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# NILU's Strategic Institute Initiatives (SIS)

2014

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**Scientific report**



## **Preface**

NILU receives basic funding from the Ministry of Climate and Environment through the Norwegian Research Council. 40 % of the basic funding is allocated to Strategic Institute Initiatives (SIS). After an initial process at NILU, the topic of each new Strategic Institute Initiative is discussed with the Norwegian Environmental Management before it is accepted as a SIS by the Norwegian Research Council.

Each SIS is required to deliver a popular science report annually to the Research Council. This report contains all of NILU's annual SIS-reports for 2014.



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## **BieBus - Bio-Ethanol in public transport: an integrated approach to evaluate the impact of climate change policies in urban areas**

**Duration:** 01.01.2011 – 31.12.2014

**Budget 2014:** 1,5 Mill NOK

**Project leader:** Susana Lopez-Aparicio

**NILU project:** B-110151

### **Description of the project and objectives**

The main aim of the BieBus project was to contribute to the understanding of the emission of new harmful compounds associated with the combustion of bioethanol via measurements, air dispersion model and integrated analysis. The evaluation of the results and the development of new methodological approaches has provided greater knowledge about the impact of climate change policies and abatement strategies on urban air quality. However, new questions and uncertainties are also raised, and they point out the need for additional research in the field of impact of alternative fuels on urban air quality. The project has been going from 2011 to 2014, when different activities were implemented. The work was originally divided in four different work packages dedicated to management and dissemination (WP1), measurements (WP2), modelling activities (WP3) and impact assessment and cost evaluation (WP4). The last year of the project (2014), the work focussed on the modelling activities.

### **Results achieved**

Innovative measurement campaigns were performed in a pilot study in Oslo to contribute to the understanding of the consequences associated with the use of bioethanol-blended fuel (E95) on a series of pollutants. The highlights from the measurement campaigns are:

- Higher ambient levels of harmful compounds (i.e. aldehydes) were measured at locations exposed to bioethanol-fuelled buses (E95) than at locations not exposed
- High acetaldehyde and acetic acid values were measured from the exhaust pipe during driving conditions and modelled at close distance to the bioethanol bus
- Human exposure to high concentration of acetaldehyde is expected, and it may involve a significantly increased chance in developing cancer. The high concentration of acetic acid will involve odour annoyance and significant material degradation or corrosion

Emission and air dispersion modelling of acetaldehyde associated with bioethanol fuel vehicles were additionally performed. Two scenarios of bioethanol implementation, both realistic and hypothetical, were considered under winter conditions; 1) realistic baseline scenario, which corresponds to the current situation in Oslo where one bus line is running with bioethanol (E95; 95 % ethanol – 5 % petrol) among petrol and diesel vehicles; and 2) a hypothetical

scenario characterized by a full implementation of high-blend bioethanol (i.e. E85) as fuel for transportation. The most relevant results from this activity are:

- The results indicate that a full implementation of bioethanol will have certain impact on urban air quality due to direct emissions of acetaldehyde. Acetaldehyde emissions are estimated to increase by 233 % and concentration levels increase up to 650 % with regard to the baseline
- In addition, formaldehyde (known carcinogenic compound) levels were modelled in the dispersion plume and at close distance to the bus, resulting in levels above 1 ppm (permissible exposure limit 8-hours) and 0.8 ppm (threshold for cytotoxic damage) at close distance to the bus. This indicates that formaldehyde associated with emissions from bioethanol fuel vehicles may be a concern for human health

An impact pathway approach for estimating an economic value on undesired pollution effects of acetaldehyde associated with the combustion of bioethanol-fuelled vehicles was also developed. The approach rests on results obtained in the measurement campaigns and by the air pollution dispersion model, followed by an impact assessment and economic valuation of the potential negative externalities.

- The carcinogenic risk from long-term inhalation exposure to acetaldehyde emissions, leads to a significant cost estimate associated with the combustion of bioethanol in vehicles in Oslo. The total cost per average incidence of oral, nose cavity, and laryngeal cancer is estimated between 9.7 and 11.9 million (2013) NOK (€ 1.3 million - € 1.6 million). Productivity and welfare loss is the dominant cost component, whereas the direct medical cost constitutes only a small part, accounting for less than 7 % of this estimate

### **Completed activities**

The results obtained in the BieBus are highly relevant for the evaluation of implementing the use of alternative fuels as mitigation measures to control greenhouse gas (GHG) emissions. The results show in general the need for additional research towards responsible decisions. Our study, as other published in the literature, points out that the use of alternative fuels may create new environmental challenges. While the GHG-emissions may be reduced, emissions of other compounds may increase (aldehydes), giving rise to local air pollution. In addition, new methodologies have been designed and implemented in the project regarding measurements, modelling tasks and the design of impact pathways. These new developments are highly important for further work in different environmental fields and supporting decision-making processes.

The results from the project have been useful for national authorities. For instance, the share for biofuels will increase in Norway from the current 3.5 % to 5.5 % from 1 July 2015. In that connection, the team at NILU – Norwegian Institute for Air Research was asked by the Norwegian Environment Agency for a brief memo describing the potential impact on air quality of increasing the share of bioethanol in fuel based on the results obtained in our study and others published in the literature. In addition, uncertainties and concerns was included.



The last year of the BieBus project has also focussed on dissemination. The main activities have included participation in national and international conferences, publication in popular dissemination magazines (e.g. Allergi i Praksis), institute magazines and webpages. Several results of the BieBus project have been published in peer-review journals:

- López-Aparicio, S., Hak, C. (2013) Evaluation of the use of bioethanol fuelled buses based on ambient air pollution screening and on-road measurements. *Sci. Total Environ.*, 452-453, 40-49. doi:10.1016/j.scitotenv.2013.02.046.
- Sundvor, I., Lopez-Aparicio, S. (2014) Impact of bioethanol fuel implementation in transport based on modelled acetaldehyde concentration in the urban environment. *Sci. Total Environ.*, 496, 100-106. doi:10.1016/j.scitotenv.2014.07.017.
- López-Aparicio S., Hak C., Sundvor I., Sundseth K. (2014) Understanding effects of bioethanol fuel use on urban air quality: An integrative approach. *Energy Procedia* 58, 215-220.
- Sundseth, K., Lopez-Aparicio, S., Sundvor, I (In review) The economic value of acetaldehyde-related health risk associated with emissions from bioethanol fuel vehicles. Submitted to *Environmental and Resource Economics*.



## GHG-Nor - Greenhouse gases in the North: from local to regional scale

**Duration:** 01.01.2011 – 31.12.2014

**Budget 2014:** 1,65 Mill NOK

**Project leader:** Cathrine Lund Myhre

**NILU project:** B-111008

### Main goal and background

CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O are the three key anthropogenic climate gases with direct emissions from human activity. Their concentrations in the atmosphere have increased strongly during the industrial era (IPCC, 2013<sup>1</sup>). All three gases have both natural and human-related sources and CH<sub>4</sub> has particularly large potential sources in the Arctic regions.

During the last years there has been an increased focus on the natural sources and sinks of these gases, and the climate feedback on the emissions and removal processes. The overall goal of GHG-Nor was to improve the knowledge of climate gas emissions and budgets on various scales at Northern latitudes, including the Arctic. In the period

2008-2011, there was considerable investments strengthening the national infrastructures on climate gas fluxes and measurements. Consequently, in GHG-Nor there was an important strategic priority to build competence and utilize the recent climate gas infrastructure investments.



The main objective of the atmospheric project was to

### ***Characterise and quantify natural and anthropogenic emissions of climate gases at northern latitudes and in the Arctic***

<sup>1</sup> IPCC, 2013: Summary for Policymakers. In: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S. K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA

Sub objectives were to:

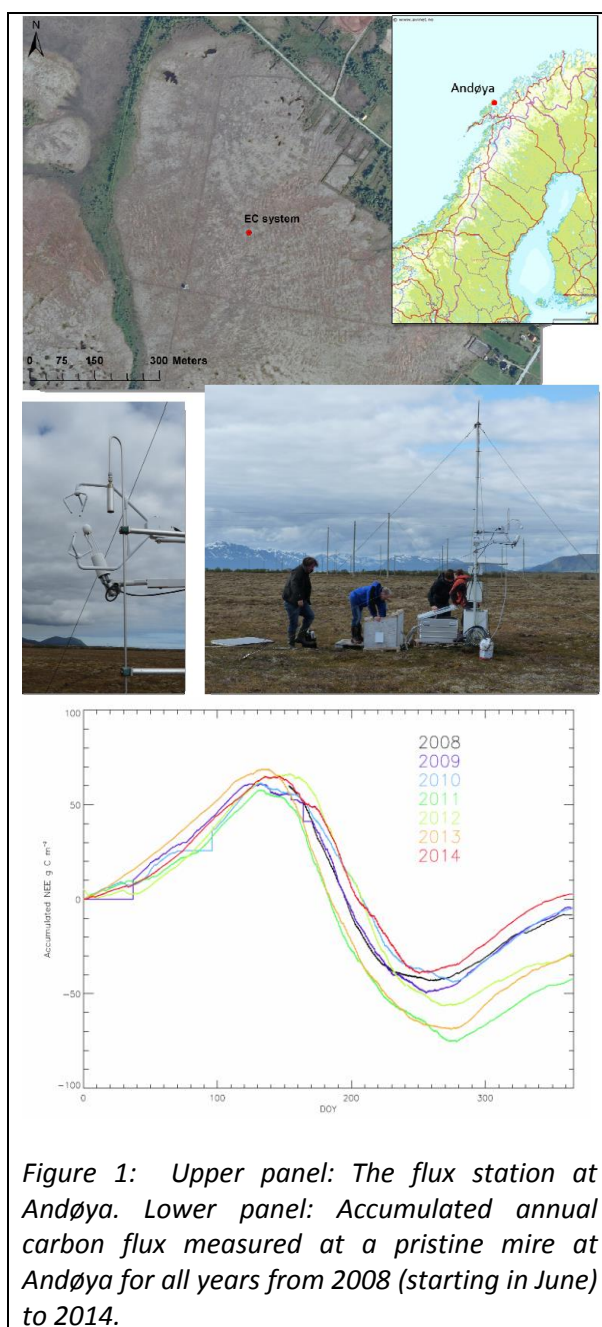
- Develop methods to integrate atmospheric measurements and inverse modelling in order to quantify the regional budgets of particularly atmospheric CH<sub>4</sub>. Attempt will also be made to quantify the regional budgets of N<sub>2</sub>O and CO<sub>2</sub>
- Ensure internationally harmonised greenhouse gas observations and CO<sub>2</sub> flux measurements linked to the international network ICOS.
- Further develop the institutional competence to fully utilize the recent strategic greenhouse gas observational investments at NILU to provide optimized results both for science and policy user communities

### **Summary of main results**

In GHG-Nor several approaches has been used to better understand the budget of natural greenhouse gases in the north and the atmospheric tasks were to:

1. Contribute to work and interpretation of the flux measurements at Andøya in close collaboration with Bioforsk
2. Measure and interpret atmospheric observations of CH<sub>4</sub>, <sup>13</sup>C-CH<sub>4</sub>, CO<sub>2</sub>, N<sub>2</sub>O
3. Combine regional observations with inversion modelling to determine the greenhouse gas emissions in the Nordic countries

## Contribute to work and interpretation of the flux measurements at Andøya in close collaboration with Bioforsk



Carbon flux measurements at the Andøya Eddy covariance flux site were continued until 31 August, 2014. Operations were terminated on 1 September because of missing external funding. All data have been analysed with the new algorithm developed in cooperation with Magnus Lund at University of Lund/University of Aarhus in early 2013. During the remaining period of the project, the flux evaluation algorithm used to evaluate the measurements is being implemented at NILU to enable future analyses of the complete Eddy covariance and high-resolution meteorological data set.

Figure 1 shows the accumulated carbon fluxes from all years between 2008 (from 3 June) and 2014 from this pristine Sub-Arctic oceanic mire. Large year-to-year variations are obvious with accumulated fluxes ranging from -41 g C m<sup>-2</sup> (uptake) to +2 g C m<sup>-2</sup> (release). So in general this mire is a sink for carbon in the Arctic environment. However, the absolute values are highly uncertain, mainly because of the instrument configuration, which causes severe uncertainties at air

temperatures below 5° C. The amount of carbon uptake through the year is very depend on the meteorological conditions (temperature, precipitation, sunlight duration) mainly during the growing season, but also winter conditions, such as freezing depth and surface icing may influence uptake in the following growing season. First results have been published in February 2015 (Lund et al., 2015); more detailed analyses are planned for autumn 2015.

## Measure and interpret atmospheric observations of CH<sub>4</sub>, <sup>13</sup>CH<sub>4</sub>, CO<sub>2</sub>, N<sub>2</sub>O in the Arctic

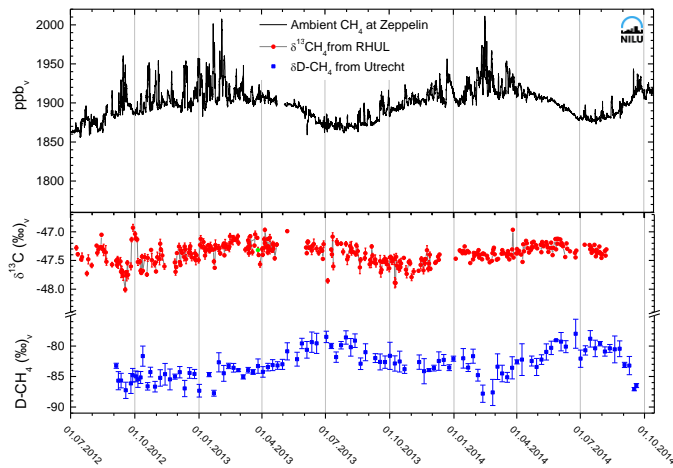


Figure 2: Upper panel; Ambient CH<sub>4</sub> measured at Zeppelin, 1 h resolution. Lower panel: Isotopic signature of CH<sub>4</sub> 5 days per week for <sup>13</sup>CH<sub>4</sub> and 2 days per week for <sup>2</sup>D-CH<sub>4</sub> since summer 2012.

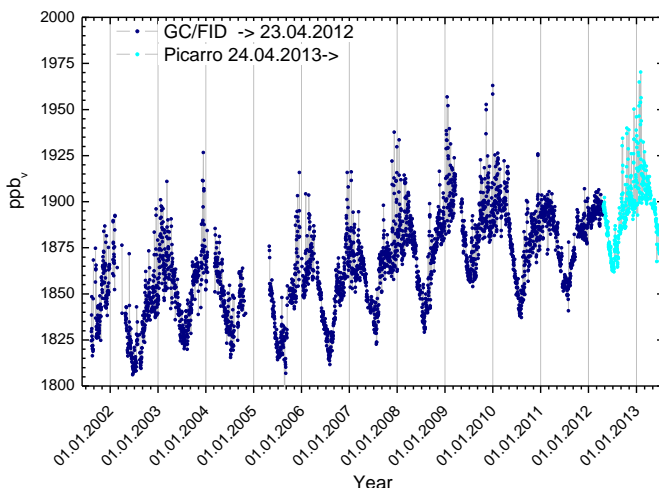


Figure 3: Daily methane observations at Zeppelin.

results show that wetlands in Siberia and changes in these seem to play a crucial role. Figure 2 shows an overview of these data.

There is considerable inter-annual variability in the CH<sub>4</sub> concentration, as shown in Figure 3, lower. This, to a large extent, represents inter-annual variability in the global background concentration. From the observations, it seems that there has been a special situation in 2010-2011 with very few episodes with high CH<sub>4</sub> at Zeppelin. This remarkable anomaly in the Zeppelin record has been investigated extensively, both by running 2 instruments in parallel and also by using an atmospheric transport model, FLEXPART. It was shown that this anomaly cannot be explained by atmospheric transport alone or instrumental

There has been a special focus on understanding the observations at Zeppelin, Svalbard. Here also measurements of <sup>13</sup>CH<sub>4</sub> and <sup>2</sup>D-CH<sub>4</sub> is a part of GHG-Nor (also supported by the NFR project GAME) to provide more information about the sources. Combined information about <sup>2</sup>D-CH<sub>4</sub> and <sup>13</sup>CH<sub>4</sub> is believed to be particularly valuable to distinguish emissions from hydrates from the ocean/sea floor, and wetlands from fossil sources. These observations have been ongoing since summer 2012, and the sampling frequency is 5 times per week. Analysing these observations together with model results, gives indications about the emissions contributing to the observed changes at Zeppelin. The work is still ongoing, but preliminary

issues, nor does the anomaly arise from a change in very local sources. Instead, it very likely indicates a change in a non-local CH<sub>4</sub> source. Since high CH<sub>4</sub> concentrations are associated with atmospheric transport from Western Siberia, it could well be that a source in this region was anomalously low in 2011. This is being further investigated using atmospheric inversion methods. New instrumentation at Zeppelin in 2010 allow for analysis of ethane together with CH<sub>4</sub>. This has also started the last year as enough data is now available. This will provide further information about sources together with the isotopes. Ethane and CH<sub>4</sub> has the same fossil sources, with a relatively stable ratio, but only CH<sub>4</sub> comes from wetlands. NILU is working on finalizing data analysis to publish this. This work is still in progress.

### **New measurements at Kjølnes Lighthouse in collaboration with UK**

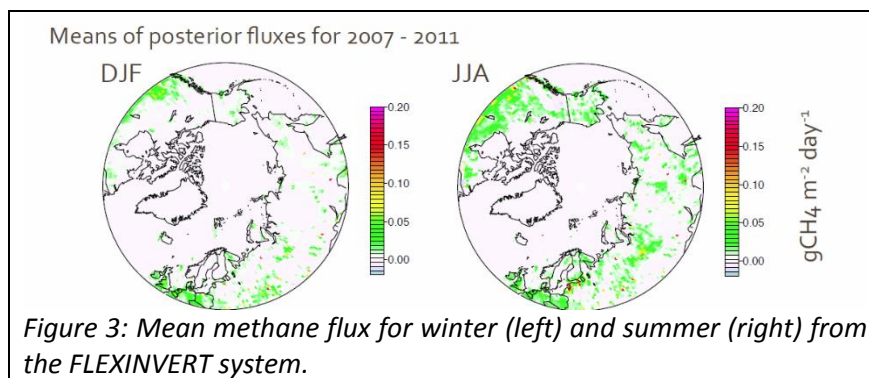


NILU has established contact with a group in UK (University of East Anglia, School of Environmental Sciences) and assisted and guided them in installation of CH<sub>4</sub>/CO<sub>2</sub> measurements at Kjølnes Lighthouse outside Berlevåg. In particular, the location selected by NILU is central with respect to input to models constraining the inverse modelling. The measurements are funded by UK, but NILU has access to these data, and information about the site is here: <http://kjolnes.co.uk>.

### **Quantify greenhouse gas fluxes on a regional scale by inverse modelling**

N<sub>2</sub>O and CH<sub>4</sub> from Zeppelin, with isotopic information, serve as central input for the model studies, together with other data from the Arctic region (e.g. Kjølnes lighthouse).

The inverse modelling system of Stohl et al. (2009) has been further developed by support of GHG-Nor. FLEXINVERT, for greenhouse gases (GHGs) and other atmospheric species, has been developed based on the work of Stohl et al. (2009). FLEXINVERT is capable of resolving fluxes temporally (e.g. weekly, monthly) and can account for temporal and spatial error correlations in the prior fluxes. These developments were necessary for GHGs that have biosphere fluxes, and considerable temporal variations, such as CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O. Additionally, the background concentration, i.e. the component of the concentration signal that is not accounted for in the period of the Lagrangian backward simulations (typically 10-20 days), can now be estimated by coupling to the output of a global Eulerian model. This combines the advantages of both the high-resolution, high accuracy Lagrangian model and the Eulerian transport model with the ability to run for many years (at much lower resolution). Inversions using FLEXINVERT have



been made for CH<sub>4</sub> in Europe (Thompson and Stohl, 2014) and East Asia (Thompson et al, 2014). Methane

inversions have also been made for the high northern latitudes for 2007 to 2011 and show higher emissions, relative to the prior, in Canada and Western Siberia, most likely owing to higher wetland emissions (paper in preparation), see also Figure 3.

Atmospheric inversions for N<sub>2</sub>O have been made globally using the PYVAR inversion framework in collaboration with the Laboratoire des Sciences du Climat et l'Environnement, Gif sur Yvette, France. PYVAR uses the Eulerian global transport model, LMDZ, and its adjoint, thus enabling the fluxes to be optimized at the resolution of the transport model, i.e., 2.5° × 3.75° latitude by longitude. The inversion was made for 1999 to 2009 and focused on understanding inter-annual variations in N<sub>2</sub>O fluxes and how these depend on climate (Thompson et al., 2014b).



## Publications

- Lund, C.M., Bjerke, J.W., Drake, B.G., Engelsen, O., Hansen, G.H., Parmentier, F.J.W., Powell, T.L., Silvennoinen, H., Sottocornola, M., Tømmervik, H., Weldon, S., and Rasse, D.P., **Low impact of dry conditions on the CO<sub>2</sub> exchange of a Northern-Norwegian blanket bog.** *Environ. Res. Lett.* 10 025004, doi:10.1088/1748-9326/10/2/025004, 2015.
- Thompson, R. L., Dlugokenky, E., Chevallier, F., Ciais, P., Dutton, G., Elkins, J. W., Langenfelds, R. L., Prinn, R. G., Weiss, R. F., Tohjima, Y., Krummel, P. B., Fraser, P., and Steele, L. P. (2013), Interannual variability in tropospheric nitrous oxide, *Geophys. Res. Lett.*, 40, 4426–4431, doi:10.1002/grl.50721
- Thompson, R. L., Stohl, A., Zhou, L. X., Dlugokenky, E., Fukuyama, Y., Tohjima, Y., Kim, S.-Y., Lee, H., Nisbet, E. G., Fisher, R. E., Lowry, D., Weiss, R. F., Prinn, R. G., O’Doherty, S., Young, D., and White, J. W. C. (2014), Methane emissions in East Asia for 2000 – 2011 estimated using atmospheric Bayesian inversion, in review, *J. Geophys. Res.*
- Thompson, R. L., and Stohl, A. (2014), FLEXINVERT: an atmospheric Bayesian inversion framework for determining surface fluxes of trace species using an optimized grid, *Geoscientific Model Development*, 7, 2223-2242, doi:10.5194/gmd-7-2223-2014
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## **SACC - Strategic Aerosol Observation and Modelling Capacities for Northern and Polar Climate and Pollution**

**Duration:** 01.01.2011 – 31.12.2015

**Budget 2014:** 1,602 Mill NOK

**Project leader:** Markus Fiebig

**NILU project:** B-111011

### **Background**

Atmospheric aerosol has a wide range of effects relevant not only for scientists, but also at the policy making level and for the general public. Atmospheric aerosol particles influence climate by scattering incoming solar radiation back into space (direct climate effect, cooling), and absorbing infrared radiation emitted by the Earth surface, thus heating the atmosphere (semi-direct effect, warming). They also influence climate by increasing cloud reflectivity and lifetime (indirect effect, cooling). In the net balance, atmospheric aerosol particles exert a cooling forcing on climate. The 5<sup>th</sup> IPCC assessment report identifies deficits in the understanding of the atmospheric aerosol climate effects as one of the most significant sources of uncertainty in climate predictions. Here, the deficits in understanding are significant for the direct aerosol climate effect, and even larger for the indirect aerosol climate effect. These uncertainties do not question the facts that climate has been warming, and that human activity is contributing significantly to this change. However, uncertainties in the magnitude of the atmospheric aerosol climate effect contribute significantly to the uncertainty in quantifying future climate change, and thus impact planning of mitigation measures.

Last, but not least, atmospheric aerosol affects human health by influencing the respiratory and cardiovascular system, leading to 300 000 premature deaths annually in Europe. While aerosol concentrations and properties are strongly influenced by local and regional sources in urbanised and industrialised areas, it is also transported on longer scales. Transport pathways go between the continents, but also pole ward from the source regions at lower latitudes.

### **Strategic Relevance**

The project answers the research needs specified in the relevant strategic documents, i.e. the “Prioritised research needs in the area of environmental management 2010 – 2015”, the 2009 – 2012 strategy of the Norwegian Research Council, and the Norwegian Parliament Announcement Nr. 30 “Klima for Forskning”:

- Better understanding of climate system, with focus on northern and polar latitudes, the effects of atmospheric aerosol, and changes in natural emissions
- Knowledge on long-range transported particulate matter (sources, chemical composition, effects) and consequences of climate change thereon

- Answer challenges on society posed by climate change, feed into national research focus on climate
- Foster international collaboration to meet challenges posed by climate change while underlining international excellence
- Extend capacities and competence in areas of strategic importance by building on areas where host institution is already strong
- Improve efficiency and international competitiveness by developing national and Nordic division of labour in climate research further while focussing on strong own areas of expertise/capacity building and national, Nordic, and international collaboration and networking
- Lasting effect of efforts by focussing on equipment and infrastructure (Forsk 2012), extend capacities for monitoring climate relevant parameters at Northern / polar latitudes for early detection of climate forcing and change signals
- Reduce uncertainty of climate predictions by focussing on largest uncertainty sources, aerosol-cloud interaction and the hydrological cycle

### **The project**

The project structure reflects the extreme range of atmospheric aerosol effects with scientific and social relevance. The project is organized in three work packages (WPs), each addressing a challenge identified as research priorities by the Norwegian Environment Agency and the Norwegian Research Council:

#### **WP1: Observations Tailored to Assessing the Indirect Aerosol Climate Effect**

Insufficient understanding of the indirect aerosol climate effect is one of the most significant causes for the uncertainty in current climate predictions. In order to improve the situation, climate models need to be constrained by data on the number concentration of cloud condensation nuclei in order to reduce their uncertainty. In regions well covered by such observations, these uncertainties will be reduced best. Before this project, corresponding measurements were conducted in Norway only intermittently, and only on Svalbard. For reducing the corresponding uncertainty specifically for continental Norway, but also elsewhere, this work package aims at establishing these observations at Birkenes observatory in Southern Norway, and collecting the corresponding data from stations in Europe and around the globe. During the previous reporting periods, the corresponding instrument, a Cloud Condensation Nucleus Counter (CCNC), was purchased, installed, and taken into operation at Birkenes. Further, templates were drafted for reporting the corresponding data from European stations and others around the globe to the WMO Global Atmosphere Watch World Data Centre for Aerosol at NILU and were iterated and accepted by community stakeholders. During the present reporting period, the first years of CCNC data were collected from stations around Europe in collaboration with the EU-FP7 research infrastructure ACTRIS. The CCN observations at Birkenes continued in side-by-side operation with an Aerosol Chemical Speciation Monitor (ACSM) instrument. An ACSM provides the chemical speciation of aerosol particles with the same high time resolution as CCNC measurements. The data are presently evaluated to quantify the connection between aerosol chemical composition and particle CCN properties.

This will be the key ingredient to reduce the uncertainty of the indirect aerosol climate in climate predictions.

**WP2: Global Transport Pathways of Particle-Bound Air Pollution with Focus on Southern Polar Latitudes**

Changes in climate become visible first at high and polar latitudes as compared to lower latitudes. With a national territory largely located at high latitudes, and also in connection with their focus on climate research, Norwegian authorities have placed special emphasis on polar climate research.

This work package addresses this emphasis by investigating data on aerosol properties collected at the NILU operated atmospheric observatory at Norway's whole-year Antarctic research station Troll. Subject of the investigation was the synchronous annual cycle seen in the baseline of microphysical (particle number size distribution) and optical (scattering coefficient) aerosol properties. The analysis showed that Central Antarctic baseline air is transported upward at mid-latitudes or in the tropics, transported to Antarctica in the upper free troposphere or lower stratosphere, and descends over Central Antarctica. The aerosol particles in Antarctic baseline air are largely produced by photochemical oxidation of precursor substances during this transport. The analysis is unique in its overview over these processes, and facilitates a better understanding of natural versus anthropogenic aerosol processes. It will contribute to a better distinction between natural and man-made climate change, and a better quantification of man-made climate change (Fiebig et al., *Atmos. Chem. Phys.* 14(6), 3083 – 3093, 2014). It was possible to achieve these results despite the fact that 80% or more of the data collected at the previous location of Troll Atmospheric Observatory needed to be disregarded due to local contamination by the station main buildings. The station was moved in January 2014 to a new location virtually uninfluenced by local contamination. The observations continue to elaborate the aerosol annual cycle further.

**WP3: Past, Present, and Future Air Pollution Transport to Norway**

Reliable source attributions of climate forcing agents and atmospheric pollutants are a prerequisite for target-oriented emission policies. The atmospheric observatory at Birkenes in Southern Norway, a cornerstone of Norway's air monitoring network, has been upgraded to an EMEP supersite and WMO GAW station in 2009. In this WP, this comprehensive set of observed aerosol properties is extended by the levoglucosan concentration. Levoglucosan is a highly specific tracer for biomass burning. In the present reporting period, the 2010 – 2012 dataset of microphysical (particle size distribution) and optical (aerosol scattering and absorption) properties at Birkenes has been analysed with cluster analysis to determine the dominating air mass types. In addition, the transport model FLEXPART has been used to determine the regions where the air masses in the detected clusters may have picked up pollution near the surface. The result reveals that at least 4 main air mass types can be distinguished: arctic/marine air, most often in autumn, winter, and spring; continental European air, most often in summer and late winter; air influenced by regional (domestic) wood burning, occurrence following the heating season; air dominated by particle formation from biogenic precursors, most often in summer. The regional wood burning cluster is present 45% of the time in winter,

and contains significant amounts of absorbing particles (black carbon). These will have a warming effect over most landscape types, especially over snow. This effect will be local, but can influence Arctic regions since Norway is a source region for Arctic air. The work in this WP will continue with looking at future transport scenarios obtained from the Nor ESM climate model.





## OrgSpec - Speciation and quantification of emerging pollutants

**Duration:** 01.01.2011 – 31.12.2015

**Budget 2014:** 1,7 Mill NOK

**Project leader:** Martin Schlabach

**NILU project:** B-111088

Analysis of complex mixtures in environmental samples is an extremely difficult task. In most cases, sample matrices require a complex sample preparation. Following that, ultra-trace analytical methods are developed for specific groups of substances. This traditional targeted approach provides excellent sensitivity and reliable identification and quantification of the analytes. However, unknowns and untargeted substances have been overlooked even when present at high concentrations. Therefore, non-target or screening methods are increasingly realized as an important tool in environmental chemistry. For organic pollutants, two complementary techniques have shown to be important and relevant. For the lipophilic and non-polar compounds, GC-based techniques like GCxGC/MS-ToF, are best suited, whereas for the more polar compounds different, LC-techniques normally give the best results. Both approaches were tested for a long range of different compounds in the OrgSpec-SIS.

In the first years of OrgSpec, NILU gave major focus to the study of ambient air samples from the Arctic that were analysed on potential new persistent organic pollutants (POPs) by GCxGC/ToF-MS. NILU analysed the samples for contaminants (e.g. chlorinated and brominated organics, PAHs, PAH analogs and nitro compounds) by applying advanced data filtration tools (VB Scripts). This technique allows detecting of compounds without a commercially available library of known mass spectra. The mass spec library, however, was used either to identify the detected compounds or to determine if further investigation was needed to identify the detected compounds. In addition, NILU examined the samples for potential new contaminants that were proposed by modelling approaches. By evaluating the model results, this can help to improve the quality of the available models, as well as develop highly sensitive “non-target” analytical methods for the identification of hitherto unknown POP-like chemical residues in the environment.

In the later years, NILU has given more focus to more polar compounds like pharmaceuticals, personal care products, and a huge range of industrial chemicals. Based on the application of LC/ToF and LC/Q-ToF techniques, NILU developed a similar non-target screening approach for these compounds of higher polarity. The separation capacity of even the most advanced LC-chromatography cannot be compared to GCxGC-separation. Furthermore, LC/MS-techniques are restricted by mass spectra with lesser structural information. Therefore, it is necessary to apply a more complex and time-consuming data treatment. In many cases, the structure of the compounds are

only tentatively assigned and more research is needed to confirm the identity of the compounds.

For a fast and effective development of the non-target screening approach, a strong international cooperation is required. NILU is collaborating in this field with other Norwegian, Scandinavian, and European universities and institutes. Most important during the last years, was the collaboration with NMBU in Ås with common PhD students and with Umeå University and NIVA in Oslo with common research projects. To improve the exchange of ideas, information, data, and mass spec libraries, NILU has also become a leading member of the European NORMAN network (Network of reference laboratories, research centres and related organisations for monitoring of emerging environmental substances).

During the last years, these established techniques has been used in a remarkable number of projects for Norwegian Environment Agency (Miljødirektoratet), Research Council of Norway (NFR), Norwegian Polar Institute, ØKOKRIM, other research institutes and different private organizations and customers. Non-target screening has proven to be a practical and useful tool for identification of unknown or new emerging environmental pollutants. NILU has been able to identify a huge number of new or earlier unrecognized contaminants in different environmental samples. The following compound classes were identified and partially quantified in these studies: pharmaceuticals and personal care products (PPCP) including perfumes and biocides, polymer additives and other compounds used in technical applications including bisphenols, phthalates/adipates, antioxidants, benzothiazoles/triazoles, pesticides, and halogenated compounds (prevailing chlorinated and brominated compounds).

Many of these compounds have been classified to be of environmental or health concern. These environmental or health concerns enforce reaction of the public, the marked and restrictions by national and international authorities. In most cases, however, these restricted compounds are replaced with compounds of similar technical properties, which often means also similar chemical, environmental, and health properties. Bisphenol A (BPA), for example, was recognised as an endocrine disruptor and there are concerns over its potential impact, particularly on the health of children and the environment. BPA was permitted for use in food contact materials in the European Union (EU). Recently, the restrictions on the use of BPA have forced the polymer industry to replace BPA with bisphenol S (BPS) in thermal paper and other products. Bisphenol F (BPF) and bisphenol B (BPB) are possible replacements in the production of epoxy resin and polycarbonate, and have already been detected in canned foods and soft drinks. In addition to these analogues, bisphenol AF (BPAF) is used in the manufacturing of phenolic resins or fluoroelastomers.

New techniques, developed under the OrgSpec-SIS, qualified NILU in performing a screening study for Norwegian Environment Agency for contaminants of

emerging concern that included new bisphenols. The overall objective of this program was to establish the occurrence and environmental impact of these new persistent organic pollutants in the Norwegian marine and freshwater environments, with particular focus on their potential to bioaccumulate. The study was conducted in collaboration with NIVA and beside bisphenol A (BPA), bisphenol F (4,4'-BPF and 2,2'-BPF), bisphenol AF (BPAF), bisphenol BP (BP-BP) and bisphenol S (BPS) were detected in comparable concentrations in effluent, sludge, leachate, sediment and biological samples. These bisphenols have a structural similarity to BPA and unfortunately, may have the same health effects as BPA.

The OrgSpec-SIS has proven that non-target screening is a practical and useful tool for identification of unknown or new emerging environmental pollutants. It is possible to identify huge numbers of new or earlier unrecognized contaminants in different environmental samples.



## **SCLF - Beskrive kilder, dannelse og transport av kortlevde klimadrivere ved bruk av nye avanserte målemetoder**

**Varighet:** 01.01.2013 – 31.12.2016

**Budsjett 2014:** 1,2 Mill NOK

**Project leader:** Wenche Aas

**NILU project:** B-113006

### **Mål**

Det overordnede målet med dette prosjektet er å karakterisere kildeopphav, transport, dannelse og prosessering av kortlevde klimapådrivere og deres forløpere ved å utnytte nye avanserte målinger på Birkenes kombinert med statistiske beregninger og modeller.

### **Bakgrunn**

Kortlevde klimadrivere, som partikler og ozon, og deres potensial for å bremse den globale oppvarmingen de førstkomende 20 år, har hatt stor oppmerksomhet de senere år. Disse komponentene er også viktige for effektstudier relatert til helse og terrestrisk natur. Effektive utslippsreduksjoner forutsetter god forståelse av hvor disse komponentene kommer fra og hvordan de omdannes og transporteres i atmosfæren. Det er store usikkerheter i dagens estimater av partikkelforurensning i Europa, spesielt siden en stor andel av partikkelmassen i stadig større grad ser ut til å bestå av karbonholdig materiale, som er dårlig karakterisert og som har et utall antropogene og naturlige kilder. Nyutviklede instrumenter gjør det mulig å spore kilder på en mer spesifikk måte enn tidligere, de gir kunnskap om andelen som er primært og sekundært dannet, samt til en viss grad fordelingen mellom naturlige og antropogene kilder.

### **Strategisk relevans**

En bedre beskrivelse av kildeopphav for kortlevde klimadrivere er av stor nytte for fremtidig politikktutforming, spesielt knyttet opp mot UNECE LTRAP og EUs AQD.

Nasjonalt er dette også et strategisk viktig tema. Økt kunnskap om klimaendringene og reduksjon av utslipp er sentrale behov slik det er beskrevet i «Miljøforvaltningens prioriterte forskningsbehov 2010 – 2015» og i forskningsmeldingen «Klima for forskning», St.meld. nr. 30 (2008-2009). Av relevans er også Miljødirektoratet nylige publiserte rapport med «Forslag til handlingsplan for norske utslipp av kortlevde klimadrivere» som beskriver tiltak for utslippsreduksjoner samt nasjonale overvåkingsbehov.

For NILU er overvåking og kjemisk analyse av luftforurensninger en av grunnpilaren i instituttets virksomhet. En strategisk satsning og videreutvikling innen dette feltet ved å utnytte nye avanserte målemetoder kombinert med

bedre statistiske verktøy er essensielt for at NILU skal beholde sin sentrale posisjon på dette feltet i Europa.

### Sammendrag av aktiviteter og resultater i 2014

Av nye avanserte instrumenter er det i hovedsak snakk om «Aerosol Chemical Speciation Monitor» (ACSM) som måler konsentrasjoner av partikulært nitrat, sulfat, ammonium, klorid og organisk karbon med høy tidsoppløsning og et «Proton-transfer-reaction mass spectrometry» (PTR-ToF-MS) instrument som måler flyktige organiske forbindelser (VOC). En ACSM ble installert på Birkenesobservatoriet i 2012 for permanent drift, mens en PTR-ToF-MS har vært brukt i to målekampanjer på Birkenes, sommer 2013 og vinter 2013 (Langebner et al., 2014).

Det har vært stort fokus på å sikre høy kvalitet på målingene som har blitt etablert. Dette innebærer å delta i feltsammenligninger, etablere kalibreringsrutiner og rapportering av data. Det har vært et nært samarbeid med EUs infrastrukturprosjektet ACTRIS på dette:

- I desember 2013 tok ACSM instrumentet fra Birkenes del i en europeisk interkalibrering i Paris med tretten andre laboratorier/stasjoner med tilsvarende instrument. To artikler med resultater fra denne interkalibreringen er sendt inn til *Atmos. Meas. Tech. Discuss* (Fröhlich et al. og Crenn et al., 2015).
- For å beskrive kildeopphav av forurensningen benyttes et statistisk verktøy spesielt utviklet for dette (Canonaco et al., 2013). Det har vært opplæring i bruk av verktøyet på en workshop i 2014 (Zürich desember 2014) hvor NILU deltok med to personer.
- Det har vært viktig å få på plass harmonisert europeisk datarapportering av ACSM observasjoner inkludert all relevant metadata til EBAS databasen som lagrer observasjoner for blant annet EMEP og ACTRIS. I samarbeid med Paul Scherrer Institute (PSI) har det blitt utviklet et templat for innlevering av data til EBAS:  
[\(<http://ebas-submit.nilu.no/SubmitData/RegularAnnualDataReporting/ACSMregular.aspx>\)](http://ebas-submit.nilu.no/SubmitData/RegularAnnualDataReporting/ACSMregular.aspx)

### Videre planer

- Det vil bli arbeidet videre med å bestemme kildekategorier ved å bruke observasjonene fra ACSM-instrumentet. Det har vist seg å være vanskelig å skille kildekategorier for de ulike oksygenerte organiske forbindelsene fra hverandre. For å bedre tolkningene, vil disse målingene kombineres med andre målinger som for eksempel levoglukosan, sukker og <sup>14</sup>C fra andre prosjekter, samt utnytte målinger som inngår i det tradisjonelle overvåkingsprogrammet på Birkenes, inklusive målinger av optiske, fysiske og kjemiske egenskaper for partikler. Dette vil kunne bidra til å si mer om omdanning og transport av kortlevde klimapådrivere og for eksempel, kvantifisere bidraget fra brenning av biomasse og skille mellom antropogene og naturlige kilder. De første resultatene fra dette vil bli presentert på European Aerosol Conference (EAC) i Milano i september 2015.
- Det vil bli arbeidet med en videre tolkning av PTR-MS-TOF målingene utført i 2012 og 2013, kombinert med kjemisk transportmodellering (EMEP), for å estimere andelen av lokale mot langtransporterte forurensninger av primære- og sekundære partikler og ozon på Birkenes.

- Utnytte Flexpart transportmodellering for å bestemme kilderegioner for biomasse brenning ved å bruke levoglukosanmålingene som er gjort på Birkenes. Det planlegges å publisere disse dataene i løpet av høsten 2015.
- Det vil bli installert et nytt ToF-ACSM instrument på Zeppelin i løpet av høsten 2015. Kvalitetssikring og tolking av disse dataene vil bli viktig i 2016.



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## AMOM - Advanced modelling of organic contaminants/Avansert modellering av organiske miljøgifter

**Duration:** 01.01.2013 – 31.12.2017

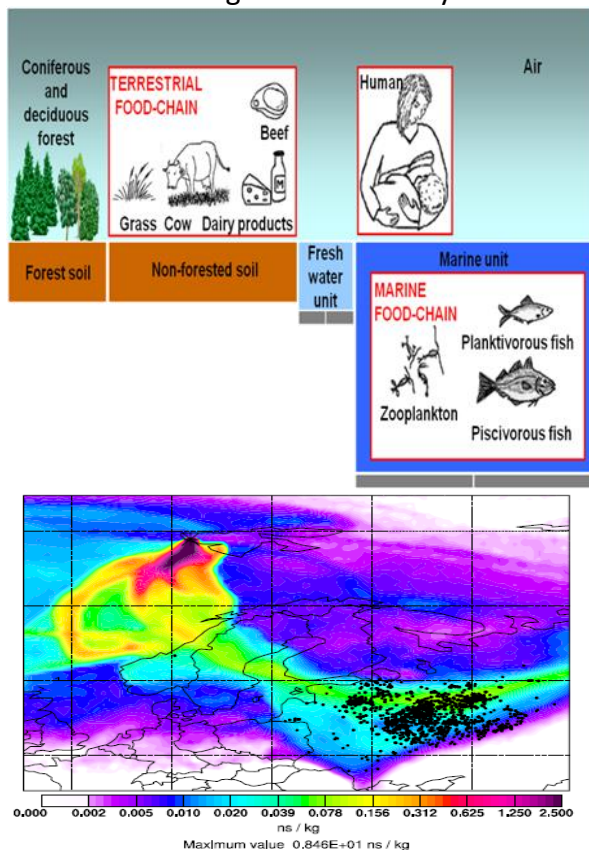
**Budget 2014:** 1,2 Mill NOK

**Project leader:** Knut Breivik

**NILU project:** B-113014

### Main objective and key modelling tools

The overall goal with the AMOM SIS is to understand and predict relationships between sources and emissions of organic contaminants and environmental and human exposures. A particular emphasis is on the long range atmospheric transport of various organic contaminants. CoZMoMAN and FLEXPART are the two core modelling tools in focus in the AMOM-project (Figure 1). Main activities and results during 2014 are briefly summarized below.



**Fig. 1.** Potential emission sensitivity (PES) footprint map (0–100 m) for air arriving at Zeppelin between 1 May 2006 at 10:14 UTC and 3 May 2006 at 08:38 UTC 2006. Black dots show MODIS fire detections on days when the footprint emission sensitivity in the corresponding grid cell on that day exceeded  $2 \text{ ps kg}^{-1}$ .

**Figure 1:** Complementary modelling tools at NILU to study organic contaminants. Left: Model structure for the CoZMoMAN multimedia model [1]. Right: Illustrative output from FLEXPART whereby record high levels of PCBs measured at Zeppelin (Svalbard) in combination with satellite data (black dots) are traced back to biomass burning events in Russia [2].

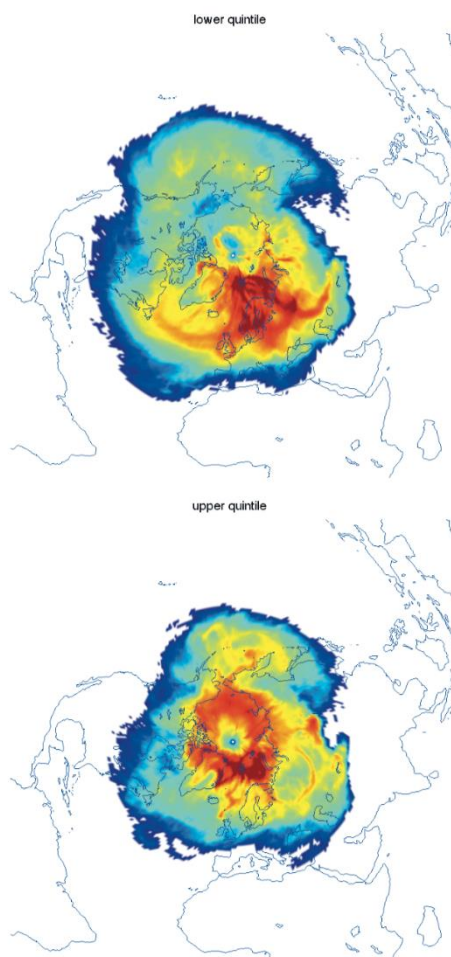
## Main activities and results during 2014

### FLEXPART

FLEXPART is a model for atmospheric transport representing the Lagrangian trajectories of a large number of particles in the atmosphere. These particles, (which can be tracked forward or backwards in time) are driven by Eulerian (three-dimensional) wind fields such as those produced by meteorological prevision or climate models (e.g. ECMWF is the main source of input data). The backward capabilities of FLEXPART can be exploited in order to analyse episodes of high and low concentrations of POPs at Zeppelin and relate those episodes to specific primary or secondary (over land, ocean or ice) sources.

The footprints produced by FLEXPART are 2D surface fields proportional to the transition probability of an air parcel from the **source** regions (over sea or land) to the location of the instrumental samplings (the **receptor**). The footprints are useful because of the property that if multiplied with the emission fluxes of a species (the source), they provide an estimate of the increase of the concentration over the background at the receptor location and time. We calculated the footprints for all available POPs measurements at Zeppelin since 2000, extending the partial case studies performed in 2013.

In the first place we have selected for a case study the footprints corresponding to the peaks and the background concentrations (higher and lower 20 % of the measurements time series respectively, Figure 2) of  $\Sigma$ -HCH at Zeppelin in 2007. The higher panel shows that the lowest concentrations are associated with air masses originated in the European continent. In contrast, the peak concentration correspond to air masses exposed to the high Arctic latitudes.



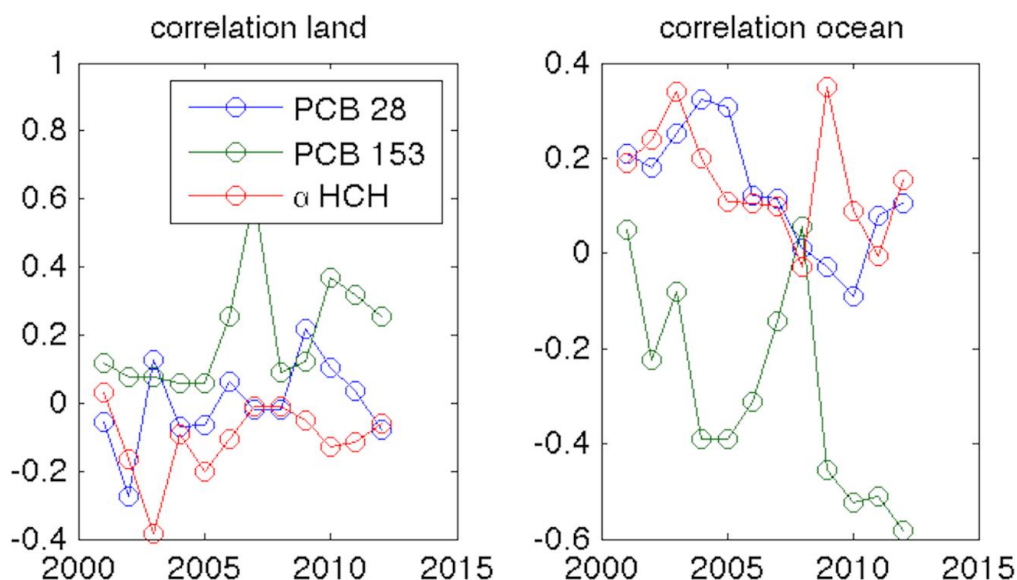
**Figure 2:** *Integrated footprints associated with the  $\Sigma$ -HCH peak and the background concentrations (higher and lower 20 % of the measurements time series respectively) at Zeppelin in 2007. Upper panel: lower quintile of concentrations or background, the transport influence is from southern continental masses. Lower panel: upper quintile of concentrations or peaks, the transport influence is from the Arctic region.*

This case study of pure transport calculations focusing on time integrated peak vs. lowest concentrations supports the hypothesis of large influence of

oceanic and/or cryospheric secondary outgassing emissions for  $\alpha$ -HCH.

In addition, in order to assess the temporal trends we multiplied the individual footprints of every available measurement with a land mask and a sea mask. The footprints multiplied with the land/sea masks represent the influence of the continents and the ocean onto the Zeppelin measurement site. For every available year the resulting time series was compared with the time series of measurements (for three representative POPs: PCB 28, PCB 153 and  $\alpha$ -HCH) yielding one correlation coefficient per species per year. Therefore we obtained six time series for the 2000s of correlation coefficients between measurements (3 species) and source region influence (land and sea, Figure 3).

The degree of confidence of the trend analysis is currently low due to a low signal to noise ratio. It doesn't nevertheless rule out the existence of trends for some POPs (e.g. PCB 28). Additional analysis with more species or measurement sites may be required to increase the level of significance (not shown) of the statistical studies.



**Figure 3:** Time-series of the correlation coefficients (one per year) between the influence of the continents and the ocean onto the Zeppelin station's measurements (calculated multiplying all the footprints and land/sea masks) of three representative POPs: PCB 28, PCB 153 and  $\alpha$ -HCH. Left panel: using land mask. Right panel: using sea mask.

In 2014, we also initiated the studies with more sophisticated analysis applying inverse methodologies for flux optimization. Our preliminary results indicate that the estimates depend strongly on the uncertainty of the POPs primary emission fluxes.

### CoZMoMAN

CoZMoMAN is a dynamic and mechanistic integrated multimedia model aiming to describe the link between environmental emissions and levels of organic contaminants in both the environment and in the human food-chain. The

CoZMoMAN model was developed at NILU in close collaboration with scientist from University of Toronto and Stockholm University.

Last year, AMOM supported the completion of a study exploring the utility of the CoZMoMAN model to predict human exposure of individuals to PCBs. We consider the results of this important as the deterministic simulation describes mechanistically the steps in a PCB molecule's journey from initial production and release into the environment to its uptake and accumulation in individual Norwegians. Equally important, a comparison with multivariate statistical approaches also helps to identify processes which should be considered included in CoZMoMAN in the future. The manuscript by Therese H. Nøst and co-workers was provisionally accepted in *Environmental Health Perspectives* in December 2014.

During 2014, AMOM supported efforts to finalize an international collaborative effort, involving scientists from the Universities of Stockholm and Toronto, to screen thousands of high production volume chemicals for their potential enrichment in the environment and the human food chain. In 2014, a paper was published in *Environmental Science and Technology*, examining 215 siloxane compounds more closely. The model identified three of them as possible “new” environmental contaminants. The researchers at Stockholm University developed the necessary analytical methods and gathered environmental samples from the field in both Sweden and Norway. Their measurements established that all three of the assumed siloxane contaminants were present in at least one sample – and one substance was detected in all samples [3]. In 2014, we have also made progress on expanding the application of *in silico* methods to screen for potential new and hitherto unrecognized organic contaminants, albeit in a Nordic context. This is approached by addressing discrete organic chemicals included in the SPIN (Substances in Preparations in Nordic countries) database ([www.spin2000.net](http://www.spin2000.net)).

The end of 2014 also resulted in a successful evaluation of a grant proposal submitted to the RCN “Økosystem” call. The new project “*Development, evaluation and application of a nested exposure assessment model for organic contaminants in the Nordic and Arctic region (NordicExposureModel)*” aims to greatly expand and further improve the CoZMoMAN modelling tool and thereby generate significant synergies with AMOM in the years to come.

## References

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## **TOXROS - Chemical and toxicological characterization of reactive atmospheric species**

**Duration:** 01.01.2013 – 31.12.2016

**Budget 2014:** 1,2 Mill NOK

**Project leader:** Elise Rundén Pran

**NILU project:** B-113064

### **Background and objectives**

The impact of emerging indoor and outdoor pollutants and newly formed functionalized products on human health has never been comprehensively studied under conditions that are close to the real situation, mainly due to the need for interdisciplinary expertise from tropospheric, analytical and quantum chemistry, as well as from cell biology and toxicology disciplines. A reliable and representative *in vitro* model for respiratory exposure has also been lacking.

The objective of this interdisciplinary project is to develop a realistic *in vitro* respiratory model for lung exposure. This model will be used for toxicity studies by inhalation exposure to study health effects of indoor and outdoor air pollutants. To obtain knowledge about health effects of emerging pollutants and functionalized mixtures of short lived organic pollutants, it is essential to develop a combined exposure-effect model, mimicking real exposure on cells of first target such as lung. The final aim is to investigate health effects of various chemical pollutants by exposure of human cells *in vitro* in an air-liquid interphase (ALI) model, under well-controlled and characterized exposure conditions. We aim to study potential toxicity of chemical pollutants as emerging pollutants, functionalized particles, nanoparticles and reactive volatile and semi-volatile organic compounds, as well as mapping underlying mechanisms of toxicity and development of disease.

### **Objectives:**

1. Develop an advanced *in vitro* human lung model for inhalation exposure that is as close as possible to the real situation (Air-Liquid Interphase (ALI)-model, CULTEX)
2. Develop an exposure system (controllable and measurable) for the lung model
3. Study toxicity and underlying mechanisms of toxicity of:
  - a. emerging pollutants
  - b. functionalized particles and reactive volatile
  - c. semi-volatile organic compounds
  - d. Nanoparticles

## Work progress in 2014

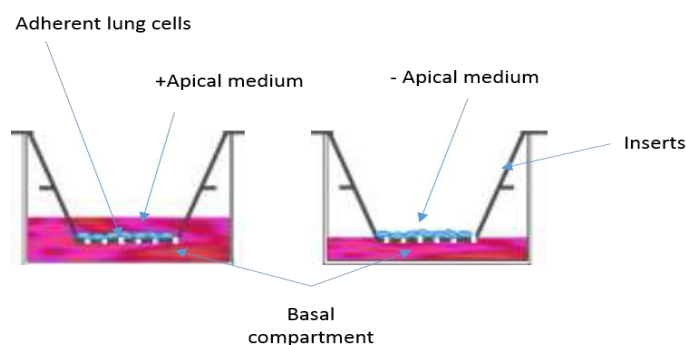
### Development of ALI model for lung exposure

#### Cultivation of human lung cells on membranes at the air-liquid interphase

To reduce toxicity testing on animals and at the same time use models as close as possible to humans, *in vitro* human cell models are widely used in toxicology. For inhalation studies, a challenge has been that the cells are cultivated and exposed submerged in cell culture medium. However, *in vivo*, the lung epithelial cells are exposed to liquid on one side and air on the other. Thus, an *in vitro* respiratory model closer mimicking the *in vivo* situation would then be comprised of cells cultivated on the interface between air and liquid, as the lung cells are separated from the air only by a thin aqueous lining layer with a surfactant film at the air-liquid interface.

Therefore, our main aim was to set up an air-liquid interphase (ALI) model based upon CULTEX<sup>®</sup> exposure system for mimicking atmospheric, respiratory exposure conditions. The ALI-exposure model is technically challenging because: i) the cells must be adapted to new conditions, ii) a defined aerosol at constant composition and flow has to be generated, iii) exposure concentrations must be measurable, and iv) the temperature and humidity conditions for the cells should be at physiological level for the whole exposure period.

We have during this period **developed and validated protocols** for cultivation of human lung epithelial cells under normal conditions, and have also adopted the selected A549 human lung epithelial cells for cultivation on transwell membranes for the ALI-system. In this system, the cells are growing at the air-liquid interface (ALI), where the upper part of the cells is directly in contact with air and the basal part of the cells is directly in contact with liquid medium, similarly as *in situ* in the lungs (figure 1). This configuration mimics the conditions found in the human airway, and drives differentiation of the cells towards a mucociliary phenotype. We have set up conditions for testing of different media and for growing cells on different semiporous transwells, in which the cells exhibit morphological and functional characteristics similar to the human epithelium.



**Figure 1.** Human adherent lung epithelial cells (A549) seeded onto porous cell culture inserts (transwell) with submerged cells (medium in both basal and apical part) (left), and cells directly exposed to air from above (apical medium removed) and fed from the medium below (air-liquid-interphase (ALI) (right).



### Testing of exposure system for the ALI-cells - CULTEX

For exposure of the cultivated ALI-cells, a sort of cultivation chamber is needed. Therefore, we developed a protocol for cultivation of A549 cells in the CULTEX device. In this set-up, transwell inserts with cells were transferred from the conventional system and placed into their corresponding position inside the CULTEX device (figure 2) before the testing.

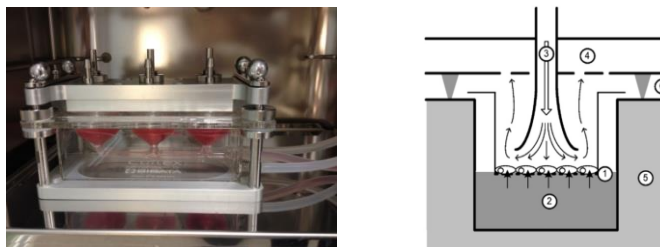


Figure 2: CULTEX device for ALI exposure of lung cells.

For adaptation of the cells to ALI conditions before exposure, the cells were cultivated with reduced serum concentration to slow growth before removal of apical medium (figure 3). In our testing, up to 6 hours, cells cultivated on transwell membranes without apical medium were found to tolerate these conditions, and their viability was above 80 %. Cytotoxicity assays were performed and validated for the A549 cells both for conventional cultivation, submerged cultivation on membranes and on membranes without apical media (ALI). We found no significant differences in cells viability with the different cultivation methods. Standard operating procedure was written for cultivation, adaptation before exposure, as well as for proper cytotoxicity assays.

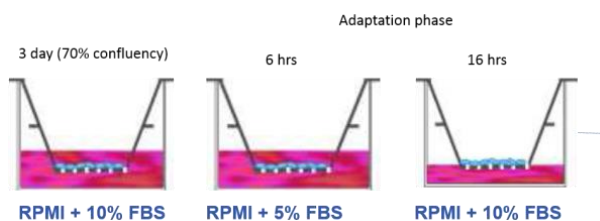


Figure 3: Adaptation of A549 cells for ALI exposure.

### Exposure of cells in the CULTEX system

The cells cultivated at the ALI needs to be exposed to the pollutants and compounds in a controllable manner. It is important to be able to measure what the cells are being exposed to, as well as the exposure concentrations. We therefore tested an exposure chamber with a flow control and a calibration unit. The cells were exposed to synthetic air as a control (figure 4).

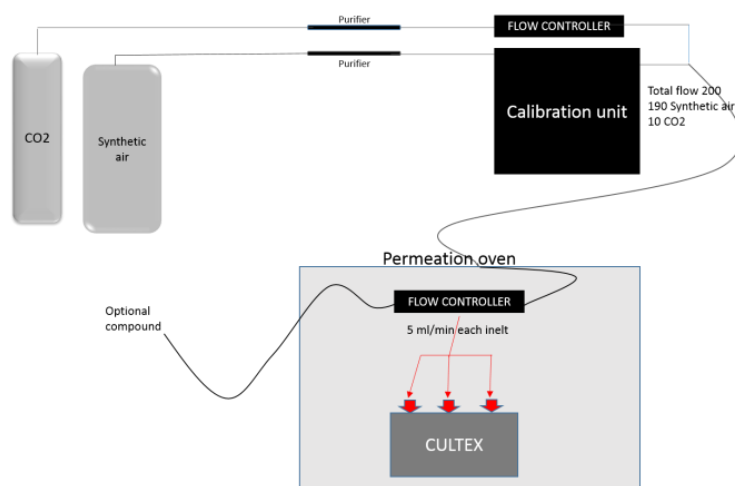


Figure 4. Preliminary exposure set up for CULTEX

Draft protocol for CULTEX exposure for this exposure system was developed. There are different possibilities for exposure systems for CULTEX. We have been visiting other laboratories working on ALI systems for getting increased knowledge on this challenging aspect of the model. We have also attended an ALI workshop in Berlin, in which many issues and challenges connected to both cultivation of cells and exposure were discussed efficiently.

### Studies of exposure and uptake of nanoparticles

For exposure of the cells with nanoparticles, uptake of the nanoparticles into the cells is an important issue. We performed some preliminary studies with submerged exposure of the A549 cells to  $\text{TiO}_2$  nanoparticles, and studied uptake by our laser scanning microscope. We were able to visualize the nanoparticles, as well as intracellular uptake in some of them. The A549 cells were clearly apoptotic after exposure, showing the cytotoxicity of the nanoparticles tested (figure 5). Procedure for both uptake of nanoparticles and measurement of oxidative stress (ROS production) by live cell imaging using Confocal microscope was developed.

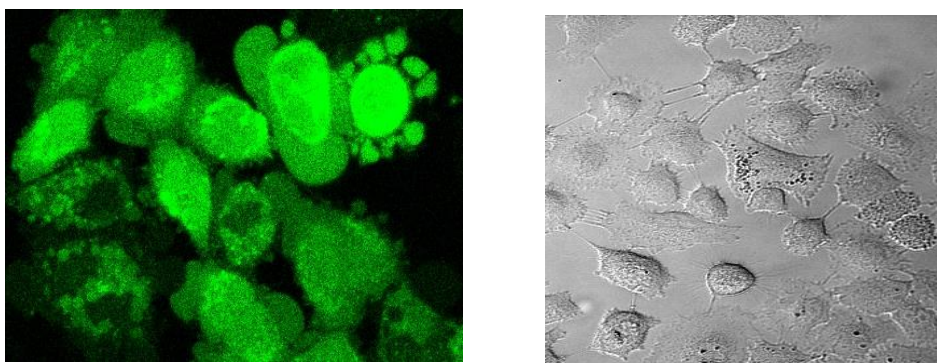


Figure 5. Uptake of nanoparticles into A549 cells. Images obtained by laser scanning microscope (Zeiss LSM 500 confocal microscope). In the left image,  $\text{TiO}_2$  nanoparticles can be seen as black dots on top of and around the cells. Also, it is seen how the cells stretch out filopodia to reach each other in a survival strategy. On the right, the cells are labeled with a fluorescent probe (cell tracker green), and are apoptotic (programmed cell death).

**Summary of results**

- Protocols for ALI cultivation of A549 human epithelial lung cells were developed and validated
- Protocols for cytotoxicity assays, oxidative stress and genotoxicity were developed and validated for the ALI model
- Different systems for exposure of the cells were studied
- Exposure chamber was set up and the cells exposed to synthetic air as control
- Exposure of A549 cells to nanoparticles under conventional submerged system was performed. Uptake of the nanoparticles into the cells, as well as ROS production, was studied by confocal microscopy
- Draft review paper on the ALI model for respiratory exposure was completed
- Attended ALI work shop in Berlin

**Further progress**

We want to develop the culture model further, to even more closely resemble the in situ situation in the human lungs, by establishing an advanced and complex co-culture system. Thus, we want to include immune cells (macrophages) in the model, and plan to set-up THP1 cell line (human peripheral blood model - monocyte-macrophage cell line) in co-culture with A549 to closer mimic the real situation. THP-1 cells have been received in the laboratory, and master cell bank is being created.

1. Develop and validate protocols for cultivation of THP-1 cells
2. Establishing co-cultures of A549 and THP-1
3. Development of improved exposure system for CULTEX
4. Selection of atmosphere and nanoparticles to test
5. Cytotoxicity testing of different chemicals on A549 cells by CULTEX system
6. Cytotoxicity testing of different chemicals on A549 and THP-1 cells in co-culture by CULTEX system
7. Uptake of NPs in the cells after exposure (confocal microscope)





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