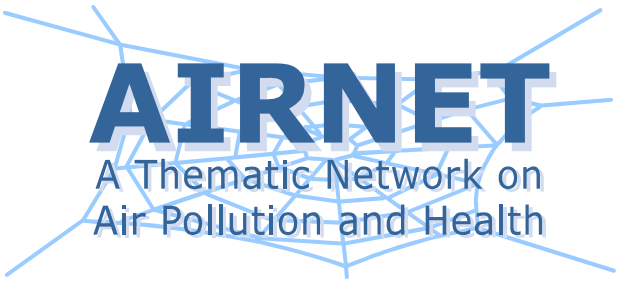

Air pollution and the risks to human health – a toxicological perspective –

AIRNET WORK GROUP 3 - TOXICOLOGY

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EXECUTIVE SUMMARY

Exposure to air pollution is a known risk to human health. However, decision-makers wishing to put in place cost-effective and health-beneficial measures to reduce air pollution are hampered by limitations in the knowledge base and a number of uncertainties. In particular, a better understanding is needed of the contribution made by air-pollution components to human health, as well as of the biological mechanisms behind the toxicity of these components.

For example, 'Are we at greater risk from short-term exposure to high pollution levels, such as diesel particulates in traffic tunnels?' 'Or is the risk greater from long-term exposure to lower levels, such as ozone in rural areas?' 'Which groups of the population are at greatest risk, for genetic and/or physical reasons?

Toxicology is the scientific discipline from which we may expect answers to these types of questions. Toxicology aims to understand the processes of how pollutants affect people's health, and to identify the factors influencing those processes.

This report from the AIRNET Toxicology Workgroup is intended to provide a relatively short, non-specialist assessment, using today's knowledge, of toxicology research findings in the field of air pollution and human health.

SIX KEY AIRBORNE POLLUTANTS

This report focuses on six ambient air pollutants that are known to provide a risk to human health. There are others, for example volatile organic compounds (VOCs) including irritating aldehydes. However for reasons of space this document must limit itself to the known major pollutants.

These six pollutants are:

- Particulate matter (PM)
- Ozone (O_3)
- Nitrogen dioxide (NO_2)
- Polycyclic aromatic hydrocarbons (PAHs)
- Carbon monoxide (CO)
- Sulphur dioxide (SO_2)

The following chapters give the current toxicological understanding of the risks from exposure to each of these key pollutants. Each chapter commences with 'Key points', a bulleted list of the key points of concern, together with an introduction to the pollutant, its biological effects and likely implications for human health.

The report also attempts to provide answers, using today's knowledge, to a number of questions on each pollutant that emerged from the AIRNET stakeholder survey. These questions and answers are given at the end of each chapter, respectively.

IMPACT ON HUMAN HEALTH

Particulate matter (PM)

Particulate matter (PM) originates partly from natural sources (wind-born soil, sea-spray and organic compounds) and from man-made activities (combustion of fossil fuels and industry) and may therefore have a heterogeneous composition depending on weather conditions, type and strengths of sources, and exposure location. Emissions by road traffic may, especially in urban environments, contribute substantially to PM, and comprise exhaust pipe emissions, friction processes and re-suspension of road dust. Inhaled PM can range in size from a few nanometres to tens of micrometres.

Different-sized particles, i.e. coarse, fine, and ultrafine PM, deposit differently on the various walls of the airways and lungs. Coarse particles tend to deposit higher up in the airways, whereas fine particles tend to deposit in the lower airways and lungs. It is not yet clear whether the health effects observed in relation to PM exposure are caused by particles of specific sizes and/or composition.

Currently both coarse and fine particles seem capable of inducing toxicity. Whether the ultrafine particles, tested at environmentally relevant levels, are also toxic remains unclear. Clarification on this issue would be a great help in the development of plans to control PM emission sources. On the issue of the chemical composition of particles and their toxicity, there are preliminary indications that primary, carbonaceous PM components may have more significant health effects than secondary components such as sulphates and nitrates.

Toxicological studies in humans and animals suggest that PM pollutants could affect the functioning of the lung, the blood vessels and the heart. Most of the results however, arise from in vitro studies using cell systems or from direct high dose administration of PM to laboratory animals into their airways and lungs. This makes it difficult to extrapolate this data to the actual human ambient exposure conditions. However, these results do indicate the potential ability of PM to induce toxicity. The few inhalation studies available from laboratory animals and human volunteers indicate that different types of PM may induce toxicity at relatively high levels.

In studies testing the inhalation exposure of PM at concentrations well above ambient levels, the results show exacerbation of symptoms in patients with mild or moderate pre-existing lung diseases such as asthma, as well as heart and blood vessel disease. Toxicological studies suggest that these effects are due to induction of lung inflammation, disturbances in heart rhythm, alterations in blood viscosity or oxygen deprivation. Remarkably, PM deposition models suggest that these individuals receive much higher doses in their airways and lungs compared to healthy individuals. It could very well be that the more severely affected individuals receive even higher doses and/or that their tissue responds more extensively to a certain dose. All these effects may partly explain why individuals with pre-existing disease are at increased risk for PM. Analogous effects, albeit at much lower intensity, have also been observed in healthy individuals.

There is also evidence linking PM exposure with lung cancer, however, the basis of PM-related cancer development is poorly understood.

Ozone (O_3)

Ground-level ozone has become one of Europe's most serious air pollutants. Despite slight reductions in peak ozone levels during the latter years, each year ozone concentrations in ambient air exceed the EU Limit Values established to protect human health and the ecosystem, and that will probably continue to occur for the next decades. Ozone is the major component in photochemical smog.

Because of its very low solubility in water, ozone is carried over into the deep lung, with the greatest deposition and the most pronounced damage where the small airway branches enter the air sacs of the lung. Health effects include reduced pulmonary function, pulmonary inflammatory processes, increased airway permeability, heightened hyper-reactivity at first, then irreversible structural changes to the airways.

Some 20% of the general population, regardless of airway disease, are more susceptible to ozone's effects. Repeated or long-term exposure to higher levels of ozone may lead to irreversible effects and perhaps increase the risk of developing chronic lung disease such as asthma.

Toxicological studies show that acute ozone exposure in humans and laboratory animals results in lung inflammation and tissue injury in the small airways and the gas exchange region of the lung, causing a reduction of the lung function. There are few long-term studies of ozone in experiments on laboratory animals, but changes in lung and airway structures and cell types have been reported.

Nitrogen dioxide (NO_2)

Nitrogen dioxide is one of the major components of air pollution in densely populated areas. Nitrogen-dioxide levels in ambient air are normally in the order of ten to 50 $\mu g/m^3$, but may reach some hundred $\mu g/m^3$ (as an 1-hour average) in pollution hot spots (such as road tunnels or street canyons) during high pollution episodes.

The gas acts as an effective indicator of (traffic-related) air pollution, although it is not considered a major causal influence on human health by itself. That said however, exposure to extremely high levels of the gas, or the mixture it represents, has been shown as able to negatively affect human health. It is therefore important to continue to monitor its ambient levels and to investigate the health effects.

Nitrogen dioxide is a highly reactive, poorly water-soluble gas that reacts with components in the lining fluid of the respiratory tract. Its effects seem to depend more on the level of exposure than on the duration. Asthmatic individuals are more sensitive than healthy subjects, reacting to high-concentration episodes of the gas with narrowing of airways and increased responsiveness to irritants and allergens.

Nitrogen dioxide can at very high levels exacerbate, independently of other pollutants, allergic reactions in asthmatics. Susceptible groups include allergic asthmatics, patients with chronic obstructive lung disease and possibly children. However, some individuals may be affected at lower concentrations. Animal studies indicate long-term effects of nitrogen dioxide on lung structure and function at much higher than ambient concentrations.

Polycyclic aromatic hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) are a large family of related organic compounds that arise mainly from the incomplete combustion of fuels. They include some powerful animal carcinogens, and therefore could well act as carcinogens in humans. In addition, recent evidence suggests that they can cause inflammation and adverse birth outcomes.

Individual PAHs present in the ambient air mixture, in the vapour phase or bound to particulate matter, have widely varying carcinogenic potencies, with particulate-bound PAHs generally having the highest carcinogenic potency. Currently it is not possible to distinguish between the toxic effects of airborne PAHs alone, or in combination with particles to which they are bound.

Benzo[a]pyrene, used as a qualitative marker of PAHs, is usually found in urban air at levels usually below 1 ng/m^3, but sometimes found at significantly higher levels. The European Union has proposed a limit value for benzo[a]pyrene of 1 ng/m^3.

PAHs are animal and human carcinogens, acting through a genotoxic mechanism (i.e. they damage DNA and cause mutations). For such compounds, it is considered that there is a human health concern without any exposure threshold. Recent studies have suggested that the soluble components of atmospheric particles, including PAHs or their derivatives, can play a role in the induction of inflammation, with a potential role both in the development of cancer and the exacerbation of asthma and heart and blood vessel effects.

Carbon monoxide (CO)

Carbon monoxide (CO) is formed mainly from the incomplete combustion of fuels, and has traffic as its main source. At ambient air concentrations of carbon monoxide, healthy individuals do not experience any adverse health effects. For individuals with pre-existing heart disease however, the heart may be affected at carbon-monoxide levels relevant in high concentration episodes.

Carbon-monoxide exposure can result in reduced oxygen supply to organs and tissues, due to binding to blood haemoglobin, thereby forming carboxyhaemoglobin (COHb). This ability of carbon monoxide to bind to haemoglobin is central to its impact on human health. The level of carboxyhaemoglobin varies in the population, depending on smoking habits, environmental exposure and reproductive state (both mother and unborn child have higher levels). Although high, accidental exposures to carbon monoxide may induce adverse health effects and even death, healthy people do not normally stay in highly polluted areas for sufficiently long periods to attain adverse levels of COHb.

Sulphur dioxide (SO_2)

Sulphur dioxide (SO_2) is a colourless gas with a pungent odour. The main man-made sources are combustion of fossil fuels containing sulphur (mainly coal and heavy oils), and the smelting of ores containing sulphur. Ambient concentration levels have fallen considerably in most parts of Europe in recent decades, and most measurements are in compliance with the air quality standards, i.e. 24 hour average of 125 µg/m^3, and hourly 350 µg/m^3.

Sulphur dioxide is an upper respiratory tract irritant. At least 95% of inhaled sulphur dioxide is absorbed in the nose and throat during resting conditions, while penetration to the lower airways is greater during mouth breathing and with exercise.

Short-term exposure to sulphur dioxide may cause mild narrowing of the airways, which is reflected as a measurable decrease in lung function and increase in airway resistance. However, among healthy individuals these reactions are only shown at concentrations markedly in excess of current ambient levels. Asthmatics, on the other hand, have shown to be more responsive than healthy subjects, and may possibly react at concentrations pertinent to hot spots, such as those that could occur near certain industrial plants without proper emission controls.

For long-term exposure, there is no experimental evidence on whether ambient concentrations of sulphur dioxide are harmful, either to healthy individuals or to asthmatics.

ROLE OF TOXICOLOGY

Environmental policymakers today are faced with the need to develop new risk management tools to incorporate toxicological findings and the growing understanding of the hazards of air pollution. Key questions for toxicology for example include:

- What are the actual risks associated with exposure to hazardous pollutants?
- To what extent will public health benefit from reduced exposures to toxicants?
- Will the costs of abatement be acceptable given the level of risk?
- How certain or uncertain are we about health-effective abatement policy?

Increases in toxicological knowledge and judgement help to answer the first of these questions. Toxicological studies can be especially valuable in reducing uncertainties over the causative role of a single pollutant appearing in a complex ambient mixture.

Improved understanding of the modes of toxicological action also helps to interpret data on air pollution and health. For ozone the toxicological database is extensive and for PM it is growing rapidly, if still limited. However, a few preliminary suggestions can be made. Primary carbonaceous PM components, for example, may be considered as more important than secondary components such as sulphates and nitrates. It will take many more years before the remaining questions can be answered in much more detail.

Policy-makers increasingly request a more integrated judgement of the source-risk relationships and abatement scenarios, both from the point of view of cost-effective abatement control and that of effective health risk reduction. Meeting the needs of such integrated judgements requires the development of better descriptions, improved evaluations and sustainable decision points on health effect and risk assessments, so that all can be accepted by policy-makers, stakeholders, the public and the scientific community.

A key objective for toxicological research on air pollution is to complement epidemiological studies as inputs for assessments of the risk to human health. In many cases there is a need to reduce uncertainty in the relationship between exposure to gaseous and particulate pollutants and observed health effects, as well as to identify the causative characteristics of complex pollutant mixtures. In certain cases, such as for PAH in air, toxicological research has a larger role, because little epidemiological data is available on PAH in common urban environments.

EU-FUNDED TOXICOLOGICAL RESEARCH INTO AIR POLLUTANTS

Toxicological research within the EU Fourth and Fifth Framework Programmes has contributed significantly to a better understanding of the health effects associated with exposure to air pollution. The programmes have funded a number of toxicological projects on air pollution (AULIS, EXPAH, HELIOS HEPMEAP, RAIAP, PAMCHAR, etc.).

The projects have also helped develop further competence in establishing risk assessments for exposure to air pollutants. In addition, they have produced extensive collaboration across Europe and established strong research networks that will be of benefit in the future.

However, there are still considerable gaps in the toxicological knowledge about the risks associated with hazardous air pollutants, which is hampering an effective and optimal preventative strategy. AIRNET has

created a wide European network among many of the scientists involved in air pollution toxicology, through better information exchange, meetings, conferences and an increased awareness of the need to further focus on toxicological research as input to policy and risk management. This network should make use of the current momentum for better structuring the science-policy interface in Europe and should further try to develop its information, interpretation, and dissemination goals.

FILLING IN THE GAPS

To meet the needs outlined above, there is a need for more short-term human and animal studies of heart and respiratory functional responses, as well as local and total-body inflammatory responses. Studies into PM from specific source environments, i.e. high traffic densities, residential heating, metal industries, etc., are required. Research into PM from urban background locations is also needed for the climate seasons with the highest adverse outcomes, as assessed by epidemiological data. The results of such toxicological research are strengthened when conducted in parallel with epidemiological panel studies.

Human clinical studies are also required into various types of PM in potentially susceptible individuals, including those with mild asthma, chronic obstructive pulmonary disease or heart and blood vessel disease. More attention needs to be paid to chemical characterisation and toxicity associated with the large organic proportion (including PAHs) of ambient air PM.

Since human exposures are to combinations of various pollutants, there is obviously a need to perform studies on the combined effects of particulate matter and gaseous pollutants. For example, it would be valuable to examine the effects of combined concentrated ambient particle and ozone exposure on the heart and blood vessel system in animal and clinical human studies.

Greater knowledge on the long-term effects of PM in animals would be helpful, especially on concentrated ambient particles of different sizes and their effect on the heart and blood vessel system, lung development and ageing. European multi-centre toxicological approaches on ambient air PM, using common PM sampling, concentration and chemical analysis instruments and protocols, can support epidemiological studies in finding explanations to geographical and seasonal differences in adverse health outcomes.

Much more may be learned from mechanistic studies with various types of PM in molecular, cellular and animal models with respect to lung, heart, inflammatory and genotoxic responses. Important mechanistic information may also be gained from examining whether particles of various sizes, especially ultrafine particles, become blood-borne from the lungs and enter and exert adverse responses in more distant organs such as the brain, liver and kidneys. Such cellular/mechanistic studies are also needed into the impact of particles on blood clotting pathways, in order to understand the involvement of particles in heart and blood vessel disease.

Finally, toxicological investigations into the effects of exhaust pollutants from different combustion systems and fuel types (vehicle engines, residential stoves, etc.) would aid in the evaluation of new fuel combustion technologies. Such investigations are necessary because new technologies may well alter the chemical and physical properties of both exhaust particles and gases. Predictive toxicology studies are needed to assess which of the various new technologies offers the most promise in reducing toxic emissions.

CONTENTS

1 INTRODUCTION

1.1 PURPOSE OF THIS REPORT

This report from the AIRNET Toxicology Workgroup offers a relatively short, non-specialist assessment of toxicology research findings in the field of air pollution and health. It also aims to provide answers, based on today's knowledge, to ten key questions that came out of the stakeholder survey organised by the AIRNET project.

The starting point for this report is the research into air pollution and health that was funded by the EU Fourth Framework Programme and some initial results from the Fifth Framework Programme. This EU-funded research has been identified and discussed within the report. Where necessary, results from the research have been complimented by other investigations conducted in Europe and elsewhere.

The following major ambient air pollutants are discussed:

- Particulate matter (PM)
- Ozone (O_3)
- Nitrogen dioxide (NO_2)
- Polycyclic aromatic hydrocarbons (PAHs)
- Carbon monoxide (CO
- Sulphur dioxide (SO_2)

Each of these pollutants is discussed in more detail in the following chapters.

The list above does not mean that other ambient air pollutants, such as volatile organic compounds (VOCs, which include irritating aldehydes, are not important. However, to ensure this report is not too voluminous, the Toxicology Workgroup chose to concentrate on the classical pollutants above.

The references given in following chapters cite are limited to only the most pertinent references selected by the chapter authors, as otherwise the document would be too lengthy. Although these references are not exhaustive, they should guide the reader to the background material most relevant for the science in to this report.

1.2 WHAT IS AIRNET?

The AIRNET network project on air pollution and health was launched in 2002 to lay the foundations for a European-wide framework for research into air pollution and its consequences for human health. Completing on 1st January 2005, AIRNET has been funded by the EU programme Quality of Life and Management of Living Resources (QoL), Key Action 4 - Environment and Health.

AIRNET's overall objective is to help create a foundation for public health policy on improving European air quality that would be widely accepted by research institutions and stakeholders alike. To this end, some 23 partners were brought into the project. Seven contractors, representing the scientific community and various stakeholders, have lead the project work. They have been supported by a further 14 organisations, all coordinators of ongoing or recently-completed EU studies on air pollution and health. Several additional institutions were introduced into the project because of their recent work in the field.

Team members have come from EU-funded projects in the field, the World Health Organisation (WHO), UN-ECE and related bodies involved in environmental health, EU institutions and selected national governments, the automobile, oil and gas industries, and some key consumer organisations and environmental NGOs.

All the organisations involved are key knowledge-holders in the field of environmental health in Europe. The project work has thus built upon proven abilities in this field from a team of highly experienced individuals. AIRNET is jointly co-ordinated by the Institute for Risk Assessment Sciences (IRAS) at Utrecht University and the Netherlands Environmental Assessment Agency (MNP) at the National Institute of Public Health and the Environment (RIVM).

A number of international agencies that focus on air pollution and its implications for human health have shown interest in AIRNET results. They include organisations such as WHO, EEA, and UN-ECE, all of which intend to use AIRNET research results as input for guiding and structuring their own assessments and policy development. AIRNET's results are also seen as a vital input to the work of the EU Clean Air for Europe (CAFE) initiative.

1.3 THE AIRNET TOXICOLOGY WORKGROUP

As one of the five workgroups within AIRNET, the Toxicology Workgroup was set up as an interactive communication and review forum to gather, discuss and interpret the findings arising from toxicology research into air pollution and health.

Together with the exposure and epidemiology workgroups, the Toxicology Workgroup aims to contribute to a better understanding of the causality and biological plausibility behind the health effects of air pollution, and also of the conditions of exposure important for such effects.

The AIRNET Toxicology Workgroup, as one of the more 'disciplinary' workgroups, consists of scientists from universities and research institutes across Europe who have met regularly throughout the AIRNET project. Feedback and communication with end-users have primarily taken place during the annual AIRNET conferences, but also through organising a joint workshop to disseminate the results of two EU projects (HEPMEAP and RAIAP[1]) to stakeholders, and the AIRNET stakeholder survey.

To this end, AIRNET Toxicology Workgroup activities have focused on the following:

- identification of key end-user issues relevant to policy-makers, industry, NGOs and the public
- writing non-specialist summaries of research findings selected as especially relevant for end-users
- making short non-specialist assessments of the science
- assessing the potential policy implications of the science

1.4 WHAT IS TOXICOLOGY?

Within the field of air pollution and health, the discipline of toxicology aims to understand the processes of how pollutants affect people's health, and to identify the factors influencing those processes. Gaining more knowledge about these issues can help in predicting and preventing problems associated with human exposure to harmful components in air.

[1] HEPMEAP – Health Effects of Particles from Motor Engine Exhaust and Ambient Air Pollution, and RAIAP – Respiratory Allergy and Inflammation due to Ambient Particles.

Toxicologists play a significant role in evaluating the potential adverse effects of chemicals, and are intimately involved in the processes of research, risk assessment and associated regulatory activities. A key challenge at present is to translate this fundamental knowledge into effective strategies for preventing environmentally induced disease.

The toxicological risk and safety assessments that are carried out are optimised through co-operation and sharing of research information with fellow researchers and scientists within other research disciplines, such as exposure research and epidemiology.

1.5 IMPORTANT AIR POLLUTION COMPONENTS AND THEIR CHARACTERISTICS

Particulate matter (PM)

Particulate matter (PM) in ambient air (i.e. air present in the immediate surroundings) originates partly from natural sources (wind-born soil, sea-spray and organic compounds) and from man-made activities (combustion of fossil fuels and industry). Emissions by road traffic contribute substantially to PM, and comprise exhaust pipe emissions, friction processes and re-suspension of road dust.

PM may be emitted directly (primary emissions) or formed in the atmosphere by conversion of gaseous precursors into secondary particles. Particles may be transported in the atmosphere over distances of hundreds or thousands of kilometres depending on particle size.

The size, surface area and chemical characteristics of the particles are important parameters in determining the potential for adverse health effects. Particles of different sizes (coarse, fine and ultrafine) often come from different sources and are of different chemical composition. Combustion particles consist of a carbon core with attached components such as metals and polycyclic aromatic compounds, and occur as ultrafine particles or after aggregation as fine particles. In contrast, coarse particles consist mainly of minerals or particles of biological origin.

Ozone

Ozone is formed in the lower atmosphere by complex reactions involving traffic-related precursors such as volatile organic compounds and nitrogen oxides in the presence of sunlight. Ozone can be transported over long distances and is regarded as a regional air pollution problem. Ozone concentrations are often lower in urban than in rural areas.

Nitrogen oxides

Nitrogen oxides are predominately generated by transport-associated combustion processes. Freshly emitted nitrogen monoxide from exhaust pipes for example reacts rapidly with ozone to form nitrogen dioxide.

Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAH)s are a group of organic compounds which contain two or more unsaturated carbon-containing rings fused together. PAHs are formed by incomplete combustion of different materials including fuels, coal and wood. More than 500 PAHs have been detected in air. Lighter PAHs are found in the gas phase, while the PAHs associated with the most serious health effects, such as benzo(a)pyrene, are usually attached to (fine) particles.

Carbon monoxide

Carbon monoxide is generated during incomplete combustion processes, and traffic is the main source.

Sulphur dioxide

Sulphur dioxide is formed during combustion of sulphur-containing fossil materials, such as oil and coal. However, the conversion to fuels with low-sulphur content and restrictions on coal burning have lead to a drastic reduction of sulphur dioxide concentrations in most areas of Europe.

1.6 THE HUMAN RESPIRATORY SYSTEM

The human respiratory system may be divided into three major regions (see Figure 1): (1) the upper airways (nasopharyngeal region; nose and throat), (2) the lower airways (tracheobronchial region; the windpipe and airway ducts), and (3) the deep lung (pulmonary region; consisting of so-called respiratory small air ducts, air sac ducts and air sacs). The nose, as the initial site of entry for gases, vapours and aerosols, is an important target site for a wide range of inhaled agents.

The throat region is also potential target tissue for inhaled toxicants, as a variety of agents have produced injury to cells lining this area. The lower airway region is responsible for delivering the inhaled air to deeper regions of the respiratory system. The surfaces of this region are covered with a layer of mucus-secreting cells and cells containing vibrating hair-like structures (cilia) that form a protective and functional blanket. The cilia and mucus work together in a co-ordinated fashion to propel particulate material away from the lung.

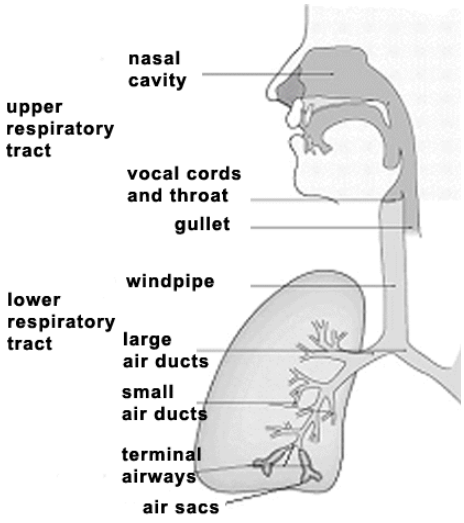


Figure 1. *Structure of the human respiratory tract.*

The 'air sac' is the functional gas exchange unit of the lung. Oxygen diffuses from the inhaled air in the air sacs to the bloodstream via extremely thin cellular membranes. A number of special cells and tissues in the respiratory tract have evolved to protect the delicate tissues of the distal respiratory tract. One of these important cell types, found in the lung, is the so-called 'macrophage', which is localised in the air sacs. These cells function in part to engulf any foreign particulate materials that are deposited in this region. The macrophages preserve the sterility of the deep lung by keeping inhaled bacteria and particles from accumulating.

1.7 THE HEART AND BLOOD VESSEL SYSTEM

In addition to the respiratory system, the heart and blood vessels seem to be key targets for health-endangering agents, such as particles and carbon monoxide, produced by air pollution. The heart is the organ that receives the blood from the respiratory system first, and thereby receives inhaled noxious substances such as carbon monoxide. In addition, the heart may receive inflammation-generating signalling substances produced in the lung.

Potential adverse effects of air pollution include heart disease related to diminished oxygen supply, or increased blood coagulation and possible clot formation in the vessels supplying the heart muscle.

Alternatively, deleterious heart rhythm effects may be induced either by disturbance of the nervous regulation of the heart or by direct stress responses on heart cells. Much attention is now focused on the so-called ultrafine particles, their possible uptake in the blood circulation system and consequent potential effects on the heart.

1.8 TOXICOLOGY STUDY METHODS

The adverse health effects shown in epidemiological studies have inspired scientists to use different techniques to study the toxicological mechanisms which form the biological background for the adverse health effects associated with gaseous and particulate pollutants.

Toxicological experiments can be categorised into either 'in vivo' or 'in vitro' experiments. In vivo experiments investigate effects in living organisms, whereas in vitro experiments are conducted in organs, tissues or cells isolated from the living organism.

In general, toxicological studies of air pollutants are shorter-term experimental approaches that tend, for ethical reasons, for the most part to study not people but research animals. These studies often also analyse the early events rather than waiting for final disease, such as reduced respiratory system development, and they may focus on cells and biochemical systems rather than whole animals.

Human studies

When investigating the effects of air pollutants on human beings, the exposure chambers used are filled in a controlled manner with the air to be investigated. The concentration of pollutants in the air that is fed into the exposure chamber can then be adjusted, to allow investigation of the health effects that follow from exposure to different pollutant concentrations.

Such studies are only suited to the examination of short-term, mild and reversible health effects in human volunteers, and cannot, for ethical reasons, be conducted with high exposure levels of air toxicants. Typical endpoints in such studies include measurements of respiratory and heart function, and contents of cells and proteins in airway wash-outs.

Increased susceptibility to inhaled pollutants may occur in individuals such as asthmatics and patients with chronic obstructive lung disease. Thus in human clinical studies, individuals with mild forms of pre-existing disease may react to lower inhalation concentrations than healthy subjects. Enhanced responses may also be demonstrated after exercise compared to rest in such investigations.

Animal studies

There are basically two approaches to studying the health effects of particulate matter on live animals: (a) placing solutions or suspensions of test substances in the nose or the windpipe, or (b) inhalation of gases, vapours or aerosols. Most commonly, rodents including the rat, mouse, guinea pig and hamster are used. Thanks to extensive studies with these rodents, investigators have generated a large body of background and historical information on responses.

Rodents have been used in toxicological studies to test vapour and particle deposition and removal, the mechanisms of pollutant-induced lung and airway injury, and as models for infection processes and the functioning of the immune system. Inhalation studies in rodents are essential to reveal significant long-term effects with the potential for major human impact, such as cancer development. However, rats in particular appear to be uniquely susceptible to chronic inflammation, fibrous tissue development and cancer from

insoluble, non-cytotoxic particles, via a process believed to involve the overwhelming of normal particle removal mechanisms (particle overload).

Dosage rates and tissue concentrations are key factors in determining toxicity. Many toxicological effects are related to inhalation exposure integrated over time, especially in those situations where exposure is longer-term. However, certain effects may be more related to peak exposures, such as local irritation in the upper and lower airways.

Extrapolation issues – animal versus human

Comparison between the effects on rodents and those on human beings can be rather difficult. This is due not least to anatomical and physiological differences, which can result in considerably lower concentrations in sensitive regions of the respiratory tract and the lungs of animals, compared to similar regions in humans.

Laboratory animals used in toxicological studies are genetically very similar within specific strains, whereas human populations are heterogeneous. Thus, extrapolating results from animals to humans must not only take strain and species differences into account, but must also consider inter-individual variation among humans.

In the case of animal studies, presumedly susceptible sections of the human population are mimicked by inducing specific cardiopulmonary diseases or focusing on older animals. For example, laboratory experiments in animal research have been carried out using a model of asthma, increased blood pressure in the lung arteries, lung inflammation and general high blood pressure. An advantage of these models is that hypotheses on the mechanisms of action and biological plausibility can be examined.

In these studies, models for respiratory infection and allergy have used diesel fuel, residual oil fly ash or ambient PM to demonstrate that disease symptoms can be exacerbated. A drawback with these disease models in animals is that they are not completely equivalent to the human disease counterpart. A second issue is that it is difficult to tell if the laboratory animal being tested is representative of the reactions of a human being, or if the endpoints being examined are sensitive and representative enough to match with equivalent human endpoints.

An additional consideration is whether the timing of exposure and observation has been correct in the animal studies. However, studies in animals continue to be the main methodology used to predict adverse health outcomes in humans, to clarify the relationship between exposure dosage and toxic effects, and to clarify modes of toxicological action.

In vitro studies

The effects of air pollutants may be studied in isolated lungs from animals, cultures of various anatomical structures of the respiratory tract from animals and humans, cultures of various cell types lining the respiratory system, and sub-cellular fractions of tissues and cells. Such approaches are good models for characterising the mode of action/mechanism for air pollutants, but findings need to be validated in whole animals.

Non-animal systems may also be used for studying biochemical aspects of qualitative and quantitative species differences in toxicity. Given the complex physiological and pathological reactions taking place in the respiratory system and elsewhere in the body when animals and humans are exposed to air pollutants via inhalation, in vitro studies can never in isolation be used for hazard characterisation purposes.

1.9 TOXICOLOGICAL EFFECTS ON THE BODY

Most toxic agents produce their effects through disruption of the cellular and molecular processes responsible for keeping body cells, tissues and organs in a relatively stable state (homeostasis). Initial reactions may be impairment of household functions such as rates of biochemical processes, cell growth and processing of the genetic material.

Further disruption of homeostatic processes can affect the basic cellular functions of particular organs, resulting for example in reduced defence against inhaled microbes or reduced removal of particles in the respiratory system. In addition, other pollutant-induced effects can include altered cellular repair mechanisms, altered cell growth and general damage of cells. The effects of pollutants on living systems are the result of multifaceted biological interactions with biochemical, cellular and molecular processes.

Toxicology studies often test single pollutant components, while in real life ambient exposures are often to complex mixtures that may have undergone different atmospheric reactions, or can result in interactive effects in the body. The complexity of air pollution is one of the great hurdles toxicologists have to overcome in study design and interpretation. As indicated both by epidemiological studies and toxicological experiments, the biological interaction of components in air pollutant mixtures may result in enhanced effects compared to those elicited by the individual components.

1.10 ASSESSING EXPOSURE RISK FOR AMBIENT AIR POLLUTANTS

Risk assessment for exposure to ambient air pollutants generally follows the classical paradigm of chemical risk assessment (Figure 2; Renwick *et al.*, 2003; EU TGD, 2003). Exposure to the pollutant(s) is assessed by determining the pollutant level in the air and the volume of air inhaled over time, in order to define the intake dose, its frequency and duration. Consideration is also given to intake in potentially sensitive population groups.

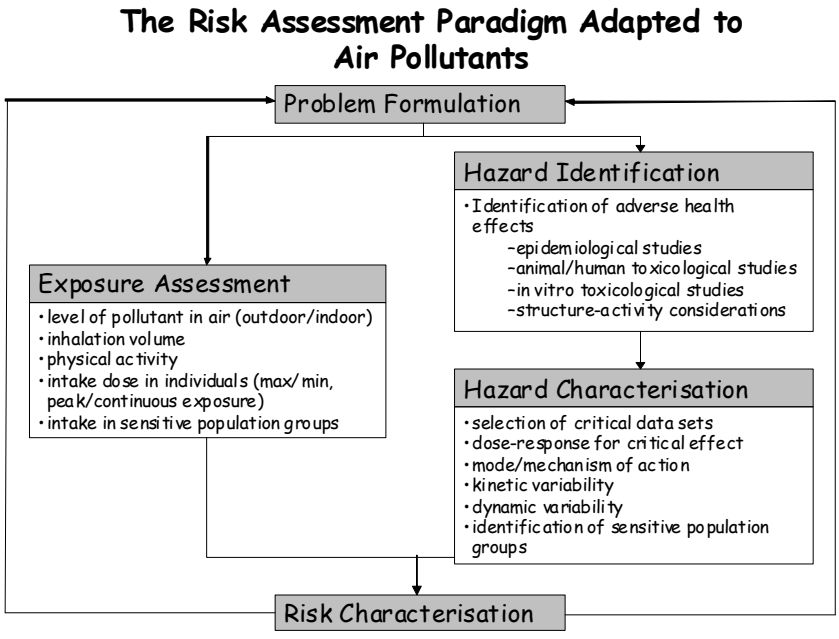


Figure 2. *Overview of the risk assessment process.*

The hazard identification step involves a *qualitative* description of adverse health effects, whereas in the subsequent hazard characterisation step a *quantitative* description of the dose-response relationships for the critical effect is given. This latter step includes a clarification of the mode or mechanism of action of the pollutant, as well as a description of human and animal variability in how the pollutant is handled in the body and how the body reacts to the pollutant. In the final synthesising step, termed risk characterisation, the exposure and hazard information is integrated.

Risk characterisation has been defined as follows (European Commission, Scientific Steering Committee, 2000): 'The quantitative or semi-quantitative estimate, including attendant uncertainties, of the probability of occurrence and severity of adverse effect(s)/event(s) in a given population under defined conditions based on hazard identification, hazard characterisation and exposure assessment'.

The risk assessment process for ambient air pollutants varies from that for general chemicals in two main aspects: (1) toxicological information usually plays a confirmatory or explanatory role for air pollutants, in that most of the critical hazard information stems from epidemiology, in contrast to the situation with general chemicals where toxicological information may play a major, or even exclusive, role in hazard identification and hazard characterisation; (2) in the air pollution field, adverse health effects may be noted at or close to current exposures, whereas in the general chemical field it may be possible to experience large enough safety margins between exposures and effects levels. Thus, for some air pollutants such as PM, it will not be possible to assign numerical guidance values, but only possible to describe dose-response functions for the associated health outcomes.

There are many difficulties in making detailed and accurate assessments of risk, as there may be uncertainty both in probability of an event occurring and in the scale and nature of its consequences (EEA and WHO, 2002). These uncertainties can arise from a variety of factors (modified from WHO Europe, 1999), such as:

- lack of (appropriate) exposure-toxicity data
- complexity of human-environment interactions
- separation of cause and effect over space and time
- cumulative and enhanced toxic effects of pollutant mixtures
- variation in susceptibilities among populations due to genetic, social or environmental factors

2 PARTICULATE MATTER (PM)

2.1 KEY POINTS

- Upon inhalation, coarse, fine and ultrafine PM is deposited on the walls of airways and lungs. Coarse particles tend to deposit higher up in the airways whereas fine particles tend to deposit in the lower airways and lungs. How such deposition, and thus how specific sizes of PM, relates to the health effects associated with PM exposure remains yet unclear.
- Toxicological studies in humans and laboratory animals suggest that PM pollutants can attack the functioning of the lung, the blood vessels and the heart.
- There is no single 'magic bullet' suggesting that only particles of a certain size or chemical composition are responsible for the adverse effects of PM. However, the amount of knowledge on how particle size, surface property or chemical composition is related to toxicity is rapidly growing.
- Coarse and fine PM fractions both seem capable of inducing toxicity. Whether the ultrafine PM fraction, tested at near-ambient levels, is also toxic, remains more uncertain. There are preliminary suggestions that primary, carbonaceous PM components may be more important for biomedical effects than secondary components like sulphates and nitrates.
- Much of the toxicological evidence indicating the potential of PM to induce toxicity arises from in vitro studies using cell systems or from direct high dose administration of PM into airway and lungs. The few inhalation studies available from laboratory animals and human volunteers indicate that different types of PM may induce toxicity at relatively high levels.

2.2 INTRODUCTION

Particulate matter (PM) in ambient air is a complex mixture of multiple components ranging from a few nanometres in size to tens of micrometres. PM develops dynamically as a reactive system in time and space, depending both on sources and weather conditions.

Primary particles may originate from a multitude of natural and anthropogenic sources. Secondary particles derive from both the gaseous phase and from interactions with primary particles in a complex manner. It needs to be emphasised that among the multiple components present in ambient PM, many toxic substances are found. However, none of these reach concentrations of toxicological relevance based on experience from occupational settings.

Hence, adverse health effects are unlikely to be related to a single PM component, but more likely to a complex, eventually synergistic interaction of multiple components with the respiratory tract and subsequent target organs.

2.3 PM LEVELS AND LOCALISATION IN THE BODY (DOSIMETRY)

Dosimetry of inhaled particles depends on particle size, but also on anatomical factors, respiration and airway disease as well as species differences. Certain identified differences between species may be used for careful dose extrapolation.

Particle measures

In the past, the most common measurement used has been 'particle mass concentration', and daily averages range nowadays from 20-50 µg/m^3 in European cities. Taking the PM size range over four decades into account, this mass concentration measure overestimates large PM in the coarse fraction and basically neglects ultrafine particles < 100 nm in size. The limitation of this metric may be illustrated by the fact that the water-solubility of ambient PM may vary from 20-80 % of PM mass, and yet the toxicity of soluble compounds is unlikely to be similar to that of the insoluble fraction.

With closer insights into particle-lung interactions, other measures such as the concentration of particle number and surface area need to be taken into account, depending on whether ultrafine or larger particles are to be considered. However, exposure measures may be inadequate, since it may be that the number of deposited particles per unit surface area of airways, bifurcations (crest at airway division) and alveoli, or dose to a specific cell such as macrophages, determine the response for specific regions. Therefore, the use of a metric depends on the specific questions posed, requiring specifically defined measures.

Fate of PM in the respiratory system

Particle deposition in the respiratory tract is determined predominantly by three mechanisms that move particles out of the streams of inhaled and exhaled air towards the airway walls, where they are deposited. These mechanisms are: (1) sedimentation by gravitational forces acting on particles > 0.5 µm aerodynamic diameter; (2) impaction caused by their inertial mass in branching airways acting on particles > 1.5 µm (aerodynamic diameter; and (3) diffusional motion of particles < 0.5 µm (thermodynamic diameter) by thermal motion of air molecules.

These mechanisms of action affecting particle deposition work through three important components (aerosol properties and physiology) during breathing: (a) particle dynamics including the size and shape and its possible dynamic change during breathing; (b) geometry of the branching airways and the alveolar structures; and (c) breathing pattern determining the airflow velocity and the residence time in the respiratory tract, including breathing through the nose in comparison to breathing through the mouth.

The human respiratory tract can be considered as a series of filters starting with the nose or mouth, via the various diameters of airways to the alveoli. Figure 3 shows that particles of different sizes deposit differently in the larger and smaller airways, as well as the alveolar region. This means that the toxicity of particles of different sizes can have dissimilar effects, according to the region of the lung involved. This may be particularly important in children with developing lungs and in individuals with asthma, since asthma mainly affects the larger airways, or in COPD (Chronic Obstructive Pulmonary Disease) which affects small peripheral airways and alveoli. These diseases can also cause a several fold increase in deposition of PM in diseased parts of the lung, which may seriously affect an individual's lung capacity.

Even though the fraction of fine PM is small in airways, note that the PM density per airway surface area may often exceed that of the gas exchange region, because of its 100-fold larger surface (adult lungs ~100 m²) when compared to that of airways. In general, particles < 1µm are well able to reach the fragile

structures of alveoli, and their deposition in the alveoli increases with decreasing diameter until they reach a size of 20 nm. The very small particles are likely to be particularly important for heart and blood vessel effects, as they may be able to penetrate the lung epithelial membrane and enter into the blood vessels.

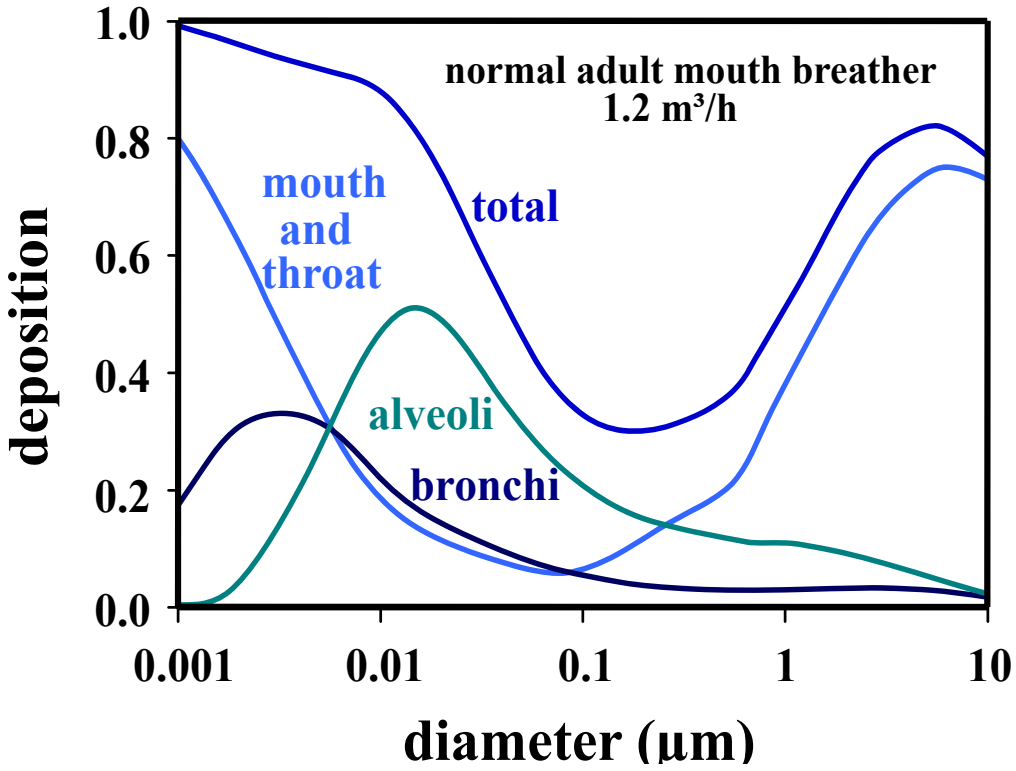


Figure 3. *Deposition probabilities of inhaled particles in the various regions mouth and throat, bronchial tree and alveoli of the respiratory tract. Adopted from International Commission for Radiological Protection, ICRP (1994).*

Fate of PM in the lungs

On the walls (epithelium) of the respiratory tract, particles first come into contact with the mucous or serous lining fluid and its surfactant layer on top. Therefore, the fate of particle compounds that are soluble in this lining fluid needs to be distinguished from that of slower-dissolving or even insoluble compounds.

Soluble particle compounds will be dissolved and often metabolised in the lining fluid, and will eventually be transferred to the blood, undergoing further metabolism. In this way they have the potential to reach any organ and to produce toxic effects far from their site of entry into the lungs.

Slower-dissolving and insoluble particles deposited on the airway wall will be mostly moved by action of ciliated cells with the mucus or by cough within 1-2 days to the throat (pharynx), where they are swallowed.

Slower-dissolving and insoluble particles deposited in the alveolar region will be taken up and digested by specialised defence cells in the alveoli called macrophages within a few hours after deposition - at least under physiological conditions in healthy lungs. Therefore, alveolar macrophages will determine the fate of these particles. However, macrophage-mediated particle removal may be impaired especially in children and elderly and people with diseased lungs. Additionally, macrophages are less able to take up ultrafine particles (< 100 nm) even in healthy lungs.

Note: Cells and solutes of body fluids like proteins interacting with an insoluble particle will not recognise what is inside the particle, but will only react with the molecules according to their structure at the particle surface. In other words, the vast amount of a reactive molecular species located only of the surface of insoluble particles and of core particles (remaining after dissolution of the soluble components) may be the ultimate metric determining adverse outcomes, although this molecule may only add a small fraction to PM mass.

Macrophage-mediated particle transport is directed:

ⓐ towards ciliated airways on the epithelium for further removal by ciliary action, passage through the gut and excretion (about a third of deposited insoluble particles).

ⓑ storage on the epithelium or uptake into the lining cells (together with (c) more than half of deposited insoluble particles).

ⓒ across the lining cells towards the spaces between underlying cells (together with (b) more than half of deposited insoluble particles).

ⓓ across the lining cells towards the lymphatic drainage system (between 1-10% of deposited insoluble particles).

ⓔ across the lining cells eventually into the blood vessels towards secondary target organs. There is evidence for ultrafine particle uptake into the blood circulation depending on the physical structure and chemical composition of their surface. Fractions and rates of uptake are currently under debate. Identified secondary target organs are: liver, spleen, kidneys, heart, brain and nerves.

Ultrafine particles are less effectively taken up by macrophages, but interact to a greater extent with lining cells than large PM and have, due to their vast numbers, a very large combined surface area, which is the interface by which they interact with cells. As mentioned above depending on the molecular surface composition, these particles may have a greater capacity to induce or mediate more adverse effects than larger particles, not only in the respiratory system, but also in the heart and blood vessels, the central nervous system and the immune system.

Do man and animals get the same PM dosages and distribution in the lungs?

The probability of any biological effect occurring in humans or animals depends on deposition and retention of particles, their inherent hazardous properties, as well as the underlying tissue sensitivity. Extrapolation of airway and lung dosages between species must consider these differences in evaluating dose-response relationships. Thus, extrapolation of deposition patterns from most healthy animal models can be performed, since the differences in anatomy and breathing conditions are widely known.

However, subsequent particle retention, redistribution within the lungs and clearance pathways towards other organs and out of the body are based on rather complex mechanisms and differ consistently between rodent models and man. As a result, extrapolation will only be possible under limited specific conditions. And yet, extrapolation from tests on dogs and monkeys is possible due to the similarities of the underlying mechanisms to human ones. Note, however, that studies on these species are less well accepted by the public. This is further discussed in section 2.5: Animal studies.

2.4 EXPERIMENTAL STUDIES IN HUMANS

Studies in which volunteers have been exposed to particulate matter including diesel exhaust particles have demonstrated airway effects and changes in the heart and blood vessel system that link to the adverse health effects found in epidemiological studies. They also provide mechanistic and other toxicological information that helps explain the adverse health effects of air pollutants. Clinical observational or intervention studies can provide additional long-term information.

Figure 4. *Exposure chamber for controlled exposure with diluted diesel exhaust.*

The adverse health effects shown in epidemiological studies have inspired scientists to use different techniques to study the toxicological mechanisms, which form the biological background for the adverse health effects associated with particulate pollution. The development of an exposure chamber set up for diesel engine exhaust was introduced as an exposure model for how traffic-related pollution may interfere with biological systems in humans. In studies investigating high kerb-side concentrations of diesel exhaust with 100-300 µg PM_{10}/m^3, the sampling of cells, fluid and small tissue specimens in the lower airways from humans, with the aid of so-called bronchoscopes, demonstrated that diesel exhaust can induce considerable airway and systemic inflammatory responses.

Both healthy and asthmatic subjects experienced a narrowing of their airways after diesel exposure, but the acute response was usually without symptoms and not in a magnitude to make the asthma worse. More importantly, asthmatics demonstrated a doubling of the propensity of their airways to narrow when challenged with an airway irritant, after diesel exhaust. This tendency in asthmatic individuals to have very responsive airways that constrict and give breathing difficulties and discomfort is commonly named bronchial hyper-responsiveness, and is a key feature in this disease. Therefore, this finding may have a relationship to the increase in asthma exacerbation and the need for asthmatic subjects of emergency-room treatment during high air pollution episodes. Additionally, some differences in airway inflammatory response have been seen between asthmatic and healthy subjects, which could also potentially relate to an increased tendency to worsening of asthma during high air pollution levels.

There is further evidence that asthmatics are sensitive to traffic-related air pollution. This stems from a study in which 20 allergic asthmatics were exposed to the air in a busy city road tunnel during 20 minutes, as compared with a day when they were breathing clean air. After the road-tunnel exposure, the asthmatics were much more sensitive when inhaling an allergen to which they were known to be sensitive. The road tunnel exposure resulted in more airway narrowing, reduced lung function and more asthma symptoms. Some experts have suggested that the enhancing effect of the tunnel air pollution on the asthmatics response to allergen was causally related to the NO_2 and PM content in the tunnel, but it was noted also that other combustion gases and particles from various origins were present. Asthmatics exposed to NO_2 levels > 300 µg/m^3 or $PM_{2.5}$ > 100 µg/m^3 tended to respond more.

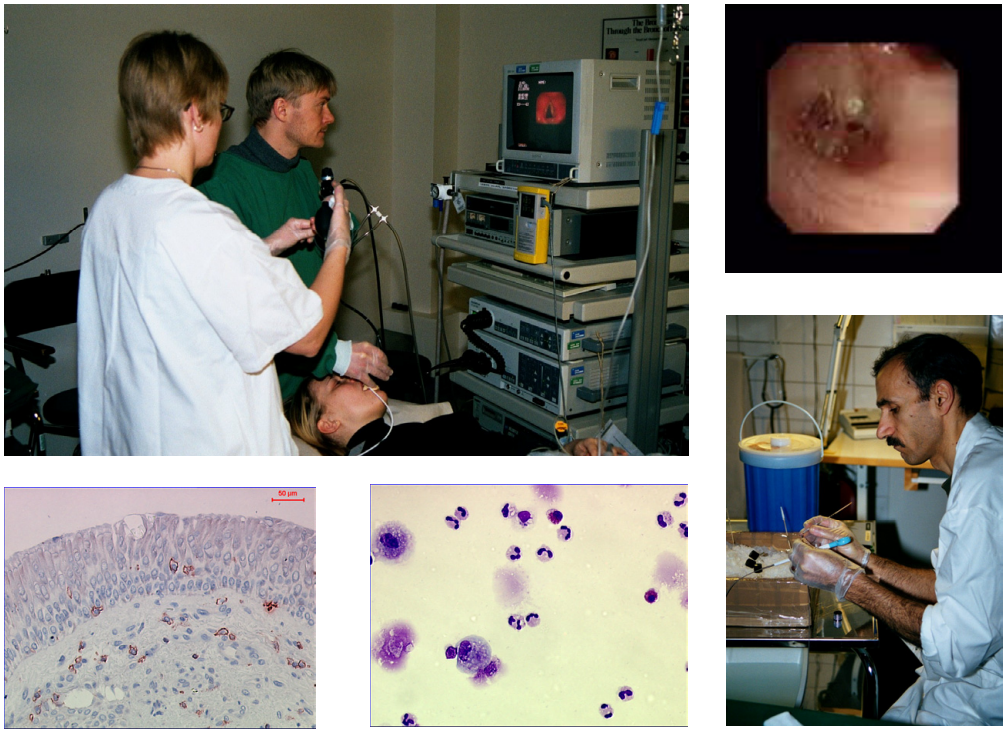


Figure 5. *Clock-wise from upper left: 1/ Bronchoscopy with a thin tube-like instrument with a video camera in the tip allowing for visual inspection, and an instrument channel for lung cell sampling in a subject anaesthetised in the throat and airways. 2/ View from an airway. 3/ Technician working with lung cells. 4/ Macrophages and other inflammatory cells sampled from air sacs and airways. 5/ Microscopy picture from a section through a small airway wall sample. The lining cells can be seen in the middle as a darker band of cells, with air on top and deeper tissue downwards. Inflammatory cells marked with darker circular colour.*

In a novel approach to studying the effects of particulate matter pollution, a particle concentrator is used to manage ambient air in such a way that the particle concentration is multiplied many times. The air is then fed in to an experimental exposure chamber where exposures of human subjects can be performed. The few studies published have all been since the year 2000. These pilot investigations performed in the US have demonstrated that concentrated ambient particles at an average of 200 $\mu g/m^3$ in healthy subjects induced a mild airway inflammation, but also an increase in a blood clotting protein called fibrinogen, which could be related to an enhanced tendency in the blood towards clotting.

In a study with healthy and asthmatic subjects, exposed to an average concentration of ambient particles of 170 $\mu g/m^3$, minor indications of inflammation were seen as well as indications of irritant responses in the airway lining cells. The latter was demonstrated by increased numbers of airway mucosal cells in sputum, the day after exposure. Recordings of electrocardiograms (ECG) indicated minor changes in the neural balance that regulates the heart rhythm. The changes were small and the relevance is difficult to interpret, but could potentially be of importance in subjects with heart illness.

A complementary study has been done with a combination of concentrated ambient particles together with Ozone. The investigators observed that in healthy subjects the major artery in the arm tended to constrict more after concentrated ambient particles and Ozone, than after filtered air. If this occurred in the heart, it could lead to increased heart symptoms in subjects with hardened and narrow vessels. All these early studies were carried out with healthy subjects, and it has not yet been established whether these findings are relevant for actual heart and blood vessel disease.

Consequently, different models have been used to evaluate experimentally what potential health effects could be associated with ambient air pollution. The material is relatively limited, and especially as regards the use of the new particle concentrators, the research is not yet conclusive. Some data supports the hypothesis that particles and co-pollutants can generate an inflammatory response both in the airways and the heart and blood vessel system. Some of these effects could be associated with increased narrowing and clotting in blood vessels, which could relate to the findings from epidemiological studies. Asthmatic subjects may appear more sensitive than healthy individuals, which is coherent with epidemiological studies.

Recent data on genetic variants in certain proteins (with antioxidant activity) that protect against reactive oxygen molecules, such glutathione-S-transferase M1 (GSTM1), indicate genetic factors to be highly important in determining sensitivity for PM responsiveness. A genetic variant of GSTM1 was found to be associated with an increased tendency for allergic airway responses. Additionally, children in Mexico City with a less functional variant of this protective enzyme were shown to benefit from intervention with antioxidants.

What is PAMCHAR?

Chemical and Biological Character of Ambient Air Coarse, Fine and Ultrafine Particles for Human Health Risk Assessment in Europe

International EU-funded project performing chemical and biological characterisation of different sizes of ambient air particles sampled in six European cities, for human health assessment.

- Aims at identifying causative physicochemical characteristics and chemical constituents of ambient air PM10 for cytotoxic, proinflammatory and genotoxic responses relevant to human cardiorespiratory health

For more information please visit the project website: http://www.pamchar.org/

2.5 ANIMAL STUDIES

Apart from crustal material (particulate matter resulting from erosion, like grains of sand) and secondary inorganic PM, the toxicological database on PM health effects is not yet able to provide definite answers regarding causal factors, whether relating to physical parameters (surface area, charge, radiation), chemical components (e.g. transition metals, organic substances) or biological aspects (viruses, moulds, spores, bacteria or products of bacteria such as endotoxins). Nevertheless, clear differences have been identified as to induction of airway inflammatory response, heart/blood vessel and allergen adjuvant effects, and between ambient PM samples from different locations. Current research is exploring the explanations for these differences.

Experimental animal studies investigating toxicity of PM are often driven by hypotheses on specific fractions of PM_{10}, acidic aerosols, ultrafine particles, organic fractions, mixtures of particles and gaseous compounds, transition metals and particle charge. Often, a single constituent is tested, while the ambient PM is a complex mixture which may have undergone different atmospheric reactions or can result in interactive effects.

In most studies, normal healthy animals have been used. During the last years however, animal models that resemble human diseases have gained considerable attention as tools for understanding how air pollution may affect the diseased and susceptible individual. These animal studies have compared responses in ageing rodents with younger ones, and also evaluated models of asthma, COPD (Chronic Obstructive Pulmonary Disease), allergy and lung infections. There is now some support for the assumption that the presence of disease increases susceptibility to PM pollutants.

Recently, animal studies have been introduced that try to explain epidemiological observations in human beings of increased heart and blood vessel problems, and even death, following peaks of PM pollution. The corresponding effects in animals are dependent on the type of PM, with for example Mount St. Helens volcanic ash being ineffective but residual oil fly ash and diesel particles being more potent. Dysfunction of vessel cells, increased plasma viscosity and vessel obstruction have all been put forward to explain clogging and obstruction in blood vessels leading to oxygen deprivation in the heart, brain or other sensitive organs. PM effects are likely to be mediated via both peripheral nerves and the central nervous system. Additionally, the inhalation of PM into the lung, resulting in widespread inflammation in the body, appears of importance.

One way of establishing the relative potency of PM is to use surrogate PM and collected PM (fraction) from ambient air sampled at different geographical areas and emission sources, and to prepare suspensions for application in the nose or the windpipe. This technique is useful for sorting out the dose-effect relationship (or relative toxicity) of PM. However, the manipulation of PM in such studies also directly changes the original particle form and size distribution. Also, the method of administration of the collected PM is quite different (hence, changing also the dose) from the typical human exposure method during normal breathing, and should at least be considered as a very short-term high exposure.

What is RAIAP?

Respiratory Allergy and Inflammation due to Ambient Particles

- International EU-funded research project on particulate matter
- Aims to assess the role of ambient particles in causing local inflammation in the respiratory tract and inducing respiratory allergies

For more information, please visit the project website: http://www.raiap.org/

Nevertheless, these studies have proven to be useful in understanding the contributing factors and confirming some of the epidemiological findings. It should also be noted that a substantial part of PM is soluble in aquous media including the fluid that covers the surface of the lung. For instance, observed adverse effects could be correlated with the contents of soluble transition metals and endotoxins. In addition, recent studies under the EU Fifth Framework Programme indicate that both coarse and fine PM will induce inflammatory responses in the lungs of rodents. Furthermore, allergy responses can be enhanced by co-exposure to an allergen and collected PM. It should be noted that urban dust samples generally show more health effects than rurally-collected dusts, with some evidence for a traffic-sourced contribution. RAIAP, HEPMEAP and PAMCHAR are three representative examples of EU projects that focus on this method of comparing the hazard potencies of Europe-wide collected PM fractions.

In general, inflammatory responses in the lung have been noted with chemically diverse PM mixtures. Since these effects have also been observed with particles of a presumed low intrinsic toxicity such as carbon black or metal oxides, other metrics such as surface area may also play an important role in the induction and development of adverse health effects.

Inhalation of particles in animals is a more normal research model, but is also often more demanding of resources. This approach can be carried out with for example resuspended ambient PM, surrogate particles like factory-produced carbon particles of different sizes, and exhaust from diesel engines. Relatively few long-term studies have been performed, due to a limitation of resources, so data is lacking for comparison with long-term exposure in diseased or otherwise vulnerable citizens. Exposure doses have often been very high, resulting in total overload of the lung cell system developed for clearing inhaled dust, which has limited applicability for human situations.

What is HEPMEAP?

Health Effects from Motor Engine Exhaust and Ambient Air Pollution

- International EU-funded research project on particulate matter
- Aims to improve the understanding of the adverse effects of ambient air particles and motor engine-generated particles

For more information, please visit the project website: http://www.hepmeap.org/

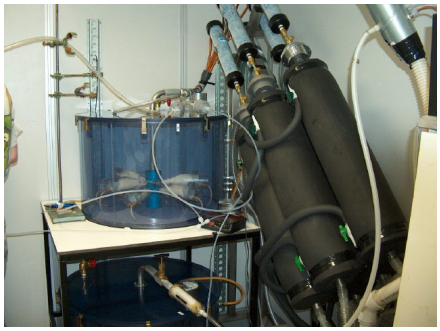
As mentioned in the human particle exposure section (p.14), concentrated ambient particle (CAP) exposures have also been carried out for animals. Specially-developed concentrator technologies have allowed an increase in PM concentration of up to 80 times normal exposure levels, reaching 3,000 µg/m^3. Specific size ranges (coarse, fine, ultra fine) can be selected.

These CAP concentrators more or less guarantee that both laboratory animals and human volunteers are exposed to the actual ambient PM mix, but in a more concentrated form so that health effects may be easier to detect. Since the PM composition and concentration varies from day to day, but also between the different locations at which the studies are performed, these studies need to be undertaken repeatedly to obtain enough statistical weight.

Studies in dogs and rodents with diseases in lung or blood vessels indicate that increased pulmonary inflammation, decreased heart rate (variability) and decreased oxygen supply are among the effects that can be seen in studies using concentrated PM. The implication is that PM may act through a cascade of inflammatory and toxic effects, or by an altered nerve system control of the heart. However, no consistent relation with the mass concentration has been observed, and there seems to be a more complex relationship than just with the mass concentration alone. The complex characterisation of the exposure atmospheres, the day-today variation of ambient PM and the large number of health indicators require sophisticated analysis techniques, which partly explains why only a few studies have been published to date.

Recently, ultrafine particles have emerged as a possible cause of PM-associated health effects. A substantial proportion of the ultrafine particles is emitted by traffic. Toxicological experiments indicate that ultrafine particles could produce serious health effects in laboratory animals. The ultrafine particles are smaller than 0.1 µm in diameter and mainly produced by combustion sources. Ongoing studies in Los Angeles, USA suggest that ultrafine particles on an equal mass basis are more potent compared to fine or coarse mode PM, and that the potency could also be related to distance from the highway. However, studies using factory-produced ultrafine carbon black could not confirm these observations, which suggests an important role for the chemical composition on the surface of PM.

Epidemiological and toxicological studies provide rather paradoxical data on the role of sulphates. While some epidemiological studies suggest associations between sulphate measurements and health effects, neither animal nor human toxicological studies indicate a probability of significant adverse health effects. This data also follows exposures which are orders of magnitude higher than ambient levels in Europe. On the other hand, epidemiology focuses on PM that may be 'marked' by sulphate but may carry other pollutants that co-locate with these sulphate particles (metals, organics, etc.) resulting eventually in interactive reactions of the respiratory system of susceptible individuals. Toxicological studies often use pure sulphates, but can also use concentrated PM that contains sulphates. Again, this may underline the the importance of carrying out toxicological studies with the complete mixture rather than one individual component.



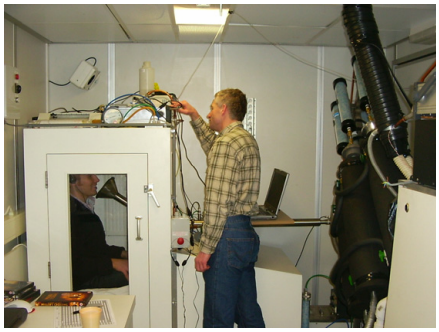


Figure 6. *Exposing animals or volunteers to concentrated PM (<2.5 μm) on site using mobile exposure facilities to get close to the source(s) of interest.*

Real-world exposure to air pollutants rarely involves single pollutants. Instead, it entails a mixture that reflects the integration of many sources, emission constituents or ongoing photochemical processes in the atmosphere. Particles can act as reactants or carriers to deliver toxicants to the deep lung.

Enhanced interactions of pollutant gases and ambient PM have been studied since the early 1950s. Fossil fuel irritants such as sulphur dioxide were shown to interact physico-chemically with soluble metal salts to generate particles intrinsically more toxic than the primary compounds. More recently, sulphur dioxide was shown to react with combustion-associated zinc oxide emission PM in a humidified atmosphere, resulting in acid sulphate that can be carried deep into the lungs of test animals.

Other experimental studies have supported the potential for combined gas-particle interactions such as fine carbon or diesel, acidic, or dispersed ambient particles combined with (in)organic gases or vapours (such as Ozone, nitrogen dioxide, sulphur dioxide, nitric acid, aldehydes). Also, prolonged exposure (four weeks) of rats to a mixture of carbon black, ammonium bisulphate and Ozone showed more inflammatory effects than with the components individually, although there were no indications of increased lung cell dam age or inflammation. Acute exposure to ultrafine and fine carbon black in combination with ammonium nitrate in healthy and compromised rats, however, did not reveal an interactive toxicological response.

Experimental animal studies have shown dose-dependent PM-induced health effects, albeit at concentrations well above ambient exposure and with a threshold. Some studies conclude that focusing not on mass but the (reactive) surface area of PM is a better method of linking health effects with PM. Also, the effects depend very much on the chemical composition of the PM. In toxicological studies (animal studies with PM) the issues of mass, particle number, surface area and durability are all important in clearance mechanisms and health outcomes (various biological endpoints).

2.6 CELLULAR AND MECHANISTIC STUDIES

Cellular models provide important mechanistic information about how various particles and components relevant to air pollution interact with cells and cellular systems. The studies describe inflammatory responses that may be important in the adverse effects seen following increases in exposure to particulate matter. The studies provide support for the theory that very small particles (ultrafine particles) are an especially harmful component, but also suggest that the more coarse and intermediate-sized PM is important. There are questions about the common use of PM mass as a description of the severity of pollution, since PM number, size and chemical composition of the PM mix are clearly important in driving biological endpoints in addition simply to PM mass.

Toxicological studies with PM in vitro

The use of in vitro (cellular and other non-animal) systems is a valuable addition to the repertoire of toxicological models. In vitro research is primarily directed towards studying early events and dissecting cellular and molecular pathways.

Among the cells that respond to particles are those that form the primary line of defence in our respiratory system, the macrophages. These macrophages are important in mobilising the inflammatory and immunological defences if there are too many particles, or if they are harmful 'living particles' as is the case with bacteria. The epithelial barrier cells that line the airspaces of the lung also encounter particles and are important in inflammation and defence, and so are also studied by toxicologists for the effects of particles.

The inflammatory response plays a central role in the adverse effects of PM and in pre-existing airways disease such as asthma and chronic obstructive lung disease, as well as in subjects with heart and circulatory diseases. Therefore toxicologists interested in this aspect study the inflammatory effects of PM. In contrast, toxicologists interested in elaborating the role of PM in cancer study DNA effects such as mutations, DNA breakage and adduct formation (irreversibly bound material).

Toxicological studies in relation to inflammation

Among the PM components thought to be harmful are coarse, fine and very fine particles (ultrafine particles), endotoxins, organics and other chemicals including metals. The metals are often found together in particles from combustion-derived sources such as traffic, and so have become a focus of attention.

Endotoxins (also called lipopolysaccharides, LPS) are fragments from dead bacteria found in biological degradation in nature, and present in the gut of animals and man. PM from ambient air around the world contains endotoxins. Laboratory studies indicate that PM with endotoxin can stimulate different cell types to produce inflammatory signalling substances relevant to asthma and other lung diseases. Endotoxin may also enhance the effects of other particle components, e.g. metals, organics, etc. Although endotoxins are present in particles of different sizes (PM_{10}, $PM_{2.5-10}$ and $PM_{2.5}$), their abundance and capability to stimulate inflammation is more important in the larger particles (PM_{10} and $PM_{2.5-10}$). This may be relevant since those particles will reach and deposit well in airways where disease processes are present in asthma and COPD.

Ultrafine particles (very small particles of mere nanometres in size) are generally derived from combustion sources, primarily traffic, and generate highly reactive 'free radical' molecules that damage and activate lung cells to produce pro-inflammatory signalling substances. The airway lining cells comprise the barrier that normally prevents the entry into the body of particles and bacteria that are inhaled, but they are also actively involved in causing inflammation. Damage or activation of these cells by ultrafine particles can lead to inflammation and also breaching of the barrier and entry of particles into the lung tissue. Particles in the tissue between the cells are unlikely to be cleared and can activate cells leading to inflammation; the particles may also become blood-borne.

If inflammation arises, the inflammatory leukocytes migrate from the blood into the lung tissue and set up an inflammatory reaction. In an individual with asthma or COPD who has low-level inflammation already, this extra inflammation may trigger an attack necessitating hospitalisation and/or increased medication usage.

Ultrafine particles have been shown to activate macrophages, leading to an increase in the intensity of inflammation. However, the coarse and intermediate size particles found in particulate air pollution have also been shown to activate macrophages, leading to inflammation, suggesting that the toxicity associated with pollutant particles is not limited to one size range.

The lung lining cells and the macrophages communicate with each other via proteins. Macrophages that have ingested PM release these mediators, which stimulate lining cells to promote inflammation. Thus, there is complex cross-talk between the important cells and together this acts to enhance the inflammatory effects of particles.

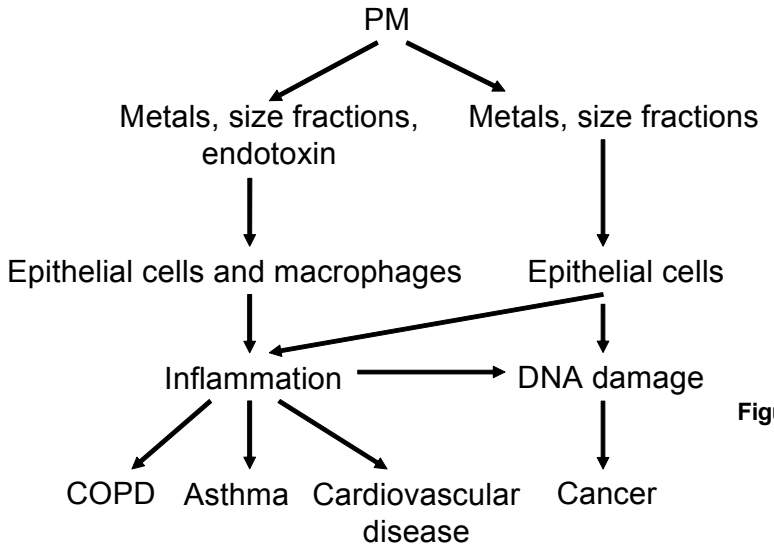


Figure 7. *Summary of the current understanding of the role of target cells in the toxicological effects of PM.*

Oxidative stress is caused by chemical reactions in the body involving the production of harmful oxygen radical molecules, which may damage cells and important molecules. This can be caused directly in the lining fluid in the airways by different particle types, depending on any metals and organic components on their surface. It can also occur after penetration into the lining cells and macrophages in the lungs. The macrophages may have especial difficulty with the very small particles. Figure 7 summarises the current understanding of the role of target cells in the toxicological effects of PM.

2.7 HEALTH IMPLICATIONS

Inhalation of PM exacerbates the symptoms of pre-existing lung disease such as asthma, as well as heart and blood vessel diseases. Toxicological studies show that these effects are brought about through factors such as lung inflammation, heart rate variability, changes in blood viscosity and oxygen deprivation. Analogous effects, albeit at lower intensity, can also be caused in healthy individuals.

There is also evidence linking PM with the induction of lung cancer. The mechanistic basis of PM carcinogenicity is poorly understood. Organic extracts of PM containing well-known carcinogens such as polycyclic aromatic hydrocarbons (PAHs), aqueous extracts containing transition metals (capable of giving rise to highly reactive species which damage DNA) and the core particles themselves may all contribute to this effect, acting individually or through interactions.

The size range of the PM responsible for adverse effects is not known with certainty. While recent evidence incriminates ultrafine particles for the induction of some health effects, especially those related to blood and heart vessel disease, other evidence suggests that larger particles may also have serious adverse effects.

As regards the components of particles which are responsible for causing these effects, it is not possible to identify with certainty any specific sub-group. Current evidence suggests that various particle components, including endotoxins, transition metals and organic substances, as well as the core particles themselves, can all contribute to different toxicological effects. In addition, it seems likely that toxicological enhancements exist between these components, as well as with other components of urban air pollution such as ozone.

Using the mass of PM as a measure of exposure to toxic components is not satisfactory. This is because some components of PM (e.g. some inorganic salts, crustal dust), which contribute significantly to the total mass of airborne PM, appear to have low toxicity. While surface area correlates with toxicity in some cases, overall the existing evidence still does not provide an adequate basis for quantitatively relating the effects of PM to any specific measure of exposure.

2.8 STAKEHOLDER QUESTIONS AND ANSWERS

1. What are the clear causal links between PM air pollution and adverse health effects?

Toxicological studies with PM of different origin demonstrate adverse health effects in the respiratory systems of healthy and asthmatic subjects, as well as heart and blood vessel effects in people with pre-existing organ diseases. The effects have been identified at concentrations comparable to air pollution episodes, but more research is needed into evaluating the lower concentration range.

2. What are the biological processes related to PM air pollution?

The mechanisms for the biological effects of PM include inflammation of the airways. Such inflammation can be induced by oxygen radical formation and antioxidant consumption in the airway fluid layer primarily, but also through interaction with cells in the lungs as well as penetration of PM into deeper tissue. There may also be a gradual spreading to the blood system and distal organs. There are many other reactions related to the highly variable chemical composition of PM, which together results in cascade activation of resident cells and inflammatory cells. Reactions like these are not only localised in the airways, they can also affect the heart and blood vessel system, bone marrow and other organs.

3. Can reduction of emissions and/or ambient concentrations of PM be shown to have a positive impact on public health?

Lower concentrations of PM appear to have less affect on human health. No threshold for effects has been shown with any certainty, although at the individual level such thresholds are biologically plausible. However, thresholds may vary between individuals with different susceptibilities.

4. What about the uncertainty in dose-effect relationships?

Deciding dose-effect relationships with certainty is difficult, due to the lack of experimental studies with PM exposure in human beings.

Toxicological studies in animals and in-vitro have often showed good dose-response relationships. The uncertainty comes from extrapolating these results both from in-vitro and animal models to humans, from higher doses to low doses and from acute studies to long-term effects.

A further source of ambiguity comes from the range of human endpoints being considered. Dissimilar thresholds and dose-response relationships may exist for different endpoints, and between populations with differing susceptibilities.

5. What is the most harmful component of 'fine dust'?

Currently there are a number of candidate chemical and physical parameters related to toxicity. The size of particulates and their surface area are important, as well as any transition metals, organic and other chemical components present on their surface.

6. At what dose and exposure time can PM be harmful, and how high is that dose compared to ambient air pollution levels?

Current human toxicological data suggests potentially adverse effects after exposure to PM at a level of hundred(s) of $\mu g/m^3$. There is not enough data to predict the effects of sub-hundred concentrations. Exposure durations have varied from 30 minutes to two hours, with no data for shorter exposures.

There is no long-term toxicological data available from testing with human beings. Susceptible subgroups should be considered as regards PM effects, as they may potentially respond to far lower levels than the healthy.

7. Is a short-term peak load more harmful than long-term exposure to a lower background concentration?

The current toxicological data rests on peak exposures, and comparisons are yet too scarce for long-term exposure. Peaks can certainly be expected to be harmful.

8. How do you quantify the effects of carcinogenic air pollutants without extrapolating the dose-response function orders of magnitude away from the original data?

The carcinogenicity data is indeed largely based on extrapolations from much higher concentrations than ambient concentrations, which makes the conclusions difficult. Methodological problems restrict the work with low doses.

9. What are the effective health policy options for PM?

With currently available scientific information on particulate matter, it is hard to answer this question. It is not yet clear which PM characteristics (such as size, mass, number concentration or chemical composition) are mainly responsible for causing health complaints. Greater knowledge about the mechanisms behind PM toxicity will help in the development of a more targeted air pollution control (e.g. removal of specific PM

components, instead of reducing the whole mass). Currently a reduction in the total mass of PM is the primary option.

10. Should we assume a threshold for PM when quantifying an effect?

So far there is no support for general thresholds of effects at a population level, but general toxicology understanding makes thresholds for individuals biologically plausible. The genetic variants of protecting enzymes suggest that different subjects have varying sensitivities; differences may also prevail between people with different diseases.

3 OZONE (O_3)

3.1 KEY POINTS

- Ozone (O_3) is the major component of photochemical smog.
- Ambient levels are usually well below 50 µg/m^3. The EU Limit Value of 120 µg/m^3 (8-hour average) is frequently exceeded and in some places during summer, ozone concentrations occasionally reach up to 400 µg/m^3 (highest peak in Europe in 2003: France: 417 µg/m^3).
- Current evidence suggests that effects of ozone may be expected even below the EU Limit Value.
- Ozone penetrates into the deep lung where it may cause damage to the lining fluid and tissue cells by oxidative stress.
- Ozone effects from short-term exposure are reduced pulmonary function, pulmonary inflammation, increased airway permeability, heightened hyper-reactivity at first, then with continuing exposure irreversible structural changes to the airways, possibly leading to reduced function.
- Evidence from the limited number of epidemiological and laboratory animal studies on repeated or long-term exposure to (high) levels of ozone suggests irreversible effects and perhaps increase the risk of developing chronic lung disease such as asthma.
- About 20% of the general population, regardless of airway disease, is more susceptible to ozone's effects. Specific risk groups for ozone include children, the elderly, and people involved in heavy outdoor physical activity. Both increased exposure and biological vulnerability may play a role here.

3.2 INTRODUCTION

Ground-level ozone (O_3) has become one of Europe's most serious air pollutants. Each year, concentrations in ambient air continue to exceed the EU thresholds established to protect human health and the ecosystem.

Ozone concentrations are much higher than a century ago, particularly during exceptionally warm summers as experienced in wide areas of Europe in 2003. Yet EU emissions of nitrogen oxides and non-methane volatile organic compounds (VOCs), the main ozone precursors, were only reduced by about 30 % between 1990 and 2000.

Ozone, nitrogen oxides and volatile organic compounds can be transported over distances of hundreds or even thousands of kilometres. Therefore under the Convention on Long-range Transboundary Air Pollution of the United Nations, countries in Europe and North America have agreed to reduce emissions according to the terms of a number of protocols. A further reduction of about 30 % is foreseen towards 2010 under the National Emission Ceilings Directive.

3.3 OZONE LEVELS AND LOCALISATION IN THE BODY (DOSIMETRY)

Because of its low solubility in water, ozone is carried over in the deep lung. Body response is determined by ozone concentration (peak concentrations in particular), exposure duration and respiratory rate. However, response can also reach a plateau and stop increasing even as ozone exposure keeps rising.

Ozone is such a reactive oxidant that it is believed that little ozone if any reacts directly with the cellular lining of the lung. Ninety percent of inhaled ozone is extracted by the respiratory tract lining layer and only reaction products reach the cells. Because of its very low solubility in water, ozone is carried over in the deep lung with the greatest deposition and the most severe damage where the small airway branches enter the air sacs.

Sophisticated models have therefore been developed which can accurately estimate the ozone dose reaching specific sites in the respiratory tract under different exposure scenarios. These models predict that ozone dose decreases slowly beyond the windpipe and subsequently falls rapidly in the lung region. The dose of ozone delivered at specific sites in the respiratory tract, calculated using such models, has successfully been used to relate to response in inter-species and intra-species comparison studies.

While the time-course of the effects caused by ozone in the respiratory system is complex, the effects of short-term exposure develop over just a few hours. This implies that the effects might be determined by peak, rather than time-averaged concentrations. Further complications can result from the fact that some lung responses to chronic ozone exposure appear to reach a plateau, beyond which they do not increase even if exposure concentration is increased. This implies that quantitative extrapolation of acute responses to longer exposure is not valid.

3.4 EXPERIMENTAL STUDIES IN HUMANS

Experimental studies of ozone effects indicate that asthmatic and healthy subjects differ with regard to inflammatory response to ozone in the lungs, while acute lung function responses do not appear to differ. Instead approximately 20% of the adult population appears to be more prone to airway narrowing, regardless of respiratory health status. New biological indicators of lung function and integrity may be used for monitoring ozone effects.

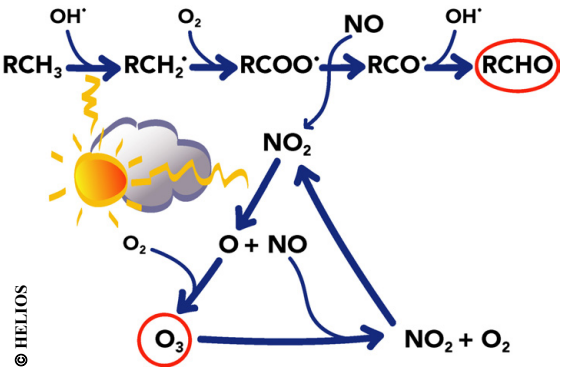


Figure 8. *Ozone formation in photochemical smog.*

The effects of ozone have been investigated in numerous experimental chamber studies. Many studies have been performed in the USA, often investigating higher concentrations of ozone which can be encountered in extreme air pollution situations with stagnant air masses during summertime, in the range of 800-1,200 µg/m^3 and often during heavy exercise.

These studies show that the strong oxidant ozone produces very pronounced inflammatory effects in the airways and that the antioxidant defence system

is highly active and involved in the protective actions. Indeed, giving antioxidant supplements to children with moderate or severe asthma in Mexico, where ozone pollution levels are high, was shown to be beneficial in modulating ozone injury to the small airways.

What is HELIOS?

Biomarkers for the Non-Invasive Assessment of Acute and Chronic Effects of Air Pollutants of the Respiratory Epithelium. Development and Application to Adults and Children along a North-South gradient

- International EU-funded project
- Aims to improve short-term and long-term health risk assessment of air pollutants by using innovative non-invasive techniques for detecting lung inflammation or damage, particularly in children

For more information and highlights of some results download the project brochure at: http://airnet.iras.uu.nl/reports_and_annexes/HELIOS/Heliosfinal.pdf

However, repeated exposure studies to 240 µg/m^3 ozone (6.6 hours/day) indicate that the peak functional response occurs on the first day then progressively declines, whilst response to methacholine (an airway-narrowing drug) challenge peaks on the second day and remains elevated for all five days of exposure. These results suggest that persistent damage to lung cells is cumulative even though functional adaptation takes place.

The pulmonary response to ozone differs markedly between species, but also among individuals. In healthy non-smoking volunteers for instance, the magnitude of changes in pulmonary function elicited by ambient ozone exposure is highly variable, with decrements in rapid exhalation during one second ranging from zero up to 38%.

Unlike with other common air pollutants, asthmatic individuals are not generally more sensitive to ozone than healthy subjects with regard to airway narrowing and increased sensitivity of airways to irritants (bronchial hyper-responsiveness). Instead, several studies show that approximately 20% of the general population, regardless of airway disease, is more prone to respond with a stronger airway narrowing, as defined by a 15-20% reduction in their ability to perform a rapid exhalation in one second. It has also been shown that the inflammatory toxicological response in the airways is not correlated with lung function decrements in the studied populations.

Figure 9. *Measurement of exhaled nitric oxide with continuous ozone monitoring (HELIOS project).*

While asthmatic subjects have not been found to be particularly sensitive to acute worsening of lung function and bronchial responsiveness, bronchoscopy investigations suggest that asthmatics develop an increase in airway inflammation, added to their already established asthmatic inflammation. Asthmatic subjects normally require treatment with drugs relaxing the airways and inhaled so-called corticosteroid medication, to control their airway inflammatory status. More recently, inhaled corticosteroid medication was shown to reduce airway inflammatory response but not lung function changes, in asthmatics exposed to ozone.

With regard to the likelihood of a threshold for ozone-induced inflammation, a recent European study at ambient ozone levels (HELIOS) could not detect any sign of inflammation in exhaled breath in adults and children below 135 µg/m^3 (1 hour) and 110 µg/m^3 (8 hours) of ozone. But ozone levels of 160 µg/m^3 have been shown to be sufficient to initiate a relatively pronounced inflammatory airway response. Studies investigating the outcome of repeated days of exposure suggest that the lung function decline is attenuated after three to four days of repeated exposure, and also that the magnitude of inflammatory response may decrease to some extent. However, there are other continuing adverse effects of repeated exposure, such as an increase in cell damage and cell death in the airways.

Monitoring air pollution effects using non-invasive techniques, such as measurements of exhaled nitric oxide, could be of use in determining any worsening of airway inflammation due to ambient ozone exposure. Although individual variations exist, measurements of certain proteins stemming from the airways, such as the Clara cell protein 16 (CC16) in blood samples, also have a potential as biomarkers of ozone effects.

3.5 ANIMAL STUDIES

There is a wealth of evidence in scientific literature that susceptibility to ozone is, remarkably, not only species-dependent but also strain-dependent, and that resistance to ozone is inherited as a dominant trait. Prenatal exposure to ozone appears to increase sensitivity to allergens and risk of asthma. Susceptibility factors to ozone are cigarette smoke, obesity, diet deficiency in antioxidants, repeated exposure and old age.

A wide range of experiments in different animal species have been conducted to assess the various acute, short-term and chronic effects of ozone exposure, using different doses and studying different end-points. Such effects can include reduced lung volume and diffusing capacity, increased lung permeability, changes in the lung lining cells and influx of inflammatory cells. One interesting feature of earlier inhalation studies in rats was the inverse linear correlation between multiplication of air sac cells and cumulative ozone exposure, indicating thereby an apparent lack of a threshold effect.

There is some evidence that prenatal ozone exposure may induce subtle medium and long-term behavioural deficits in mice, and that ozone inhalation in young rats irreversibly alters lung growth and development. Recent studies in sensitised and non-sensitised infant Rhesus monkeys, believed to be the animal model closest to the human lung, indicate that repeated ozone exposure at levels of the same magnitude as has been found in Mexico City or Los Angeles (1,000 µg/m^3), amplifies the allergic and structural remodelling effects of allergen sensitisation and inhalation. Such repeated exposures can also lead to atypical development of the basement membrane zone in the windpipe.

Long-term changes in lung development include a thickening of the lining of the lung and cell proliferation in lung tissue, but also a disruption of nerve and airway branch formation leading to a loss of airway branches, as well as smooth muscle cell remodelling in sensitised infant Rhesus monkeys. All of these changes can make the exposed animals more sensitive to allergens and increase their risk of suffering from severe asthma attacks.

Interactions between ozone and other air pollutants including nitrogen dioxide indicate enhanced effect on lipid peroxidation and on lung permeability at rest, and even more so during exercise. Similar effects are found with other co-pollutants such as acidic particles (e.g. acid gas-particle air pollutant mixture), and fine particles such as elemental carbon and ammonium bisulphate. Studies on combinations of ozone with

particles, diesel exhaust particles and volatile organic compounds are particularly important research areas in air pollution toxicology. This is because ambient ozone is often associated with particles in urban atmospheres, particularly in the summer or in areas where smog formation is enhanced.

Ozone has been known for over 35 years to increase susceptibility to bacterial infection in mice, even at low urban ozone levels under 200 µg/m^3. However, there are marked inter-species variations, indicating again that caution is needed in extrapolating animal data to humans.

Susceptibility studies with ozone

- *transgenic mice which do not produce CC16 (natural anti-inflammatory factor in airways):*
  - ↑ airways damage by Ozone and high oxygen
  - early appearance of fluid in the lung
  - ↑ mortality
- *cigarette smoke, obesity (hormone leptin ↑ airway inflammation), old age:*
  - ↑ susceptibility (mice)
- *diet: vitamin C and urate in lining fluid: role in adaptive response to Ozone:*
  - vitamin A deficiency: ↑ Ozone-induced lung injury
  - vitamin E: good protection against Ozone damage
  - selenium: age-dependent effects: ↑ antioxidant activity at first, ↓ it in 6-month-old mice
- *previous, repeated or chronic exposure:* shift: acidic mucus secretion + ciliary damage
- *interactions with other air pollutants*

Short-term exposure to ozone has also been shown to increase the effective dose of insoluble particles which may have toxic and/or carcinogenic effects. Asbestos fibres are a good example, as they were found to be three times more numerous in the lungs of rats pre-exposed to up to 500 µg/m^3 ozone than in control rats. This effect, not immediately present post-exposure, suggests an impaired clearance of fibrous tissue-producing and possibly carcinogenic materials, and may be of importance with regard to lung cancer.

Although animal experiments may be limited in relation to human risk assessment, there is no reason to believe that humans are less sensitive to ozone than rats or monkeys. On the contrary, humans inhale a larger dosage of ozone into the deep lung than rats do at the same exposure concentration. And with outdoor exercise during summer months, effective exposure to ozone is even greater. Finally, humans are exposed to ozone in ambient air-pollutant mixtures, which may contribute to an enhancement of characteristic ozone responses.

3.6 CELLULAR AND MECHANISTIC STUDIES

Ozone causes direct oxidative damage to the protective lining fluid and cells of the airways. This induces lung inflammation, increased airway permeability with fluid accumulation, with ensuing decreases in lung function and increased reaction to broncho-active challenges. Depending on ozone concentration, repeated or long-term exposure and individual susceptibility factors, irreversible changes in the airways later take place, including DNA damage.

A variety of whole animal and cellular studies have investigated the sequence of acute, short-term and chronic events that take place in the lung during ozone inhalation. Initially, ozone acts by direct oxidative stress injury to lining cells and to surfactant (the protective lining fluid – see Figure 10). Lung-tissue injury then results from secondary oxidation products of lung lipids and cellular inflammation signalling substances with tissue-damaging, fibrous tissue-producing potential. The classic description of the biphasic lung response to ozone inhalation dates back to only 1991. Acutely, there is lung inflammation, fluid accumulation and influx of macrophages, all of which subside after three weeks of exposure, leading thereafter to multiplication of lung-lining cells, proliferation of fibre-producing cells and accumulation of material between the lung cells (with irreversible thickening of the barrier between oxygen and blood). The final air ducts show first a loss of airway ciliated cells, changes in the Clara cells producing the natural anti-inflammatory protein called CC16, then a stabilisation before structural injury occurs to both ciliated and Clara cells.

Time studies indicate that whereas protein and inflammatory cell influx in the lung return to control levels within a few days of continuous exposure to 800 $\mu g/m^3$ ozone, the macrophage response and structural changes persist or increase during continued exposure. The recruited inflammatory cells were previously thought to release toxic signalling substances increasing lung injury. However, recent comparison studies of ozone-resistant and ozone-susceptible rats suggest that high numbers of blood and tissue inflammatory cells actually confer a certain degree of protection against subsequent damage.

The initial damaging oxidation of lung lipids can be prevented by both vitamin C and E; vitamin C being more effective against nitrogen-dioxide oxidation whilst vitamin E is more effective against Ozone damage. Other natural anti-oxidant protective factors are urate and the Clara cell secretory protein (CC16), a major secretion product of airway Clara cells in humans and animals, which is also a natural inhibitor of the immune system. Increases in the number of Clara cells in distal airways and in intracellular CC16 production also play a major role in adaptation to long-term ozone exposure. Individual differences (genetic, dietary, …) in levels and types of antioxidant factors and enzymes may partly explain the high inter-species and intra-species variations observed.

The mechanism by which ozone damages the lining cell barrier involves both an increased passage through the cell and the disruption of tight junctions existing between them, by direct oxidative damage and by inflammation-stimulating signalling substances. Several mechanisms are then probably involved in the increased flux of proteins across the air/blood barrier.

Acute effects of ozone on the respiratory system

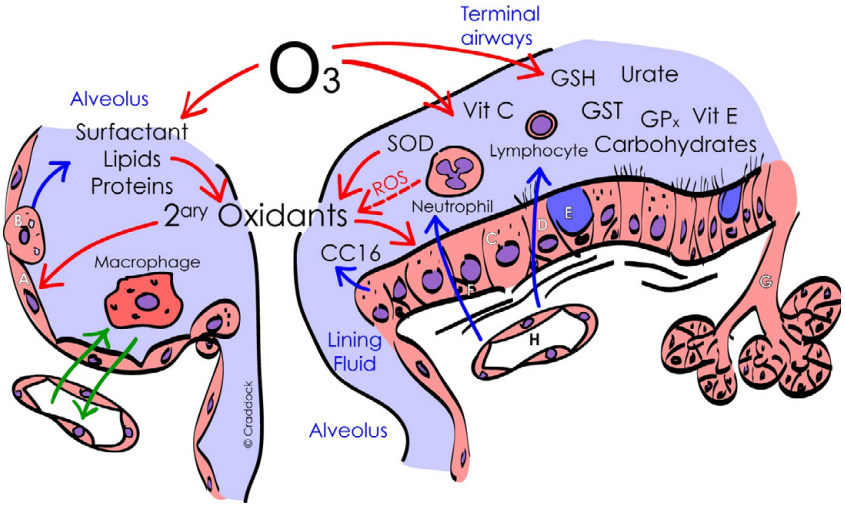
- lung function impairment
- bronchial hyper-reactivity
- an inflammatory reaction:
  - influx of inflammatory cells
  - increased permeability of the air/blood barrier
- can be followed by permanent loss of function due to:
  - tissue damage
  - fibrous tissue formation

There have been conflicting data up to recently on ozone's potential mutagenicity.

A recent French study demonstrated the potent genotoxicity of ozone in human cells, characterising its mutation spectrum occurring in a specific pattern, defining thus the first molecular fingerprints of ozone in human cells. Comparison of DNA damage to human lining cells of the nose between citizens of Florence and Sardinia demonstrated a positive correlation between atmospheric ozone levels and DNA damage. This effect appears to be confirmed when human populations are studied over time.

3.7 HEALTH IMPLICATIONS

Ozone is associated with several adverse health effects. Although there are considerable variations in responses between species and between individuals, acute ozone exposure causes reduced pulmonary function, pulmonary inflammation, increased airway permeability and heightened hyper-reactivity. These effects and ensuing tissue injury in the small airways and the gas exchange region, depending on exposure concentration and duration as well as individual susceptibility, may lead to irreversible changes in the airways and worsen lung disease.



Letter codes:

A: type I pneumocyte (gas exchanges air/blood)

B: type II pneumocyte (produces surfactant and regenerates lining)

C: Clara cell (secretes CC16)

D: ciliated airway cell (brings particles up to the throat/nose)

E: goblet cell producing mucus

F: basal regenerative cell

G: bronchial gland producing proteins and a little mucus

H: blood vessel (gas exchanges in air sacs, cell migration into lining fluid and surrounding tissues)

Figure 10. *Ozone interactions with the terminal airway lining fluid and cells (only 10% of inhaled ozone gets there). The lining fluid contains 12 times more lipids than proteins (over 150 proteins identified to date, some of which are shown here), and acts as first line of defence against oxidative stress caused by ozone. The resulting secondary oxidants then react with the air sac (alveolus) and airway lining cells.*

Some health effects such as lung function decline, and inflammatory reaction first shows an increase followed by attenuation during repeated ozone exposure. This attenuation phenomenon should not be seen as an intrinsic positive response, as it may impair or prevent the normal physiological response needed in order to react effectively to new challenges such as infectious or allergic agents. In addition, evidence of combined and enhancing effects with allergens, viruses and particles has raised concerns that ozone may worsen airway and lung diseases.

Furthermore, ozone-induced morphological, biochemical and inflammatory changes in lung tissue and lower airways do not show this attenuation, and even increase during ongoing exposure. Some acute effects may therefore lead to long-term ozone effects such as the development of structural lung tissue changes, with impaired clearance of fibrous tissue-producing, possibly carcinogenic materials and accelerated decline in lung function.

Data on the long-term health effects of ozone is scarce but striking. Laboratory animal studies show changes in anatomical lung and airway structures and cell types. Studies in infant primates have shown altered lung development possibly leading to the onset of irreversible asthma. Whether similar effects occur in humans is not yet known.

There is ample evidence that ozone exerts its toxicity in a dose-dependent way. An important question remaining to be addressed is whether the above mechanisms are triggered above a specific threshold or not. From a mechanistic point of view, it is conceivable that if the main mechanism of injury is mediated by oxidative stress, there should be a threshold related to the oxidant/anti-oxidant balance either within the lung or at the precise site of ozone toxicity. However, this would imply that the threshold could vary from individual to individual, as suggested by the above-mentioned findings.

Recent results in healthy children (HELIOS project) exposed to various ambient ozone levels provide no evidence of short-term inflammatory effects of ozone below 135 µg/m^3 (1 hour) and 110 µg/m^3 (8 hours), with significant rises in exhaled markers of lung inflammation above those levels. As far as long-term effects are concerned, very few studies have assessed the effects of repeated exposure to ozone at different air concentrations with the objective of determining a possible threshold.

EU Air Quality Guidelines for Ozone (WHO, 2000)

- 8-hour average value: 120 µg/m^3 (not to be exceeded on more than 25 days per year, averaged over a 3-year period)
- 1-hour peak Ozone information threshold: 180 µg/m^3
- 1-hour alert threshold: 240 µg/m^3 (US: 235 µg/m^3)

We can therefore conclude that important health effects linked to acute ozone exposure alone are unlikely below 120 µg/m^3 in healthy individuals. However, repeated exposure particularly combined with other air pollutants may lead to long-term health effects, especially in more susceptible individuals such as asthmatics, old or obese individuals, those with pre-existing lung disease or suffering from antioxidant deficiency.

3.8 STAKEHOLDER QUESTIONS AND ANSWERS

1. What are the clear causal links between air pollution and health?

At ozone concentrations above 120 µg/m^3 a variety of endpoints reflecting airway inflammation or damage have been causally related to the inhaled concentration of ozone in experimental studies conducted in humans or animals.

2. What are the biological processes related to air pollution?

Ozone-induced lung injury is largely the consequence of oxidative damage produced by ozone in the lung with the production of secondary oxidants and pro-inflammatory signalling substances.

3. Can reductions of emissions and/or ambient concentrations of ozone be shown to have positive impacts on public health?

For short-term effects, any reduction of ambient ozone levels is expected to have a positive impact in accordance with the dose-effect/-response curves.

4. What about the uncertainty in dose-effect relationships?

Currently the major uncertainties concern the precise ozone effect threshold, particularly in sensitive populations that for ethical or practical reasons can not be studied, such as infants and young children, the elderly, individuals with severely compromised heart and respiratory systems. Further studies remain to be carried out concerning susceptible populations, genetic, dietary and other unknown causes of inter-individual differences in ozone response, and the interactions between ozone and other air pollutants.

Finally, considerable uncertainty still remains concerning long term exposure to ozone, especially for sensitive groups, taking into account the fact that background levels may continue to rise.

5. At what dose and exposure time can ozone be harmful and how high is that dose compared to ambient air pollution levels?

For short-term effects, early pro-inflammatory changes are likely to occur from an ambient ozone concentration of about 135 µg/m^3 (1 hour) and 120 µg/m^3 (8 hours). This is at levels that are currently at least two to four times above ambient levels in European cities, although higher levels are often observed during summer episodes of photochemical smog, where levels occasionally reach the EU information threshold of 180 µg/m^3 or even the warning threshold of 240 µg/m^3. Current EU regulations have adopted an eight-hour average value of 120 µg/m^3 that is not to be exceeded on more than 25 days per year (averaged over a three-year period). As several countries already have difficulty complying with this threshold, investigations concerning long-term exposure to ozone are crucial.

6. Is a peak load more harmful than long-term exposure to a lower background concentration?

The question is difficult to answer, as it is evident that peaks of ozone produce acute effects in the airways. However, lung inflammation induced by ozone is likely to be more detrimental upon repeated and sustained exposure, even though some adaptative changes occur. This is likely to be more harmful than exposure to lower background levels, provided that the latter remain largely below a certain level such as 100 µg/m^3.

7. Should we assume a threshold for ozone when quantifying an effect?

As far as short-term effects of ozone are concerned, all the toxicological evidence available so far points to the existence of a threshold above which the lung's natural defence mechanisms can no longer cope with the oxidative stress induced by ozone.

4 NITROGEN DIOXIDE (NO_2)

4.1 KEY POINTS

- Nitrogen dioxide levels in ambient air are normally of the order of ten to 50 µg/m^3 (daily average), but may reach some hundred µg/m^3 (1-hour average) in pollution hot spots during high pollution episodes.
- Nitrogen dioxide is a highly reactive, poorly water-soluble gas that reacts with components in the lung lining fluid of the respiratory tract.
- Nitrogen dioxide is a representative indicator of (traffic-related) air pollution, although it is not considered as a major causal factor for inducing health effects alone. Exposure to extremely high levels of the gas, or the mixture it represents, has been shown as able to negatively affect human health.
- For toxicological effects, the level of nitrogen dioxide exposure (inhalation concentration) plays a more important role than the duration (time). Compared to similar mass levels of Ozone, nitrogen dioxide is less potent in inducing toxicity.
- Asthmatic individuals are more sensitive than healthy subjects, reacting to high-episode concentrations of the nitrogen dioxide gas with increased narrowing of airways and responsiveness of the airways to irritants and allergens. Low ambient levels seem to be without an effect.
- Animal studies show an augmented allergy development and exacerbation of allergy sensitivity and infection. Also, changes in lung structure have been observed in long-term exposure studies. These effects were observed at much higher than ambient levels, and interpreting them for the human situation is difficult.

4.2 INTRODUCTION

Nitrogen dioxide (NO_2) is one of the major components of air pollution in densely populated areas. Since the gas acts as an effective indicator of pollution exposure, and has also been shown as able to negatively affect human health, it is important to continue to monitor its ambient levels and to investigate the health effects.

4.3 NITROGEN-DIOXIDE LEVEL AND LOCALISATION IN THE BODY (DOSIMETRY)

Nitrogen dioxide is a highly reactive, poorly water-soluble gas that reacts with components in the lung lining fluid of the respiratory tract. Its effects seem to depend more on the level of exposure than on the duration. Though mathematical models describe correctly the area of strongest effects as the region of the junction between airways and gas exchange zone, these calculations still are not accurate enough to predict effects from the total inhaled dose, or to make quantitative extrapolations from the animal to the human situation.

Nitrogen dioxide is a highly reactive, poorly water-soluble gas. Its biological effects result primarily from its reaction with components (lipids, proteins, etc.) in the cell-lining fluid at various sites of deposition in the respiratory tract. Studies into the dependence of biological responses on the level (concentration) and the duration of exposure show that, for both short-term and long-term exposure, this dependence is complex. The level of exposure plays a more important role than duration, especially under conditions of intermittent exposure. Consequently, it is not possible to predict in a quantitative way the effects of nitrogen dioxide from knowledge of the total inhaled dose alone.

Mathematical modelling of nitrogen-dioxide distribution and absorption in the respiratory tract correctly identifies the predicted region of maximal deposition (i.e. the junction of the conducting airways with the gas-exchange region) as that where maximum structural effects are observed. It also predicts that the amount of nitrogen dioxide reaching the distal airways and air sac space, and therefore the toxic effects in that region, would be substantially increased by exercise. For an impression of the area affected by nitrogen dioxide, see the respiratory tract illustration in Chapter 1. However, there are major uncertainties about the quantitative predictions for the distribution of nitrogen dioxide in the respiratory tract using mathematical modelling, and these predictions cannot be utilised for animal-to-human extrapolations.

High levels of exposure to nitrogen dioxide have been used in animal studies. Such levels are only observed in special outdoor areas such as poorly ventilated road tunnels. However, the exposure time to such levels in tunnels would normally be a matter of minutes. This indicates that the results in animal studies are of limited use, since they demonstrate the potential of nitrogen dioxide to elicit effects, but not the risks when humans are exposed for example in cities with dense traffic.

However, the dosage comparison between rodents and humans is rather difficult given the complexity of the rat nasal passages, which can result in considerably lower doses to sensitive regions in the deep lung of animals compared to similar regions in the human lung. A recent study looked at the effects induced by brief, high-dose exposure of experimental animals. The results suggest that using as measurement the dose per mass of lung, or per area of air sac surface, may predict more accurately the intensity of acute effects than the total inhaled dose per body size.

An additional factor complicates the quantitative prediction of nitrogen-dioxide effects. It is the fact that these effects may be modified by interactions of nitrogen dioxide with other air mixture components, notably Ozone and sulphuric acid.

4.4 EXPERIMENTAL STUDIES IN HUMANS

Experimental studies with nitrogen-dioxide exposure in human beings clearly demonstrate that asthmatic individuals are more sensitive than the healthy. Asthmatics may experience greater airway narrowing and enhanced bronchial hyper-responsiveness to irritants and allergens, which are reactions which relate to increased symptoms and worsening of asthma. The effects have been shown after exposure to levels much higher than in average ambient air, occurring only rarely in very polluted locations. The effects of low ambient levels of nitrogen dioxide have not yet been studied.

Experimental exposure chamber studies with human subjects have been used as a complement to experimental data from animal and cell culture studies. The aim is to study a possible toxicological rationale for the health effects thought to be associated with nitrogen dioxide in epidemiological studies.

These exposure chamber studies have clearly demonstrated that subjects with asthma are far more likely than healthy people to develop lung function impairment or increase in bronchial hyper-responsiveness after nitrogen-dioxide exposure. Asthmatic subjects are more prone than the healthy to experiencing a narrowing of their airways, resulting in breathing difficulties, impaired lung function and physical incapacity. The asthmatic airways are many times more responsive to inhaled irritants and may therefore respond with constriction and shortness of breath after inhalation of extremely low levels of various irritants. This tendency for the airways to be very sensitive is a key feature in asthma.

The tendency in asthmatic subjects to be bronchially hyper-responsive after nitrogen-dioxide exposure was evaluated in a well done so-called meta-analysis, where all published reports were evaluated. The results showed that a statistically significant increase in bronchial hyper-responsiveness in asthmatics occurred after exposure to 180 µg nitrogen dioxide per m^3, whereas healthy subjects required nitrogen-dioxide levels that were ten times higher in order to elicit the same response.

Asthmatic subjects are a very heterogeneous population, and it is clinically well known that bronchial hyper-responsiveness may vary a hundred-fold within this population. Therefore the possibility cannot be excluded that part of the population of asthmatics may well respond to much lower concentrations of nitrogen dioxide than referred to in this report. Subjects with chronic obstructive lung disease, which is a frequently occurring smoking-induced disease in the airways and alveolar sacs, may be generally sensitive to adverse health effects of air pollutants in epidemiological studies. There have been few studies of this population with regard to nitrogen dioxide. It has been demonstrated that asthmatics' lung function may decline after exposure to 540 µg nitrogen dioxide/m^3, but there has been little research into the threshold levels for this population.

Human toxicology studies on inflammatory effects have utilised airway wash-outs and airway lining biopsies to sample fluid cells and tissue from the lower airways. Manifestations of the oxidative potency of nitrogen dioxide have been demonstrated on antioxidant levels, while inflammatory changes have been considerably less pronounced than after ozone and diesel exhaust. Most recently, a shift towards an allergic immune cell response in the cells of the airway wall has been demonstrated in non-allergic subjects after nitrogen-dioxide exposure.

Generally, these effects have only been studied at nitrogen-dioxide levels that are considerably higher than those encountered in traffic situations. These levels, more likely to occur in occupational settings, have started from a thousand µg/m^3 up to several thousand µg/m^3, often with exposures of several hours. Apart from the more general inflammatory responses by nitrogen dioxide, American scientists demonstrated an impaired defence against viruses after exposure to 1,080 µg nitrogen dioxide/m^3. This was demonstrated in a laboratory situation, showing that alveolar macrophages had a suppressed capacity to deactivate viruses in the test tube situation after the subject had inhaled nitrogen dioxide. The finding has yet to be confirmed by other investigators.

A question that has attracted interest is whether nitrogen dioxide could enhance the response to allergens in allergic asthmatic subjects. Several studies have confirmed this reaction. However, the concentrations of nitrogen dioxide were generally higher than those encountered in traffic situations, but did occur in homes with gas stoves. Following studies demonstrating an enhanced allergen response in the airways of asthmatics after exposure to 500-800 µg/m^3 for 30-60 minutes, repeated short 15-minute exposures to 500 µg /m^3 nitrogen dioxide on sequential days have also been suggested as enhancing the allergic

asthmatic response. In a study with asthmatics exposed to traffic-polluted air in a tunnel for 30 minutes, an exacerbated reaction to allergens was found several hours later. Nitrogen dioxide and $PM_{2.5}$ were associated with the effect (see chapter 1). The levels of exposure with effect were above 300 µg/m^3 nitrogen dioxide and above 100 µg/m^3 for $PM_{2.5}$.

4.5 ANIMAL STUDIES

In animal studies nitrogen dioxide elicited inflammatory and allergy-enhancing effects, and exacerbated infection. Long-term exposure studies have also revealed changes in lung structure. High levels of exposure in the animal studies make the extrapolation of the results to human exposure situations difficult.

In animal studies, nitrogen dioxide can be described as having inflammatory effects, allergy enhancing effects, effects on lung infections and other cellular or biochemical changes that may disrupt lung function. Some of the same effects as in humans have been found in animal studies and cellular experiments.

Inflammation is an important factor in the development of many lung diseases. The inflammatory reaction comprises attraction of cells to combat inhaled intruders such as viruses or particles, preceded and maintained by the switching on of a network of signalling substances alerting cellular defence. Oxidative compounds (such as nitrogen dioxide) or an inflammatory reaction that cell-protection systems such as antioxidants are not able to handle, may lead to toxic effects such as cell death, which in turn may contribute to more inflammation. Nitrogen dioxide has been shown to damage type I alveolar lung cells important for the gas exchange in the lung. Compensatory cell growth has also been observed. Nitrogen dioxide (10 to 15 mg/m^3) elicits an inflammatory reaction in the lungs of rats and mice, including the entry of defence cells into the lung and an increase in signalling substances that attract defence cells. In addition, the loss of ciliary function, which aids in the removal of particulate material or dead cells, and expansion of the air sac space, which may result in reduced gas exchange efficiency, has been observed. Some studies indicate that short exposures to high concentrations are more hazardous than longer exposures to lower concentrations.

Certain studies show that exposure to nitrogen dioxide after or before a challenge with an allergen (dust mites or ovalbumin) increases the allergic response measured as antigen-specific antibody levels. A couple of other studies reveal a decreased allergy-related inflammatory cell influx after nitrogen-dioxide exposure. The different findings might be due to differences in nitrogen-dioxide concentrations or time of nitrogen-dioxide exposure.

In general, nitrogen dioxide increases the susceptibility to infection in the lung. The studies in mice discovered impaired intrapulmonary killing of bacteria, decreased resistance to infection with mycoplasma micro-organisms and increased virus replication in the presence of nitrogen dioxide (10 to 15 mg/m^3). However, at lower concentrations some of the nitrogen-dioxide effects were no longer observed.

Nitrogen dioxide is an oxidant and through its mechanism of action it induces oxidative stress and a compensatory response. Lipid peroxidation has been observed after nitrogen-dioxide exposure and short-term treatment depleted antioxidants such as glutathione in the lung (exposure to one or more mg/m^3). The oxidative stress elicited by nitrogen dioxide impairs the function of extra-cellular surfactant, which is an important component of the airway/air sac lining fluid. Components of the surfactant play an important role in the protection of the lung against oxidants, particles and microbes.

4.6 CELLULAR AND MECHANISTIC STUDIES

Nitrogen dioxide exerts its effects through the formation of reactive oxygen structures that can damage or stress cells to release pro-inflammatory signalling substances or disrupt their tissue-defence functions.

The dosage issue for the cellular and mechanistic studies is approximately the same as for animal studies. Although concentrations of some hundred µg/m^3 have been shown to have effects in inhalation studies, 1,000-fold higher concentrations have also been used.

Nitrogen dioxide may induce or modulate an inflammation-stimulating response in airway lining cells or macrophages. Human epithelial lung cells from asthmatics, exposed in cultures, released several different inflammation signalling substances, whereas only one of those substances was found to be released from similar cells in non-asthmatic subjects. This might indicate an increased sensitivity of asthmatics to nitrogen-dioxide exposure. In contrast, non-stimulated human macrophages did not exhibit these increases in signalling substance levels, whereas bacterial toxin (LPS)-stimulated macrophages even showed a reduction in mediator release. Particle-induced signalling substance release from human macrophages was reduced by concomitant exposure to nitrogen dioxide. This result might indicate that agents with similar mechanisms of action do not necessarily lead to enhanced effects.

A reduced cellular response may also have consequences for the organism's reaction to microbial infections. However, apparently contradictory results have been found so far, with nitrogen dioxide exerting an inhibitory effect on signalling substance release in one study and a stimulatory effect in another. The difference in response may well be due to the different viruses used. In a study with human macrophages from smokers and non-smokers nitrogen dioxide did not affect signalling substance release from non-smoker cells, but inhibited the release of all such substances tested from smokers' cells. A similar result was obtained with bacterial toxin treated cells.

Nitrogen dioxide has been found to exert oxidant effects also in cell culture systems. Its effects on surfactant remain unclear in cell-culture experiments. Permeability of the epithelial cells, which indicates cellular damage, increased on exposure to nitrogen dioxide. This increased permeability was more pronounced in human airway lining cells from asthmatic subjects than from healthy subjects.

Exposure to nitrogen dioxide was shown to induce DNA strand breaks in macrophages from mice at high concentrations. Combined exposure to butadiene and nitrogen dioxide resulted in a significant increase in strand breaks. Nitrogen dioxide enhanced carcinogen activation in white blood cells.

The results from animal and cellular experiments alone are not sufficient to point to particular aspects of susceptibility in the population.

4.7 HEALTH IMPLICATIONS

At the kind of nitrogen-dioxide concentrations that can occur in certain pollution hot spots, there is a link between nitrogen-dioxide levels and health effects in asthmatics. Some particularly sensitive individuals within susceptible groups such as asthmatics, and possibly children and patients with chronic obstructive lung disease, may be affected at lower concentrations than found in hot spots.

Controlled human exposure studies show that nitrogen dioxide can elicit effects on asthmatics independently of other pollutants. The concentrations used in these studies (several hundred µg/m^3), rarely occur, even in areas of pollution hot spots during short-term episodes. Higher concentrations may be observed in road tunnels, but the exposure time in tunnels is usually short. Human studies on subjects with mild disease show that nitrogen dioxide can sometimes exacerbate an allergic reaction in allergic asthmatics. Thus, susceptible groups are asthmatics and possibly subjects with chronic obstructive lung disease and children.

Animal studies may indicate a long-term effect from nitrogen dioxide on lung structure and function at much higher than ambient concentrations. No-one yet knows whether these effects may be elicited in humans. Animal studies also indicate an exacerbation of microbial lung infection by high concentrations of nitrogen dioxide. The gas is an oxidant and diminishes antioxidant concentrations. This mechanism also plays an important role for the effects of Ozone and particles, and could indicate possibilities for interaction at the cellular level.

4.8 STAKEHOLDER QUESTIONS AND ANSWERS

1. What are the clear causal links between air pollution and health?

At the kind of nitrogen-dioxide concentrations that can occur in pollution hot spots, there is a causal link between nitrogen-dioxide levels and health effects in asthmatics. There is no toxicological data on the effects at concentrations generally observed in ambient air. However, susceptible groups might comprise individuals sensitive to lower concentrations than those used in the studies. At very high concentrations, animal studies show long-term effects such as changes in lung structure and increased susceptibility to infection. At lower concentrations these effects were no longer observed in healthy animals.

2. What are the biological processes related to air pollution?

The mechanism through which nitrogen dioxide can affect human health is the formation of reactive oxygen and nitrogen components, and the consumption of protective anti-oxidants. The oxidative activity of nitrogen dioxide can elicit an inflammation response in the lung. A strong enough or sustained inflammation may exacerbate disease.

3. Can reductions of emissions and/or ambient concentrations of nitrogen dioxide be shown to have positive impacts on public health?

Human experimental data indicate that a reduction of high concentration episodes may have a positive impact on the health of certain subgroups in the population. There is presently no toxicological data on the effects of ambient concentrations. Since mildly affected subjects can respond to exposure, it is conceivable that more severely affected people may respond at lower concentrations.

4. What about the uncertainty in dose-effect relationships?

Human experimental studies do not show clear dose-effect relationships in the range of nitrogen-dioxide concentrations that might occur in ambient air.

5. At what dose and exposure time can nitrogen dioxide be harmful and how high is that dose compared to ambient air pollution levels?

Short-term effects in human beings have been measured in the concentration range 300 µg/m^3 to 3,000 µg/m^3 nitrogen dioxide (lowest concentration: road tunnel study with other pollutants present; even

higher concentration have been tested and effects have been demonstrated). Exposure times have varied from a few minutes (highest concentrations) up to an hour. The highest concentrations referred to here may be found in certain longer tunnels.

The concentrations used in a number of other studies, 300 to 500 µg/m^3 nitrogen dioxide, have been measured in pollution hot spot areas, but these are not general, ambient levels (some tens of µg/m^3). Animal studies have used 10 to 15 mg/m^3 in long-term exposure studies. The general population is not usually exposed to such levels. However, doses in the rat are difficult to compare to humans.

6. Is a peak load more harmful than long-term exposure to a lower background concentration?

An effect of peak nitrogen-dioxide concentrations has been documented. Effects of lower, longer-term concentrations have been reported from indoor studies, in which many other air pollution components were present. However, nitrogen dioxide very likely functioned only as an indicator of pollution in the indoor studies. Thus, at present there is insufficient data to answer the question.

7. Should we assume a threshold for nitrogen dioxide when quantifying an effect?

Given the mechanism of nitrogen dioxide mediating its effects through the formation of reactive oxygen and nitrogen components, and given the complex antioxidant defence system in animals and humans, it seems likely that human individuals have some defence against the effects of nitrogen dioxide. This would indicate the existence of a threshold.

5 POLYCYCLIC AROMATIC HYDROCARBONS (PAHS)

5.1 KEY POINTS

- PAHs are carcinogenic air pollutants that are found in ambient air as a mixture of related compounds, present in the vapour phase as well as bound to particulate matter (PM).
- PAHs include some powerful carcinogens, and toxicological evidence indicates that they have the potential to contribute to lung carcinogenesis at ambient air pollution levels (for which also epidemiological evidence exists in relation to long-term ambient PM exposure).
- Recent findings suggest that PAH exposure also may contribute to adverse birth effects as well as cardiovascular and respiratory disease, via inflammation. These are issues that merit further investigation.
- Individual PAHs present in the ambient air mixture have widely varying carcinogenic potencies, with particulate-bound PAHs generally having the highest carcinogenic potency. At the present time, no corresponding distinction can be made regarding inflammatory activity.
- Currently it is not possible to distinguish between the toxic effects of airborne PAHs alone or in combination with particles to which they are bound.
- The carcinogenicity of PAHs is probably mediated by their ability to damage DNA, implying that there is a health concern without any exposure threshold.
- Benzo[a]pyrene, used as a qualitative marker of PAHs, is usually found in urban (background) air at levels usually below 1 ng/m^3, but at hotspots significantly higher levels (up to a few micrograms) may occur. The EU proposed Limit Value for benzo[a]pyrene is 1 ng/m^3.

5.2 INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a large family of related organic compounds, which arise mainly from the incomplete combustion of fuels. They include some powerful animal carcinogens and therefore could well act as carcinogens in humans. Recent evidence suggests that they can also cause inflammation and birth defects, and can give rise to the production of damaging reactive oxygen species.

This chapter presents information on the toxicological properties of PAHs. Because there are no studies in which human beings were deliberately exposed to PAHs, toxicological information on PAHs comes almost exclusively from animal or in vitro studies. The only data of toxicological relevance concerning humans comes from studies on biomarkers measured in humans environmentally exposed to PAHs (studies of molecular epidemiology), and for this reason selected data of this type is also included here.

5.3 PAH LEVELS AND LOCALISATION IN THE BODY (DOSIMETRY)

The quantitative effects of PAHs depend on their rate of liberation from the airborne particles (PMs) to which they are bound, and on their lipophilicity, which affects their local accumulation in the lung. Once free, PAHs cause DNA damage in the form of complexes (DNA adducts), which may serve as a measure of their biologically relevant carcinogenic dose. There is evidence that the dose-response curve for the formation of such adducts is not linear. The quantitative prediction of the effects of PAHs is further complicated by:

ⓐ *the fact that in addition they give rise to reactive oxygen species which cause additional damage to DNA as well as inflammation*

ⓑ *their occurrence in the air as highly complex mixtures which may exhibit interactions in their toxicity.*

Depending on their chemical structure, airborne PAHs are found bound to particles (especially the carcinogenic ones), as well as free in the vapour phase. The bio-availabilty of PAHs bound to airborne particles constitutes a major factor influencing the biological dose acting on the target cells. Furthermore, the high lipophilicity of many PAHs results in their selective retention in the tracheobronchial epithelium, their local metabolism and subsequent delivery of relatively high biological doses, a process which could make a particularly important contribution to the damage to target cells in the lung at ambient levels. The overall consequence of these effects may be deviation from linearity of the dose-response curves.

All the available information regarding PAH dosimetry relates to carcinogenesis. PAHs owe their carcinogenicity to their ability to interact, following metabolism, with DNA and form DNA adducts which play a critical role in the carcinogenic process. Measurements of PAH-DNA adducts underline the extent of metabolism of PAHs to their active species, i.e. they serve as a dosimeter for the critical interaction of PAHs with target tissues. Indeed, a good correlation between the formation of such adducts and carcinogenicity has been observed in some animal studies, and for this reason PAH-DNA adducts have been proposed as a suitable biomarker of internal dose. It appears that the dose-response relationship for adduct formation in experimentally exposed animals as well as in environmentally exposed humans is not linear, and that saturation of adduct formation occurs at high doses.

It is recognised that, in addition to PAH-DNA adducts, damage caused by reactive oxygen species may also contribute to the carcinogenic and inflammatory effects of PAHs. However there are still no examples of where such damage has been used as a dosimeter for PAH exposure.

A major problem with quantitatively predicting the toxic effects of PAHs relates to the fact that they are always found in the environment as mixtures consisting of a large number of related species of varying carcinogenic potencies. Two approaches to the dosimetry of PAH mixtures are commonly employed.

The first uses benzo[a]pyrene (B[a]P), one of the most potently carcinogenic PAHs, as a measure of the dose of the PAH mixture, by assuming that its contribution to the carcinogenic potency of most environmental PAH mixtures is roughly constant. There are some concerns about the appropriateness of this approach. These relate to the variability of the composition of different PAH mixtures, the presence in some of them of high concentrations of low-potency PAHs (e.g. fluoranthene), and the recent discovery of

minor PAH components possessing very high carcinogenic potency (e.g. dibenzo[a,l]pyrene, with a potency 1-2 orders of magnitude higher than B[a]P).

Because of these concerns, a second approach to PAH mixture dosimetry has been proposed. This method uses the sum of the concentrations of different mixture components, weighted for their carcinogenic potency relative to that of B[a]P.

Both of the above approaches assume independence of action for the individual components. However, there is evidence that interactions between mixture components may take place which can result in significant deviation from risk additivity (see the section below on Animal studies).

5.4 EXPERIMENTAL STUDIES IN HUMANS

There are no human clinical studies on inhaled PAH. For this reason, information on the effects of inhaled PAHs on human beings comes only from epidemiological biomarker studies (workplace, urban environment) and concerns carcinogenicity and embryotoxicity. No analogous information referring specifically to induction of inflammation by PAHs is available.

PAHs as a family are carcinogenic in humans, as indicated by the increased risk of lung cancer observed among highly exposed workers (e.g. coke-oven workers). On the other hand, while urban air pollution is also associated with increased risk of lung cancer, the specific role of PAHs in this case is not clear. However, there is information relevant to assessing the biological effects of PAHs in connection with the effects of air pollution on PAH-related biomarkers. Thus, increased levels of various biomarkers (e.g. PAH-DNA adducts, which, as mentioned in section 5.3, may reflect the level of cancer risk) have been found in people exposed to urban air pollution. Furthermore, such biomarkers can correlate with adverse birth outcomes in urban populations, implicating airborne PAHs in the causation of embryotoxicity.

Extensive studies of the kind indicated above have been conducted on populations living in heavily polluted regions, such as certain areas in Poland and the Czech Republic (where ambient air concentrations of B[a]P ranged up to a few tens of ng/m^3). Such studies have also been carried out on populations living in less polluted areas (B[a]P concentrations lower than 5 ng/m^3), but suffering extensive exposure to ambient air pollution because of their occupational activities (e.g. policemen in Genoa, bus drivers and postal workers in Copenhagen).

While the results of these studies are not always consistent, the overall picture that emerges indicates that exposure to PAHs present in polluted urban atmosphere, even at the relatively low concentrations found in cities of Western Europe, results in increased levels of various biomarkers specific for PAHs (e.g. PAH-DNA adducts). Further information on the relationships observed between population exposure to ambient air PAHs, PAH-specific biomarkers and adverse health effects is given in the AIRNET Epidemiology report.

Additional important observations from human biomarker studies suggest the existence of populations with an increased susceptibility to PAHs as a result of their genetic makeup. For example, people who carry a specific form of cytochrome P4501A1, a gene known to play an important role in the metabolism and the biological effects of PAHs, tend to suffer higher levels of DNA damage when exposed to PAHs, and also have higher risks of lung cancer.

5.5 ANIMAL STUDIES

Most animal carcinogenesis studies with PAHs have been conducted using routes of administration other than inhalation. A major problem, on which animal studies may throw some light, relates to the carcinogenic potency of PAH-containing mixtures. Studies with mixtures of PAHs have given contradictory results. Some suggest that mixture potency does not deviate significantly from the sum of the potencies of individual components, while others imply strong synergistic or antagonistic interactions. Furthermore, as far as lung carcinogenicity is concerned, animal studies have not so far clarified conclusively the role of PAHs adsorbed on diesel exhaust particles.

The animal carcinogenicity of PAHs has been extensively studied. Most studies have involved administration of the test agents by routes other than inhalation, the route of interest in terms of airborne PAHs. An old study, which examined animal carcinogenicity by inhalation using pure B[a]P, has provided the quantitative animal potency data which are employed in some risk assessments.

Subsequent research examined inhalation carcinogenesis by mixtures containing PAHs, such as coal tar. Although these studies indicated that the carcinogenicity of such mixtures cannot be simply related to their PAH content, there have been few systematic efforts to examine interaction phenomena in PAH mixtures. The most systematic study of this kind, utilising mixtures with varying content of five selected PAHs, showed that mixture potency for the induction of mouse lung tumours did not deviate more than twofold from the sum of the potencies of the individual components.

The result of this study was surprising in view of the results of other studies which suggest significant synergistic or antagonistic interactions during PAH-mixture carcinogenesis. For example, B[a]P caused six-fold more DNA adducts when it was administered orally to mice together with coal tar than when administered by itself. Yet the skin tumour inducing potencies of B[a]P and dibenzo[a,l]pyrene were substantially lower when they were administered together with coal tar than when they were administered by themselves.

Most of the carcinogenic PAHs present in ambient air are found adsorbed in particulate matter. Hence the interest in the carcinogenicity of ambient-air particulates, and in particular the role of PAHs in such activity. Studies with diesel-exhaust particles show that their lung carcinogenicity in rats after inhalation did not parallel their ability to cause PAH-DNA adducts in the lung, while removal of the PAHs from the particles did not reduce their carcinogenicity. This implies that PAHs might not be important for the experimental carcinogenicity of these particles, which may have caused cancer via inflammation. However, the possibility that PAHs may play a role in particle carcinogenicity is still open, since recent studies suggest that the organic extracts of atmospheric particles, including PAHs, can also play a role in the induction of inflammation (see ‘Cellular and mechanistic aspects’).

5.6 CELLULAR AND MECHANISTIC STUDIES

Mechanistic studies, for example related to the role of specific DNA adducts in the induction of genetic changes and carcinogenesis, can provide tools for the improved understanding of the etiologic role of environmental PAHs in human carcinogenesis. Recent findings that PAHs can play a role in the production of reactive oxygen species and induction of inflammation raise new questions on their carcinogenic mechanism and also on their possible contribution to respiratory and cardiovascular disease.

Following their metabolic activation, PAHs react with different sites on DNA and give rise to different kinds of DNA adducts. Although such adducts are generally accepted to be important in PAH carcinogenesis, their exact role is not well understood. Some light is being shed on this role through comparison of the structures of adducts formed by different PAHs, their effects on the accuracy of DNA replication and the kinds of genetic changes observed in experimental tumours.

When toxicologists have a firmer grasp on the function of specific adducts, they will better understand the role of environmental PAHs in cancer etiology, and will be able to produce improved tools for the quantitative prediction of effects. An example of the potential of such an approach is given by recent studies which show that sites on DNA (e.g. in the p53 tumour suppressor gene) at which B[a]P metabolites bind and cause mutations preferentially, coincide with sites at which similar mutations are observed in lung tumours of tobacco smokers. This implies an etiologic role of B[a]P in smoking-induced lung cancer.

Recent studies suggest that PAHs bound to atmospheric particles, as well as free in the vapour phase, can play a role in the induction of inflammation which can contribute to respiratory and cardiovascular disease, as well as to carcinogenesis, through the production of reactive oxygen species. For example, it has been shown that diesel-exhaust particles, components extracted by organic solvents (which include PAHs), and benzo[a]pyrene alone, mobilise to a comparable degree inflammatory processes and generate reactive oxygen species in treated cells. Yet particles that had been washed to remove their organic components were much less active in this respect. Furthermore, the production of reactive oxygen species by ambient air ultrafine particles was reported to correlate with their PAH content.

These observations imply the existence of novel pathways by which ambient air PAHs might contribute to carcinogenesis, as well as a worsening of respiratory and cardiovascular disease by ambient air particulates.

5.7 HEALTH IMPLICATIONS

Certain PAHs are animal carcinogens and probable human carcinogens also, causing damage to DNA. Hence, a non-thresholded mode of action may be anticipated. Furthermore, recent studies suggest that ambient air PAHs may adversely affect birth outcomes, and may also contribute to respiratory and cardiovascular disease via the induction of inflammation. Biomarker studies suggest that cellular damage of relevance to carcinogenesis is induced by exposure to PAHs in the ambient air at environmentally relevant levels. However, the significance of such damage in terms of cancer risk cannot be evaluated at this stage.

Concern over the health effects of ambient air PAHs has traditionally focused on their potential carcinogenicity. PAHs are genotoxic carcinogens and low-dose exposure to them might be expected to lead to cancer in a non-thresholded manner. Experimental and human field studies utilising biomarkers support the notion that cellular damage related to carcinogenesis may be caused by exposure to PAHs in the ambient atmosphere. However, the significance of such damage in terms of additional cancer risk cannot be easily assessed quantitatively at this stage.

Recent evidence suggesting that PAHs may potentially cause carcinogenic damage, via the induction of inflammation and the generation of reactive oxygen species, introduces new complications to these questions. PAH-mediated inflammation may also be connected with the induction by ambient air pollution of respiratory and cardiovascular disease.

Contemporary biomarker (molecular epidemiology) studies suggest that perinatal exposure to ambient air PAHs may have adverse effects on birth outcomes. Further data is required to support this suggestion, and to provide the quantitative information that would support a risk assessment.

Human beings are exposed to mixtures of PAHs present in the ambient air, free as well as bound to particles. Current approaches to risk assessment of PAH mixtures are based on a number of assumptions, including the assumption that mixture components do not influence each other's activity. Current evidence does not permit an assessment of the degree of uncertainty which these assumptions introduce into risk estimates.

Benzo[a]pyrene is often used as a representative marker compound to express the levels of PAHs in ambient air. It is found in the urban air at concentrations usually in the range 0.1-1 ng/m^3, although in some European cities with relatively high levels of air pollution it is found at higher concentrations. An EU limit value of 1 ng/m^3 for this PAH has been proposed.

5.8 STAKEHOLDER QUESTIONS AND ANSWERS

1. What are the clear causal links between ambient air PAHs and health?

Certain PAHs are animal carcinogens, and also probably cause lung cancer in humans exposed to high (occupational) air concentrations. Although they act via a genotoxic mechanism (i.e. damage DNA), and hence a non-thresholded mode of action may be anticipated, no direct evidence exists that certain PAHs contribute to lung carcinogenesis associated with exposure to urban air pollution.

Furthermore, while carcinogenesis-related cellular damage is caused by exposure to airborne PAHs at environmentally relevant levels, the significance of such damage in terms of cancer risk cannot be evaluated. There is evidence that ambient air PAHs may adversely affect birth outcomes, but the mechanistic basis of this effect is not clear. Finally, there is experimental evidence that PAHs may cause inflammation, implying that they might contribute to the induction of respiratory and cardiovascular disease by air pollution.

2. What are the biological processes related to ambient air PAHs?

The mechanism of PAH carcinogenesis involves induction of DNA damage and mutagenesis, directly as well as via the generation of reactive oxygen species, but it is possible that additional biological pathways may also be important. The production of reactive oxygen species by PAHs may also lead to the induction of inflammation and related diseases.

3. From a toxicological point of view, can reductions of emissions and/or ambient concentrations of PAHs be shown to have positive impacts on public health?

Because PAHs are genotoxic carcinogens, it must be assumed that no carcinogenesis threshold exists. This means that a reduction in airborne PAH levels would result in a reduction of risk.

4. What about the uncertainty in dose-effect relationships?

Existing data on the carcinogenic potency of PAHs concerns workplace exposures to high concentrations of PAH mixtures, while no data exists on PAH carcinogenicity at ambient levels. This introduces substantial uncertainty into dose-response relationships. On the other hand, available dose-response relationships with regard to the induction of DNA damage at ambient levels of exposure, while suggesting a linear relationship, also suffer from significant uncertainty owing to limitations in the assessment of exposure.

5. At what dose and exposure time can PAH be harmful and how high is that dose compared to ambient air pollution levels?

Direct evidence of PAH carcinogenesis in human beings exists only after exposure of many years to occupational levels which exceed ambient air concentrations by orders of magnitude. On the other hand, changes in cellular damage related to carcinogenesis (DNA adducts) have been shown to occur at ambient air levels, and to correlate with levels of recent (few days) exposure. However, that there is not enough data to predict the health consequences of such damage.

6. Is a peak load more harmful than long-term exposure to a lower background concentration?

At the low levels of PAHs found in the ambient air, PAH carcinogenicity depends on long-term exposure. However, peaks may be of importance for inflammation.

7. How do you quantify the effects of PAHs (carcinogenic air pollutants) without extrapolating the dose-response function orders of magnitude away from the original data?

The carcinogenicity data is based on extrapolations from substantially higher (occupational) concentrations, which makes it difficult to draw conclusions. Data on biological indicators, such as DNA adducts, is available at environmental levels, but at present it cannot be used directly for risk estimation.

8. What are health-effective policy options for PAH?

Because there is no evidence to suggest that environmental PAH mixtures arising from different combustion sources have significantly varying carcinogenic potency, the only option appears to be reducing overall emissions regardless of source.

9. Should we assume a threshold for PAH when quantifying an effect?

Because PAHs are genotoxic carcinogens, no threshold can be assumed for carcinogenicity. With respect to inflammation, individual thresholds are biologically plausible.

6 CARBON MONOXIDE (CO)

6.1 KEY POINTS

- The binding of carbon monoxide to haemoglobin in blood is critical to its adverse impact on human health, as this binding reduces the supply of oxygen to peripheral tissue.
- At ambient air concentrations of carbon monoxide, healthy individuals do not experience any adverse health effects.
- In individuals with pre-existing heart disease, the effects of carbon monoxide on heart symptoms may occur at concentrations relevant for hot spots/episodes of ambient carbon-monoxide pollution.

6.2 INTRODUCTION

Carbon monoxide (CO) is formed mainly from the incomplete combustion of fuels, and has traffic-related activities as its main source. Acute carbon-monoxide poisoning is known to induce adverse health effects in human beings and even cause death. However, there is less certainty about the effects of carbon monoxide at ambient air levels.

6.3 CARBON-MONOXIDE LEVELS AND LOCALISATION IN THE BODY (DOSIMETRY)

The binding of carbon monoxide to haemoglobin is critical for its adverse health effects. The level of carboxyhaemoglobin (COHb) varies in the population, depending on smoking habits, environmental exposure and reproductive state (both mother and unborn child).

Carbon monoxide is rather stable in the atmosphere, and the lungs are the only significant route for environmental exposure. Carbon monoxide binds readily with haemoglobin in red blood cells to form carboxyhaemoglobin (COHb), which can be measured in a blood sample. As a biomarker of carbon-monoxide exposure, COHb is specific and closely related to the mechanisms of carbon-monoxide toxicity (see section 6.6).

In healthy subjects, small amounts of carbon monoxide are formed because of natural processes in the body. At rest and without environmental exposure, this results in a COHb level of 0.4-0.7%. The COHb levels in non-smoking general populations are usually higher at 0.5-1.5%, owing to production within the body and environmental exposures.

In certain situations, endogenous carbon-monoxide production may be considerably higher, which increases the risk of enhanced exposures in polluted environments. During pregnancy, increased COHb levels for the mother of 0.7-2.5% have been reported, and the unborn child (foetus) of non-smoking mothers have also shown elevated levels (0.4-2.6%). Smokers have high COHb levels (3-4%), and heavy cigarette smokers may have COHb levels of up to 10%.

After reaching the lungs, carbon monoxide diffuses rapidly across the lining of the air sacs and blood-vessel membranes. It also readily crosses the placenta of pregnant women to the foetus. The uptake and elimination of carbon monoxide have been described in various mathematical models. The most important variables determining the COHb level are carbon-monoxide concentration in inhaled air, duration of exposure and degree of breathing. At a fixed concentration of carbon monoxide, the COHb concentration increases rapidly at the onset of exposure and starts to level off after three hours. The elimination rate is slow and depending on the COHb level, the time to 50 per cent reduction ranges from two to 6.4 hours. Furthermore, the elimination rate of COHb is much slower in the foetus than in the pregnant mother.

In real life situations, prediction of individual COHb levels is difficult because of large spatial and temporal variations in both indoor and outdoor carbon-monoxide concentrations.

6.4 EXPERIMENTAL STUDIES IN HUMANS

The toxic effects of carbon monoxide on humans are due to low supply of oxygen to peripheral organs and tissues. Individuals with pre-existing heart disease and the foetus seem to be most sensitive. The adverse effects of carbon monoxide on heart symptoms may occur at concentrations relevant for hot spots/episodes of ambient pollution.

Effect on the nervous system including behaviour. It is well known that low levels of oxygen in the brain, due to acute carbon-monoxide poisoning, may cause both reversible short-lasting deficits in the nervous system and severe, often delayed, brain damage. At a COHb level of about 10%, only observed after accidental exposure or heavy smoking, carbon monoxide is likely to cause headache.

However, the effect of carbon monoxide on human behaviour may occur at lower levels. Psychomotor effects, such as reduced co-ordination and driving ability, and impaired wakefulness have been revealed at COHb levels as low as 5.1-8.2%. Furthermore, the effects of carbon monoxide on intellectual performance are uncertain at COHb levels of 5-20%. Thus, the effects of carbon monoxide on neurological functions seem to occur at carbon-monoxide concentrations observed after accidental exposure or smoking, whereas ambient carbon-monoxide concentrations are of no or only marginal significance.

Heart effects. Numerous human clinical studies have been conducted, with both healthy subjects and patients with heart disease, in order to characterise the effects of low-level carbon-monoxide exposures on the responses of the heart and lungs to exercise. In apparently healthy subjects, the maximal exercise time and the maximal oxygen consumption have been decreased at COHb levels as low as 5%. Patients with disease, especially ischaemic heart disease (insufficient oxygen supply to the heart and blood vessels) are expected to be particularly sensitive to carbon monoxide. During exercise, these subjects experience heart ischaemia, which can impair heart contractility, affect heart rate and rhythm, and cause angina pectoris (chest pain due to narrowing of the heart vessels). Several studies have suggested that low-level carbon-monoxide exposures resulting in COHb levels of 2.5-3% shorten the time to onset of exercise-induced chest pain in patients with angina pectoris.

The potential effects on heart rate and rhythm associated with low-level carbon-monoxide exposures have not been fully resolved. However, an effect has been reported at 5% COHb, but not at lower levels. In patients with severe ischaemic heart disease, carbon-monoxide poisonings have been lethal at COHb levels of 10-30%, while COHb levels common in lethal poisonings are around 50-60%. In conclusion, the adverse

effects of carbon monoxide on heart symptoms may occur at carbon-monoxide concentrations relevant for the ambient air.

Developmental effects. The pregnant mother, the foetus and the new-born infant may be at risk of adverse health effects from carbon-monoxide exposures. However, the possible health effects of carbon-monoxide concentrations in ambient air are poorly understood. The increased risk in pregnant mothers and foetus can be due both to higher COHb levels and to an enhanced sensitivity of the foetus and the developing organs to carbon monoxide. The developing brain seems to have the highest sensitivity of all organs. A relationship between maternal smoking and low birth weight at foetal COHb levels of 2-10% has been established.

Furthermore, maternal smoking is associated with death around birth and behavioural effects in infants and young children. Carbon monoxide is probably one of the most important aetiological factors for these effects, although there are numerous other hazardous substances in tobacco smoke. Even though it cannot be excluded that carbon monoxide in ambient air may represent a risk to the foetus, carbon monoxide from smoking is unquestionably a much more prominent risk factor.

6.5 ANIMAL STUDIES

Experimental studies in laboratory animals demonstrate that carbon monoxide can induce adverse effects on the heart and blood vessel system. Furthermore, maternal carbon-monoxide exposure also produces reductions in birth weight, enlarged heart size, delays in behavioural development and disruption in intellectual function. However, most of the animal studies, have been conducted at very high levels of carbon monoxide (i.e. levels not found in ambient air).

Heart effects. There is evidence from experimental studies in laboratory animals that carbon monoxide can adversely affect the heart and blood vessel system. Although disturbances in heart rhythm have been seen in healthy and heart-dysfunctional animals, results from these studies are not conclusive. The lowest level at which effects have been observed varies, depending upon the exposure regimen used and the species tested. There is conflicting evidence that carbon-monoxide exposure will enhance development of arteriosclerosis (blood vessel disease with hardening and thickening of arterial walls) in laboratory animals, and most studies show no measurable effects.

Development effects. Studies in several laboratory animal species provide strong evidence that maternal carbon-monoxide exposure of 170-230 mg/m^3, leading to approximately 15-25% COHb, produces reductions in birth weight, enlarged heart size, delays in behavioural development and disruption in brain function. Isolated experiments suggest that some of these effects may be present at concentrations as low as 70 mg carbon monoxide/m^3 inhaled air (approximately 6-11% COHb) maintained during pregnancy.

Cancer. No evidence is available on damaging effects on genes and development of cancer in relation to carbon-monoxide exposure.

6.6 CELLULAR AND MECHANISTIC STUDIES

Carbon monoxide will displace oxygen from haemoglobin and reduce the transport and release of oxygen to the tissues. This seems to be the crucial mechanism for carbon-monoxide-induced toxicity.

Carbon monoxide binds reversibly to a group of iron-containing proteins in the body, named haem proteins. Approximately 80-90% of the absorbed carbon monoxide binds with haemoglobin in red blood cells.

Normally, oxygen is bound to haemoglobin in these cells after uptake in the lungs, and is transported to the different tissues in the body and released for energy-producing processes in the cells. As carbon monoxide has a much higher affinity (200-300 times) for haemoglobin than oxygen, carbon monoxide will displace oxygen from haemoglobin and reduce the transport and release of oxygen to the tissues. The low levels of oxygen (hypoxia) in the tissues, seems to be the determining factor for carbon-monoxide-induced toxicity, both at low concentrations usually found in ambient air and at high concentrations during accidental exposure.

However, other studies show some support for a direct action of carbon monoxide, e.g. on the heart. Carbon monoxide might bind to other types of haeme proteins, but with much lower affinity. Such mechanisms may contribute to carbon monoxide induced toxicity at the high concentrations that only occur during accidental exposure, but not much is known about this.

In mechanistic studies, in which isolated cultured cells have been exposed to high concentrations, carbon monoxide has been reported to exert anti-inflammatory effects, and to counteract cell death. However, these studies are probably of little relevance because of the very high concentrations used.

Recent studies have shown that carbon monoxide formed in the body operates as a signalling substance in different cells. There is increasing evidence that endogenous carbon monoxide plays a physiological role in a number of biological systems. Thus, carbon monoxide has been reported to induce smooth muscle and blood vessel relaxation and to inhibit blood platelet aggregation. These physiological effects of carbon monoxide are presumably not relevant for the toxic effects induced by inhaled carbon monoxide.

6.7 HEALTH IMPLICATIONS

Healthy people do not normally stay in highly polluted areas for sufficiently long periods to attain adverse levels of carbon monoxide bind to haemoglobin (COHb). Individuals with inherent heart disease might experience heart symptoms at carbon-monoxide concentrations that may occur in ambient air. There is little knowledge of the adverse health effects induced by carbon monoxide at ambient air concentrations on foetal development. However, carbon-monoxide exposure due to mothers smoking is much more important.

Although it has been unequivocally shown that acute carbon monoxide poisoning induces adverse health effects and death, it is more uncertain to what extent carbon monoxide at ambient air levels exerts non-lethal health effects. The impact on human health from carbon monoxide exposure in ambient air depends on the concentration, the exposure time, the individual's physical activity and whether sensitive individuals are exposed. Some individuals are sensitive due to a pre-existing illness (e.g. heart disease), leading to adverse responses at lower carbon monoxide concentrations than for healthy subjects. Such risk groups with pre-existing heart diseases have been intensively studied. An increased probability of health effects (heart symptoms such as chest pain) has been observed at ambient or near-ambient carbon monoxide concentrations that result in COHb levels down to 3%.

Normally carbon monoxide concentrations in ambient air do not reach a critical threshold level (giving 2.5% COHb). However, in some exposure situations/ hot spots, such as inside motor vehicles, tunnels and garages, carbon monoxide concentrations are generally higher than normal, and susceptible individuals may acquire COHb levels that could be of concern.

Other risk groups are pregnant women and foetuses who have enhanced COHb levels formed in their bodies. In addition, foetuses and young infants deserve special attention, because their developing brains are especially susceptible to adverse effects. However, due to limited knowledge, it is uncertain whether serious, irreversible, effects in the central nervous system may be elicited at ambient carbon-monoxide concentrations, therefore further research is required.

6.8 STAKEHOLDER QUESTIONS AND ANSWERS

1. What are the clear causal links between air pollution and health?

At high accidental carbon-monoxide concentrations there is a clear causal link between carbon-monoxide levels and health effects. However, at the concentrations generally observed in ambient air, the link between the carbon-monoxide levels and health effects is more uncertain. It may be that only individuals with pre-existing heart disease are at risk.

2. What are the biological processes related to air pollution?

The mechanism for the health effects of carbon monoxide is oxygen deprivation in sensitive tissues (such as brain, heart), due to displacement of oxygen by carbon monoxide from haemoglobin in the red blood cells.

3. Can reductions of emissions and/or ambient concentrations of carbon monoxide be shown to have positive impacts on public health?

In special micro-environments (garages, etc.) with high carbon-monoxide concentrations, reduction of carbon monoxide may reduce the adverse health impact on sensitive individuals. At concentrations normally occurring in ambient air, giving a COHb concentration below the threshold for adverse effects, reductions of carbon monoxide will not have any positive impact.

4. What about the uncertainty in dose-effect relationships?

The exposure dose for carbon monoxide is easy to quantify, due to measurement of COHb (an internal dose indicator). The health effects are, however, more difficult to assess and are based on animal studies and a limited number of experimental studies on human beings. Although the threshold for some of the adverse effects (heart symptoms) are established, others are more uncertain (brain functions and development). Furthermore, the quantitative dose-response relationships are not completely characterised in the different sensitive groups.

5. At what dose and exposure time can carbon monoxide be harmful and how high is that dose compared to ambient air pollution levels?

The concentrations of carbon monoxide that induce adverse health effects will vary with the different health effects. Exercise-induced chest pain in heart disease patients is established as the most sensitive parameter, as effects are observed from 2.5% COHb. To reach this level of COHb, both carbon-monoxide concentration in ambient air and the time of exposure are important. Exposure to approximately 40 mg/m^3 for one hour with moderate to high physical activity, is sufficient to give such levels of COHb. In urban traffic environments of large European cities, the eight-hour average carbon-monoxide concentrations are generally lower than 20 mg/m^3 with short-lasting peaks up to 60 mg/m^3.

6. Is a peak load more harmful than long-term exposure to a lower background concentration?

For carbon monoxide it is necessary to reach a critical adverse level of COHb (>2.5%). This will only be reached during peak episodes of air pollution. The duration of the exposure will also determine the COHb

level. Furthermore, the periods with enhanced COHb levels inducing oxygen deprivation have to be sufficiently long to give adverse effects in sensitive organs. Long-term exposure to lower background concentrations will not reach high enough COHb concentrations to induce any adverse effects. In contrast to carbon-monoxide exposure in ambient air, smokers will more easily reach the critical threshold for adverse health effects.

7. Should we assume a threshold for carbon monoxide when quantifying an effect?

For carbon monoxide the mechanism is clearly known and based on its ability to reduce oxygen supply to sensitive tissues. The tissue will tolerate a certain oxygen deprivation, and thus it is a critical threshold for the carbon-monoxide-induced effect. However, the threshold will vary for different tissues and effects, as well as among individuals (with lower thresholds in sensitive persons). The most critical threshold that has been established is 2.5% COHb (for exercise-induced chest pain in heart patients).

7 SULPHUR DIOXIDE (SO_2)

7.1 KEY POINTS

- The basic respiratory response to inhaled sulphur dioxide (SO_2) is a dose-dependent narrowing of the airways.
- Reactions to sulphur dioxide among healthy individuals are only shown at concentrations much in excess of current ambient levels.
- Asthmatics, however, can be more responsive than healthy subjects, and may possibly react at concentrations pertinent to hot spots, such as those that could occur near certain industrial plants without proper emission controls.

7.2 INTRODUCTION

Sulphur dioxide (SO_2) is a colourless gas with a pungent odour. The main anthropogenic sources are combustion of fossil fuels containing sulphur (mainly coal and heavy oils), and the smelting of ores containing sulphur. Ambient concentration levels have fallen considerably in most parts of Europe in recent decades, and most measurements are in compliance with the air-quality standards, i.e. 24 hour average: 125 µg/m^3, and hourly: 350 µg/m^3.

7.3 SULPHUR-DIOXIDE LEVELS AND LOCALISATION IN THE BODY (DOSIMETRY)

Sulphur dioxide is an upper-respiratory tract irritant. At least 95% of the inhaled sulphur dioxide is absorbed in the nose and throat during resting conditions, while penetration to the proximal airways is greater during mouth breathing and with exercise.

Sulphur dioxide is a highly water-soluble gas and is rapidly absorbed in the moist regions of the upper respiratory tract. Sulphur dioxide is therefore primarily an upper respiratory tract irritant. More than 95% of the inhaled sulphur dioxide is absorbed in the nose and throat during resting conditions, while penetration to the proximal airways (trachea and bronchi) is greater during mouth breathing and with increased physical activity. Changes with exercise are due to shorter residence time of the inhaled air in the upper airways, increased ventilation volume per unit time and a shift from nose breathing to breathing through both the mouth and nose. Very little sulphur dioxide reaches the lungs directly; its transport to the deep lung is generally accomplished primarily by adsorption onto respirable particles.

It is estimated that 12-15% of sulphur dioxide absorbed to the mucus membrane of the airways is eliminated unchanged in the exhaled air. The remaining is converted into ions (bisulphite ion and sulphite ion), which may enter the blood circulation and be distributed throughout the body and excreted, primarily in the urine as sulphate. Inhaled sulphur dioxide can also dissolve in the saliva and be swallowed to enter the gut in the form of sulphurous acid.

7.4 EXPERIMENTAL STUDIES IN HUMANS

The basic airway response to inhaled sulphur dioxide is a mild narrowing of the airways. Asthmatics are more responsive than healthy subjects, but it is not clear that severe asthmatics are more sensitive than mild asthmatics. Patients with chronic obstructive pulmonary disease show responses similar to healthy subjects.

The acute airway narrowing by sulphur dioxide is rapid, dose-dependent, and tends to reach a peak after 5-10 minutes. The response does not increase with prolonged exposures. Spontaneous recovery often occurs within about 30-60 minutes. Controlled human studies show high variability in response among healthy individuals. Since individuals with hay fever and asthma often experience nasal congestion, mouth breathing is practised at a greater frequency in these individuals, perhaps making them more vulnerable to sulphur dioxide. However, sulphur dioxide does not appear to increase airflow resistance in the nose in either asthmatics or individuals with hay fever and would therefore not alter the proportion of mouth versus nose breathing.

It is difficult to draw a consistent picture of exposure-response relationships for sulphur dioxide. Most healthy subjects seem to develop increased airway resistance first at exposure concentrations above 14,000 $\mu g/m^3$, orders of magnitude higher than found in ambient air concentrations. Occasionally, sensitive subjects, reported to be non-asthmatic, have been found to react slightly after exposure to 2,856 $\mu g/m^3$, still well above ambient concentrations. This higher level seems to be a threshold for most healthy individuals if exercise is involved.

When exposed to sulphur dioxide, asthmatics or others with hyper-reactive airways may show a striking acute response, characterised by airway narrowing associated with increased airway resistance and decreased expiratory flow rates, as well as the clinical symptoms of wheezing and shortness of breath. The magnitude of response, however, is quite variable among individual asthmatics. A two-minute exposure to 1,140-2,856 $\mu g/m^3$ elicited airway narrowing in exercising asthmatics within five to 10 minutes. There is also one example of small changes in airway resistance in two sensitive subjects out of six at 286 $\mu g/m^3$.

The potential development of tolerance to the airway effects of sulphur dioxide has been studied in asthmatics and is a recognised event in workers. Repeated exposures of asthmatics to sulphur dioxide may result in a diminished responsiveness. The diminished response is evident in about 30 minutes, but initial responsiveness is restored within six hours after exposure to clean air.

Sulphur dioxide exposure at concentrations well above ambient levels has also been associated with airway inflammation in controlled human studies. Healthy people exposed to 22,848 $\mu g/m^3$ sulphur dioxide for 20 minutes showed elevated numbers of inflammatory cells in airway lavage fluid.

It is not known whether asthmatic children are more sensitive to sulphur dioxide than asthmatic adults, and there is no experimental data to support the theory that asthmatic children do react to current ambient concentrations.

7.5 ANIMAL STUDIES

Dose-related airway narrowing has been demonstrated in guinea pigs and dogs. Animal studies have also shown impaired defence mechanisms and tissue injury in the airways following inhalation exposure to high concentrations of sulphur dioxide.

Prolonged exposure to sulphur dioxide at high concentrations may affect various aspects of removal and defence mechanisms in the airways. Rats exposed to sulphur dioxide for 170 hours and dogs exposed for a year, both to concentrations orders of magnitude higher than ambient levels, exhibited reduced removal of particles and diminished ciliary transport in the windpipe.

Sulphur dioxide is also capable of impairing macrophage-dependent bacterial killing in mouse models, and exposed mice had greater frequency and severity of infections, which was proposed to be associated with this effect. The evidence of effects after extended exposure periods is not clear, however, as some studies showed no effects. Guinea pigs and monkeys showed no effects on lung function or structure after a year of continuous exposure to high concentrations of sulphur dioxide.

Exposure of animals to sulphur dioxide concentrations several orders of magnitude higher that ambient exposures has been used to produce laboratory animal models of bronchitis.

7.6 CELLULAR AND MECHANISTIC STUDIES

Sulphur dioxide is converted into ions in the airway lining fluid that may interact with nerve receptors to cause irritation and airway narrowing.

Sulphur dioxide is converted into ions (bisulphite ion and sulphite ion) after absorption into the surface lining fluid of the airways. When these ions interact with nerve receptors in the airways, primarily in the proximal airways (trachea and bronchi), they may initiate irritation and airway narrowing (bronchoconstriction). The bronchoconstriction is due to contraction of the muscles that surround the large airways, and that normally help maintain airway tone and diameter during expansion and contraction of the lung. These airway muscles may contract during much less provocation in people with asthma than in normal subjects.

7.7 HEALTH IMPLICATIONS

Reactions to sulphur dioxide in healthy individuals are only shown at concentrations markedly in excess of current ambient levels. Asthmatics, however, may possibly react with reversible health effects at concentrations down to the highest ambient levels/ hot spots.

Sulphur dioxide is an upper respiratory tract irritant. Inhalation exposure to sulphur dioxide may cause a decrease in lung function due to airway narrowing. Reactions among healthy individuals are only shown at concentrations markedly in excess of current ambient levels. Asthmatics, however, are shown to be more responsive than healthy subjects, and the most susceptible subgroup of asthmatics may possibly react with acute airway narrowing at concentrations down to the highest ambient levels, e.g. those living near certain industrial plants.

There is no experimental evidence that long-term exposure to ambient concentrations of sulphur dioxide will be harmful, either to healthy individuals or to asthmatics.

The highest measured one-hour average sulphur dioxide concentration in Europe between 1990-1999 was 587 $\mu g/m^3$ and the highest 24-hour average concentration was 327 $\mu g/m^3$. These levels are now declining, and most measurements were found to be in compliance with the air quality standards.

Long-term exposures to sulphur dioxide have recently been associated with excess effects on mortality and morbidity in epidemiological studies. However, current ambient levels of sulphur dioxide are much lower than levels (2 mg/m^3) known to cause reversible lung-function effects in most individuals. Sulphur dioxide is

therefore considered not to be responsible for effects on survival in epidemiological studies, but to act as a surrogate for some other air pollutant, possibly ambient particulate matter.

However, sulphur dioxide may still persist as a potential hazard for reversible health effects in susceptible individuals in some locations in Europe, in particular those near power plants or industrial plants.

7.8 STAKEHOLDER QUESTIONS AND ANSWERS

1. What are the clear causal links between air pollution and health?

A number of laboratory studies in healthy volunteers have associated exposure to sulphur dioxide with acute effects including upper respiratory tract and eye irritation, reduced lung function, and enhanced pulmonary and nasal resistance. These symptoms have been reported in high accidental sulphur dioxide exposure.

2. What are the biological processes related to air pollution?

The basic pulmonary response to inhaled sulphur dioxide is mild broncho-constriction, which is reflected as a measurable decrease in lung function with increase in airway resistance due to narrowing of the airways. The mechanism of response is not completely understood, but it is anticipated that sulphur dioxide causes its irritation effects by stimulating the nerve endings of airways.

3. Can reduction of emissions and /or ambient concentrations of sulphur dioxide be shown to have positive impacts on public health?

Current ambient levels of sulphur dioxide do not represent a health hazard, except from inhalation to the highest concentration hot spots close to certain industries (e.g. smelters). Only in extreme situations may certain asthmatics respond with broncho-constriction. A further reduction of ambient concentrations at these hot spot sites may have a positive impact on public health. Technical solutions could eliminate the few remaining hot spots.

4. What about uncertainty in dose-effect relationships?

It is difficult to draw a consistent picture of exposure-response relationship for sulphur dioxide. There is a threshold, an exposure concentration below which there will be no response. This threshold concentration is different from person to person, and lower for asthmatics than for healthy people. In healthy subjects no acute effects on the respiratory system have been described below 2 mg/m^3. For chronic exposure little valid human data are available.

5. At what dose and exposure time can sulphur dioxide be harmful and how high is that dose compared to ambient air pollution?

Most healthy non hyperreactive subjects seem to develop increased airway resistance at exposure concentrations above 14,000 µg/m^3, several orders of magnitude higher than ambient concentrations. Occasionally, sensitive subjects, reported to be non-asthmatic, have been found to react slightly after exposure to 2,856 µg/m^3, still well above ambient concentrations. This latter concentration and higher seems to be a threshold for most if exercise is involved.

The highest measured 1-hour average concentration in Europe between 1990-1999 was 587 µg/m^3 and the highest 24-hour average concentration was 327 µg/m^3, and levels are still declining. Most measurements were in compliance with the air quality standards, 24 hours average: 125 µg/m^3 and hourly: 350 µg/m^3.

6. Is a peak load more harmful than long-term exposure to a lower background concentration?

The current data indicate that sulphur dioxide induces acute effects mainly on the respiratory tract, and that these effects need to be prevented.

8 POTENTIAL POLICY IMPLICATIONS OF TOXICOLOGICAL FINDINGS

8.1 KEY POINTS

- In contrast to regulation for other chemical exposure hazards, toxicological information plays a supportive role as an input to regulatory policy.
- Toxicology contributes to policy by describing effect types and delineating the modes of biological action.
- Toxicological studies of human disease models may give important information for preventative measures.
- Toxicological investigations may explain reasons for differences in susceptibility towards air pollutants.

8.2 INTRODUCTION

A key role for policymakers engaged in the field of air pollutants is taking action to protect human health and the environment from significant negative impacts. The policymaker will probably also need to add sustainable development and the general development of society to the equation. Inherent in managing any risk is consideration of the precautionary principle and assessment of the cost-benefit of any remedial action.

Policymakers need to know both the type and magnitude of risk related to specific air pollutant sources, as well as the integrated risk related to all sources of air pollutants. Sometimes knowledge is required on single pollutants, but more often the policymaker is faced with a need to understand the risks from a mixture of differing air pollutants.

This is clearly the case with particulate matter, where it is important for instance to differentiate between the various sources of fine and ultrafine particles. A key question to answer is if there are special groups in the population that are at greater risk from air pollutants.

It is also important to be aware of the uncertainties involved in risk assessments, including where the research only reports associations, in contrast to situations where causality is documented in the relationships between exposure and health effects. When legislation is enacted and air quality standards are introduced, a central question is the level of protection provided by regulatory measures. To answer this, it is necessary to monitor both the levels as well as the health impact of air pollution.

Three main categories of scientific information are employed by agencies in the evaluation and regulation of ambient air pollutants: ① epidemiology, ② human clinical studies and ③ animal toxicology studies. In the air pollution field, epidemiological data are primarily used as input into risk management. This is in contrast to the situation for many other chemical hazards, where toxicological data are in fact the main source for regulatory decisions.

Toxicological research on air pollutants contributes to the overall risk assessment and risk management processes associated with air pollutants, especially in describing specific effect types and the modes of biological action of the various pollutant components. Also, toxicological data derived from studies with models of human disease may supply important contributions to the risk management process.

Further, toxicological studies may explain why there are differences in susceptibility towards pollutants. In vitro studies including cell culture studies and assessment of structure-activity relationships are typically used as supportive information in the interpretation of epidemiological and human and animal toxicology data, and are very seldom used as primary sources of information. However, in vitro data may yield valuable information on the mechanisms involved in pollutant-induced health effects.

8.3 CONCLUSIONS FROM TOXICOLOGY OF AIR POLLUTANTS

The following conclusions from toxicological studies of the main air pollutants show the key aspects for each pollutant that should be considered in any regulatory policy:

PM – inhalation of PM exacerbates symptoms of pre-existing lung disease such as asthma, as well as those of heart and blood vessel disease. Toxicological studies (mainly from in vitro studies) suggest that these effects are due to lung inflammation, disturbances in heart rhythm, alterations in blood viscosity and oxygen deprivation. Analogous effects, albeit at much lower intensity, can also be caused in healthy individuals. There is also evidence pointing to a link between PM exposure and lung cancer, however, the mechanistic basis of PM cancer development is poorly understood. The size range and chemical constituents of the PM responsible for adverse effects are not clearly defined. Coarse and fine PM are both capable of inducing toxicity (the evidence for ultrafine PM is less clear). The chemical composition of PM seems to play an important role, although the view on specific, if any, causative components is far from clear. Primary carbonaceous PM seems more toxic than secondary PM. Deposition of PM in airways and lungs suggest that larger particles might be important for upper airway effects and smaller particles for lower airway and lung effects. Population groups at increased risk for PM appear to receive much higher PM doses in airway and lungs, in addition to their possibly enhanced biological vulnerability to tissue effects.

Ozone – toxicological studies show that acute ozone exposure in humans and laboratory animals can reduce lung function and result in lung inflammation and tissue injury in the small airways and the gas exchange region. Current evidence suggests that effects of ozone may be expected even below the EU Limit Value. There are few long-term studies on ozone in laboratory animals, but changes in lung and airway structures and cell types have been reported. About 20% of the general population, regardless of airway disease, is more susceptible to ozone's effects. Specific risk groups include children, the elderly, and people involved in heavy outdoor physical activity. Both increased exposure and biological vulnerability may play a role here.

Nitrogen dioxide – can exacerbate, independently of other pollutants, allergic reactions in sensitised asthmatics at concentrations that occur in pollution hot spot episodes. Susceptible groups include allergic asthmatics, patients with chronic obstructive lung disease and possibly children. Others may include individuals who are affected at lower concentrations. Animal studies indicate that the long-term effects of nitrogen dioxide on lung structure and function are only of concern at much higher than ambient concentrations.

Polycyclic aromatic hydrocarbons – are animal and human carcinogens, acting through a genotoxic mechanism (i.e. they damage DNA). For such compounds, it is considered that there is a human health concern without any exposure threshold. Recent studies suggest that the soluble components of atmospheric particles including PAHs or their derivatives can play a role in the induction of inflammation, with a potential role both in cancer development, and in the exacerbation of asthma, and heart and blood vessel effects.

Carbon monoxide – exposure to carbon monoxide may result in reduced oxygen supply to organs and tissues due to binding to blood haemoglobin, thereby forming carboxyhaemoglobin (COHb). Although accidental exposure to high carbon-monoxide levels may induce adverse health effects and even death, healthy people do not normally stay in highly polluted areas for sufficiently long periods to attain adverse levels of COHb.

Sulphur dioxide – is an upper respiratory tract irritant. Short-term exposure to this gas may cause mild narrowing of the airways, which is reflected as a measurable decrease in lung function and increase in airway resistance. However, these reactions are only shown at concentrations markedly in excess of current ambient levels. There is no experimental evidence that long-term exposure to ambient concentrations of sulphur dioxide will be harmful, either to healthy individuals or to asthmatics.

8.4 POTENTIAL POLICY IMPLICATIONS

Inhalation of PM exacerbates the symptoms of pre-existing lung disease such as asthma, as well as heart and blood vessel diseases. Toxicological studies show that these effects are brought about through factors such as lung inflammation, heart rate variability, changes in blood viscosity and oxygen deprivation. Analogous effects, albeit at lower intensity, can also be caused in healthy individuals.

Ozone is associated with several adverse health effects. Although there are considerable variations in responses between species and between individuals, acute ozone exposure causes reduced pulmonary function, pulmonary inflammation, increased airway permeability and heightened hyper-reactivity. These effects and ensuing tissue injury in the small airways and the gas exchange region, depending on exposure concentration and duration as well as individual susceptibility, may lead to irreversible changes in the airways and worsen lung disease.

At the kind of nitrogen-dioxide concentrations that can occur in certain pollution hot spots, there is a link between nitrogen-dioxide levels and health effects in asthmatics. Some particularly sensitive individuals within susceptible groups such as asthmatics, and possibly children and patients with chronic obstructive lung disease, may be affected at lower concentrations than found in hot spots.

Certain PAHs are animal carcinogens and probable human carcinogens also, causing damage to DNA. Hence, a non-threshold mode of action may be anticipated. Furthermore, recent studies suggest that PAHs in ambient air may adversely affect birth outcomes, and may also contribute to respiratory and cardiovascular disease via the induction of inflammation. Biomarker studies suggest that cellular damage of relevance to carcinogenesis is induced by exposure to PAHs in the ambient air at environmentally relevant levels. However, the significance of such damage in terms of cancer risk cannot be evaluated at this stage.

Healthy people do not normally stay in highly polluted areas for sufficiently long periods to attain adverse levels of carbon monoxide bound to haemoglobin (COHb). Individuals with inherent heart disease might experience heart symptoms at carbon-monoxide concentrations that may occur in ambient air. There is little knowledge of the adverse health effects induced by carbon monoxide at ambient air concentrations on foetal development. However, carbon-monoxide exposure due to the mother's smoking is much more important.

Reactions to sulphur dioxide in healthy individuals are only shown at concentrations markedly in excess of current ambient levels. Asthmatics, however, may possibly react with reversible health effects at the concentrations found in the highest ambient levels/ hot spots.

9 EU-FUNDED STUDIES INTO AIR POLLUTANTS – AND FUTURE RESEARCH NEEDS

9.1 KEY POINTS

- The EU Fourth and Fifth Framework Programme toxicological research projects have contributed significantly to a better understanding of the health effects associated with exposure to air pollution.
- AIRNET has created a wide European network among many of the scientists involved in air pollution toxicology, through better information exchange, meetings, conferences and an increased awareness of the need to further focus on toxicological research as input to policy and risk management. This network should make use of the current momentum for better structuring the science-policy interface in Europe and should further try to develop its information, interpretation, and dissemination goals.
- There are still considerable gaps in the toxicological knowledge about the risks associated with hazardous air pollutants, which is hampering an effective and optimal preventative strategy.
- A number of important, policy relevant toxicological research needs on air pollutants are outlined in this chapter.

9.2 EU-FUNDED RESEARCH INTO AIR POLLUTANTS

The Fourth and Fifth Framework Programmes of the European Union have funded a number of toxicological projects on air pollution. The AULIS and EXPAH projects focused on the genotoxicity of polycyclic aromatic hydrocarbons (PAHs). The AULIS project has recently provided new data that links the expression of genetic variants of one metabolism enzyme with increased DNA adduct formation and the frequency of aberrant chromosomes in human lymphocytes. The link was found at exposure to relatively high levels of environmental tobacco smoke and moderate levels of benzo(a)pyrene.

The **EXPAH** project has just finished, and not all analyses are finalised. A genetic variability parameter is still under investigation. People from three central European cities exposed to high levels of PAHs had higher levels of DNA adducts in lymphocytes than less exposed subjects, and so had smokers compared to non-smokers. In addition, increased oxidative DNA damage was detected in samples from two cities. In overall air pollution, PAHs seemed to be most important in inducing DNA damage.

The **HELIOS** project (FP 5 'Quality of Life' Key Action 4 'Environment and Health' QLK4-CT-1999-01308) has recently been completed. Its goal was to improve short-term and long-term health risk assessments of air pollutants by using innovative non-invasive techniques for detecting lung inflammation or damage in children and adults of three main European cities along a north-south European gradient (in Sweden, Belgium and Italy).

The RAIAP, HEPMEAP and PAMCHAR projects were all designed to detect differences in the toxic potential of particles collected in various European cities. All three projects were able to characterise the different particles extensively using physical and chemical methods.

The **RAIAP** project emphasised the importance of different seasons and the ability of particles to induce airway inflammation or a respiratory allergic reaction. Particles from different locations and seasons had varying potential to induce inflammation, with the coarse particle fraction shown as most potent. With respect to allergy the results were ambiguous. One assay found the fine particles to be more potent than coarse, but with little difference between locations or seasons. The other found differences with respect to these parameters, but mainly different from those variations observed in the inflammation assays. The analysis of the importance of different chemical compounds has not yet been completed.

The **HEPMEAP** project showed, similarly to the RAIAP project, significant variations between different particle samples with respect to inflammatory markers, and in addition observed differences in oxidative potential. The project also focused on human experimental studies, including subjects with suspected susceptibility to air pollution exposure. The correlation with chemical composition has not yet been completed.

The **PAMCHAR** project is still in progress. It includes endpoints that are partly similar to RAIAP and HEPMEAP, but also comprises assays on oxidative DNA damage and the effects of ultrafine particles.

9.3 WHY A TOXICOLOGICAL APPROACH?

Modern environmental policy is faced with the need to develop new risk management tools to incorporate growing knowledge about the hazards of air pollution. The key questions to be answered include the following:

- What are the actual risks associated with exposure to hazardous pollutants?
- To what extent will public health benefit from reduced exposures to toxicants?
- Will the costs of abatement be acceptable given the level of risk?
- How certain or uncertain are we about effective policy?

Increases in toxicological knowledge and judgement will, at least in part, help in answering the first of these questions. Toxicological studies can be especially valuable in reducing uncertainties over the causative role for adverse effects of a single pollutant appearing in a complex ambient mixture. Also, improved understanding of the underlying assumptions and limitations in toxicological research will aid in reducing uncertainty, both in the toxicological database and the way toxicological data is interpreted.

To achieve a sustainable advance and an optimal cost-benefit policy, policy-makers increasingly request a more integrated judgement of the source-risk relationships and abatement scenarios, both from the point of view of abatement control and that of effective health risk reduction. Meeting the needs of such integrated judgements requires the development of better descriptions, improved evaluations and sustainable decision points on health effect and risk assessments, so that all can be accepted by policy-makers, stakeholders, the public and the scientific community.

9.4 RESEARCH NEEDS

In the current situation, where toxicology plays a substantial role in risk assessments of exposure to air pollutants, toxicologists are faced with a considerable number of research efforts that still remain to be carried out. For example:

- What is the nature and strength of the evidence for an adverse health effect and for the role of specific exposure factors in that effect?
- What is the nature of the impact (trivial or serious, reversible or irreversible, immediate or long-term, large or small numbers affected)?
- Is the potential for increased sensitivity in vulnerable groups taken into consideration?
- What is the quantitative relationship between exposure and risk associated with actual or predicted exposures?
- Are the specific circumstances considered that may increase exposure as well as the likelihood of cumulative exposure?

A key objective for toxicological research into air pollution is to complement epidemiological health effect studies as inputs for assessments of the risk to human health. In many cases there is a need to reduce uncertainty in the biological plausibility of the relationship between exposure to gaseous and particulate pollutants and observed health effects, as well as to identify causative physico-chemical characteristics of complex pollutant mixtures. In certain instances, such as for PAH in air, toxicological research has a larger role, because little epidemiological data is available on PAH in common urban environments.

There is a need for more short-term human and animal studies (both in healthy and susceptible individuals) of heart and respiratory functional responses, as well as local and total-body inflammatory responses, to PM from specific source environments, i.e. high traffic densities, residential heating, metal industries, etc. Studies of PM from urban background locations are also needed in seasons with the highest adverse outcomes, as assessed by epidemiological data. The results of such toxicological studies would be strengthened when conducted in parallel and in connection with epidemiological panel studies and air quality and source apportionment studies.

More attention should be paid to chemical characterisation and toxicity associated with the large organic fraction (including PAHs) of ambient air PM. There is also a need to study the long-term effects of PM in animals, especially with different size fractions of concentrated ambient particles, with respect to the heart and blood vessel system, lung development and ageing. European multi-centre toxicological approaches, using common PM sampling, concentration and chemical analysis instruments and protocols, on ambient air PM can support epidemiological studies in finding explanations to geographical and seasonal differences in adverse health outcomes.

Much more may be learned from mechanistic studies with various types of PM (including different types of source PM, concentrated ambient PM, size-fractionated ambient PM) in molecular, cellular and animal models with respect to lung, heart, inflammatory and genotoxic responses. Important mechanistic information may also be gained from examining whether particles of various sizes, especially ultrafine particles, become blood-borne from the lungs and enter and exert adverse responses in more distant organs such as the brain, liver and kidney. Cellular/mechanistic studies of the impact of particles on blood clotting pathways are also needed, in order to understand the involvement of particles in heart and blood

vessel disease. The oxidative stress hypothesis should be further investigated in animal and clinical human studies using concentrated ambient particulate samples.

Also, more human clinical studies are required into various types of PM in potentially susceptible individuals, including those with mild asthma, chronic obstructive pulmonary disease or heart and blood vessel disease. The endpoints considered should include sensitive measures of heart effects.

Toxicological investigations of exhaust pollutants from different combustion systems and fuel types (vehicle engines, residential stoves, etc.), using both cell culture and whole animal techniques, would aid in the evaluation of new fuel-combustion technologies. These investigations are necessary because such new technologies may well alter the chemical and physical properties of both exhaust particles and gases. Predictive toxicology studies are needed to assess which of the various new technologies offers the most promise in reducing toxic emissions.

Since human exposures are to combinations of various pollutants, there is obviously a need to perform studies on the combined effects of particulate matter and gaseous pollutants. For example to examine the effects of combined concentrated ambient particle and Ozone exposure on the heart and blood vessel system in animal and clinical human studies.

Additional human clinical studies need to be performed, with exposure regimens more typical of those occurring in ambient air, for potentially susceptible subgroups (mild asthmatics, and those with chronic obstructive lung disease, heart and blood vessel disease) and with endpoints that include more sensitive measures of heart effects.

Animal studies with Ozone should also be carried out using models more sensitive to heart function and inflammatory responses. In addition, long-term studies of lung growth in healthy young animals are warranted both with Ozone and nitrogen dioxide alone and in combination with each other or ambient particles. Important information supporting risk assessment could come from long-term inhalation studies with nitrogen dioxide in rats using sensitive measures of lung responses.

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