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Hair and feathers as indicator of internal contamination of ^{210}Po and ^{210}Pb

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March 2010

Abstract

The activities of the NKS-B HAIRPOL project is summarised in this report. The objective was to investigate if hair and feathers were suitable matrices for the estimation of the intake of ^{210}Po. Human hair from people of different sex and age was analysed for ^{210}Po showing concentrations between 0.4 to 11 Bq/kg dry weight. Samples from horses, mane, fur and tail showed concentration from 6 to 17 Bq/kg with no significant difference between the different sample types. Musk ox from Greenland showed much higher concentrations since the animal has to graze a large surface. In fur the concentration was 260 Bq/kg. A considerable fraction of the total ^{210}Po in this animal is contained in the hair. Also different organs were analysed and the highest concentration was found in kidney, 2 700 Bq/kg. The ^{210}Pb concentration in hair was estimated to about 20 Bq/kg. Three different seabirds from Svalbard were analysed. Feathers from all three seabird species show increasing activity concentrations of ^{210}Po and ^{210}Pb from the base to the tip of the feather, but it was difficult to relate feather concentrations to muscle concentrations due to a number of complicating factors.

Key words

Hair and feathers as indicators of internal contamination of ^{210}Po and ^{210}Pb

Final Report from the NKS-B HAIRPOL activity (Contract: AFT/B(08)9)

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1. Introduction.

Polonium-210 is a radio-ecologically interesting natural element to investigate due to its high radiotoxic characteristics. This radioactive element is, together with radon, the natural radioactive material delivering the highest natural dose to human. Several studies have been conducted to investigate the human response and coupling to ^{210}Po in nature (Thomas *et al*. (2001); Santos *et al*. (1994)). Several suggestions have been made that urine and feces should be collected when the body burden of polonium is to be analyzed. Not much work has been done considering to analyze alternative material, to monitor polonium in man and animals. Several radio analysts consider using urine and feces as analyze object when an internal contamination of an alpha emitting radio isotope has occurred. It is commonly known that urine and feces are considerably difficult to sample, store and handle, and hard to digest and homogenize in the radiochemical preparations. An easier approach to study internal contamination has been suggested to use hair as analyze object. Hair is easier to collect and store, and the preparations and procedure for radiochemistry is considerably easier and less time consuming than foregoing suggestions.

Human hair consists of 45% Carbon, 7% Hydrogen, 28% Oxygen, 15% Nitrogen and 5% Sulphur. The composition of bird feathers is quite similar. Under normal conditions hair contains 20-220 ppm Fe, 10-20 ppm Cu, 190 ppm Zn and 0.6 ppm I. The water content is about 12% at room temperature. The thickness of hair is 20-180 μm and the growth rate about 1 cm per month. The data vary to some extent with sex, race, hair color, age and season of the year. Hair contains a kind of keratin which is insoluble in water. Keratin consists of a large number of amino acids (18 different) where cysteine is the most important one.

The central core consists of polypeptide chains with hydrogen and disulphide bindings. The disulphide bindings are the strongest ones and can not be broken by heat or water. Keratin is also present in bird feathers, hoofs, nails, claws, and antlers.

Human hair has been used as indicator for internal contamination especially for heavy metals at industrial exposure (Bencko, 1995, Nowak, 1998, Patra *et al*., 2007, Rodushkin 2000 and others). The hypothesis is that the contamination sis mainly through oral intake but that direct incorporation is possible.

Natural radioactivity has been shown to be present in wool with a good correlation to concentrations in grass and soil (Kulwich *et al.,* 1960, Saracevic *et al*., 2003).This leads to the possibility that radioactivity is present on clothes. Schreckhise and Watters (1969) reported a remarkably high fraction of total Polonium in the hair from goats (96%). It has been shown that bird feathers can contain relatively high concentrations of ^{210}Po depending on species (Skwarzec and Fabisiak, 2007)

Sulphur in hair has been used as detector of fast neutrons through the reaction ^{32}P(n,p)^{32}P (Lebaron-Jacobs *et al., 2007*).

Natural ^{210}Po constitute the highest dose from alpha emitters to man. ^{210}Po is very radiotoxic, with a specific activity of 166 TBq/g and a dose factor of about 7×10^{-7} Sv/Bq at oral intake. A deadly dose of 2 Sv would then correspond to 3 MBq of Polonium or 0.02 ug. The recent murder of a Russian person by putting ^{210}Po in his tea motivates to think about Polonium in term of emergency situations. ^{210}Po is a daughter product of ^{210}Pb. The specific activity of ^{210}Pb is lower, 3.5 TBq/g and has the same dose factor. At the analysis and assessments the build up of ^{210}Po from ^{210}Pb must be considered.

If we can relate an activity concentration in hair to a certain activity level in an internal soft tissue or an organ, it could be possible to use human hair as a bio indicator to trace irregular amounts originating from a high polonium intake. It might be possible that human hair and hair from animals and bird feathers in general constitute good indicators for internal contamination and can quantify the general environmental levels of certain radioactive elements. A hypothesis is that sulphur plays an important role if there is a chemical similarity or they can form sulfides. Several heavy metals have such characters i.e. Zn, Cd, Hg, Cu, Ag, and also Po, Pb, U, and Tc. The presence in hair and feathers would then give a quantification of the internal

contamination. Also elements associated to proteins, i.e. Sr is present in hair and might reflect the internal contamination of radioactive Strontium. (Della Rosa *et al.,* 1966, Morita *et al.*, 1986). While it is not likely that it is a good indicator for radiocaesium.

From sampling, analytical and hygienic point of view it would be an advantage to study hair instead of urine and faeces. In an ongoing project funded by SSI, Polonium is taken orally by 3 persons (^{209}Po) and urine and faeces is analyzed. Hair from these persons will also be analyzed.

Po-210 in human hair

Fredrik Henricsson, Elis Holm

Introduction

Polonium-210 is a radiological interesting natural element to investigate due to its high radiotoxic characteristics. This radioactive element is, together with radon, the natural radioactive material delivering the highest natural dose to human. Several studies have been conducted to investigate the human response and coupling to ^{210}Po in the environment (Thomas *et al.,* 2001, Santos *et al.,* 1994). The Uranium-238 decay chain present in the earth's crust continuously produces ^{222}Rn which may be transferred to the atmosphere where it moves with continental air masses and eventually forms ^{210}Pb, ^{210}Bi and ^{210}P.e.g. radioactive lead and polonium into the biosphere. These radionuclides, are then deposited by wet and dry deposition onto terrestrial vegetation and is therefore present in vegetation and animal products at much higher concentrations than if the only pathway would be direct root-uptake through soil. Due to a combination of the long half-life of ^{210}Pb (22y), relatively high concentrations in food products and in air and the relatively high adsorbed fraction in the gastrointestinal tract and the alpha emitting ^{210}Po the Pb-Bi-Po is responsible for a considerable fraction of our annual radiation dose. The reason for the high accumulation from direct uptake of polonium into the body is polonium's affinity for protein and hence passes easily through the food chain into the human body. Protein rich food contains the highest amount of activity which is reflected in results from measurements of people with diet of this category (Hunt et al., 1993). The most of the activity which enters the body orally and reaches the gastrointestinal tract is eliminated via excreta (Stannard, 1964). The amount of polonium and radioactive lead activity adsorbed into the blood stream is transported though the body system and distributed throughout the soft tissues of the body (Fellman et al., 1994). Since it is the ^{210}Po, which delivers the main dose of the Pb-Bi-Po due to its alpha decay, the behavior of this isotope is of major interest. Human biomonitoring has been a subject since the early 1930's with the main matrices being urine and blood (Angerer et al., 2007). The advantage in using blood is the contact of blood with all the metabolic active parts of the body. Changes in the polonium body burden should thus be reflected in blood. The disadvantage of using blood is the small amount of sample available. Suitable analogs to help understand the pathways in the human body are lacking. Group VI elements including selenium are having same chemically characteristics as ^{210}Po and hence is believed to have the same characteristic pathways and behavior in biological systems (Waska *et al.,* 2007). The pathways of Po-210 in the body is however complicated. The concentration of ^{210}Po in hair is partly dependent on the biokinetics of polonium in the body but also on the biokinetics of lead where Pb-210 is providing supported levels of ^{210}Po.Apart from direct uptake of ^{210}Po through food, water and air it may be produced in the body by decay of ^{210}Pb which in turn is produced by Ra-226, both mainly residing in the skeleton. Although lead accumulates in bone, the bones may also constitute a source for lead due to an increase in bone turnover. Analysis of lead in bone have been compared with those in blood but diseases related to adverse effects of lead are better reflected by the concentration in bone than in blood (Esteban and Casteno, 2009). Barbosa *et al.* (2005) estimated that between 45-75% of the lead found in blood originated from bone in a population not excessively exposed to lead. If assuming the biokinetics of ^{210}Pb to be the same as stable lead the available studies on lead in hair and its correlation with body content may be used. Earlier data on lead in blood may however be questionable due to poor detection limits. Recent data, all based on analysis using ICP-MS, shows that non-exposed individuals in general have lead concentrations in blood-plasma less than 1 ppb (Barbosa *et al.,* 2006). Contamination during sampling and sample treatment also introduce increased blank levels. Hair to blood ratios of lead has varied extensively in studies where both matrices have been included.
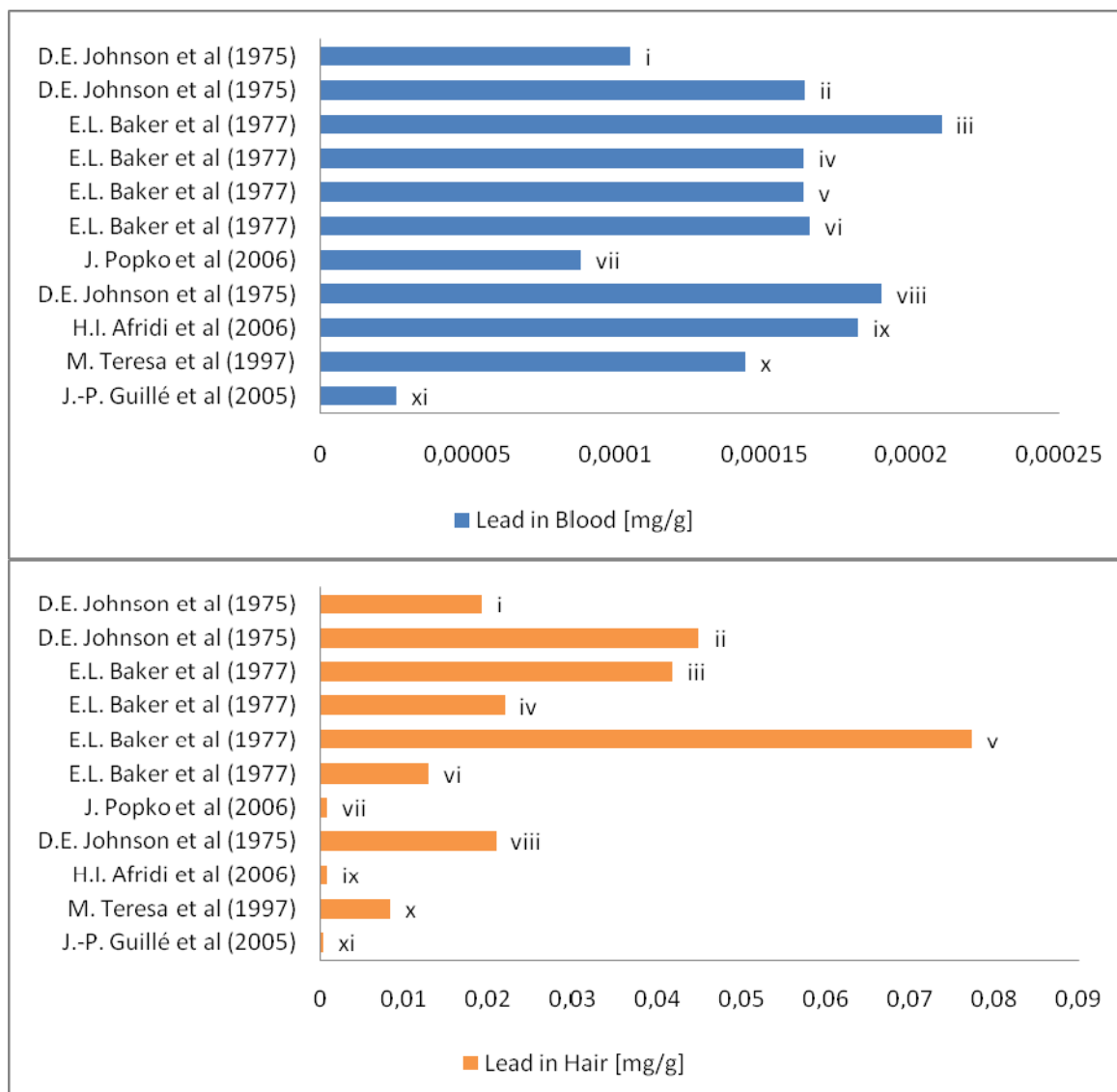
Figure 1. *Measurements of stable lead in human blood (blue bars) and human hair (yellow bars) from different studies.*

The studies in figure 1, where stable lead has been investigated in hair and blood originating from different kind of healthy average population and healthy industry workers, shows a very fluctuating hair to blood ratio. Conclusions based on these studies may be difficult due to the resulting differences in hair-blood ratio. A hair-blood correlation is hard to determine since hair concentrations reflect a long time period (growth rate of hair about 1 cm per month) while blood concentrations may be sensitive to transient changes in food intake. Rodrigues *et al.* (2008) found only a weak correlation (r = 0.22; p<0.001, n=280) between lead in hair and blood in a Brazilian population. On the average, concentrations where about 22 times higher in hair than in blood on a weight basis. Weak correlations between lead in hair and blood was also reported by Stupar *et al.* (2007) and Wilhelm *et al.* (2002).

Several suggestions have been made that urine and feces should be collected when the body burden of polonium is to be analyzed. Not much work has been done considering reviewing alternative analyze material to monitor polonium in human beings. It is commonly known that urine and feces are considerably difficult to store and handle, and relatively hard to digest and homogenize in the radiochemical preparations when only using wet-ashing. Due to the volatile nature of polonium dry-ashing in a muffle furnace is not possible. An easier approach to study internal contamination has been suggested to use hair as analyze object. Hair is an excellent matrix in human biomonitoring. It prevents numerous advantages such as easy collection,

transportation, storage and procedure for radiochemistry is considerably easier and less time consuming than foregoing suggestions. The only real major disadvantage is the difficulty in distinguishing between internal and external exposure. Other disadvantages are potential difficulties in variation with hair colour, race, curliness etc. which may reflect different kinetics and incorporation of trace elements in hair due to variation in hair protein content. In general, the lack of knowledge about kinetics of trace element incorporation in hair and correlations between hair and other body organs, especially correlation with hair content and health effects. The risk from external exposure introduces the need to clean the hair extensively before analysis. This step to some extent limits hair as a simple matrix to work with. Sources of exogenous contamination are deposits of serbum, sweat, aerosols and residue of cosmetic or pharmaceutical products. There are numerous studies on inorganic as well as organic pollutants in hair. Relatively few studies have focused on building correlations to other biomonitoring matrices such as blood and urine or to body content. In spite of this, hair has been used widely to assess trace element exposure through analysis of wild life and human hair (Schmacher *et al.*, 1991 and 1996, Wilhelm *et al.*, 1994 and 2002). The idea behind this study is to investigate if hair can be used to monitor the activity level of the polonium body content. If we can relate an activity concentration in hair to a certain activity level in an internal soft tissue or an organ, it could be possible to use human hair as a bio indicator to trace irregular amounts originating from a high polonium intake. The incorporation of polonium in hair is however not widely studied. The correlation between body burden and export to hair should be preferably being known. Also if some pools in the body are more important than others in transferring polonium to hair.

Methods
Human hair samples were collected from 4 females and 22 males in ages between 3-65 years. 4-5 grams of sample were prepared for each sample and then carefully washed in soap and alcohol to separate them from fats and different forms of pollution. They were completely dried in oven in 60-80 ˚C for 24 hours before homogenization. After the samples were collected from the drying process they were weighted to identify the dry weight of the samples. Approximate 0.1 Bq of ^{209}Po 209 was added as a yield determinant to each sample pre-analysis. To digest the samples further they were preheated with 10 ml nitric acid. When temperature 80-90 degrees was maintained, the additional acids were used to further dissolve the hair matrix. 10 ml of hydrochloric acid and 5 ml of hydrogen peroxide were added to the sample solution. The mix of reagents and homogenized sample were left to react in a glass beaker on heater for several hours. When the appropriate clear solvent solution was reached, the samples were considered ready for polonium extraction via electrochemical deposition. Plating procedure was conducted according to plating method adopted by Flynn (1968). Polished silver discs were used as plating material. 1 gram of ascorbic acid was finally added to the prepared solutions to reduce the ionizing state of the competing iron ions and to ensure maximum plating output of polonium onto the discs. The silver discs were arranged and mounted in plastic disc holders' in contact with the solutions and heated to 60 degrees to initiate the plating process. Plating was conducted for 2 hours by the spontaneous auto deposition process. The silver discs with the extracted polonium were analyzed by alpha spectrometric system CANBERRA SiO_2 *passivated implanted planarsilicon* (*PIPS*™) model A detectors installed in CANBERRA vacuum chambers. The active area of the detectors is 300 mm^2 with a minimum depletion depth of 140 microns. Information sent from MCA in the system is handled and analyzed by computer software *Genie 2000*.

Results
The activity concentration of Po-210 in analyzed hair was in the range of 0.5 – 4.8 Bq/kg for humans in age 3 – 32 and in the range 0.5 – 11.5 Bq/kg for humans in age 32 – 60 (Table 1). These results indicate slight higher activity content in hair from the older group of humans.

This may be due to the parent nuclide ^{210}Pb, which is known to accumulate in the body over time and should give an extra contribution of ^{210}Po to the body. However, the numbers of study objects are far too few to draw any conclusions on this. The age is only one factor affecting the polonium concentration and should be considered together with issues concerning eating habits, smoking, drinking etc. No particular investigation has been conducted in this study to connect the different results with the individuals living environment, diet or smoking habits.

Age	Gender	^{210}Po activity [Bq/kg]	Uncertainty ± [Bq/kg]
3	F	4.18	0.50
22	M	1.49	0.10
22	M	2.57	0.16
22	M	1.97	0.13
22	M	2.96	0.14
22	M	2.03	0.15
22	M	1.33	0.16
22	M	4.09	0.61
22	F	1.99	0.15
22	M	3.73	0.33
25	F	0.40	0.35
28	M	3.01	0.21
28	M	3.40	0.66
30	M	1.44	0.26
30	F	4.76	0.30
30	M	3.05	0.49
30	M	3.68	0.65
32	M	4.87	0.81
32	M	2.30	0.69
32	M	0.53	0.14
32	M	8.02	0.23
40	M	7.08	0.80
60	M	11.48	0.41
60	M	3.69	0.18
60	M	5.78	0.60
60	M	4.97	0.69

Table 1. Po-210 concentration in hair from different individuals ordered in age of participants

From this study we can establish that hair is a highly qualified material to analyze, which makes it a very good complement to urine and feces as bio indicators to indicate the polonium content in the human body. The aspects on the fact that hair is good as an analysis material relies on background from present study where hair has been investigated to complement earlier established matrices used to monitor bioaccumulation of radio nuclides (H.E. Silberstein *et al,* 1950, G.J. Hunt *et al,* 1993, W. B. Li *et al*, 2008). The transfer factor of supported polonium from in-growth of ^{210}Pb in human bone is known and is responsible for 60 % of the total polonium body burden (Santos *et al*, 1994). An important parameter to further analyze is the blood-hair transfer coefficient for polonium. A study of analyses of time integrated blood samples correlated with hair analysis gives information that can be used to calculate the transfer rate of polonium from blood to hair. From these facts it should be possible to calculate the polonium amount in hair that is accumulated due to an internal build up from incorporated lead in human bone. Further animal analyses are required to be able to couple the hair activity to i.e. internal organ of the body. Investigations have been made in a biokinetic study where a

given oral intake has been connected with the activity output in nails, urine and feces. From these results it can be clarified that measurable quantities can be established that can be reflected in an internal body contamination. If an unintended internal intake of polonium has occurred by suspicion, one can later by donating hair for a radiochemical analyze, settle by measurements the average activity quantity in the body. Further radiobiological investigations could, based on these results, deliver a radiological assessment, which includes dose calculations.

Po-210 in hair from musk ox and horses

Per Roos

Introduction

Hair analysis as a non-destructive monitoring tool for metal exposure assessment has a long tradition in human toxicology (e.g. Coleman *et al.*, 1967; Bencze, 1990) but has had only limited use in wild mammals. Consequently, reports on the use of hair as a monitor for radioisotopes in mammals are very rare. Advantages and disadvantages in analysing hair from animals are otherwise roughly similar as when using human hair. Advantages being high concentrations relative to what are found in urine or blood, easy way of analysis and no need to sacrifice the animal. A further advantage compared to humans is that absolute concentrations are generally higher and more hair is available for sampling. Also, due to the huge collection of preserved animals in museums worldwide the possibility of doing retrospective studies using hair from animals is relatively simple (e.g. Horton *et al.*, 2009) although the effect of the tanning process on elements in the hair should be evaluated. The main disadvantage is just as with human hair the difficulty in interpreting the results and relating them to body burden and the difficulties to separate external contamination from what is carried inside the hair strands. Just as with human hair other mammalian hair is predominantly made up of keratin, a fibrous structural protein partly composed by the amino acid cysteine which is rich in thiol groups with high binding affinity for many metals. Even though each hair shaft is in contact with the blood stream at the hair root, and thus may incorporate metals circulating in the blood during growth, external contamination may dominate for some elements. This is a particular problem when sampling hair from animals where the general loading of soil and dust is far worse then in human hair. Washing procedures for animal hair may thus need to be more extensive than for human hair. Also the sampling strategy for animal hair must include what type of hair that has a minimum risk of external contamination.

In spite of the risk with external contamination sufficient evidence exists to suggest that mammalian hair is an appropriate indicator of metal bioavailability. Even though literature on radioisotopes in animal hair is lacking we may use available data on stable lead as a proxy for ^{210}Pb behaviour. This may not be correct since the stable lead and ^{210}Pb in the body may originate from slightly different pools due to a fraction of ^{210}Pb being produced from ^{226}Ra and also due to that ^{210}Pb intake through respiration is more pronounced relative stable lead. There are however studies showing that there is a correlation between stable lead in hair and body organs such as liver and kidney among mammals. Beernaert et al. (2007) found a significant linear correlation between hair and liver and kidney for lead in Wood mouse. Similarly D'Have *et al.* (2006) found a positive linear relation between liver and hair for several metals, including lead, in European hedgehog. The concentration ratios of lead in hair to liver in the animals studied were in the range of 2-5 for wood mice and 0.2 for hedgehog. Clearly, hair concentrations of lead may be comparable to concentrations even in the most exposed organs in animals. If the same holds for ^{210}Pb is difficult to say but information from stable lead may be indicative.

Materials and Methods

In this study samples from horse and musk ox were analysed. Samples of hair from tail, fur and mane from 2 horses (2, and 7 years old) were collected during spring 2009. Hair from tail were cut close to the root, fur was brush-collected while pieces of mane were pulled out directly. The tail and mane were cut in intervals from 2 to 7 centimetres depending on distance from the root. All samples were cleaned using soap and water before analysis. Polonium-210 was analysed using digestion of 2-3g material in HNO_3 in the presence of ^{210}Po- tracer. Following digestion of the samples plating was done on silver discs which were then subject to solid state alpha spectrometry. All results are decay-corrected back to the day of sampling.

A 3 year old female musk ox in the Thule area was collected by local hunters during spring 2009 (1 April). Internal organs and other body-parts were separated and placed in plastic bags on site. In late April samples arrived to Risø laboratory were they were identified and later freeze-dried but not grounded and homogenised. From each sample analysis of ^{210}Po were done using sample weights 0.2-10 g depending on sample. Hair and hooves were thoroughly washed using soap and water. For bone-samples all flesh was carefully removed by manual means. Digestion of samples was done first using nitric acid at progressively higher heat and then adding hydrogen peroxide and Fe^{2+} as catalyst. After digestion samples were plated on silver discs and counted for 1-3 days on PIPS-detectors. ^{209}Po was used as yield tracer. All results were decay corrected to 1 April 2009.

Results and Discussion

The results for ^{210}Po analysis in hair samples from the two horses are shown in Table 1 below. Somewhat surprisingly no clear trend in ^{210}Po concentrations were seen in tail and mane. Also concentrations are rather low. The fact that the animals had spent about 6 months indoor feeding mainly on hay and fodder with unknown age and Po/Pb content may obscure trends in the hair. No data on ^{210}Pb are yet available. From the data it is unclear if the long tail and mane hair can be used to monitor past exposure to Po. A study requiring details on intake and/or body content is required.

2 yr horse	**Po-210 [Bq/kg] dry weight**
Fur	7
Tail 0-2cm	9
Tail 2-4cm	11
Tail 4-7cm	8
Tail 7-12cm	10
Tail 12-17cm	7
Mane 0-4	6
Mane 4-8	6
7 yr horse	
Fur	10
Tail 0-2cm	17
Tail 2-4cm	11
Tail 4-7cm	9
Tail 7-10cm	14
Tail 10-13cm	9
Tail 13-17cm	12
Tail 17-21cm	16
Tail 21-26cm	9
Tail 26-33cm	10
Tail 33-40cm	11
Mane 0-4	21
Mane 4-8	16
Mane 8-12	12
Mane 12-16	13

Table 1. *Po-210 in fur, mane and tail from horse. Errors are typically between 8-15%.*

Concentrations of ^{210}Po in the musk ox studied are shown in table 2. Compared to the samples analysed from horse the musk ox data show concentrations in hair nearly two orders of

magnitude higher. Due to the very low area density of food (kg/m^2) in the area musk oxen need to feed over large areas thus reflecting the high body content of ^{210}Po. Obviously a large quantity is transferred to the fur which has a considerable size of these animals and therefore probably holds a significant part of the total animal inventory of ^{210}Po. The concentration of ^{210}Pb in hair was determined to be around 20 Bq/kg as determined from older hair collected in the region from mixed animals. Analysis of ^{210}Pb in the various samples except hair still remains to be done. This overestimates the concentrations presented in Table 2 somewhat. However, preliminary measurements done using gamma spectrometry on ashed samples indicates concentrations less than 50 Bq/kg of ^{210}Pb in kidney and liver which should mean a minimal influence on the ^{210}Po content reported

Organ/Tissue	Po-210 [Bq/kg] dry weight
Hair	260
Hoof	110
Kidney	2669
Liver	570
Flesh	90

Table 2. *Po-210 in organs and tissue from a 3 yr old musk ox from the Thule area, NW Greenland.*

The concentration of ^{210}Po found in the studied musk ox kidney are among the highest recorded for terrestrial mammals of any kind. Traditionally high levels of both ^{210}Po and ^{210}Pb are found in Reindeer or Caribou. The food chain lichen-reindeer-man is one of the most studied simply because the habit of reindeer to eat lichens and the great interception ability of lichen for atmospheric deposition. Typical values of ^{210}Po in flesh and liver/kidney have been around some tens of Bq/kg and some hundreds of Bq/kg respectively. High concentrations of ^{210}Pb are found in bone where it has not been unusual to find levels up to a kBq/kg and even above. Studies of Reindeer or Caribou where fur have been included are rare. Thomas and Gates (1999) included fur in their studies of natural radioisotopes in Caribou living around areas with uranium mineralization in Canada but the data on fur should possibly be taken with some care. The average concentrations in fur, meat, kidney and liver of the studied animals were 126, 42, 710 and 948 Bq/kg respectively (dry weight). Handling of the fur was not mentioned which introduce some doubts regarding the risk due to external contamination, which the authors also mention. In Greenland musk ox and reindeer share the same habitat. Two previous studies on ^{137}Cs and ^{90}Sr transfer to these two animals in Greenland showed that transfer was lower to musk ox than reindeer (Aarkrog *et al*., 2000; Strandberg, 1997). Something, which was believed to be related to the different food habits where reindeer had a higher proportion of lichens included and also that musk oxen are less selective in their food habits.

Interestingly, the musk ox is considered genetically mainly related to the goat and to the authors knowledge the only study on transfer too hair from administrated polonium published in peer reviewed literature is a study of polonium in animal hair on a goat (Schreckhise and Walters, 1969). In this unusual study a single ingestion of 4.2 μCi (155 kBq) ^{210}PoO$_2$ were administrated to a lactating goat and the concentration of polonium in various body organs and hair was analysed after 20 days. The results showed that hair was the tissue having the highest concentration of polonium, more than 6 times higher than was found in kidney and nearly 15 times the concentration found in liver. The collected hair in the study was allowed to grow on a 400 cm^2 area on the side of the animal where the older hair was shaved off in connection with the polonium being administered. The goat is a ruminant and the risk of the ingested polonium

partly coming back to the mouth is large. This in connection with the probable interest in licking at the newly shaved area may significantly overestimate the internal transfer to hair.

As a comparison to the levels found in the studied musk ox we may compare with an animal from the same area which normally is considered to hold relatively high levels of ^{210}Po due to its food habits. Analysis of ^{210}Po in meat and liver from seal collected in Bylot Sound at Thule resulted in concentrations of only 10 and 40 Bq/kg respectively (Nielsen, 2006). These are relatively low concentrations and significantly lower than what is found in seals from the Baltic Sea where Holm *et al* (2006) measured 50-400 Bq/kg of ^{210}Po in liver. Hair is found also on seals and studies have been conducted where selected heavy metals have been analysed both in body organs as well as fur. In a study by Medvedev *et al.* (1997) a range of metals were studied in seals from Lake Ladoga and the White Sea, from their data on lead in ringed seal it can be calculated a ratio hair to liver as high as 10. In fact, for all element studied except mercury the highest recorded concentrations were found in hair even though organs such as liver and kidney were included.

Also hair from other animals were analysed (Table 3). There is an assumption that the body burden of ^{210}Po depend on type of food, the metabolism but also how large area an animal must graze in order to obtain enough food.

Animal	Po-210 [Bq/kg] dry weight
Moose (n=2)	3.0
Hare (n=1)	2.3
Wild boar (n=1)	16.6
Roe deer	14.7
Sheep (lamb) (n=3)	6.9

Table 3. Po-210 in hair from different animals

Conclusions

Results from analysis of hair from horse showed no trend along hair strands which either indicate mainly supported ^{210}Po, a highly variable intake through food or transfer to the hair with time or mobility within the hair strands. Potentially a combination of all causes. For musk ox concentrations in hair is about two orders of magnitude higher than horse and ^{210}Po/^{210}Pb ratio around 10. If levels in hair can reflect concentration in meat a ratio hair to meat of about 3 can be used.

On the possible use of feathers as indicators of ^{210}Po body burdens in seabirds

Justin Gwynn, Agata Zaborska, Torbjørn Gäfvert

Introduction

Birds have been utilised as biomonitors for environmental pollution for decades as they are sensitive to environmental changes, are susceptible to bioaccumulation due to their position in food chains and are generally well studied with regard to their ecology, physiology and behaviour. Seabirds are particularly useful as biomonitors of marine pollution because they are exposed to a wide range of chemicals, occupy high trophic positions and may show lower coefficients of variation for contaminants than fish or marine mammals (Gilbertson *et al.*, 1987).

As interesting target bird species are often protected, non-invasive techniques are preferred to minimise any potential detrimental impacts on the birds sampled. In this regard, feathers may provide a useful non-invasive biomonitoring tool as they can be sampled easily without long lasting harm to the bird. Numerous studies have documented the use of feathers from various bird species for the biomonitoring of heavy metals (e.g. Goede & de Bruin, 1984; Burger, 1993; Hahn *et al.*, 1993; Denneman and Douben, 1993; Dauwe et al., 2002, 2003; Muralidharan et al., 2004) and more recently for organic pollutants (Dauwe et al., 2005; Jaspers *et al.*, 2006, 2009; Van den Steen et al., 2007). In particular, Monteiro and Furness (1995) noted a high correlation between levels of contaminants in the diet of seabirds and levels in their feathers.

For ^{210}Po, little data on activity concentrations in birds exists in the literature. Gwynn *et al.* (2008) reported an inverse relationship between trophic level and muscle activity concentrations of ^{210}Po in five different Arctic seabird species. Elsewhere, Skwarzec & Fabisiak (2007) reported ^{210}Po activity concentrations for various tissues, including feathers, for 10 seabird species from the southern Baltic Sea. Activity concentrations of ^{210}Po in feathers from this study were stated as being between 0.47 and 5.70 Bq/kg (w.w.), with lower muscle activity concentrations observed in each case. However, all activity concentrations reported in this study were decay corrected only to the date of deposition and not the date the bird died as this was unknown or not recorded. Since digestion of samples took between 1 and 6 months in this study, significant decay of ^{210}Po would have occurred in these samples. Additionally, no consideration of the contribution from supported ^{210}Po from the in situ decay of ^{210}Pb is given in this study. While typically it is safe to assume negligible amounts of ^{210}Pb in muscle, the situation with regard to feathers is unclear.

In this study we aim to analyse feather and muscle samples from Arctic seabird species to determine whether any relationship exists between ^{210}Po activity concentrations in muscle (i.e. the body burden) and feathers from the same bird.

Materials and Methods

In this study, activity concentrations of ^{210}Po were determined in muscle and feathers of kittiwakes (*Rissa tridactyla*), little auks (*Alle alle*) and common eiders (*Somateria mollissima*) sampled in the high arctic Svalbard archipelago (76° to 81° North and 10° to 35° East). All 3 species migrate to Svalbard for the summer months, overwintering in Norway (eider), Greenland (little auk) or at sea (kittiwake). Eider feed predominantly on bivalves (Varpe, 2009), little auk are specialised planktonic feeders, feeding primarily on copepod species (Karnovsky *et al.*, 2003), whilst kittiwake typically have a mixed diet of various invertebrate species and small fish (Mehlum and Gabrielsen, 1993). Only data for female eider are presented, whereas data for little auk and kittiwake is based on male and female birds.

Tissue samples were taken from 2 examples of each seabird species in July 2008 from both the Kongsfjorden area and the Liefdefjorden area (Figure 2). The birds were shot opportunistically

for scientific purposes under license from the Governor on Svalbard using stainless steel ammunition.

Figure 2. Sampling sites for seabird species on Svalbard

All seabirds were stored frozen at -20 ºC from the time of collection until analysis. Muscle samples from all individual birds generally represent breast muscle only and were analysed as fresh weight. Primary flight feathers (n = 4 to 6) from kittiwakes and common eiders were removed from the bird in the field. For little auks, an entire wing was removed in the field, with primary and secondary flight feathers (n = 10 to 12) removed in the laboratory. All feathers were washed with acetone (x1) and distilled water (x3) twice to remove loosely adhering materials. Feathers were then dried overnight at 60 °C in a fan assisted oven. All feathers were cut into 3 segments; 1 segment representing the calamus (from base of feather to start of barbs) with the remainder cut into two equal lengths (Figure 3).
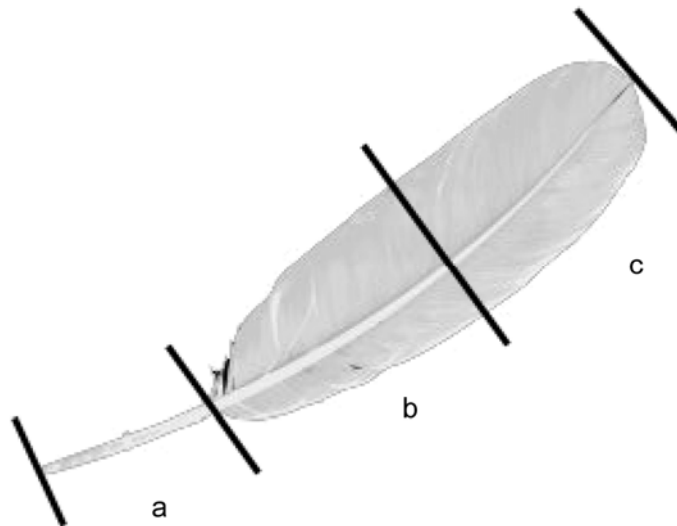
Figure 3. *Feathers were divided into 3 segments (part a, b and c) prior to analysis.*

For each individual bird, identical segments from each feather were pooled in order to provide sufficient sample mass for analysis. Po-210 was determined in muscle and feather samples by alpha spectrometry based on the method of Chen *et al*. (2003). Activity concentrations of ^{210}Pb in seabird muscle were assumed to be negligible, based on data from two previously analysed samples. Po-210 and ^{210}Pb in feather samples were determined by alpha spectrometry based on the method of Chen *et al*. (2003). Po-209 was used as a yield tracer for all determinations. Recovery yields were typically between 70 and 80%. NIST-4359 seaweed reference material was used as an internal control.

All activity concentrations were decay corrected to sampling dates. Contributions from unsupported and supported ^{210}Po were determined using Bateman's equation (Ivanovich and Harmon, 1992). Expressed errors (given at the 95% confidence level) are the standard accumulation of uncertainty sources for radiometric measurements. All muscle results are expressed as fresh weight, while feather results are expressed as dry weight.

Results and Discussion

From the seabirds sampled in 2008, common eider showed higher activity concentrations of ^{210}Po in muscle than was observed in kittiwake. This difference can probably be interpreted as a reflection of differences in diet between the two seabird species with recommended concentration factors for Po in molluscs, the preferred prey of common eider, one order of magnitude higher than the recommended concentration factor for fish, the preferred prey of kittiwake (IAEA, 2004).

Activity concentrations of ^{210}Po in little auk in 2008 were similar to those for kittiwake and lower than values reported for this species when sampled in the summer months in Svalbard in 2005, 2006 and 2007 (Gwynn *et al*., 2009). Previously, higher activity concentrations of ^{210}Po in muscle in little auk compared to kittiwake have been interpreted as resulting from a difference in the ability of prey groups of these seabirds to assimilate ^{210}Po (Gwynn *et al*., 2008); i.e. recommended concentration factors for Po in zooplankton, the preferred prey of little auk, are one order of magnitude higher than the recommended concentration factor for fish, the preferred prey of kittiwake (IAEA, 2004).

Seabird species and location	Muscle ^{210}Po (Bq/kg f.w.)	n	Feathers					
			^{210}Po (Bq/kg d.w.)			^{210}Pb (Bq/kg d.w.)		
			Part a	Part b	Part c	Part a	Part b	Part c
Common eider								
Kongsfjorden1	45.4 ±5.0	5	31.4 ±5.0	46.6 ±6.3	76.0 ±10.3	1.24 ±0.53	4.52 ±1.02	25.5 ±4.1
Kongsfjorden2	20.2 ±2.3	5	17.8 ±3.4	37.0 ±5.3	29.3 ±4.5	1.14 ±0.53	3.82 ±0.93	13.8 ±2.7
Liefdefjorden1	15.9 ±1.9	4	161.8 ±19.4	181.6 ±21.0	207.9 ±26.1	2.97 ±0.95	5.32 ±1.21	19.6 ±3.6
Liefdefjorden2	13.4 ±1.7	4	136.4±16.8	175.8 ±20.6	192.3 ±23.7	1.69 ±0.86	3.13 ±1.00	21.7 ±4.0
Little auk								
Kongsfjorden1	5.44 ±0.70	10	18.1 ±7.5	36.0 ±7.1	92.0 ±14.4	5.22 ±3.89	30.0 ±7.5	136.5 ±22.0
Kongsfjorden2	4.36 ±0.63	10	11.6 ±5.0	4.30 ±0.82	139.3 ±21.5	8.70 ±4.72	53.5 ±12.3	155.2 ±24.0
Liefdefjorden1	1.48 ±0.27	12	Sample lost	38.4 ±7.6	60.8 ±9.5	Sample lost	25.9 ±6.5	132.4 ±22.0
Liefdefjorden2	3.01 ±0.56	11	24.1 ±9.1	40.2 ±8.1	93.5 ±14.3	5.03 ±3.77	28.1 ±6.7	148.3 ±23.6
Kittiwake								
Kongsfjorden1	2.17 ±0.30	4	31.3 ±6.1	75.1 ±9.0	176.7 ±20.6	1.73 ±0.86	42.9 ±6.1	139.9 ±18.0
Kongsfjorden2	5.78 ±0.70	4	53.8 ±8.6	98.8 ±11.7	201.9 ±22.7	1.88 ±1.05	31.2 ±4.1	125.4 ±15.4
Liefdefjorden1	1.96 ±0.33	4	9.24 ±2.64	34.5 ±4.7	169.5 ±19.1	3.27 ±1.58	24.2 ±3.5	182.8 ±21.1
Liefdefjorden2	3.40 ±0.45	4	100.1 ±14.2	168.9 ±19.0	346.6 ±39.7	5.30 ±1.79	55.5 ±6.9	254.4 ±28.9

Table 5. Activity concentrations of ^{210}Po in muscle and ^{210}Po and ^{210}Pb in feathers from individual seabirds sampled in Kongsfjorden and Liefdefjorden.

That activity concentrations of ^{210}Po in muscle of little auk show large variations from year to year may indicate variations in prey (zooplankton) availability or even phytoplankton assemblages. Indeed, Stewart and Fisher (2003) demonstrated that assimilation efficiencies of ^{210}Po by copepods from different marine algal species could vary from 19% to 55%.

Feathers from all three seabird species show increasing activity concentrations of ^{210}Po and ^{210}Pb from the base to the tip of the feather (Figure 4), as has been observed in other studies with various metals (e.g. Goede, 1991). For common eider, ^{210}Po appears in excess of ^{210}Pb, with the ratio decreasing towards the tip of the feather, whereas for little auk and kittiwake, ^{210}Po/^{210}Pb ratios tend towards 1 (Figure 5).

Several factors make the interpretation of the observed activity concentrations of ^{210}Po and ^{210}Pb in feathers difficult. Sources of ^{210}Po and ^{210}Pb to feathers may arise from incorporation during feather formation, direct deposition from the environment and through the application of preen oil. Furthermore, once feathers are formed they are disconnected from the bird's blood system, so that feathers sampled in this study may comprise newly formed feathers or feathers of up to one year in age, as the species studied moult their feathers in the summer months. In the case of heavy metals, feathers that are exchanged first may exhibit higher concentrations than feathers that are exchanged last (Dauwe et al., 2003). To further confuse the situation, feathers and parts of feathers that have a higher degree of exposure can have receive higher levels of atmospheric deposition (Dauwe et al., 2003) while mechanically or sensory important feathers may receive greater preening attention than other feathers (Bartels et al., 1994). Therefore the influence and degree of external contamination as well as the degree of internal contamination may vary both by feather and with time.

That the feathers analysed were all from the wing, should ensure a similar exposure to environment, although the tips of the feathers may be exposed to a greater degree than the base of the feather, which may help explain the observed trends. Preen oil should be removed through washing with acetone, but as only flight feathers were analysed one may in any case assume a similar application of preen oil, although again, application may vary along the length of a particular feather.

Given the aforementioned issues, it is perhaps not surprising that no clear relationship could be discerned between activity concentrations of ^{210}Po in muscle with activity concentrations of ^{210}Po observed in feathers from the same bird when considering all three species. Indeed for common eider, higher activity concentrations of ^{210}Po were observed in muscle from seabirds sampled in Kongsfjorden compared to Liefdefjorden, with the reverse true for activity concentrations of ^{210}Po in feathers from the same individuals.

In the course of these investigations, the possible role of the preen gland and the application of preen oil to feathers was considered as a pathway for dietary ^{210}Po to be transferred to feathers. The preen gland has been shown to play a role in selenium excretion in birds (e.g. Goede and de Bruin, 1984) along with the liver and kidney. As an analogue of selenium, ^{210}Po may follow the same metabolic pathways as selenium in birds. Initial investigations reveal that activity concentrations of ^{210}Po in preen glands in eider and kittiwake from 2008 show similar trends to that observed in muscle.

As washing of the feathers with acetone, removed between 40% and 60% of the ^{210}Po on the feather, it is possible that at least part of the ^{210}Po removed was associated with preen oil. These observations, open up the possibility of using preen oil as an indicator for body burdens of ^{210}Po. As it is possible to obtain samples of preen oil from live birds without the need for destroying the bird in question, preen oil may
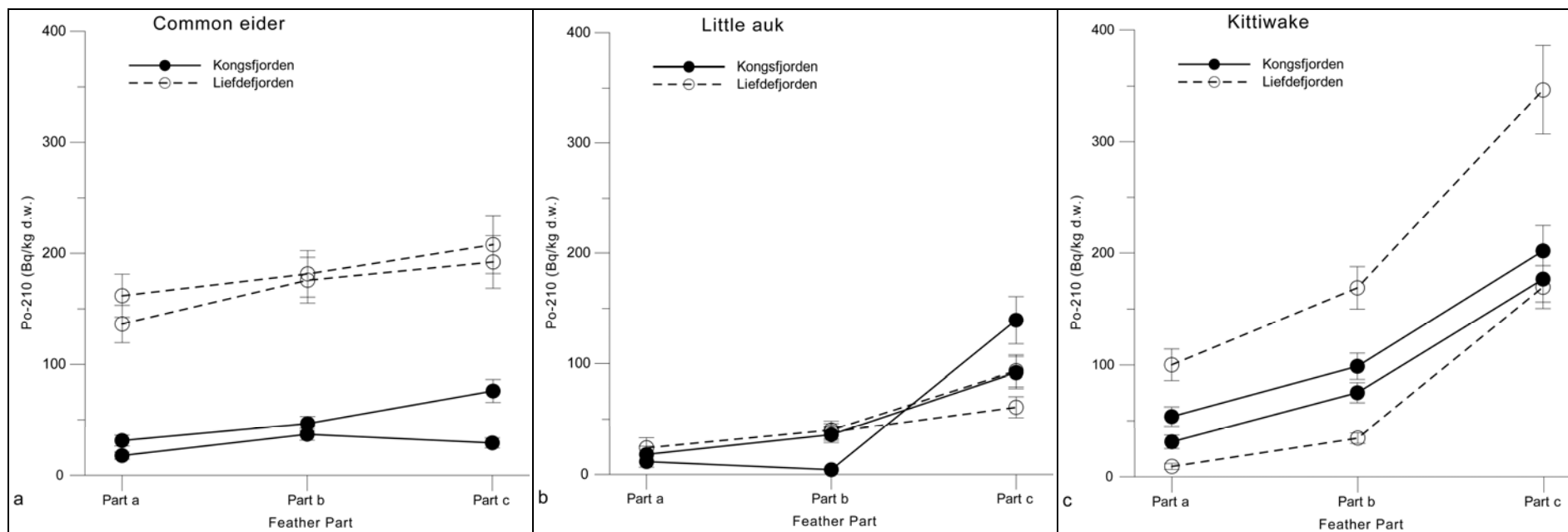
***Figure 4.** Activity concentrations of ^{210}Po in sections of pooled primary flight feathers from individual seabirds sampled in Kongsfjorden and Liefdefjorden; a) common eider, b) little auk and c) kittiwake.*

Figure 5. *Activity ratios of ^{210}Po /^{210}Pb in sections of pooled primary flight feathers from individual seabirds sampled in Kongsfjorden and Liefdefjorden; a) common eider, b) little auk and c) kittiwake.*
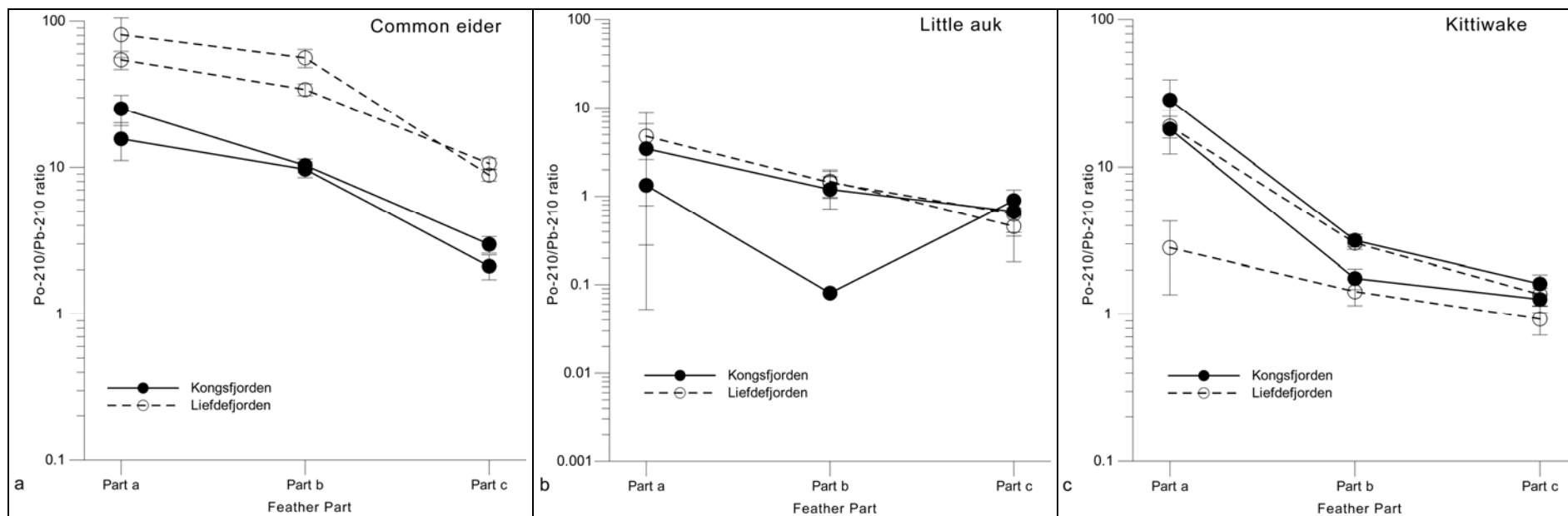
| Species | Location | ^{210}Po Bq/kg f.w. | |
		Muscle	Preen Gland
Common Eider	Kongsfjorden1	45.4 ±5.0	60.6 ±8.4
	Kongsfjorden2	20.2 ±2.3	17.7 ±3.1
	Liefdefjorden1	15.9 ±1.9	10.4 ±2.1
	Liefdefjorden2	13.4 ±1.7	25.9 ±4.3
Kittiwake	Kongsfjorden1	2.2 ±0.3	3.5 ±2.8
	Kongsfjorden2	5.8 ±0.7	15.3 ±4.7
	Liefdefjorden1	2.0 ±0.3	3.6 ±2.8
	Liefdefjorden2	3.4 ±0.5	4.8 ±3.1

Table 6. *Activity concentrations of ^{210}Po in muscle and preen glands from individual seabirds sampled in Kongsfjorden and Liefdefjorden.*

offer a non-destructive method to monitor body burdens of ^{210}Po. However, typically only very small amounts (mg quantities) of preen oil may be sampled, depending on the size of the bird in question, which may cause some analytical problems.

Conclusions

Given the varied and complex issues that may lead to the observed activity concentrations of ^{210}Po on feathers at any particular time, feathers appear not to be suitable as possible indicators for ^{210}Po body burdens in seabirds. In particular, the uncertainty over the age of the feathers (i.e. when they were formed and removed from the blood supply), raises the most concern due to the relatively short half life of ^{210}Po and the in-growth of supported ^{210}Po from ^{210}Pb present in the feathers. Selective removal of feathers and analysis of new replacement feathers may circumvent this problem, but issues concerning the degree of external contamination would still need to be resolved. However, the observations of ^{210}Po in preen oil raises the possibility of utilising preen oil as an indicator of body burdens of ^{210}Po. However, further study is required to fully understand the biological fate of ^{210}Po in birds and to qualify what role the preen gland may play in the excretion of dietary ^{210}Po.

Overall Conclusions

Human hair certainly reflects the intake of polonium and the concentration in hair. The activity concentration in hair is about 100 times the daily intake. Food intake is the major source and not external contamination, smoking or radon inhalation. The mechanism is probably due to the high sulphur content in hair and sulphide bindings with ^{210}Po. However, there is an uncertainty concerning the role of ^{210}Pb in hair and any subsequent the *in vivo* in growth of ^{210}Po.

Studies on the activity ratio ^{210}Po/^{210}Pb are of interest. Especially along the hair if it is long. Considering the growth rate of hair (1 cm per month) this ratio would change with the age of the part of the hair.

Further studies on the relation between ^{210}Po in hair and feathers in different organs, such as liver and kidney, should be undertaken.

Seasonal variations in animals, due to change in food habits, should be investigated.

Hair from seals and reindeers should be analysed since we know they have high intake of Po-210, with high concentrations reported in the liver and kidneys.

Sequential extraction techniques applied to hair would show if ^{210}Po is associated with sulphides.

Assuming external contamination can be removed properly, musk ox hair could be a possible indicator of ^{210}Po body burden. The relatively high ^{210}Po/^{210}Pb ratio of about 10 would indicate that ^{210}Pb is not a major issue, although this observation is only based on one herd. Mechanisms for incorporation in hair and the correlation to changes in body distribution remain to be investigated. With respect to hair from horses, the nearly even ^{210}Po concentration along the length of hair needs clarification. Studies should be performed in the autumn when horses have been acquired food from the outdoor environment

Due to the number of apparent complicating factors it seems unlikely that feathers could be used routinely as an indicator matrix for the intake of ^{210}Po. Although selective removal of feathers and analysis of new replacement feathers may circumvent the problem of not knowing when a particular feather was isolated from the bird's circulatory system, issues concerning the degree of external contamination and the contribution from ^{210}Pb would still need to be resolved.

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Title	Hair and feathers as indicator of internal contamination of ^{210}Po and ^{210}Pb
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ISBN	978-87-7893-287-7
Date	March 2010
Project	NKS-R / HAIRPOL
No. of pages	28
No. of tables	6
No. of illustrations	5
No. of references	71

Abstract   The activities of the NKS-B HAIRPOL project is summarised in this report. The objective was to investigate if hair and feathers were suitable matrices for the estimation of the intake of ^{210}Po. Human hair from people of different sex and age was analysed for ^{210}Po showing concentrations between 0.4 to 11 Bq/kg dry weight. Samples from horses, mane, fur and tail showed concentration from 6 to 17 Bq/kg with no significant difference between the different sample types. Musk ox from Greenland showed much higher concentrations since the animal has to graze a large surface. In fur the concentration was 260 Bq/kg. A considerable fraction of the total ^{210}Po in this animal is contained in the hair. Also different organs were analysed and the highest concentration was found in kidney, 2 700 Bq/kg. The ^{210}Pb concentration in hair was estimated to about 20 Bq/kg. Three different seabirds from Svalbard were analysed. Feathers from all three seabird species show increasing activity concentrations of ^{210}Po and ^{210}Pb from the base to the tip of the feather, but it was difficult to relate feather concentrations to muscle concentrations due to a number of complicating factors.