NATUR VÅRDS VERKET

Forskningsprogram
SNAP X **REPROSAFE** ☐ **FLIPP** Inriktning: Ekonomiska styrmedel ☐
Inriktning: Informationssystem och indikatorer IPP ☐

Projekttitel (svensk): Lungpermeabilitet, deposition och distribution av 30 nm förbränningspartiklar hos friska och astmatiker

Projekttitel (engelsk): Lung permeability, deposition and distribution of 30 nm combustion particles in healthy and asthmatic subjects

Huvudsökande

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En stor andel av partiklarna i luftföroreningar återfinns i den ultrafina fraktionen, <100 nm. Luftföroreningar och främst partiklar mindre än 2,5 μm har associerats med systemiska effekter. Mekanismerna för detta är ännu inte klarlagda. Möjligen spelar upptag av partiklar som sådana en roll. Den globala ökningen av allergi och astma anses av en del forskare vara orsakad av exponeringar för luftföroreningar. Det finns få humanstudier om vad som händer med förbränningspartiklar efter att de har andats in i lungorna. Möjligheterna har hittills begränsats av svårigheter att producera läckagefria partiklar, dvs där märkisotopen följer partikeln.
En unik metod för att följa partiklar i människokroppen har tagits fram av forskargruppen med stöd av SNAP. Preliminära resultat från forskargruppen tyder på att ultrafina partiklar (100 nm) som har deponerats i lungan stannar kvar fullständigt under flera dagar utan att transporteras bort. Hos personer med skador på lungslemhinnan som hos rökare och astmatiker, deponeras en större andel av partiklarna i lungan än hos friska icke-rökande personer. Distributionen av partiklar i lungan tenderar också att skilja sig mellan de olika grupperna.
Vi har i förförsök lyckats göra läckagefria 30 nm partiklar. Dessa motsvarar mer storleken hos färska förbränningspartiklar. Med ett upprepat försök med inandning av mindre partiklar, 30 nm, kan resultaten också jämföras med studier som visar att små partiklar t.ex. Technetium DTPA transporteras genom lungbarriären ut i blodet.
Projektet kommer att bidra med kunskap om vad som händer med ultrafina partiklar (30 nm) när de inandas av friska försökspersoner och försökspersoner med astma. Målet är att bestämma lungpermeabilitet, deposition och distribution. Aktiviteten från de radioaktiva partiklarna mäts direkt efter inhalation och sedan en gång dagligen under ca 5 dagar med gamma kamera. Vi vill svara på om partiklarna uppför sig på samma sätt som 100 nm partiklar, d v s om de deponerade partiklarna stannar kvar fullständigt i lungan under flera dagar eller om de translokerar till blodet. Vi vill också ta reda på om skillnaden i deponeringsfraktion och distribution mellan grupperna påverkas av partikelstorlek.
Partiklarna som ska inandas tillverkas i en Technegasgenerator, en metod som normalt används för att diagnostisera lungsjukdomar. Technegas är en ultrafin torr aerosol bestående av kolpartiklar märkta med en kortlivad radioaktiv isotop, ^{99m}-Tc ($t_{½}$=6h). Vi har modifierat metoden och är den första forskargrupp som tillverkat läckagefria partiklar, d v s att all märknukleid är bunden till partiklar samt att isotopen och partiklarna håller ihop under hela försöket. Dessutom späder vi ut aerosolen direkt efter generering för att hindra partiklarna från att öka i tillväxt p g a hög partikelkoncentration. För att kontrollera att ingen märknukleid släpper från partiklarna undersöks både aerosolpartiklar som samlats upp på filter, och om aktivitet samlats i urinen hos försökspersonerna som inandats partiklarna. Fria märknukleider passerar snabbt från lungan till blodet och utsöndras med urinen. Dessutom ska förekomsten av fri respektive partikelbunden aktivitet undersökas i blodprov med TLC-teknik.
Resultaten kommer att presenteras enligt planen för resultatspridning inom SNAP.

	År 2004	År 2005
Summa sökta medel per år i kr:	620 090	430 700

Sökta projektmedel fördelade på kostnadsslag	År 2004 (kr)	År 2005 (kr)
Personalkostnad inkl. soc. avgifter *		
Pernilla Wiebert, doktorand 6 mån/år	186 500	192 000
Ingenjör, 2 månader		77 000
Klas Philipsson, kemist 2 månader		77 000
Övriga omkostn exkl moms (förbrukningsmtrl, analyser, resor etc)**		
Partikelmätningsinstrument, CPC 3022A	325 000	
Filter	4000	
Argon 5.0, 3 tuber á 1000 kr/st	1000	2000
Gasreningssystem (3 delar)	5000	
Isotoplösning, NaTcO4		1000
Försökspersonsersättning 1000 kr/pers, 16 pers		16 000
Filterhållare 20 st á 200 kr	4000	
Delsumma av ovanstående poster:	525 500	365 000
Förvaltningspåslag: 18 %	94 590	65 700
Totalsumma per år: (införs sid. 1):	620 090	430 700

*) Specificera namn, tjänst **) Specificera

Samtliga övriga miljörelaterade projekt för vilka de sökande har beviljats anslag eller söker anslag för 2004-2006. OBS Även EU-finansiering.

Projekttitel	Finansiär	Tidsperiod	Sökt kr	Beviljat kr
Is there a difference in the uptake fo ultrafine particle healthy subjects, asthmatics and smokers	Naturvårdsverket	2004	682 000	

Miljörelaterade projekt för vilka sökande har beviljats anslag för 2000-2003
OBS Även EU-finansiering

Projekttitel	Finansiär	Tidsperiod	Beviljat Kr
Is there a difference in the uptake of ultrafine particles in healthy subjects, asthmatics and smokers	Naturvårdsverket	2002-2003	120 000
Stockholms akut effekt studie av exponeringar för bilavgaser i en vägtunnel	Naturvårdsverket	2001-2003	150 000
Hästallergen, <Equ c1, häststöv samt omgivande luft	Astma/allergiförbundet	2000	75 000
Clearance from small ciliated airways in patients with Cystic Fibrosis	Hjärt/lungfonden	2000	60 000
Allergi i yrkeslivet	Astma/allergiförbundet	2000	50 000
A pilot study of the interaction between amoking and genetic factors in the development of COPD	AstraZeneca	2003	212 600

Datum och sökandes underskrift, vilken samtidigt ger Naturvårdsverket tillåtelse att publicera sökandes namn på sin webbplats:	Datum och underskrift av prefekt eller motsvarande med namnförtydligande:

Application for funding from the Swedish Environmental Protection Agency's Environmental Research appropriation

Lung retention, deposition and distribution of 30 nm combustion particles in healthy and asthmatic subjects

Background

Traffic related air pollution is held to contribute to pulmonary diseases and reactive airway effects. It has been proposed that the world-wide increased prevalence of allergy and asthma might be caused by exposure to air pollution. There are a great number of studies published during the last years on human exposure to air pollution and their consequences. Effects have been demonstrated both for children and adults. Several studies in the last decades have confirmed that ambient air pollution levels covaries with pulmonary function, consumption of asthma drugs, daily variations in hospital morbidity as well as mortality. Moderate levels of air pollution have also been shown to be associated with increased mortality in long-term follow up studies.

The health effects found in epidemiological studies have mainly been of cardiovascular and pulmonary origin [1, 2]. The mechanisms mediating cardiovascular effects are to a large extent unknown. Proposed mechanisms concern autonomic regulation of the heart, inflammation and coagulation effects and possible direct metal toxicity to the heart muscle.

Particles less than 10 μm in diameter (PM_{10}) are known to cause inflammation and injury to airway epithelium with an increased epithelial permeability as a consequence. Li et al. found that ultrafine carbon black particles (<100 nm) induced an even greater inflammatory response than PM_{10} [3]. Subjects with pulmonary diseases and smokers have a significantly higher alveolar epithelial permeability than that of healthy non-smokers [4-7]. Asthmatics, who today represent about 5-9% of the adult Swedish population [8], have an increased bronchial permeability, but only during acute attacks [9].

In clinical examinations the ventilation in various regions of the lung is estimated by measuring radioactivity over the chest with a gamma camera after the subject has inhaled a radioactive aerosol. The main aim of these clinical examinations has not been to study clearance of the ultrafine carbon particles, although a few studies suggest a prolonged pulmonary retention [10, 11].

Only a few human studies have investigated lung permeability for ultrafine particles. The majority have used Technegas, which is a radioactive combustion aerosol of ultrafine particles that is normally used for lung scintigraphy. However the method is not immediately applicable to study the permeability. After inhalation of the particle aerosol there is an initial phase with a rapid decrease in activity over the lungs and a second phase with a much slower decrease in activity, approximately corresponding to the decay of the isotope [12]. The initial phase probably consists of "leached" free isotopes, not bound to particles. In clinical examinations these leaching particles does not pose a problem, but when the aim is to quantify the translocation of particles the free activity is not easily distinguished from activity bound to particles. Nemmar et al. [13] conclude in a Technegas study with human volunteers,

that inhaled particles pass rapidly into the systemic circulation. More likely is that they have leaching particles, which means that the "particles" they find in the blood actually is free activity.

We have, with funding from SNAP, modified the Technegas method and are the first research group to produce leaching free particles. In a recent study on healthy, asthmatic and smoking subjects we showed that there was no translocation of 100 nm particles from the lung to the blood circulation (writing in process). Once the particles were deposited in the lung they stayed there during the 4 days the activity was followed, and the decrease in activity corresponded to the decay of the isotope. We could verify that there was no leaching from the particles by testing them both in vitro and in vivo. Further we found differences between the three subject groups regarding both particle deposition fraction and distribution in the lung. The deposition fraction was highest in smokers, somewhat lower in asthmatics and comparatively low in healthy subjects. When comparing the distribution apex to basal there was a tendency to differences between the three groups.

These results show the clear advantage of our modified Technegas method, and the natural continuation is to investigate if there is a difference between 100 nm and smaller particles, f ex 30 nm, regarding translocation, deposition and distribution in the human lung. A study on smaller particles is especially interesting since newly generated combustion particles are in the rage of 30 nm. We have in a pilot study generated 30 nm particles free from leaching, and most of the necessary equipment for the study is available. The results from a study on 30 nm particles will also be comparable to studies showing that small particles, f ex Technetium DTPA, translocates from the lung to the blood circulation.

The project is a part of the thesis study plan for PhD student Pernilla Wiebert.

Objective and questions

The objective is to study lung permeability, deposition and distribution in an inhalation study with healthy and asthmatic subjects, using leaching free 30 nm particles generated with our modified Technegas method.

Questions we want to answer is:
Is the lung more permeable to 30 nm particles than 100 nm particles and, if this is true, is there a difference in lung permeability between healthy and asthmatic subjects?
Is the difference in deposition fraction and distribution between healthy and asthmatic subjects, seen in the 100 nm study, dependent on particle size?

Methods and working plan

The study will be performed at the Department of Occupational and Environmental health and at the Department of Nuclear Medicine at the Karolinska Hospital.

The measurement of lung retention of inhaled ^{99m}Tc-labelled carbon particles, Technegas, will be used to assess the permeability of the lungs. The particle size distributions will be measured by the particles electrical mobility. This measurement technique is commonly used for particles less than 1 μm., and will be done with a TSI scanning mobility particle sizer, SMPS model 3934. The SMPS consists of two parts, an electrostatic classifier and a

condensation particle counter CPC 3010. Size distribution and concentration will be monitored simultaneously. For the concentration measurements we are in need of a CPC 3022, which will be used in this and forthcoming studies.

To prevent the particles from aggregating due to high particle concentration, the aerosol will be diluted into a conducting flexible bag immediately after generation.

The activity in the lungs will be measured with a gamma camera during 4-5 days after inhalation. In our previous experiment with 100 nm particles we measured retention during the first 24 hours with a gamma camera, and for the following 3 days we used the more sensitive equipment at the low-activity laboratory at SSI (Swedish Radiation Protection Authority). During the study we noticed that the activity probably was high enough to use the gamma camera during the whole measuring period. In the present study we therefore consider to use only the gamma camera.

Leaching will be tested with three methods, which will give us an accurate control of possible leaching. One in vitro test on particles from the diluted aerosol, and two in vivo tests, on urine and on blood. Free isotopes readily translocate from the lungs to the blood circulation and are very rapidly excreted with urine [14]. With TLC (Thin Layer Chromatography) it is possible to separate bound activity from free activity. Particle bound activity stays at the origin while free activity moves with the solvent front.

Working plan:

- Generation of a stable aerosol of ^{99m}Tc-labelled ultrafine particles 30 nm by dilution of the aerosol generated by our modified Technegas method.
- In a study on voluntary humans follow the retention, deposition and distribution of inhaled ultrafine particles in two test groups, healthy subjects and subjects with asthma. Each group will consist of 6-8 subjects. Healthy subjects are characterised as non-smokers without a history of respiratory illnesses. Subjects with asthma shall be non-smoker and have a diagnosis of asthma based on reversible attacks of dyspnoea and bronchial hyper responsiveness to histamine.
- Estimation of the leakage, i.e. the activity not bound to particles in the aerosol in order to measure the correct uptake of particles. This will be studied both in vitro: on particles collected on filter, and in vivo: in urine and blood.

Dissemination of results

According to the plan for dissemination within SNAP.

Principal investigator

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Scientific knowledge available

Rolf Falk, B.Sc., Swedish Radiation Protection Authority

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Latest original work published with referee system

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Curriculum Vitae

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