Project information

Project title
Contaminant effects on energetics
Year
2013/2014
Project leader
Heli Routti, NPI
Participants

Project leader(s)/institution:

- Heli Routti (NP)

Project participants/institutions **(during 2013):**

- Mikael Harju
- Dorte Herzke (NILU)
- Lisa Helgason (NILU)
- Ingebrigt Sylte (UiT)
- Anders Goksøyr (UiT)
- Roger Lille-Langøy (UiB)

MSc-students: Mari K. Berg (UiB), Camilla Aas (UiT)

Flagship

Hazardous substances, Theme: The impact of climate change on transport and fate of contaminants in the Arctic.

Funding Source
Fram Centre, UiB, NPI, UiT
Summary of Results

Highlights:

- In silico methods indicate that several brominated and fluorinated compounds bind to human and polar bear PPARγ
- In vitro results show that several POPs have potential to activate human and polar bear PPARs
- In vitro results show that extracted and composed mixtures of contaminants have different effect on differentiation of mouse preadipocytes

In silico:

We have modelled binding affinity of over 600 chemicals to polar bear and human PPARγ. The results indicate that several brominated and fluorinated compounds bind with similar or higher affinity than known ligands to polar bear and human PPARγ. Models for polar bear PPARα are run but we still have to process the results.

In vitro:

In vitro reporter gene assays: Polar bear PPARγ and PPARα have been sequenced. Luciferase reporter gene assays have been established for human and polar bear PPARγ and PPARα. The results of testing 15 individual POPs and 4 mixtures of POPs with luciferase assay show that several POPs have potential to activate human and polar bear PPARs.

Mouse preadipocytes:

We have tested the effect of the mixtures extracted from polar bear tissues and mixtures composed of standards (equivalent to polar bears) on preadipocyte differentiation in vitro. Results indicate that exposure with extracted mixtures have an increasing effect on fat accumulation, while the effect mixtures composed of standards varies.

Polar bear preadipocytes:

Fat biopsies of three polar bears from Svalbard were taken in September 2012 during the fieldwork for the NFR project BearEnergy.

Isolation of fat cells for further in vitro investigations has been done in collaboration with Aalborg University.

In vivo:

Polar bears: We have collected samples of 112 polar bears as a part of NFR project “Synergistic effects of sea ice-free periods and contaminant exposure on energy metabolism in polar bears (BearEnergy)”, nr. 216568/E10, in spring and autumn 2012 and 2013. Metabolomics is going to be run next year.

Arctic fox (funding applied for 2012): MSc-student Camilla Aas has performed PFAS analysis to look at the effect of fasting on PFAS distribution in tissues using Arctic fox as a model species. Statistical analyses are going on.

For the Management

The results of this project will provide information about the two main threats to the arctic ecosystem, namely climate change and contaminants. The results are highly relevant for the environmental monitoring of Svalbard and Jan Mayen (MOSJ) program and AMAP. Knowledge about the effects of climate change and contaminants will give input to international conventions (Stockholm Convention, REACH) as well as national acts and regulations (Climate and Pollution Control Agency) to prohibit the use and production of chemicals and to slow down emission of greenhouse gases.

The results of the project will be of high interest for human health issues. Recent reports indicate that these disorders are associated with increased human exposure to contaminants, and emerging need for further research is. Our results will strongly contribute to understanding about relationships between contaminant exposure and health problems in humans.

Published Results/Planned Publications

We have several planned publications based on the in silico and in vitro testing.

Research papers:

Castelli MG, Rusten M, Goksøyr A, Routti H. mRNA expression of genes regulating lipid metabolism in ringed seals (*Pusa hispida*) from differently polluted areas. Revised version with minor revisions submitted to Aquatic Toxicology.

Berg MK, Lille-Langøy R, Goksøyr A, Rusten M, Routti H. Characterisation of PPARs in polar bears (Ursus maritimus) and their role as target receptors for environmental pollutants. Manuscript under preparation.

MSc-thesis:

Mari K. Berg. Peroxisome proliferator-activated receptors (PPARs) in polar bear (*Ursus maritimus)* as target receptors for environmental pollutants. 2013. University of Bergen

Martina Galatea Castelli. Expression of genes related to lipid metabolism in ringed seals. 2012. Università Politecnica delle Marche, Italy

Conferences:

Routti H, Rusten M, Harju M, Berg MK, Lille-Langøy R, Goksøyr A. Effect of contaminant mixtures found in polar bears on fat cell differentiation *in vitro.* Norwegian Environmental Toxicology Symposium. Tromsø, Norway, 16-18.10.2012. Presentation

Castelli MG, Rusten M, Goksøyr A, Routti H. Expression of genes regulating lipid metabolism in ringed seals (*Pusa hispida*) from differently polluted areas. Norwegian Environmental Toxicology Symposium. Tromsø, Norway, 16-18.10.2012. Presentation

Helgason LB, Kristiansen K, Gabrielsen M, Routti H, Lille-Langøy R, Berg M, Sylte I. In silico modeling of the peroxisome proliferator-activated receptor gamma and emerging persistent organic pollutants. Norwegian Environmental Toxicology Symposium. Tromsø, Norway, 16-18.10.2012. Presentation

Berg MK, Rusten M, Eidsheim S, Lille-Langøy R, Goksøyr A, Routti H. Cloning of peroxisome proliferator-activated receptors in polar bear (*Ursus maritimus*) - target receptors for environmental pollutants? Norwegian Environmental Toxicology Symposium. Tromsø, Norway, 16-18.10.2012. Poster

Routti H. Arukwe A, Gabrielsen GW, Jenssen BM, Kanerva M, Letcher R, Nyman M, Rusten M. Contaminant effects on multiple endpoints in ringed seals - conclusions from a 5 year study. IPY 2012, Montréal, Canada, 22-27.4.2012. Poster

Routti H, Rusten M, Harju M, Goksøyr A. Effect of contaminants on polar bear fat metabolism *in vitro.* IPY 2012, Montréal, Canada, 22-27.4.2012. Poster

Communicated Results

Invited speaker (Heli Routti) at the Institute of Medical Biology, UiT. 5.10.2012. Effects of environmental contaminants in arctic wildlife – from mechanisms to ecological relevance

Invited speaker (Heli Routti) at Sysselmans kunnskapseminar, Svalbard. 13.11.2013. Effects of pollutants – from novel mechanisms to ecological relevance

Interdisciplinary Cooperation

This project absolutely benefits of the inter-disciplinary cooperation. The different techniques (in silico, in vitro, in vivo) are completing each other in order to get a wider understanding about contaminant effects on energetic and mechanisms involved. This project includes both physical and analytical chemistry, molecular and cell biology, physiology and ecology.

Budget in accordance to results

Fram Centre funding (2013: 285 000) has covered the salary costs for Mari K. Berg for 3 months and a small part of the helicopter costs. Own financing (UiB) has covered 2 months salary for Mari K. Berg and NP and NFR have covered the majority of the sampling costs of polar bears 2 000 000 and 700 000, respectively + costs of salaries at NP. UiB has covered the salaries of Lisa Helgason and Ingebrigt Sylte. Fram Centre funding has been necessary for the in vitro part of the study.

Could results from the project be subject for any commercial utilization

No

Conclusions

a) Indicate future research and/or perspectives which the project results have led to

Our future plans are

1. finish the ongoing work on in silico modeling and test the highest ranked compounds in vitro
2. Test in vitro how mixtures of contaminants affect differentiation and gene expressions of polar bear preadipocytes
3. Screen a wide range of current used chemicals in polar bears

Relate the contaminant exposure in free-ranging polar bears to the metabolome.

b) List and describe new methods or techniques that have been developed during the project or that the project has revealed a need for

In silico:

We have built up an extensive library of new chemicals for this project that can now been easily used for other in silico studies.

In vitro:

The extraction method used in this study by NILU is a new combination of known methods. The methods used in *in vitro* preadipocyte studies have previously been used by other laboratories; the methods have been implemented to UiB due to this project. A new method of has been established at UiB, namely luciferase reporter assay to include polar bear PPARs. These methods are both valuable tools to assess the metabolic effects of pollutants.