TRANSFER OF PERSISTENT CHLORINATED AND BROMINATED COMPOUNDS FROM ADULT SEMI-DOMESTICATED REINDEER (RANGIFER TARANDUS TARANDUS L.) TO FETUS AND CALF

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Introduction

Levels of Persistent Organic Pollutants (POPs) bioaccumulating to organisms are net result of many different factors: 1) feed, 2) uptake, 3) distribution, 4) metabolism, 5) excretion, and 6) stability. Thus, there may be inter-individual differences between POPs concentrations. Several biological characteristics, like species differences in lipid distribution and lipid dynamics, physiological condition, age, sex, and reproductive status, may affect to these functions, too. Also the structure and halogenation pattern of compounds affect their physicochemical and metabolic properties[1,2].

Fatty and energy rich milk of reindeer is the main food of calves for their neonatal life[3]. Intestinal functions and lipid-metabolism of calves are similar to those in monogastric animals, until the microbial function of rumen is on the level allowing a digestion of plant derived carbohydrates[4]. A reindeer calf can therefore easily utilize incoming lipids. However, also lipophilic contaminants may easily absorb into the calf. When POPs contaminated fat sources are used for different metabolic processes of animals, contaminants achieve a new steady-state situation between the lipids in blood and tissues[5]. This may result in transfer of compounds from the reindeer hind to its fetus. Reindeer fetuses have been observed to have measurable concentrations of POPs in their tissues, indicating placental transfer of these compounds[6]. POPs have been analyzed also from reindeer milk, reindeer calves, and adult reindeer[6,7,8]. Due to the high fat content of reindeer milk lactational transfer of lipophilic POPs is also likely to be significant.

In this study PCDD/Fs, PCBs and PBDEs were analyzed from milk and tissues of reindeer fed with different feed and lichen. The aim of the study was to study the transfer of POPs from feed to reindeer and from reindeer hind to fetus, milk and calf.

Materials and methods

Two pregnant reindeer hinds were captured and kept in the pounds over a period of 4.5 months in an experimental zoo of the University of Oulu, Finland in April-August 2010. At first both of them were fed on the same diet; free hay, lichen and reindeer feed. Hinds were individualized to hind #1 and #2. About two weeks after calving, control milk samples were taken from both hinds and the hind-calf-pairs were placed into the separate pounds for different diets: Pair #1 was given lichen and reindeer feed, and pair #2 green plants and reindeer feed, but no lichen. Milk samples were collected in June about six weeks from calving and at the end of August 2010, after which the hinds and their calves (age about 3.5 months) were slaughtered.

Secondly, two randomly assigned pregnant reindeer hinds were slaughtered in May in 2010. These animals were fed with lichen and reindeer feed according to normal herding practise. Hinds were individualized as hind #3 and hind #4. Unborn fetuses (gestation day 200; reindeer gestation is on average 224 days) of these hinds were dissected out with the placentas and individualized to fetus #3 and fetus #4. In addition, formerly in February in 2008 slaughtered reindeer hind (individualized to hind #5) and its fetus (#5) (gestation day 100) were included in the study. They were gathered from a more southern herd than the other reindeer in this study. However, the diet (hay, lichen and reindeer feed) was the same.

Liver and muscle samples from rump, shoulder and rib/chest were taken from hinds and fetuses using clean instruments and stored in polyethylene bags. Blood from the jugular vein was sampled from hinds #3 and #4 to the glass bottles. All samples were stored at temperature of -20°C until the analyses. Placentas from the hind #3 and #4 were taken and stored at -20°C until the preparation before the analyses. The inner lumps of uterine cones were separated for the analyses. The placenta of hind #5 was analysed as complete with uterus. Milk samples (30 ml in each) were taken from hinds #1 and #2 to glass bottles after Oxytocin injection (10 IU, i.m.). The samples were stored at -20°C until the analyses.

After homogenization solid samples were freeze dried and fat was extracted with ethanol and toluene using Accelerated Solvent Extractor (ASE 300) equipment. Milk samples were extracted with diethylether-hexane. The extraction solvent was evaporated and the samples were transferred into hexane, from which the fat content was measured gravimetrically. The samples were defatted on an acidic silica column and purified and fractionated on alumina and carbon columns. High resolution gas chromatography - high resolution mass spectrometry (HRGC/HRMS) method was used to analyze PCDD/Fs, PCBs and PBDEs. The analyzed PCDD/F congeners included 17 toxic 2378- substituted congeners. 37 PCB congeners included 12 dioxin-like PCBs. PBDEs consisted of congeners BDE-28, -75, -71, -47, -66, -77, -100, -119, -99, -85, -154, -153, -138, -183, and -209. Analyzes were performed at the National Institute for Health and Welfare (THL), the Unit of Chemical Exposure. The Unit is an accredited testing laboratory (by FINAS, No T077) according to EN ISO/IEC 17025 requirements. POP concentrations in reindeer tissues, milk and feed are reported as fat based and those in lichen as dry weight based levels. WHO-PCDD/F- and WHO-PCB-TEQs (WHO, 2005) are reported as upper bound concentrations (EU requirement) for the food safety purpose. PBDEs are reported as fat based upper bound concentrations.

Results and discussion:
Figure 1 shows the fat based WHO-PCDD/F- and WHO-PCB-TEQ concentrations in reindeer tissue and milk samples, and in reindeer feed and lichen.

Both WHO-PCDD/F-TEQ and WHO-PCB-TEQ concentrations were higher in muscle and liver of reindeer calves than in their corresponding hinds. Also the levels in milk of hinds (especially in hind #1) were higher than in their muscle. These findings suggest that PCDD/Fs and especially dioxin-like PCBs are transferred efficiently into the calf via lactation. This is in agreement with earlier findings indicating that lactational transfer of dioxin-like compounds is quantitatively much more important than placental transfer[9].

WHO-PCDD/F-TEQs and WHO-PCB-TEQs were lower in the muscle and liver of fetuses (#3 and 4) than in their corresponding hinds. In adult animals, high relative concentrations of dioxin-like compounds in liver is considered to result from their high binding affinity to cytochrome P450 (CYP) 1A2 expressed in the liver[9,10]. However, it has been shown that CYP1A2 is not detectable in fetal human or rat liver[11,12], and therefore the likely explanation for low PCDD/Fs and PCB levels in the liver of reindeer fetuses is the lack of CYP1A2.

In hinds #3 and #4, highest PCDD/F and PCB concentrations were found in liver followed by blood, placenta and muscle. Hind-fetus-pair #5 deviated from the pairs #3 and #4 as the WHO-PCDD/F-TEQs were clearly higher in the fetus (analyzed as a whole fetus sample) and in placenta + uterus than in the muscle of the hind, although the sampled fetus was younger (about 100 days) than fetuses # 3 and #4 (about 200 days).

WHO-PCDD/F-and WHO-PCB-TEQ concentrations in reindeer-calf pair #1 (lichen diet) show that these compounds are transferred from lichen to the tissues and milk of reindeer hind resulting in high concentrations in the muscle and especially in the liver of calf.

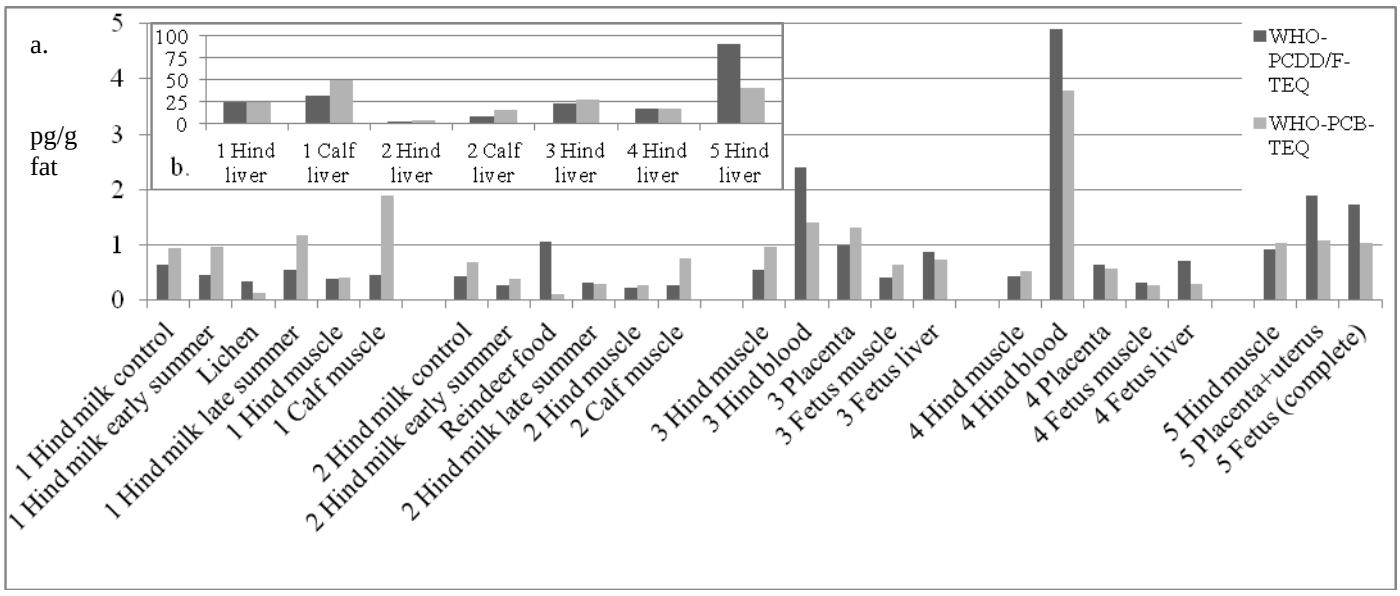

Figure 1. WHO-PCDD/F- and PCB-TEQs (pg/g fat, except lichen pg/g dry weight) in a. reindeer milk, tissue, reindeer feed and lichen, and b. in reindeer liver samples.

Figure 2 shows PBDE sum concentrations in the studied samples. In reindeer muscle samples calves #1 and #2 had lower PBDE levels than their corresponding hinds). PBDE sum (23 ng/g fat) was clearly highest in fetus #5. Fetus #5 had clearly more PBDEs than the corresponding placenta and uterus (3.4 ng/g fat), and also the hind's muscle (2.0 ng/g fat) and liver (4.1 ng/g fat). Also other fetuses (fetus #3: 2.9 ng/g fat in muscle, 0.7 ng/g fat in liver, and fetus #4: 6.4 ng/g fat in muscle, 1.2 ng/g fat in liver) contained generally more PBDEs than their corresponding placentas (placenta #3: 0.5 ng/g fat, placenta #4: 1.3 ng/g fat), but clearly less than hind's blood. This suggests that PBDEs are transported to some extent through the placenta..

In reindeer liver samples the highest PBDE sum concentration was detected in hind #5 (4.1 ng/g fat). In this sample a proportion of BDE-209 was as high as 96%. It is worth to notice that in hinds, foetuses and calfs PBDE sum concentrations were generally lower in liver than in muscle. An exception was hind #5, whose liver contained more PBDEs than the muscle. An effective transport of PBDEs from reindeer nutriment to reindeer muscle has been supposed in earlier studies, too.[6]

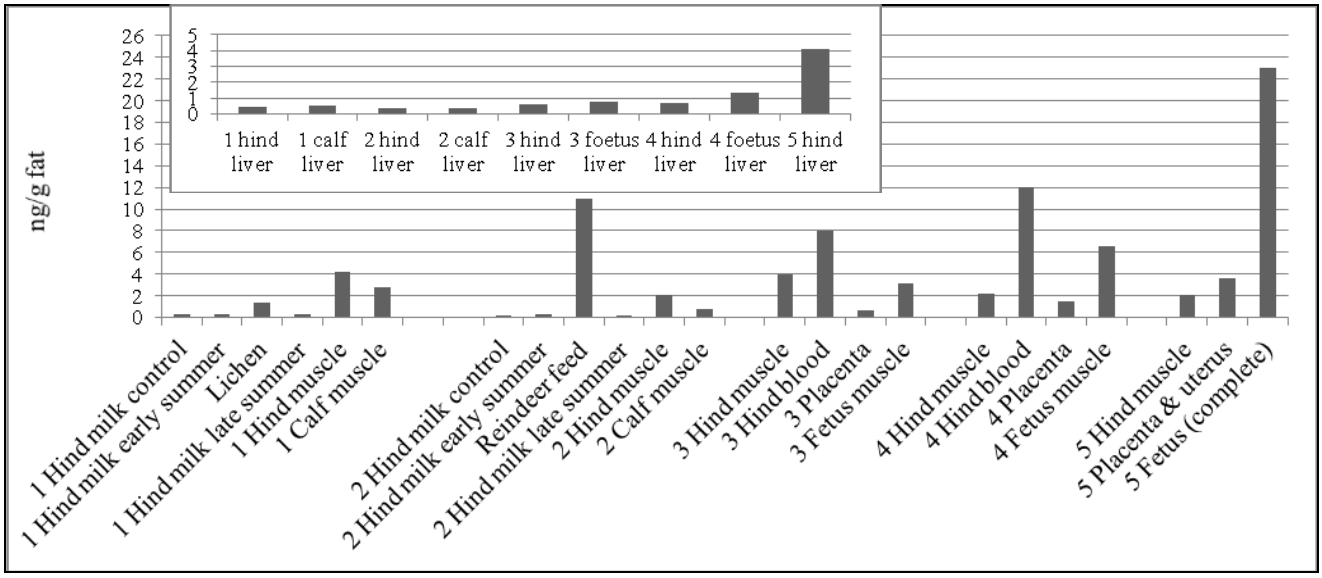

Figure 2. PBDE sum concentrations (ng/g fat, except lichen ng/g dry weight) in the studied samples.

In conclusion, concentrations of 17 PCDD/Fs, 37 PCBs (including 12 dioxin-like PCBs), and 15 PBDEs were analyzed in reindeer muscle, liver and milk, as well as in reindeer feed and lichen. In the muscle of calves, PCBs were the dominating compounds in calves. PCDD/Fs and PCBs were higher in muscle and liver of calves than in their hinds, but they were lower in the fetuses than in their corresponding hinds. Opposite to PCDD/Fs and PCBs, calves had lower muscle PBDE levels than their corresponding hinds.

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