Park E, Choi J, Park Y and Park K (2008) Oxidative stress induced by cerium oxide nanoparticles in cultured BEAS-2B cells, **Toxicology**, 10.1016/j.tox.2007.12.022, 245:1-2, (90-100), Online publication date: 1-Mar-2008.
Park E, Yi J, Chung K, Ryu D, Choi J and Park K (2008) Oxidative stress and apoptosis induced by titanium dioxide nanoparticles in cultured BEAS-2B cells, **Toxicology Letters**, 10.1016/j.toxlet.2008.06.869, 180:3, (222-229), Online publication date: 1-Aug-2008.
Xia T, Kovochich M, Liong M, Mädler L, Gilbert B, Shi H, Yeh J, Zink J and Nel A (2008) Comparison of the Mechanism of Toxicity of Zinc Oxide and Cerium Oxide Nanoparticles Based on Dissolution and Oxidative Stress Properties, **ACS Nano**, 10.1021/nn800511k, 2:10, (2121-2134), Online publication date: 28-Oct-2008.
Ayres J, Borm P, Cassee F, Castranova V, Donaldson K, Ghio A, Harrison R, Hider R, Kelly F, Kooter I, Marano F, Maynard R, Mudway I, Nel A, Sioutas C, Smith S, Baeza-Squiban A, Cho A, Duggan S and Froines J (2008) Evaluating the Toxicity of Airborne Particulate Matter and Nanoparticles by Measuring Oxidative Stress Potential—A Workshop Report and Consensus Statement, **Inhalation Toxicology**, 10.1080/08958370701665517, 20:1, (75-99), Online publication date: 1-Jan-2008.

VALAVANIDIS A, FIOTAKIS K and VLACHOGIANNI T (2008) Airborne Particulate Matter and Human Health: Toxicological Assessment and Importance of Size and Composition of Particles for Oxidative Damage and Carcinogenic Mechanisms, **Journal of Environmental Science and Health, Part C**, 10.1080/10590500802494538, 26:4, (339-362), Online publication date: 2-Dec-2008.
Stern S and McNeil S (2007) Nanotechnology Safety Concerns Revisited, **Toxicological Sciences**, 10.1093/toxsci/kfm169, 101:1, (4-21), Online publication date: 1-Jan-2008., Online publication date: 1-Jan-2008.
Stevens T, Krantz Q, Linak W, Hester S and Gilmour M (2008) Increased Transcription of Immune and Metabolic Pathways in Naïve and Allergic Mice Exposed to Diesel Exhaust, **Toxicological Sciences**, 10.1093/toxsci/kfn006, 102:2, (359-370), Online publication date: 1-Apr-2008., Online publication date: 1-Apr-2008.
Mühlfeld C, Rothen-Rutishauser B, Blank F, Vanhecke D, Ochs M and Gehr P (2008) Interactions of nanoparticles with pulmonary structures and cellular responses, **American Journal of Physiology-Lung Cellular and Molecular Physiology**, 10.1152/ajplung.00442.2007, 294:5, (L817-L829), Online publication date: 1-May-2008.
Ghio A (2014) Mechanism of asthmatic exacerbation by ambient air pollution particles, **Expert Review of Respiratory Medicine**, 10.1586/17476348.2.1.109, 2:1, (109-118), Online publication date: 1-Feb-2008.
Enomoto M, Tierney W and Nozaki K (2008) Risk of human health by particulate matter as a source of air pollution -Comparison with tobacco smoking-, **The Journal of Toxicological Sciences**, 10.2131/jts.33.251, 33:3, (251-267), .
Jung E, Avliyakulov N, Boontheung P, Loo J and Nel A (2007) Pro-oxidative DEP chemicals induce heat shock proteins and an unfolding protein response in a bronchial epithelial cell line as determined by DIGE analysis, **PROTEOMICS**, 10.1002/pmic.200700377, 7:21, (3906-3918), Online publication date: 1-Nov-2007.
Groenewold G, Cortez M, Gianotto A, Gresham G, Olson J, Fox R, White B, Bauer W, Avci R, Deliorman M and Williams E (2007) Surface analysis of particulates from laboratory hood exhaust manifold, **Surface and Interface Analysis**, 10.1002/sia.2554, 39:6, (547-553), Online publication date: 1-Jun-2007.

Biswas S, Ntziachristos L, Moore K and Sioutas C (2007) Particle volatility in the vicinity of a freeway with heavy-duty diesel traffic, **Atmospheric Environment**, 10.1016/j.atmosenv.2006.11.059, 41:16, (3479-3493), Online publication date: 1-May-2007.
Sklorz M, Schnelle-Kreis J, Liu Y, Orasche J and Zimmermann R (2007) Daytime resolved analysis of polycyclic aromatic hydrocarbons in urban aerosol samples – Impact of sources and meteorological conditions, **Chemosphere**, 10.1016/j.chemosphere.2006.11.006, 67:5, (934-943), Online publication date: 1-Mar-2007.
Li H, Haberzettl P, Albrecht C, Höhr D, Knaapen A, Borm P and Schins R (2007) Inhibition of the mitochondrial respiratory chain function abrogates quartz induced DNA damage in lung epithelial cells, **Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis**, 10.1016/j.mrfmmm.2006.12.001, 617:1-2, (46-57), Online publication date: 1-Apr-2007.
Kennedy I (2007) The health effects of combustion-generated aerosols, **Proceedings of the Combustion Institute**, 10.1016/j.proci.2006.08.116, 31:2, (2757-2770), Online publication date: 1-Jan-2007.
Sandström T and Brunekreef B (2007) Traffic-related pollution and lung development in children, **The Lancet**, 10.1016/S0140-6736(07)60038-5, 369:9561, (535-537), Online publication date: 1-Feb-2007.
Unfried K, Albrecht C, Klotz L, Von Mikecz A, Grether-Beck S and Schins R (2009) Cellular responses to nanoparticles: Target structures and mechanisms, **Nanotoxicology**, 10.1080/00222930701314932, 1:1, (52-71), Online publication date: 1-Jan-2007.
Eiguren-Fernandez A, Avol E, Thurairatnam S, Hakami M, Froines J and Miguel A (2007) Seasonal Influence on Vapor-and Particle-Phase Polycyclic Aromatic Hydrocarbon Concentrations in School Communities Located in Southern California, **Aerosol Science and Technology**, 10.1080/02786820701213511, 41:4, (438-446), Online publication date: 1-Mar-2007.
Jalava P, Salonen R, Pennanen A, Sillanpää M, Hälinen A, Happo M, Hillamo R, Brunekreef B, Katsouyanni K, Sunyer J and Hirvonen M (2008) Heterogeneities in Inflammatory and Cytotoxic Responses of RAW 264.7 Macrophage Cell Line to Urban Air Coarse, Fine, and Ultrafine Particles From Six European Sampling Campaigns, **Inhalation Toxicology**, 10.1080/08958370601067863, 19:3, (213-225), Online publication

date: 1-Jan-2007.
Grahame T and Schlesinger R (2008) Health Effects of Airborne Particulate Matter: Do We Know Enough to Consider Regulating Specific Particle Types or Sources?, **Inhalation Toxicology**, 10.1080/08958370701382220, 19:6-7, (457-481), Online publication date: 1-Jan-2007.
Samet J, Graff D, Berntsen J, Ghio A, Huang Y and Devlin R (2008) A Comparison of Studies on the Effects of Controlled Exposure to Fine, Coarse and Ultrafine Ambient Particulate Matter from a Single Location, **Inhalation Toxicology**, 10.1080/08958370701492706, 19:sup1, (29-32), Online publication date: 1-Jan-2007.
Brook R Is air pollution a cause of cardiovascular disease? Updated review and controversies, **Reviews on Environmental Health**, 10.1515/REVEH.2007.22.2.115, 22:2
Dykens J, Marroquin L and Will Y (2014) Strategies to reduce late-stage drug attrition due to mitochondrial toxicity, **Expert Review of Molecular Diagnostics**, 10.1586/14737159.7.2.161, 7:2, (161-175), Online publication date: 1-Mar-2007.
Liu Y, Sklorz M, Schnelle-Kreis J, Orasche J, Ferge T, Kettrup A and Zimmermann R (2006) Oxidant denuder sampling for analysis of polycyclic aromatic hydrocarbons and their oxygenated derivates in ambient aerosol: Evaluation of sampling artefact, **Chemosphere**, 10.1016/j.chemosphere.2005.07.049, 62:11, (1889-1898), Online publication date: 1-Mar-2006.
Singh M, Phuleria H, Bowers K and Sioutas C (2005) Seasonal and spatial trends in particle number concentrations and size distributions at the children's health study sites in Southern California, **Journal of Exposure Science & Environmental Epidemiology**, 10.1038/sj.jea.7500432, 16:1, (3-18), Online publication date: 1-Jan-2006.
Valavanidis A, Fiotakis K, Vlahogianni T, Papadimitriou V and Pantikaki V (2006) Determination of Selective Quinones and Quinoid Radicals in Airborne Particulate Matter and Vehicular Exhaust Particles, **Environmental Chemistry**, 10.1071/EN05089, 3:2, (118), .
Su Y, Sipin M, Spencer M, Qin X, Moffet R, Shields L, Prather K, Venkatachari P, Jeong C, Kim E, Hopke P, Gelein R, Utell M, Oberdörster G, Berntsen J, Devlin R and Chen L (2006) Real-Time Characterization of the Composition of Individual Particles Emitted From Ultrafine Particle Concentrators, **Aerosol Science and Technology**, 10.1080/02786820600660887, 40:6, (437-455),

Online publication date: 1-Jul-2006.
Ning Z, Moore K, Polidori A and Sioutas C (2006) Field Validation of the New Miniature Versatile Aerosol Concentration Enrichment System (mVACES), **Aerosol Science and Technology**, 10.1080/02786820600996422, 40:12, (1098-1110), Online publication date: 1-Dec-2006.
Soberanes S, Panduri V, Mutlu G, Ghio A, Bundinger G and Kamp D (2006) p53 Mediates Particulate Matter–induced Alveolar Epithelial Cell Mitochondria-regulated Apoptosis, **American Journal of Respiratory and Critical Care Medicine**, 10.1164/rccm.200602-203OC, 174:11, (1229-1238), Online publication date: 1-Dec-2006.
Schwarze P, Øvrevik J, Låg M, Refsnes M, Nafstad P, Hetland R and Dybing E (2016) Particulate matter properties and health effects: consistency of epidemiological and toxicological studies, **Human & Experimental Toxicology**, 10.1177/096032706072520, 25:10, (559-579), Online publication date: 1-Oct-2006.
Gehr P, Rothen-Rutishauser B and Schv°rch S (2006) Interaction of Particles with Membranes **Particle Toxicology**, 10.1201/9781420003147.ch7, (139-160), Online publication date: 21-Dec-2006.
Ghio A and Samet J (2006) Particle-Associated Metals and Oxidative Stress in Signaling **Particle Toxicology**, 10.1201/9781420003147.ch8, (161-181), Online publication date: 21-Dec-2006.
Stern S, McNeil S, Patri A and Dobrovolskaia M (2006) Preclinical Characterization of Engineered Nanoparticles Intended for Cancer Therapeutics **Nanotechnology for Cancer Therapy**, 10.1201/9781420006636.ch7, (105-137), Online publication date: 19-Dec-2006.
Papkovsky D, Hynes J and Will Y (2006) Respirometric Screening Technology for ADME-Tox studies, **Expert Opinion on Drug Metabolism & Toxicology**, 10.1517/17425255.2.2.313, 2:2, (313-323), Online publication date: 1-Apr-2006.
Gilmour M, Jaakkola M, London S, Nel A and Rogers C (2006) How Exposure to Environmental Tobacco Smoke, Outdoor Air Pollutants, and Increased Pollen Burdens Influences the Incidence of Asthma, **Environmental Health Perspectives**, 114:4, (627-633), Online publication date: 1-Apr-2006.
Künzli N, Mudway I, Götschi T, Shi T, Kelly F, Cook S, Burney P, Forsberg B, Gauderman J, Hazenkamp M, Heinrich J, Jarvis D,

Norbäck D, Payo-Losa F, Poli A, Sunyer J and Borm P (2005) Comparison of Oxidative Properties, Light Absorbance, and Total and Elemental Mass Concentration of Ambient PM2.5 Collected at 20 European Sites, **Environmental Health Perspectives**, 114:5, (684-690), Online publication date: 1-May-2006.
Lanki T, de Hartog J, Heinrich J, Hoek G, Janssen N, Peters A, Stölzel M, Timonen K, Vallius M, Vanninen E and Pekkanen J (2006) Can We Identify Sources of Fine Particles Responsible for Exercise-Induced Ischemia on Days with Elevated Air Pollution? The ULTRA Study, **Environmental Health Perspectives**, 114:5, (655-660), Online publication date: 1-May-2006.
Wang M, Xiao G, Li N, Xie Y, Loo J and Nel A (2005) Use of a fluorescent phosphoprotein dye to characterize oxidative stress-induced signaling pathway components in macrophage and epithelial cultures exposed to diesel exhaust particle chemicals, **ELECTROPHORESIS**, 10.1002/elps.200410428, 26:11, (2092-2108), Online publication date: 1-Jun-2005.
Meyer J, Volz D, Freedman J and Giulio R (2005) Differential display of hepatic mRNA from killifish (Fundulus heteroclitus) inhabiting a Superfund estuary, **Aquatic Toxicology**, 10.1016/j.aquatox.2005.03.022, 73:4, (327-341), Online publication date: 1-Jul-2005.
Geller M, Biswas S, Fine P and Sioutas C (2005) A new compact aerosol concentrator for use in conjunction with low flow-rate continuous aerosol instrumentation, **Journal of Aerosol Science**, 10.1016/j.jaerosci.2004.11.015, 36:8, (1006-1022), Online publication date: 1-Aug-2005.
Krug H (2005) Auswirkungen nanotechnologischer Entwicklungen auf die UmweltImpact of nanotechnological developments on the environment, **Umweltwissenschaften und Schadstoff-Forschung**, 10.1065/uwsf2005.08.103, 17:4, (223-230), Online publication date: 1-Jul-2005.
Cassee F, Boere A, Fokkens P, Leseman D, Sioutas C, Kooter I and Dormans J (2005) Inhalation Of Concentrated Particulate Matter Produces Pulmonary Inflammation and Systemic Biological Effects in Compromised Rats, **Journal of Toxicology and Environmental Health, Part A**, 10.1080/15287390590930171, 68:10, (773-796), Online publication date: 1-May-2005.
Singh P, Madden M and Gilmour M (2008) Effects of Diesel Exhaust Particles and Carbon Black on Induction of Dust Mite Allergy in Brown Norway Rats, **Journal of Immunotoxicology**, 10.1080/15476910590952458, 2:1, (41-49), Online publication

date: 1-Jan-2005.
Valavanidis A, Fiotakis K, Bakeas E and Vlahogianni T (2013) Electron paramagnetic resonance study of the generation of reactive oxygen species catalysed by transition metals and quinoid redox cycling by inhalable ambient particulate matter, **Redox Report**, 10.1179/135100005X21606, 10:1, (37-51), Online publication date: 1-Feb-2005.
Sioutas C, Delfino R and Singh M (2005) Exposure Assessment for Atmospheric Ultrafine Particles (UFPs) and Implications in Epidemiologic Research, **Environmental Health Perspectives**, 113:8, (947-955), Online publication date: 1-Aug-2005.
Penn A, Murphy G, Barker S, Henk W and Penn L (2005) Combustion-Derived Ultrafine Particles Transport Organic Toxicants to Target Respiratory Cells, **Environmental Health Perspectives**, 113:8, (956-963), Online publication date: 1-Aug-2005.
Will Y and Dykens J (2009) Introduction to Mitochondrial Toxicity **General and Applied Toxicology**, 10.1002/9780470744307.gat021
Krug H and Klug P (2010) Impact of Nanotechnological Developments on the Environment **Nanotechnology**, 10.1002/9783527628155.nanotech019
Gehr P, Clift M, Brandenberger C, Lehmann A, Herzog F and Rothen-Rutishauser B (2011) Endocytosis of Environmental and Engineered Micro- and Nanosized Particles **Comprehensive Physiology**, 10.1002/cphy.c100035
Xia T, Zhu Y, Mu L, Zhang Z and Liu S (2016) Pulmonary diseases induced by ambient ultrafine and engineered nanoparticles in twenty-first century, **National Science Review**, 10.1093/nsr/nww064, (nww064)
Vecchio G, Galeone A, Brunetti V, Maiorano G, Sabella S, Cingolani R, Pompa P and Chin W (2012) Concentration-Dependent, Size-Independent Toxicity of Citrate Capped AuNPs in Drosophila melanogaster, **PLoS ONE**, 10.1371/journal.pone.0029980, 7:1, (e29980)
Huang H, Lai C, Chen G, Lin Y, Jaakkola J, Liou S, Wang S and Singh K (2012) Traffic-Related Air Pollution and DNA Damage: A Longitudinal Study in Taiwanese Traffic Conductors, **PLoS ONE**, 10.1371/journal.pone.0037412, 7:5, (e37412)
Kahru A, Dubourguier H, Blinova I, Ivask A and Kasemets K (2008) Biotests and Biosensors for Ecotoxicology of Metal Oxide Nanoparticles: A Minireview, **Sensors**, 10.3390/s8085153, 8:8,

(5153-5170)
Eom H and Choi J (2011) SiO<sub>2</sub> Nanoparticles Induced Cytotoxicity by Oxidative Stress in Human Bronchial Epithelial Cell, Beas-2B, **Environmental Health and Toxicology**, 10.5620/eht.2011.26.e2011013, 26, (e2011013)
Nageh A, ThannaaA E, Magdaa E, Shimaa M, Sally E and Karima I (2015) Effects of rice straw burning products on guinea pig lungs, **African Journal of Pharmacy and Pharmacology**, 10.5897/AJPP2014.4142, 9:26, (645-661)