

Forskningsprogram Detta gäller SNAP (går ej att kryssa för)			
SNAP <input type="checkbox"/>		REPROSAFE <input type="checkbox"/>	
FLIPP <input type="checkbox"/>		Inriktning: Ekonomiska styrmedel <input type="checkbox"/>	
Inriktning: Informationssystem och indikatorer IPP <input type="checkbox"/>			
Projekttitel (svensk): Inflammatorisk och genotoxisk potential i humana luftvägsceller av tätortspartiklar - en relativ riskjämförelse			
Projekttitel (engelsk): Inflammatory and genotoxic potency in human airway cells of urban particles - a relative risk comparison			
Huvudsökande	Efternamn: Möller		Förnamn: Lennart
			Födelseår: 1954
			Kvinna <input type="checkbox"/>
			Man <input type="checkbox"/>
		X Man	
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Medsökande	Efternamn, förnamn, tjänst, organisation, institution: Ljungman, Anders, Docent, Linköpings Universitet, Yrkes- och miljömedicin		
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Sammanfattning på svenska strukturerad enligt följande:			
Projektets betydelse för programmet			
Projektet kommer att ge underlag för att mellan partikeltyper/fraktioner insamlade i tätortsmiljö kunna göra riskbedömningar avseende potential att orsaka inflammatoriska effekter, oxidativ stress och genotoxisk verkan via resultat på humana celler från andningsvägarna. Undersökta partikelprover kan jämföras kvantitativt mellan varandra avseende analyserade effekter. I stadsmiljön kommer barn/vuxen exponeringen (andningshöjd) samt olika rörelsemönster vid olika årstider att undersökas.			
Miljörelevans och förväntad betydelse för miljöpolitiken			
En minskning av partikelexponeringen är önskvärd men kan komma att bli svår att genomföra generellt annat än med stora kostnader. I det läget är det av betydelse att prioritera insatser där partiklar/partikel fraktioner från olika källor har störst potential att inducera inflammatoriska effekter, oxidativ stress samt genotoxisk verkan på humana celler. Detta projekt syftar till att ge ett underlag för sådana bedömningar.			
Mål och hypotes			
Hypotesen är att partiklar varierar i inflammatorisk och genotoxisk respons beroende på källa och storlek på partiklarna (visat i preliminära resultat). Projektet syftar till att undersöka partiklar från gata, vedeldning, t-bana, däckslitage ± sand och ± dubb avseende genotoxiska, inflammatoriska och oxidativ stress relaterade effekter i humana celler från andningsvägarna. Vidare kommer i gatumiljö andningshöjd (barn/vuxen) avseende PM10 och PM2.5 påverkan på humana lungceller in vitro att undersökas.			
Metodik och genomförande			
Genom ett nätverk av Karolinska Institutet, Linköpings Universitet, VTI, Stockholms Miljöförvaltning samt Inst för tillämpad miljöforskning vid Stockholms Universitet kommer partikelprover att insamlas (olika miljöer/källor och partikel fraktioner). Dessa prover analyseras sedan avseende inflammatoriska mediatorer (IL-6,8,10, TNF alfa), oxidativ stress på DNA (8-oxo-dG), genotoxisk och oxidativ DNA-påverkan (comet assay) via in vitro studier på humana celler från andningsvägarna. Målsättningen är att dessa prover relativt varandra skall kunna jämföras avseende inflammatorisk, oxidativ stress samt genotoxisk verkan uttryckt i effekt/µg partiklar. Partiklarna som sådana kommer att karakteriseras avseende org/oorg kol, grundämnen (metaller), PAH samt bildanalys med högupplösande elektronmikroskopi (ner till nm).			
Kommunikationsinsatser i relation till programmet:			
Vetenskapliga artiklar, SNAP's slutrapport, dr-avhandling, internat/nat konferenser.			
		År 2004	År 2005
Summa sökta medel per år i kr:		1.289.000	859.000

Miljöforskningsnämnden
Ansökan om projektbidrag inom Naturvårdsverkets forskningsprogram

Sökta projektmedel fördelade på kostnadslag	År 2004 (kr)	År 2005 (kr)
Personalkostnad inkl. soc. avgifter* Hanna Eriksson, doktorand, Karolinska Institutet, inkl lkp, 360 tkr, 50% John Lindbom, doktorand, på Linköpings Universitet, inkl lkp, 360 tkr, 50%	180.000 180.000	185.000 185.000
Övriga omkostn exkl moms (förbrukningsmtrl, analyser, resor etc)** Laboratoriedrift, Karolinska Institutet, 240 tkr, 50% Laboratoriedrift, Linköpings Univ, 120 tkr, 50% Projektledning/publikationskostnader, 150 tkr, 50% Utlagda analyskostnader Hyra high volume samplers Inköp provinsamlare, 2 st x 75.000 Inköp personburen provinsamlare, 2 st x 38.000 Driftskostnader, provtagning, filter, transporter mm Framtagning av vedeldningsprover Framtagning av däckslitageprover	120.000 60.000 75.000 55.000 20.000 150.000 76.000 18.000 73.000 85.000	120.000 60.000 75.000 35.000 10.000 0 0 8.000 0 50.000
Delsumma av ovanstående poster:	1.092.000	728.000
Förvaltningspåslag: 18.... %	197.000	131.000
Totalsumma per år: (införs sid. 1):	1.289.000	859.000

*) 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
Fn inga sökta förutom denna ansökan				

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

Projekttitel	Finansiär	Tidsperiod	Beviljat Kr
Förprojekt, karakt av tätortspartiklar	Landst Miljöfond	2003	170.000
Nedreglering av miljörelaterad oxidativ stress	Vinnova	2001-2002	275.000
Nedreglering av oxidativ stress (människa)	Vinnova	2003-2004	400.000
Läkem utv reglering av oxidativ stress	OxyPharma	2002-2003	2.200.000
ESCODD (EU-proj/28 lab), oxidativ stress *Möten/4 interlab trials/år/nätverkskostnader	EU	-2003	*
Genotyp/miljöexponering/DNA-addukter	MISTRA	-2001	250.000
Nydetekterad luftförorening/DNA-add	Japanska Miljöministeriet	årsbasis	100.000
Weartox, däckslitagegenererade partiklar	Vägverket	löpande	1.500.000

Datum och sökandes underskrift, vilken samt ger Naturvårdsverket tillåtelse att publicera sökandes namn på sin webbplats: 2003-10-15, Lennart Möller	Datum och underskrift av prefekt eller motsvarande med namnförtydligande: 2003-10-15, Ingvar Lennerfors
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Miljöforskningsnämnden
Ansökan om projektbidrag inom Naturvårdsverkets forskningsprogram

Ansökan skall bestå av detta formulär jämte högst sex sidor lång projektbeskrivning på **engelska** (strukturerad som den svenska sammanfattningen samt en redovisning av kunskapsläget). Referenser till egna publikationer ges med sifferhänvisning till CV. Andra referenser ges i löpande text. Sökandes och eventuell medsökandes CV får omfatta högst två sidor. Inga bilagor kommer att beaktas vid bedömningen. Ansökan (max 10 A4-sidor, 12 punkters teckenstorlek) skall inlämnas i **original + 15 kopior samt elektroniskt** till ansok@naturvardsverket.se. Häfta ihop ansökan och använd hålat papper. Ansökan skall ha inkommit senast den 15 oktober 2003 till Naturvårdsverket, Forskningssekretariatet, 106 48 STOCKHOLM.

Inflammatory and genotoxic potency in human airway cells of urban particles - a relative risk comparison

1. Importance for the program

This project will generate data that will make it possible to compare particles from different sources and different particle size fractions with one another regarding genotoxic and inflammatory properties. Human cells and several different methods will be used to get an extensive picture. Further, by using portable samplers the exposure to PM10 and PM2.5 respectively, at different heights at street level will be compared (child/adult exposure) and by using sensitive methods the genotoxicity of the particles will be evaluated.

The results from this study will be important in order to elucidate whether particles from different sources should be risk assessed separately. Further, knowledge about several particulate characteristics as well as toxicity will give clues to the question regarding what makes particles toxic?

The investigated particle/particle fraction samples will be compared on a quantitative basis concerning inflammatory and genotoxic potentials.

2. Relevance and expected importance for environmental policy

The environmental policy aims for reducing the particle exposure. In many cases this can be hard to achieve unless very strong measures are applied. From that perspective it is important not to consider particles as a homogeneous group. There is a need for specific knowledge of to what extent particles from different sources contribute to possible health risks. It is also of importance to understand the health effects from different particle size fractions in order to consider a size-based regulation of particles.

Particles on the road/street level have a number of sources like sand, salt, outlet from subways, mechanical removal from tires, studs and engine emissions. Knowledge of the toxicity of particles from tires, studs and sand could be of importance for decisions regarding the use of studs in the city, cleaning of the streets etc. To optimize reduction of particle exposure there is a need for knowledge regarding the contribution from different sources as well as these sources potential to affect human health, regarding inflammatory mediators, oxidative stress and genotoxic effects.

3. Hypothesis and aims

The hypothesis is that particles differ in genotoxic as well as inflammatory effects depending on source and size. The main aims of this collaborative study are to:

- Characterize particles and particle size fractions with different origin by image analysis, analysis of basic elements (metals) and polyaromatic compounds (PACs).

- Investigate effects of particles from different sources regarding cytotoxicity, genotoxicity, inflammatory mediators and oxidative stress.
- Study the relative concentrations of PM10 and PM2.5 at different heights (child/adult inhalation) at street level and compare the genotoxicity of the different size fractions.
- Investigate season and different high/low exposure sites in the urban environment regarding the PM10 and PM2.5 contribution to effects on human airway cells.

4. General design and methods

To be able to deal with the aim, a network has been formed by the Karolinska Institute (Environmental Medicine), the Swedish National Road and Transport Research Institute (VTI), the city of Stockholm Environment and Health Administration (Miljöförvaltningen), University of Linköping and the Institute for Applied and Environmental Research at the University of Stockholm (ITM). The project is co-ordinated by Lennart Möller, prof in Environmental Medicine at Karolinska Institute.

Together these groups collect particulate samples from the street level, wood combustion, subway and different forms of mechanical break down of tires (particles) ± sand or ± studs. The particles will be analyzed regarding size, inorganic/organic coal, PACs, basic elements (mainly metals) as well as by image analysis of high resolution electron microscopy (up to 500,000 times magnification). The potency of the particles damage DNA, *i.e* to cause DNA strand breaks, to oxidize DNA and to form DNA adducts will be investigated as well as their potency to form and relase cytokines from macrophages and airway epithelium. All cells to be used are of human origin.

5. Communication of results

The results will be published as scientific papers. In addition, presentations will be performed at relevant meetings on a national and international level. The data will be available in a form to be included in the final report of the program in 2006. It will also be a part of a PhD thesis.

6. Project plan and methods

6.1. Background

Exposure to particulate matter (PM) has been reported to be associated with acute mortality and morbidity as well as long-term effects such as lung cancer (Hemminki and Pershagen 1994; Pope et al 2002). Even though the interest in the health effects of particles has increased during the recent decades, the mechanisms behind the adverse effects are not fully understood. It is believed that a particulate-induced oxidative stress could have an important role for diseases such as cancer (Tokiwa et al 1999; 15) and for triggering an

asthmatic attack (Donaldson et al 2000). There is a lack of knowledge regarding how different types of particles differ in toxicity as well as what properties of the particles that are most important for its effects on health *e.g.* size, surface area or chemical composition (Harrison and Yin 2000).

In Sweden, road transport and residential wood burning are estimated to be the major sources of particles in the ambient atmosphere (Areskoug 2000). Further, recent investigations have shown that the levels of particles are very high in the Stockholm underground (Johansson and Johansson 2003). Some particle-types, such as diesel particles, have been studied rather extensively during recent decades. However, less is known about health effects of particles generated from wood combustion (Zelikoff et al 2002; Boman et al 2003) and from tire wear. Further, no studies have been made on health effects of particles from the Stockholm underground.

The mechanisms for the genotoxicity of particles are not fully elucidated. Since the particle mixture is extremely complex it is challenging to identify which components that cause a particular adverse effect. Some studies suggest that the genotoxic and inflammatory effects of particles are due to polycyclic aromatic compounds while other studies suggest that metals in the particles can catalyze reactions involved in oxidative stress and DNA damage (Donaldson et al 1997; Ghio et al 1998). In addition, it might be a mechanical damage to the cell caused by the absorbed particle.

A specific characteristic of particles in relation to genotoxicity is the role of particle uptake by the cells. A relation has been shown between DNA strand break formation and uptake of quartz particles (Schins et al 2002) as well as urban dust particles (24). Thus, the ability of the particles to enter a cell, which may depend *e.g.* on size and surface characteristics, could be an important factor for the toxicity.

6.2. Collection of particles from different sources

There will be seven main categories of samples;

1. Street level of Stockholm, **2.** Wood combustion, **3.** Subway, **4.** Winter tires, **5.** Winter tires with studs, **6.** Winter tires + sand, **7.** Winter tires with studs + sand. Samples will be collected by high volume samplers to achieve enough particles for the described biological and chemical analyses.

In addition portable samplers will collect PM10 and PM2.5 at different heights (at street level). This is done to compare the exposure for children and adults. Particles closely to the ground are probably bigger and originate to a higher extent from resuspension of road dust. Further, different seasons and locations in the urban environment will be studied.

6.3. Lung cell exposure and the comet assay

To investigate the ability of the different particles to damage the DNA, experiments will be performed on a human lung cell line. These cells

have structural and biochemical characteristics of type II cells and have been used by several research groups to study effects of particulate matter.

The cells will be exposed to the different particle types and the comet assay will be used to measure DNA strand breaks. The assay will be performed according to Singh et al (1988) with some modifications. In short, cells are embedded in agarose on a microscopic slide, lysed with detergent and electrophorised in alkali. DNA is negatively charged and will during electrophoresis migrate towards the anode. The more DNA strand breaks that is present in the cells, the more DNA will move out from the cell. When the DNA thereafter is visualized in a microscope, an image of a comet with a "head" and a "tail" appears. The bigger tail the more DNA damage was present in the cell's DNA. Preliminary results from our lab indicate that particles from two different sources in Stockholm differ in the ability to induce DNA strand breaks (Figure 1).

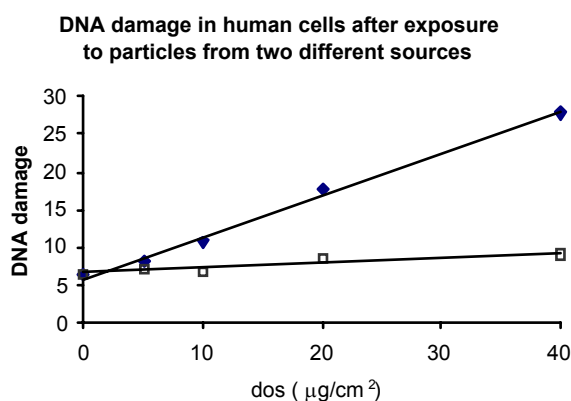


Figure 1. DNA damage in human cells after exposure to different concentrations of particles originating from two different sources. Values are means of 4 independent experiments.

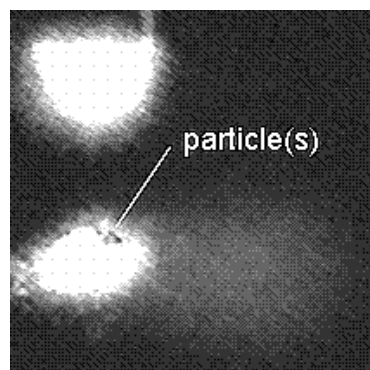


Figure 2. A cell with undamaged DNA (upper) and a cell with damaged DNA (lower) is seen. A correlation between particles in a cell and DNA damage in the same cell was seen.

A modified version of the comet assay will be used to analyze oxidative DNA damage. The enzyme formamidopyrimidine glycosylase (FPG) nicks the DNA at oxidative damaged sites, increasing the number of DNA strand breaks. The difference in tail intensity before and after treatment gives a measure of the oxidative damage on DNA.

The comet assay has been proposed as a useful tool for assessing genotoxicity of particles (Schins 2002). It has to some extent been validated within the ESCODD (European Standards Committee on Oxidative DNA damage) cooperation (ESCODD 2003). The assay has gained widespread use during the last decade and has been developed as a standard tool in the pharmaceutical industry for the assessment of the safety of potential new drugs (Wiklund and Agurell,

2003). The advantage is that the effects are studied on the cell level. Data are generated by computerized image analysis.

6.4. *In vitro* DNA reaction and ³²P-HPLC analysis

Reactive substances can react with DNA and form DNA adducts, *i.e.* molecules covalently bound to DNA, and there is a link between DNA adducts, mutations and cancer development. The ability of the different particles to form DNA adducts will be investigated *in vitro*. The different particles will be extracted with DMSO and the extracts will be reacted with DNA according to a method previously described (24).

After the reaction, the samples are ³²P-labeled and analyzed by using ³²P-HPLC according to Möller et al (1993) (2) with some modifications. In short, the ³²P-labeled samples are injected into the ³²P-HPLC system, which mainly consists of a pump, two serial C18 columns and an on-line radioactivity detector. Computerized calculations determine peaks that are significant.

6.5. dG reaction and analysis of 8-oxo-dG

The potency of particles to oxidize molecules and cause oxidative stress in the body is believed to be an important factor for various health effects caused by particles. To study the oxidation potency of the different particles, an incubation of particles and the DNA nucleoside deoxyguanosine (dG) will be done. The formation of the oxidative damage 8-oxo-2'-deoxyguanosine (8-oxo-dG) will be measured using HPLC with electrochemical detection as previously described (18).

6.6. Human cell exposure and analysis of particle induced cytokine release

Two cell lines that originates from human airway and nose epithelium will be used together with monocytes/macrophages from human blood to investigate the potency to initiate inflammatory pathways. The different particles collected will in that way be compared regarding inflammatory potency and character.

Particles from outdoor as well as indoor air at concentrations between 10–300 micrograms/ml culture medium have been found to induce cytokine (IL-6, 8 and TNF- α) release from exposed macrophages and/or epithelial cells after 4–24 hours of exposure (Monn and Becker 1999; Hetland et al 2000; Becker et al 2003). In this study, the human cells will be *in vitro* exposed for the particles and the concentration of the inflammatory mediators TNF α , IL-6, IL-8 and IL-10 released into the cell medium will be analyzed using commercially available assay kits (Quantikine[®], Fluorokine[®] R&D Systems).

Preliminary data suggests that both TNF α as well as IL-8 are released in a dose dependent manner after particle exposure to epithelial cells. Endotoxin (from Gram-negative bacteria) is a potent stimuli that induces the release of several inflammatory mediators,

including cytokines, from macrophages as well as epithelial cells. Therefore endotoxin will be used as positive control stimuli. Previous studies have indicated that endotoxin is a component of the PM₁₀ fraction of ambient air particles and therefore polymixin B (an inhibitor of endotoxin) treated particles will be used in a subset of experiments.

6.7. Investigation of particles and particle-cell interaction by using electron microscope

An electron microscope with a resolution of approx. 5 Angstrom that enable image analysis on the level of nm will be used. It is the highest resolution that is possible and the instrument is used to look into virus particles. Further, this instrument will most likely (planned for 2004) be updated with a unique set-up to characterize basic elements in very small samples. There will be two projects involving this instrument.

The first is to characterize the particles from the different locations of sampling to look for size, character, crystals, organic and inorganic particles etc.

The second set-up of experiment will be human cells. Particles are taken up into the cells and that has been shown (prel data Eriksson et al at KI) to be correlated to DNA damage. The cells will be characterized in terms of what types of particles that can enter into cells to see if the different sources of particles behave in a different manner which the preliminary data suggests. Further it will be investigated if the different particles that enter into the cells damage cell structures in a way (as asbestos for instance) that can explain the toxic effect. One can speculate that a particle can activate inflammatory pathways by damaging internal cell structures.

6.8. References

- Areskoug H (2000) Particles in the ambient atmosphere. Scand J Work Environ Health. Vol 26 suppl 1: 5-22.
- Becker S, Soukup M, Sioutas C, Cassee F. (2003) Response of human alveolar macrophages to ultrafine, fine, and coarse urban air pollution particles. Exp. Lung. Res 29:29-44.
- Boman BC, Forsberg AB, Jarvholm BG. (2003) Adverse health effects from ambient air pollution in relation to residential wood combustion in modern society. Scand J Work Environ Health. Aug;29(4):251-260.
- Donaldson K, Brown DM, Mitchell C, Dineva M, Beswick PH, Gilmour P and MacNee W. (1997) Free radical activity of PM10: iron-mediated generation of hydroxyl radicals. Environ Health Perspect. 105 Suppl 5, 1285-1289.
- Donaldson K (2000) Asthma and PM10. Respir Res. 1: 12-15
- Ghio AJ, Stonehuerner J, Dailey LA, and Carter JD (1999) Metals associated with both the water-soluble and insoluble fractions of an ambient air pollution particle catalyze an oxidative stress. Inhal Toxicol. 11, 37-49.
- Harrison RM and Yin J. (2000) Particulate matter in the atmosphere: which particle properties are important for its effects on health? Sci Total Environ. 2000 Apr 17;249(1-3):85-101.

- Hemminki, K., and Pershagen, G. (1994) Cancer risk of air pollution: epidemiological evidence. *Environ Health Perspect.* 102 Suppl 4, 187-192.
- Hetland R, Resnes M, Myran T et al. (2000) Mineral and/or metal content as critical determinants of particle-induced release of IL-6 and IL-8 from A549 cells. *J of Tox and Environ Health* 60(A):47-65
- Johansson C and Johansson P-Å. (2003) Particulate matter in the underground of Stockholm. *Atmospheric Environment.* vol 37:3-9.
- Monn C, Becker S. (1999) Cytotoxicity and induction of proinflammatory cytokines from human monocytes exposed to fine (PM_{2.5}) and coarse particles (PM_{10-2.5}) in outdoor and indoor air. *Toxicology and Applied Pharmacology.* 155:245-252.
- Pope, C. A., 3rd, Burnett, R. T., Thun, M. J., Calle, E. E., Krewski, D., Ito, K., and Thurston, G. D. (2002) Lung cancer, cardiopulmonary mortality, and long-term exposure to fine particulate air pollution. *Jama.* 287, 1132-1141.
- Singh, N. P., McCoy, M. T., Tice, R. R., and Schneider, E. L. (1988) A simple technique for quantitation of low levels of DNA damage in individual cells. *Exp Cell Res.* 175, 184-191.
- Tokiwa, H., Sera, N., Nakanishi, Y., and Sagai, M. (1999) 8-Hydroxyguanosine formed in human lung tissues and the association with diesel exhaust particles. *Free Radic Biol Med.* 27, 1251-1258.
- Wiklund, SJ and Agurell, A. (2003) Aspects of design and statistical analysis in the Comet assay, *Mutagenesis*, Vol. 18, No. 2, 167-175.
- Zelikoff JT, Chen LC, Cohen MD, Schlesinger RB. (2002) The toxicology of inhaled woodsmoke. *J Toxicol Environ Health B Crit Rev.* 2002 Jul-Sep;5(3):269-282.

CV Lennart Möller

Lennart Möller (Dr Med Sci) is professor in Environmental Medicine at The Karolinska Institute (KI) and group leader of the research group in Analytical Toxicology at KI. L. Möller is deputy executive director of Center for Nutrition and Toxicology (approximately 140 scientists) at KI. Möller was co-ordinator for the Swedish Urban Air project from 1982-1993. This project involved government, industry and academia and produced some 110 scientific papers, 17 doctoral theses and many international conferences, meetings with users of data as well as SNV-reports.

Möller has initiated the EU-project ESCODD (European Standard Committee on Oxidative DNA Damage) involving 28 different laboratories and is a member of the Executive Committee. Four international trials are performed every year to establish quality guidelines for measurements of oxidative stress, which is a complicated issue. Möller is also a member of the executive committee and task group leader in the EU-project EUROFEDA that deals with regulation of oxidative stress. EUROFEDA involves 30 different laboratories.

The research group has been and is involved in several research programs related to this application. Examples are modifications of distillation processes to produce lower levels of genotoxins from crude oil (sponsored by Swedish petroleum industry, US EPA, US NIOSH etc), analyses of DNA damage (including oxidative stress) after exposure to particles (sponsored by the Japanese EPA) from diesel emissions, analyses of DNA damage after exposure to bitumen (IARC, Fraunhofer, petroleum industry), DNA damage after exposure to nitro-benzanthrone - a recently discovered air pollutant (Japanese EPA and Saitama Medical School, Japan) and down-regulation of oxidative stress in healthy humans (The Academic University Hospital). A study (sponsored by MISTRA) performed together with IMM has just been finished concerning human genotype, in vitro exposure to aromatic hydrocarbons and genotoxic effects. In December 2001 a dissertation was presented from the research group by Tim Hofer entitled "Method Development for Analysis of 8-oxodG as a Biomarker for Oxidative Stress" (supervisor L Möller). The early development of measuring oxidative stress of DNA was sponsored by The Swedish Medical Research Council and The British Ministry of Agriculture.

L. Möller has held 41 invited lectures in 15 different countries including a 5-week series of lectures at US universities, research institutes and governmental agencies. Möller is a WHO-expert in the area of air pollution. L. Möller is editor and author of the international edition of the book Environmental Medicine (English and Japanese) including 17 international authors.

Relevant selected papers for this application, published by the research group;

1. L. Möller and M. Zeisig. (1993). DNA-adduct formation after oral administration of 2-nitrofluorene and N-acetyl-2-aminofluorene, analyzed by ³²P-TLC and ³²P-HPLC. *Carcinogenesis*, 14, 1, 53-59.
2. L. Möller, M. Zeisig and P. Vodicka (1993). Optimization of an HPLC method for analyses of ³²P-postlabelled DNA adducts. *Carcinogenesis*, 14, 7, 1343-1348.
3. L. Möller, I Lax and LC Eriksson (1993). Nitrated polycyclic aromatic hydrocarbons: a risk assessment for the urban citizen. *Environ Health Perspect.* 101 Suppl 3:309-15.
4. M. Zeisig, and L. Möller, (1995). ³²P-HPLC suitable for characterization of DNA adducts formed in vitro by polycyclic aromatic hydrocarbons and derivatives. *Carcinogenesis*, 16, 1-9 (*accelerated paper*).
5. X-S Cui, U-B. Torndal, Eriksson, L.C. and Möller, L. (1995). Early formation of DNA adducts compared with tumor formation in a long term tumor study in rats after administration 2- nitrofluorene. *Carcinogenesis* 16, 2135-2141.
6. Möller, L., Grzybowska E., Zeisig, M., Cimander, B., Hemminki, K. and Chorazy, M. (1996). Seasonal variations of DNA adduct pattern in human lymphocytes analyzed by ³²P-HPLC. *Carcinogenesis*, 17, 61-66.
7. T. Hofer and L. Möller (1998). Reduction of oxidation during the preparation of DNA and analysis of 8-hydroxy-2'-guanosine. *Chem Res Tox*, 11, 882-887.
8. ESCODD (1998): European Standards Committee on Oxidative DNA Damage (several authors, international interlaboratory trial). *Free Rad Res*, 29, 601-608.
9. L. Möller, T. Hofer and M. Zeisig (1998). Methodological considerations and factors affecting 8-hydroxy-2'-deoxyguanosine analysis. *Free Rad Res*, 29, 511-524.
10. M. Zeisig, T. Hofer, J. Cadet and L. Möller (1999). ³²P-postlabeling high-performance liquid chromatography (³²P-HPLC) adapted to analysis of 8-hydroxy -2'-deoxyguanosine. *Carcinogenesis*, 20, 1241-1245.
11. X. Cui, L.C. Eriksson and L. Möller (1999). Formation and persistence of DNA adducts during and after a long term administration of 2-nitrofluorene. *Mut Res*, 442, 9-18.
12. Y Yang, WJ Griffiths, M Nordling, J Nygren, L. Möller, J Bergman, E Liepinsh, G Otting, JA Gustafsson, J Raftar and J Sjøvall. (2000) Ring opening of benzo[a]pyrene in the germ-free rat is a novel pathway for formation of potentially genotoxic metabolites. *Biochemistry*. 39, 15585-15591.
13. J. Bananszewski, Z. Szymeja, W. Szyfter, K. Szyfter, P. Baranczewski and L. Möller (2000) Analysis of aromatic DNA adducts in larynx biopsies. *Laryngology. Eur Arch Otorhinolaryngol.* 257, 149-153.
14. ESCODD (European Standards Committee on Oxidative DNA Damage) (2000). Comparison of different methods of measuring 8-oxoguanine as a marker of oxidative DNA damage. *Free Radic Res.* 2000 Apr;32(4):333-41.

15. K. Iwai, S. Adachi, M. Takahashi, L. Möller, T. Udagawa, S. Mizuno and I. Sugawara (2000). Oxidative DNA damages in diesel exhaust-exposed rats and late development of lung tumors. *Environ Res.* 84, 255-64.
16. K. Hirayama, P. Baranczewski, T. Midtvedt, L. Möller and J. Rafter (2000). Effects of human intestinal flora on mutagenicity of and DNA adduct formation from food and environmental mutagens. *Carcinogenesis*. 21, 2105-2111.
17. Akkineni LK, M. Zeisig, P. Baranczewski, L-G, Ekström and L. Möller (2000). Formation of DNA adducts from oil-derived products analyzed by ³²P-HPLC. *Arch Toxicol.* 2001 74 720-731.
18. T Hofer and L. Möller (2002) Optimization of the Workup Procedure for the Analysis of 8-Oxo-7,8-dihydro-2'-deoxyguanosine with Electrochemical Detection. *Chem. Res. Toxicol.* 15(3), 426-32.
19. R. Touminen, P. Baranczewski, M. Warholm, L. Hagmar, L. Möller and A. Rannung (2002) Susceptibility factors and DNA adducts in peripheral blood mononuclear cells of aluminium smelter workers exposed to polycyclic aromatic hydrocarbons. *Arch Toxicol.* 76, 178-186.
20. R. Touminen, M. Warholm, L. Möller and A. Rannung (2003) Constitutive CYP1B1 mRNA expression in human blood mononuclear cells in relation to gender, genotype, and environmental factors. *Environmental Research.* 93, 138-148.
21. European Standards Committee on Oxidative DNA Damage (ESCODD), (2002) Comparative analysis of baseline 8-oxo-7,8-dihydroguanine in mammalian cell DNA, by different methods in different laboratories: an approach to consensus. *Carcinogenesis*, 23:12, 2129-2133.
22. European Standards Committee on Oxidative DNA Damage (ESCODD), (2003) Measurement of DNA oxidation in human cells by chromatographic and enzymatic methods. *Free Radic Biol Med.* Apr 15;34 (8):1089-99.
23. H. Eriksson, M. Zeisig, L-G Ekström and L. Möller. ³²P-postlabeling of DNA adducts arising from complex mixtures: HPLC versus TLC separation, applied to adducts from petroleum products. *Arch Toxicol.* *In press*
24. H. Eriksson, J. Nygren and L. Möller (2003) The role of particle-cell interactions, polyaromatic compounds and soluble iron for the genotoxicity of particulate matter. *Submitted.*

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Relevant selected papers for this application, published by the research group;

25. A.R. Nosratabadi, A.G. Ljungman, M. Lindahl, R. Welch, A. Pilon, C Tagesson. Clara Cell 10-kDa protein inhibits endotoxin-induced airway contraction in isolated perfused rat lungs. *Exp Lung Res.* 29(7):455-473, 2003.
26. Lindbom J, Ljungman AG, Lindahl M, Tagesson C. Increased gene expression of novel cytosolic and secretory phospholipase A2 types in human airway epithelial cells induced by tumor necrosis factor-alpha and interferon-gamma. *Journal of interferon & cytokine research.* 22:947-955, 2002
27. Lindbom J, Ljungman AG, Lindahl M, Tagesson C. Expression of members of the phospholipase A2 family of enzymes in human nasal mucosa. *Euro. Respir. J.* 18:130-138, 2001.
28. A.G. Ljungman, P. Leanderson, C. Tagesson, (1→3)-β-D-glucan stimulates nitric oxide generation and cytokine mRNA expression in macrophages. *Environ. Toxicol. Pharmacol.* 5:273-281, 1998.
29. A.G. Ljungman, C. Tagesson, M. Lindahl. Endotoxin stimulates the expression of group II phospholipase A₂ in rat lung *in vivo* and in isolated perfused lungs. *Am J Physiol* 270:L752-L760, 1996.
30. A.G. Ljungman, M. Lindahl, C. Tagesson. Asbestos fibres and manmade mineral fibres: Induction and release of tumor necrosis factor-alpha from rat alveolar macrophages. *Occupational and Environ. Med.* 51:777-783, 1994.
31. A.G. Ljungman, C.M. Grum, G.M. Deeb, S.F. Bolling, M. L. Morganroth. Inhibition of cyclooxygenase metabolite production attenuates ischemia-reperfusion lung injury. *Am Rev Respir Dis* 143:610-617, 1991.
32. M. Lindahl, A.G. Ljungman, R. Bruhn, R. Hede C. Tagesson. Calcium ionophore-activated neutrophils prestimulated with endotoxin increase pulmonary arterial pressure and vascular permeability in isolated perfused rat lungs. *Exp Lung Res* 17:77-89, 1991.
33. C.M. Riva, M.L. Morganroth, A.G. Ljungman, S O. Schoenich, R M. Marks, R.F. Todd III, P.A. Ward, L A. Boxer. Iloprost inhibits neutrophil-induced lung injury and neutrophil adherence to endothelial monolayers. *Am J Respir Cell Mol. Biol* 3:301-309, 1990.
34. G.M. Deeb, C.M. Grum, M.J. Lynch, T.P. Guynn, K.P. Gallagher, A.G. Ljungman, S.F. Bolling, M.L. Morganroth. Neutrophils are not necessary for induction of ischemia-reperfusion lung injury. *J Appl Physiol* 68(1):374-381, 1990.