

RIVM report 650010 021

**Progress report on PM toxicity using
concentrated ambient particulate matter (CAPs)**

F.R. Cassee, A.J.F. Boere, P.H.B. Fokkens, E.
Buringh, L. van Bree and A. Opperhuizen

February 2001

This investigation has been performed by order and for the account of Ministry of Housing, Spatial Planning and Environment, The Hague, the Netherlands within the framework of project 650010, Risks in relation to Air Pollution.

Abstract

This progress report has been compiled within the framework of project M/650010, “Risks in relation to air pollution”, to outline recent developments and progress within the project. It does not have a scientific status. A significant part of the RIVM toxicology research programme on “the relationship between particulate matter (PM) and health effects” is devoted to the use of an Ambient Fine Particle Concentrator (AFPC) situated in a mobile laboratory. The aim of this laboratory is to facilitate performance of inhalation toxicity studies with (concentrated) ambient PM (< 2.5 μ m) in experimental animals. In the future, also studies with human volunteers will take place in locations that differ in air quality and sources of PM emission. The AFPC allows us to enrich (by 15-60 times the ambient levels) the test atmosphere with particles having a diameter of less than 2.5 μ m. These are generally referred to as concentrated ambient particles (CAPs). Exposure levels up to 3000 μ g/m³ can be achieved. The research has been focused on acute health effects of PM in rodents with a cardiopulmonary disease (asthma, pulmonary hypertension), which are likely to reflect human risk groups. The studies performed in 1999 and 2000 showed that CAPs can provoke health effects, even though they were marked as mild and reversible. Sound conclusions on the causal relationship between PM and health effects cannot yet be drawn.

Samenvatting

Dit voorgangsrapport is samengesteld in het kader van project M/650010, “Risico's in relatie tot luchtverontreiniging”, met als doel de recente ontwikkelingen en voortgang binnen dit project te schetsen. Een belangrijk deel van het RIVM toxicologisch research programma omtrent “the relationship between particulate matter (PM) and health effects” is gewijd aan het gebruik van een Ambient Fine Particle Concentrator (AFPC) in een mobiel laboratorium. Het doel van dit laboratorium is het faciliteren van inhalatie toxiciteitsstudies met (geconcentreerd) buitenluchtstof (PM) in proefdieren. In de toekomst zullen ook studies in vrijwilligers worden uitgevoerd op locaties die verschillen in luchtkwaliteit en bronnen van PM emissies. De AFPC maakt het mogelijk om verhoogde concentraties (15-60 keer de buitenluchtniveaus) te genereren waarbij 50% van de deeltjes op massabasis kleiner of gelijk is dan 2.5 µm (CAPs). Er kunnen hierdoor blootstellingsconcentraties tot 3000 µg/m³ worden bereikt. Het onderzoek heeft zich gericht op de acute gezondheidseffecten van PM in knaagdieren met een cardiopulmonaire aandoening (astma, pulmonaire hypertensie), waarvan wordt aangenomen dat deze humane risicogroepen representeren. De studies die in 1999 en 2000 zijn uitgevoerd laten zien dat CAPs gezondheidseffecten kan veroorzaken, hoewel deze als mild en reversibel zijn aan te merken. Eenduidige conclusies over de causale relatie tussen PM en gezondheidseffecten zijn echter nog niet te trekken.

Contents

1. Introduction	5
2. RIVM Toxicity studies with ambient PM	8
2.1 <i>Study results of 1999</i>	9
2.2 <i>Study results of 2000</i>	12
3. Other institutes	15
4. Conclusion and planning	16
Appendix 1: Illustrations	18
Appendix 2 Mailing list	22

1. Introduction

Current levels of ambient particulate matter (PM) have consistently been associated with health effects in the general population in epidemiological studies. These observations have been reported from all over world and from locations that differ in their sources that contribute to the air pollution. This has recently resulted in proposals to revise the current PM limit value for in the EU. A clear understanding on sources and the toxicity (causality) on PM is needed for the debate on the revision of the current PM standards in the EU in 2003. However, the essential knowledge is far from complete. The ministry of VROM has formulated five key-questions for the RIVM of which the answers should provide information to fill the knowledge gaps (RIVM report 650010 020). The following two questions are addressed in the research described in this report:

1. How do the various indicators of PM compare as relevant for the causation of health effects?
2. What is the relationship between concentrations of ambient PM and health effects in order to make a substantiated choice for a PM standard?

Recently, RIVM has published a critical review on ambient particulate air pollution (PM) toxicity, particle hypotheses, and mechanisms (RIVM report 650010 015). The current data were evaluated to investigate causality and plausibility of acute health effects associated with ambient exposure.

High-dose studies indicate that PM: 1) induces oxidative pulmonary inflammation and cardiorespiratory malfunctioning, which could contribute to a disease exacerbation mechanism. PM surface reactivity seems more important than PM mass, thereby cautiously suggesting an important role for the anthropogenic (carbonaceous) fine fraction. A limited number of low-dose PM inhalation studies support this suggestion. Coarse PM may still be important in health effects related to upper airways (like worsening of asthma); however, a role for secondary components (sulphates, nitrates) or ultrafine PM at levels occurring in ambient air have not yet been established. The contribution of (diesel) exhaust particles in acute PM health effects is far from clear yet. In addition, mixtures of particles and gases (e.g.

ozone, nitrogen dioxide) have been shown to result in increased adverse health effects under laboratory condition than each of the single constituents did on their own, suggesting a combined action of these two groups of pollutants. Dosimetry models predict that people with cardiorespiratory diseases receive a significantly higher dose in their airways compared to healthy individuals. Although world wide a noteworthy effort has been put in PM toxicity studies no satisfactory support is yet present to identify: 1) a specifically important and causal role for one form of PM fraction or composition and 2) mechanisms explaining PM health effects in people considered to be at increased risk.

At RIVM, a toxicology programme has been developed to try to confirm the epidemiological associations and thus to gain knowledge about the possible causal relationship, responsible components and fractions, and their sources. The PM10 toxicity programme, directed to (oxidative) cardiorespiratory toxicity as an important pathogenic mechanism, is focussing on the following aspects.

- Determination of cardiorespiratory toxicity in laboratory animals after short-term inhalation of carbonaceous or ammonium sulphate/nitrate particles as representative components for primary and secondary fractions of ambient.
- Determination of respiratory and cardiac toxicity (in vitro/in vivo) of collected (ultra)fine and coarse mode PM at various urban, rural, industrial, and traffic locations and from motor vehicle (diesel) exhaust.
- Determination of the relative importance of particle mass versus particle size/number for PM10 health effects
- Determination of differences in cardiorespiratory toxicity between healthy animals and animal models mimicking various cardiopulmonary diseases of specific human risk groups for PM₁₀
- Development and use of ambient PM deposition models and exposure-dose relationships for quantitative risk assessment in healthy people and risk groups with compromised airways
- Determination of cardiorespiratory toxicity in laboratory animals following short-term inhalation of concentrated "real-world" PM_{2.5} at various urban and rural locations

These studies have been and still are conducted in collaboration with a number of national and international institutes (e.g. TNO, Universities of Nijmegen, Leiden and Delft, CIIT, Health Canada, US-EPA) and have been designed in accordance with the simplified strategy of the health risk assessment model for ambient PM, i.e. the "Pentagon" model of possibly main critical fractions with respect to particle size and chemical composition. The strategy for these studies has been verified on consulting an international group of experts in this field.

Within the toxicological programme on PM health effects of the RIVM, performed in the scope of 650010 Health risks in relation to air pollution, a substantial effort is put into studies using an ambient fine particle concentrator (AFPC) installed in our mobile exposure laboratory. The AFPC allows us to enrich (15-60 times ambient levels) the test atmospheres with particles with a 50% cut-off diameter of 2.5 μm . These are generally referred to as concentrated ambient particles (CAPs). The concentration of particles smaller than 0.15 μm are not increased and present at ambient levels, whereas particles $> 2.5 \mu\text{m}$ are efficiently removed.

The purpose of a mobile exposure laboratory is:

- On-line toxicity studies with concentrated ambient $\text{PM}_{2.5-0.1}$ in experimental animals and human volunteers
- Toxicity of $\text{PM}_{2.5-0.1}$ at various locations and smog situations with differences in air quality, emissions, and sources
- Tool to study ambient PM (also in combination with gases) in stead of model aerosols
- Possibility of hybrid toxico-epidemiological studies
- Possibility of hybrid toxico-air quality studies

2. RIVM Toxicity studies with ambient PM

Progress of AFPC studies has been reported in December 1998 by means of a letter. The conclusion of this report was that particulate matter (PM) has the potential to induce minimal effects on the airways. Before 1998 was an exploratory phase with an AFPC in a mobile facility and also for the development of useful toxicological test protocols. In addition, an extensive network with other institutions has been established allowing fast and detailed exchange of research information, needed for a comprehensive answer of the policy questions that need to be addressed with the framework of project 650010. The present letter report outlines the preliminary results of studies performed with the AFPC from early 1999 until July 2000. These studies will be published in the international scientific literature in more detail.

One of the main conclusions of RIVM report 650010 017 (in preparation) is that the use of a so-called 3-stage AFPC seldom leads to PM levels higher than 0.75 mg/m^3 inside the inhalation chamber used. Although this seems a relative high level of PM, it has not shown to be sufficient to establish concentration-dose-effect relationships in experimental animals. These high concentrations are needed due to the airway deposition differences between humans and experimental animals. These higher levels will lead to a dose in the lower airways that is equivalent to the dose that humans can receive (RIVM report 650010 018/019). Therefore, an 4th stage has been added leading to exposure levels that will usually be above 0.75 mg/m^3 at which level it may be expected to observe adverse health effects in experimental animals. In addition, in anticipated human studies these relatively high levels will make it possible to dilute the test atmosphere to fixed exposure level with adjusted humidity and gas concentrations.

In light of the complexity of and the extent of data needed for the exposure characterisation, a considerable effort has been put in the development of a database to process all the data efficiently. The database makes it possible to consistently collect, summarise and to evaluate the data. An extensive description of the air quality during and exposure characteristics of the studies performed in 1999 and 2000 will be presented in RIVM report 650010 022.

The data base on health effects of ambient particulate matter points to specific host responsiveness. These risk groups may include 1) elderly people, presumably with a weak physical condition, 2) children, and 3) people with pre-existing cardio-respiratory diseases like congestive heart disease, pulmonary hypertension, asthma, chronic bronchitis, emphysema, as well as airway infections. Exposure to PM is suggested to result in an exacerbation of these diseases, possibly resulting in excess morbidity and mortality. The responses of the diseased or compromised cardiorespiratory system to inhaled PM might therefore be stronger than those in healthy lungs. For this reason, toxicological research should also use animals that mimic the health status of human risk groups. RIVM has tested the use of several animal models in the past few years (asthma, pulmonary hypertension, heart failure, chronic bronchitis, aged).

2.1 Study results of 1999

In 1999 a series of 3- day inhalation exposures in healthy and compromised rat and mice has been performed thereby mimicking possible human risk groups. About half of the studies have been performed in an industrialised area in the city of Utrecht, whereas the other half was performed on the RIVM premises in Bilthoven. This would very likely lead to differences in chemical composition and absolute mass concentrations of ambient PM, also originating from different sources of emission.

a. Asthma (9901¹)

A mouse model has been applied in which asthmatic symptoms such as increased IgE levels and increased airway reactivity upon exposure to a non-specific challenge (ovalbumin) are induced. Mice were exposed in two independent experiments to concentrated ambient PM (CAPs) that was concentrated by a factor of about 20 for 4 hr/day and during 3 consecutive days. The smooth muscle contraction of isolated trachea due to increasing concentrations of metacholine exposure was measured 1- and

¹ Number refers to research protocol as used with the Laboratory of Health Effects Research

4-day post CAPs-exposure. The CAPs mass concentration varied significantly between each experiment (mean mass concentration of 1161 and 556 $\mu\text{g}/\text{m}^3$) but also within the 3 exposure days (350 up to 2 mg/m^3). Sulphate levels are roughly 15-20% and nitrate levels between 40-50% of the total mass. Histopathological examination showed that asthmatic mice had an inflammation in lung tissue (as shown by increase number of eosinophils) as well as a hypertrophy of the bronchiolar epithelium. However, CAPs did not seem to affect the pathological conditions of the bronchoalveolar regions. The ovalbumin-treatment resulted in a low hyperreactivity of the trachea by metacholine, as compared to healthy controls. This difference did not gain statistical significance and was lower than observed in previous studies. No biological significant effect of CAPs on the tracheal reactivity could be observed. We concluded that CAPs used in this study did not affect airway pathobiology. Neither a significant hyperreactive nor hypersensitive response was observed despite the relative high exposure levels of CAPs. These data did not provide support to the hypothesis that $\text{PM}_{2.5}$ can exacerbate a pre-existing asthmatic condition in a relevant experimental animal model neither did they refute the hypothesis. The latter was concluded since CAPs themselves seem to have been ineffective and in addition, the asthmatic effects are present though not very observable.

In addition to a mouse model, another asthma model has been developed in rats. Brown Norway rat were sensitised with house dust mite antigen (HDM) as model for atopic asthma and subsequently exposed to CAPs for 6 hrs (9903). Specific lung function parameters like pulmonary resistance (R_L), dynamic compliance (C_{dyn}) and airway responsiveness were measured after exposures to CAPs. Only in one of two independent experiments marginal ($0.05 < p < 0.02$) effects on R_L and no effects on C_{dyn} were observed after CAPs exposures that ranged from 80 - 400 $\mu\text{g}/\text{m}^3$. The effects, however, were rather mild and most probably not clinically relevant. Notably, while mass concentrations of CAPs in two studies were comparable, these effects could not be related to the mass concentrations of CAPs.

These studies were also one of the first at the RIVM that showed moderate effects on health effects indicators found in blood rather than in the lung. Increased blood

fibrinogen, decreased numbers of red blood cells and haemoglobin levels were observed in rat exposed to two different types of CAPs.

It was concluded from these studies that CAPs could result in small systemic effects and small effects in compromised airways of an animal model mimicking a human PM risk group, but that similar effects were observed in healthy animals. This means that this study design could not provide evidence for increased sensitivity of people with asthma (as a possible risk group) toward PM, although PM itself provokes toxicity.

b. Pulmonary hypertension

Two identical studies have been carried out in which rats were pre-treated with monocrotaline to induce pulmonary inflammation followed by muscularization of the vascular system of the lung leading to pulmonary hypertension (9902). This type of hypertension can result in right ventricular hyperplasia and thus heart failure. The adverse effects were studied in healthy and compromised rats 4 and 11 days after short-term (4 hr/day for 3 consecutive days) exposure. Exposure levels were on average 350 en 1100 $\mu\text{g}/\text{m}^3$ respectively ranging between 200 and 1700 $\mu\text{g}/\text{m}^3$. This range illustrates the significant differences between exposure days. Health effects were studied using bronchiolar lavage fluid analysis and histopathological examinations. However, no statistically significant differences were observed between sham and CAPs exposure groups regardless the time after exposure or the treatment with monocrotaline.

During the autumn of 1999, a number of studies (other than the ones mentioned above) using CAPs exposures were performed in supposedly healthy rats. However, during these studies we found out that a significant number of the supplied animals were diseased as shown by the high number of red blood cells in bronchiolar lavage fluid and histopathological examination. However, a substantial number of health indicators are measured in bronchiolar lavage fluid, which was not possible in these rats. For this reason all studies carried out in that period were eliminated from our database.

2.2 Study results of 2000

Early 2000, a critical evaluation has been performed on the study design and the animal models that mimic human diseases. In addition, the applied health indicators and the time after exposure to establish adverse health effects have been evaluated. This evaluation has partly been performed in light of a project of the research school of Environmental Chemistry & Toxicology funded by the Ministry of VROM. We have applied animal models for cardiopulmonary disorders (marked by inflammation) to study the health effects of ambient concentrated particulate matter (CAPs) in 1998 and 1999. Although the animal models for asthma show signs of asthma, the models are neither robust nor show a variation that is small enough to detect the effect of CAPs. In additions, the signs of asthma seem to be relatively mild, not reflecting the clinical signs like wheezing and tightness of the chest. The relatively long pre-treatment to induce the diseases limits the flexibility in the performance of the exposures to CAPs (which is highly weather dependent). In addition, the rat asthma model appeared to be useless due to an extreme high level of red blood cells in lavage fluid of rats that were not yet exposed to CAPs (most likely due to interference with some unknown infection). This experimental problem has resulted to the rejection of all the results of studies performed during the autumn of 1999.

Alternatively, ozone-exposed rat was considered as an inflammation model. This type of inflammation can be related to a type of pneumonia. It is our experience that the variation of the effects within a group is smaller compared to the above-mentioned animal models. The inflammatory time-response curve has been shown to reach an optimum at 24 hr post-exposure. Therefore, a single 8-hr exposure to 0.8 ppm ozone (24 hr before CAPs exposure) has been applied in this model.

For the 2000 studies the following hypothesis has been studied using rats pre-treated with either ozone or monocrotaline:

Acute exposure to CAPs will exacerbate induced respiratory inflammatory processes and will subsequently lead to changes in mediators that influence blood pressure and heart and lung function.

This hypothesis is supported in a number of experimental studies that combined exposure to fine (acidic) particles and (in) organic gases or vapours, like e.g. O₃, NO₂, SO₂, HNO₃, aldehydes, caused acute effects in lower airways.

Since our previous studies have shown limited effects due to CAPs exposures, and the reviewers of this research have expressed the need for a positive control, these studies performed in 2000 have paid special attention to the effects of diesel exhaust particles. This (in potential) positive control should help us to validate and to test the usefulness of the effect parameters to determine effects caused by particulate matter. For this purpose, the exhaust of a diesel generator has been placed near the inlet of the particle concentrator. This has resulted in concentrated diesel engine exhaust PM (CDPs) mass concentrations of approximately 8500 µg/m³ inside the inhalation chamber. The contribution of the gases in the diesel exhaust were minimised (and at least 1-2 orders lower than in the exhaust pipe also due to dilution with ambient air) due to the fact that only PM levels will be enriched, and also below the no-observed effect level. Effect parameters have been measured after 1, 2 and 4 days of the exposure.

In a series of studies with ozone pre-treated rat effects of CDPs after different times of a single 4-hr exposure were investigated (2001; 2002). Do to limitations in the experimental set up no group of healthy rat exposed to CDPs was added. Preliminary results indicate that CDPs exposures may induce small increases of the number of red blood cells and fibrinogen levels that increase during the 4-days post CDPs exposure. These results point in the direction of a more systemic effect of ambient PM. In addition, figure 1 shows that the lungs were affected by oxidants and have responded by increasing the antioxidant status. This phenomenon was stronger for the animals that were exposed to CDPs. Also, histopathological examinations revealed a significant increase in cell turn over in bronchiolar epithelium (fig. 2), clearly showing that CDPs affects the airways. This increase could be a response to cell death (cytotoxicity). In addition, the results of these studies also indicated that the chances to observe an effect caused by PM on the range of health indicators are the highest at 2-days post-exposure, although effects were sometimes more pronounced after longer duration. The need to reduce the number of experimental animals in these studies to a minimum resulted in choice for measuring PM health effects 2 days post-exposure.

In a similar study applying the ozone inflammation model (2005) using only ambient air without added diesel exhaust, similar results were observed (fig. 3). Increased levels of antioxidant shortly after CAPs exposure and enhanced levels of markers for toxicity at 1000 - 2000 $\mu\text{g}/\text{m}^3$ indicate that in potential CAPs can cause health effects. So far, these are the most prominent effects that were observed in the RIVM PM-toxicity studies.

In addition to the inflammation model using ozone, a more or less identical study with added diesel exhaust was performed using the above-mentioned model for pulmonary hypertension in rat (2003). Apart from small changes in body, lung and heart weight, no significant changes were observed within 4 days after the CAP exposures. A number of health indicators still need to be analysed. Among these are sensitive markers for blood pressure modulation as part of our collaborations with Health Canada.

3. Other institutes

Our results reflect similar results obtained by other research groups, primarily in the USA (Harvard School of Public health, Boston; US EPA, Research Triangle Park and Chapel Hill, University of Southern California, Los Angeles, New York University, Tuxedo) and Canada (Health Canada, Toronto), which are using the concentrator technologies. Only minor changes are observed in both experimental animal (e.g. small changes airway reactivity asthmatic mice after co-exposure of ozone and CAPS, changes in blood cell numbers in aged rat, exacerbates the effects of myocardial ischemia in canines) and human-clinical studies (elderly subjects developed small but significant and persisted decreases heart rate variability). However, exposure of healthy subjects to similar mass concentrations (100 to 200 $\mu\text{g}/\text{m}^3$) CAPs for 2-hr periods in Los Angeles with intermittent exercise did not result in physiological changes. A detailed overview of these studies will be provided in a second toxicological review next year.

4. Conclusion and planning

In conclusion, the RIVM CAPs (PM_{2.5}) studies during 1999-2000 have been shown to induce slight, most likely reversible, effects in experimental animals including models mimicking human diseases of groups at risk. However, a significant amount of collected data has not been evaluated yet. The high exposure levels needed results in pulmonary doses, which are comparable to the human situation. Despite the fact that the intra group variation is much smaller for experimental animals compared with human subjects, the health indicators in the used species might not be sensitive enough to detect changes that can explain the low relative risks found in epidemiological studies. As described in RIVM report 650010 015, a number of underlying biological mechanism are being studied but so far none of them have been completely elucidated. RIVM is still searching for new sensitive markers for health effects that also can be applied in human clinical and epidemiological studies. RIVM has plans to start a new line of research called micro array assays which will identify parallel expression of thousands of genes and allow associations to define key players in signalling pathways and to try to better understand the molecular mechanisms of health endpoints.

The variation in ambient air conditions and meteorology requires a substantial number of studies to relate a fast number of exposure parameters (mass and number concentrations, chemical constituents, temperature, pressure drop, humidity etc.) with effect indicators but also with the contributing sources of emissions. For this reason, a database has been developed to be able to do multivariate and factor analysis. Similar studies as in the first half of 2000 have been and will be performed in the second half of this year. An overall analysis of the CAPs will be performed early 2001. A new location within the Netherlands is currently under investigation. This site is located very close to a busy highway to focus on traffic emissions.

Dosimetric modelling and human extrapolation should provide answers to the relevance of the CAPs exposure studies for the human situation. The RIVM CAPs studies are presently only focussed on the fine fraction of PM₁₀. Recently, a coarse fraction concentrator has been installed in the RIVM mobile laboratory in order to investigate this fraction of PM for its

ability to induce toxicity. It has already been shown that this fraction affects macrophage function and induces free radical formation. However, because of the size of these particles rodents do not inhale these particles very efficiently. In fact, it is predicted that less than 1% will reach the lower airways. This means that relatively high exposure levels should be used in rodent studies. In collaboration with the Leiden University Medical Centre, RIVM is presently investigating the possibilities to carry out studies in human volunteers like currently performed by US EPA, Health Canada and the University of Southern California. This would make it possible to study coarse and fine fraction PM at the same time for their health effects. Human clinical studies demand that experiments be executed near a hospital.

This report summarises the following research protocols:

9901, 9902, 9903, 9905
2001, 2002, 2003, 2005

Appendix 1: Illustrations

Effect of ozone and CDPs

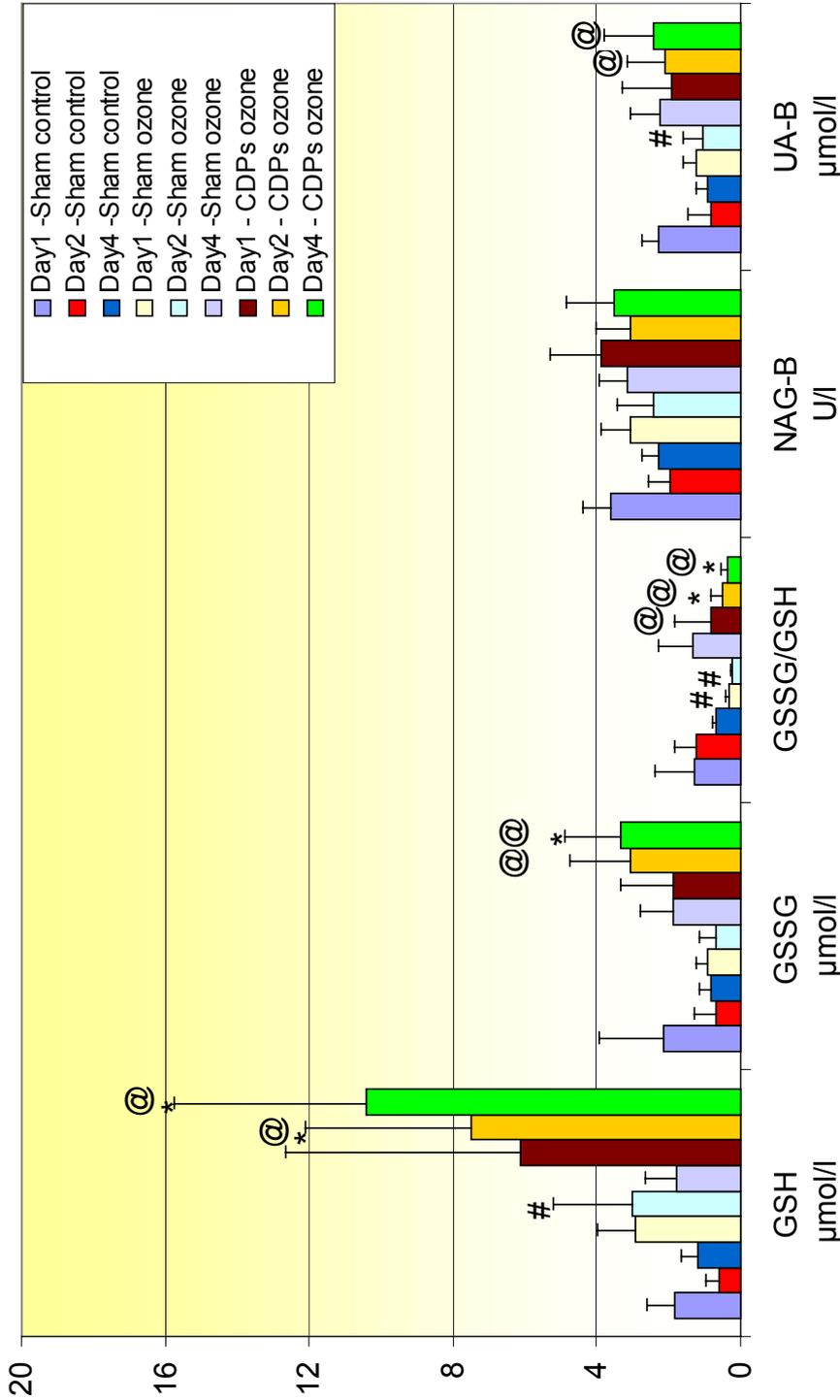


Fig.1 The effect of concentrated diesel particulates (CDPs) on sham and ozone pre-exposed rat on antioxidant status (GSH, GSSG, and the ratio), N-acety glucosaminidase (macrophage activation) and uric acid showing an slightly stronger effect in animals exposed to ozone followed by CAPs. Markers indicate p<0.05: @ CDPs-ozone vs sham ozone; # sham ozone vs sham control; * CDPs+ ozone vs. control sham.

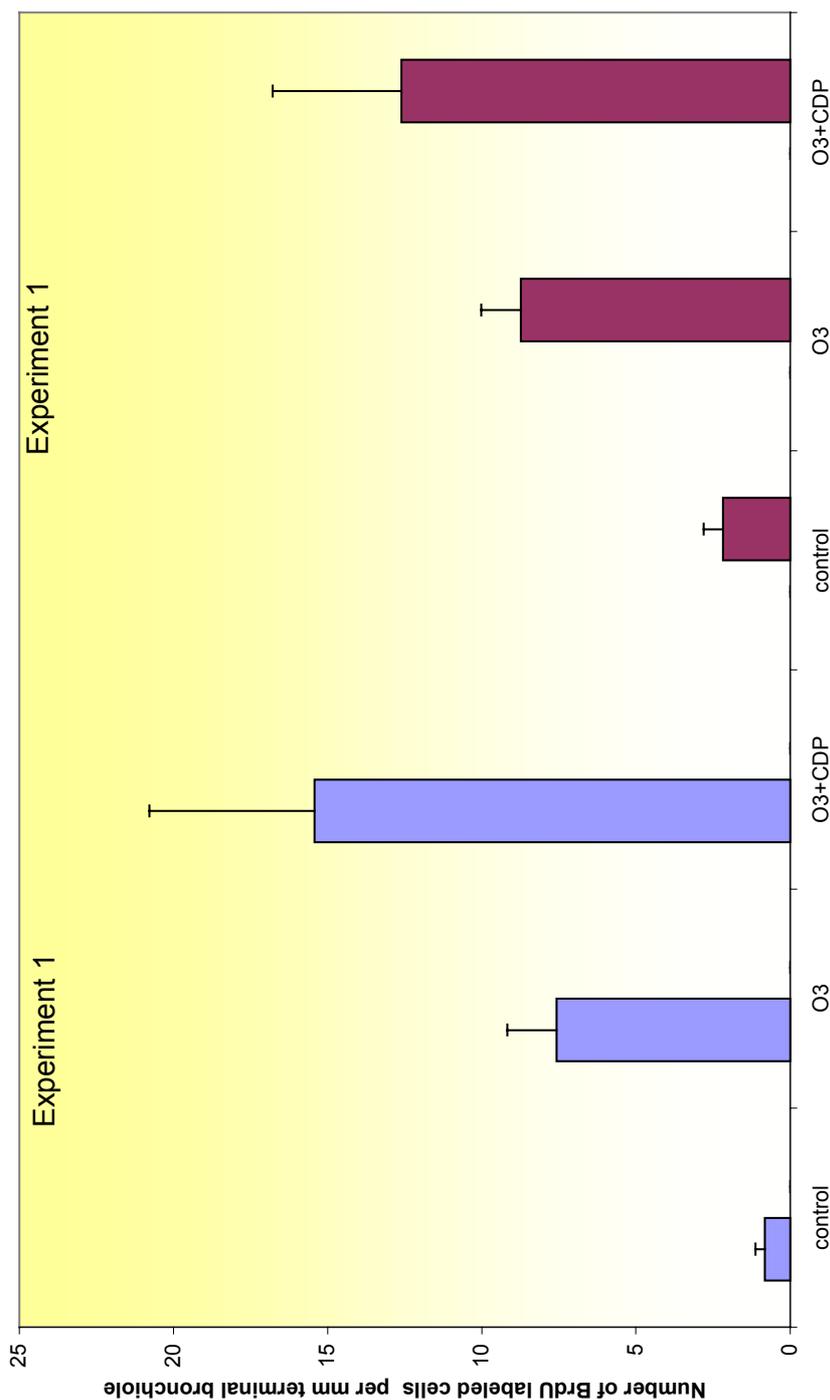
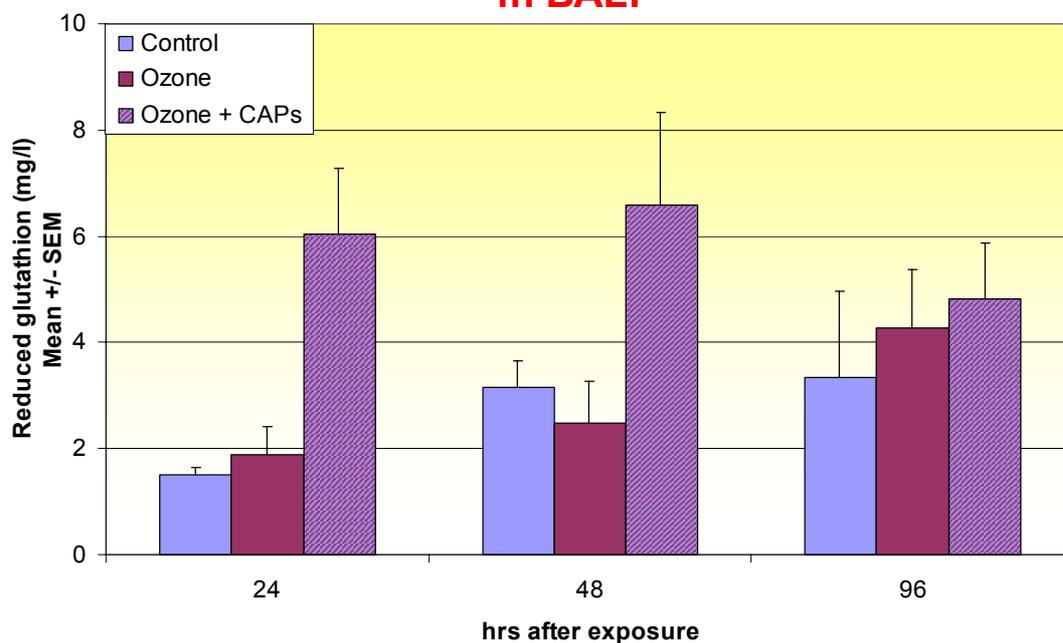


Fig 2. Cell proliferation in terminal bronchioli of rat exposed to ozone alone or with the addition of concentrated diesel exhaust particles (CDP) showing an enhanced cell proliferation due to CDP exposure.

Effect of CAPs on reduced glutathion (GSH) in BALF



Effect of CAPs on total protein in BALF

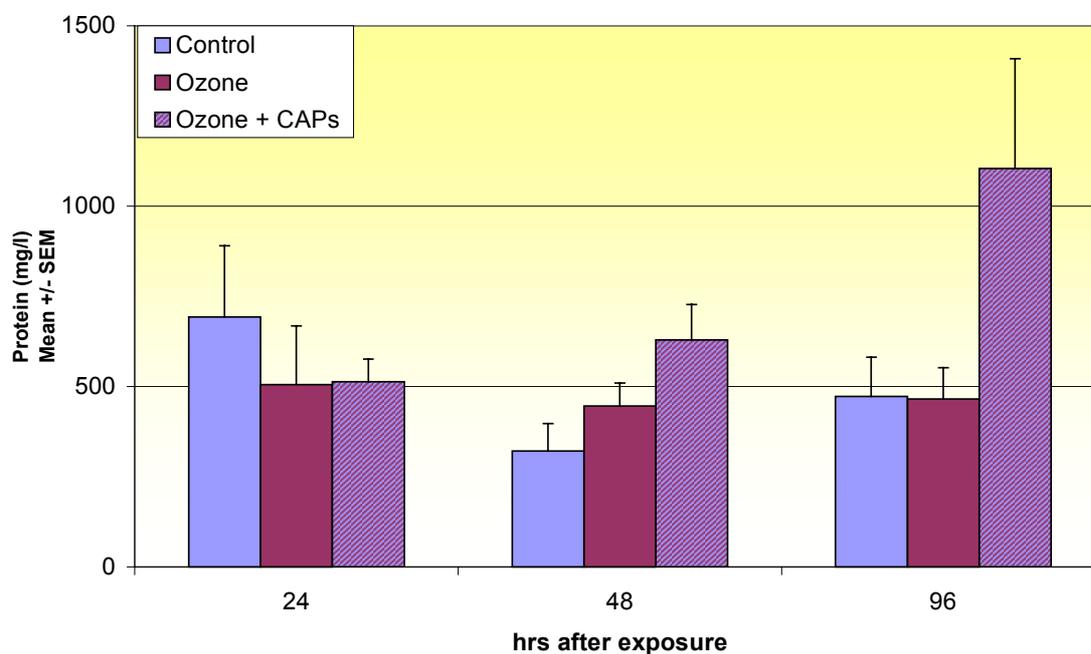


Fig. 3 Effect on CAPs on healthy and pulmonary hypertensive rat on antioxidant status (GSH) and protein (toxicity) in the lung. Note that CAPs levels were different for each time period: 24 hr 960; 48 hr 1750; 96 hr 1030 $\mu\text{g}/\text{m}^3$.

Appendix 2 Mailing list

1. Dr.ir.B.C.J.Zoeteman, Directeur Generaal
2. Directeur Directie Klimaatverandering en Industrie
3. Ing.M.M.J.Allessie, Directie Klimaatverandering en Industrie
4. Dr. K. Krijgsheld, Directie Klimaatverandering en Industrie
5. Ir J.A. Herremans, Directie Klimaatverandering en Industrie
6. Ir A. Blom, Directie Klimaatverandering en Industrie
7. Directie Rijksinstituut voor Volksgezondheid en Milieu
8. Depot Nederlandse Publikaties en Nederlandse Bibliografie
9. Prof.dr. P.J. Sterk, LUMC
10. Dr. P.S. Hiemstra, LUMC
11. Prof. Dr. R. Maynard, Department of Health, UK
12. Prof. Dr. R.M. Harrison, University of Birmingham, UK
13. Dr. P. Koutrakis Harvard School of Public Health, USA
14. Dr. J. Godleski, Harvard School of Public Health, USA
15. Dr. T. Gordon, New York University Medical Center, USA
16. Dr. L.-Y. Chen, New York University Medical Center, USA
17. Dr. M.T. Kleinman, University of California- Irvine, USA
18. Dr. D.L. Costa, Environmental Protection Agency, USA
19. Dr. R.B. Devlin, Environmental Protection Agency, USA
20. Dr. K. Dreher, Environmental Protection Agency, USA
21. Dr. J.Heyder, GSF, München
22. Dr. R.E. Wyzga, Electric Power Research Institute, USA
23. Dr. J. Brook, Environment Canada, Canada
24. Dr. R. Vincent, Environment Canada, Canada
25. Dr. W. Linn, USC, USA
26. Dr. H. Gong, USC, USA
27. Dr.J. Froines , USLA, USA
28. Dr. T. Sandström, Umea University Hospital, Sweden
29. Dr. T. Kobayashi, NIES, Japan
30. Dr. A. Kato, JARI, Japan
31. Dr. ir G. de Mik, directeur sector Stoffen en Risico's
32. Ir F. Langeweg, directeur sector Milieu
33. Dr. A. Opperhuizen, hLEO
34. Dr. C.F. van Kreyll, LEO
35. Dr.ir. E.H.J.M. Jansen, LEO
36. J. Bos, LEO
37. Dr. W.H. Könemann, hCSR
38. Dr. Ir E. Lebret, hLBM
39. Dr. ir D. van Lith, hLLO
40. Dr. A. van der Meulen, LLO
41. Ir. H.S.M.A. Diederer, LLO
42. Prof.dr.J.G. Vos, hLPI
43. Dr. H. van Loveren, LPI
44. Dr. P.A. Steerenberg, LPI
45. Dr. J.A.M.A. Dormans, LPI
46. Dr. J. Hoekstra hLAE

- 47. Bureau Rapportenregistratie
- 48. Bibliotheek RIVM
- 49. SBD/Voorlichting & Public Relations
- 50. auteurs
- 51.-64 Bureau Rapportenbeheer
- 65.-87 Reserve exemplaren