EFFECTS OF *IN UTERO* AND LACTATIONAL EXPOSURE TO PCB 118 AND PCB 153 ON BONE TISSUE IN LAMBS (*Ovies aries*)

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Abstract
In the last decade, several studies in experimental as well as free-ranging animals have shown that the bone tissue is a target for a number of persistent organic pollutants. The aim of the present study was to describe effects of low dose exposure during gestation and lactation to the mono-ortho PCB 118 and the di-ortho PCB 153 on bone composition and dimensions in male and female sheep offspring. The ewes were dosed orally with PCB 118 (49 µg/kg body weight/day) and PCB 153 (98 µg/kg body weight/day) throughout gestation. Bone composition and dimensions were determined on the femoral bones of the offspring by peripheral quantitative computed tomography (pQCT) and a three-point bending test was conducted to determine the strength of the bones. The result of this study indicate a disturbance of the developing bone tissue in female and male sheep offspring after *in utero* and lactational exposure to PCB 118 but not PCB 153.

Introduction
Endocrine disrupting compounds (EDCs) such as polychlorinated biphenyls (PCBs) have been shown to cause alterations of bone tissue in mammals and such effects may have wide implications for all exposed species including humans. Previous experimental findings in goats [1], rodents [2, 3] as well as findings in free ranging animal [4-6] supports the hypothesis that EDCs are able to alter bone tissue homeostasis. The present study is part of a larger project on "Endocrine disrupters: Risk assessment for food quality and animal health" with focus on effects on reproductive endpoints, immune function and behaviour in the offspring.

Aim
The aim of the present study was to investigate if low levels of the mono-ortho PCB 118 and the di-ortho PCB 153 cause changes in bone composition and dimensions in male and female sheep offspring, Norwegian breed (Dala) lambs *(Ovies aries)*, exposed *in utero* and during lactation.

Animals, Materials and Methods
In short, fifty adult female sheep were housed at the University farm of the Norwegian University of Life Sciences in Ås, Norway. The ewes were mated on a natural oestrous after being synchronised in the previous oestrus. The ewes were allocated into three groups (2 groups of 17 and one of 16 ewes) using block randomization. The ewes were orally administered either PCB 153 (98 µg/kg body weight/day), PCB 118 (49 µg/kg body weight/day), or corn oil (0.1 ml/kg body weight/day) 3 times a week throughout gestation until delivery that took place 146 days later. The pregnant does in the PCB 118 and PCB 153 treatment groups and the control animals gave birth to 18, 21 and 21 female and male lambs, respectively. The lambs were euthanized at 21 weeks of age. Due to erroneous handling of animals, single events of cross-contamination occurred resulting in a mixed exposure scenario, of both PCB 118 and PCB 153 in all groups instead of the planned exposure to single compounds. Therefore, a chemical analysis of PCB 118 and PCB 153 of the adipose tissue at 60 days of age in the lambs was performed. The material in this study on bones has thus been divided for both PCB 118 and PCB 153 in two groups; one considered to be "low" exposed and one to be "high" exposed (table 1).

Table 1. *The limits (ng/g) in the group "low" and "high" exposed animals of PCB 118 and PCB 153 in adipose tissue in the lambs at approximately 21 weeks of age. The number of the animals in each group is shown in the parenthesis. a = range 16.7-73.7, b = range 724-14951, c = range 528-917, d = range 1079-100956*

	"Low" exposed	"High" exposed
PCB 118 (ng/g)	x < 100 (18)[a]	x > 100 (42)[b]
PCB 153 (ng/g)	x < 1000 (12)[c]	x > 1000 (48)[d]

Bone composition and dimensions were determined at three different locations of the femoral bones of the offspring by peripheral quantitative computed tomography (pQCT, (Stratec XCT 960A with software version 5.20; Norland StratecMedizintechnik, Pforzheim, Germany). The measure points were placed at a distance of 2, 35 and 50 % of the total bone length distal to trochlea ossis femori in the metaphysis at the distal end of the bone. For the analysis trabecular BMD (mg/cm3), trabecular CSA (cross-sectional area) (mm2), trabecular BMD (mg/mm), total BMD (mg/cm3), total CSA (mm2), total BMC (mg/mm) and periosteal circumference (mm) cortical BMD (mg/cm3), cortical CSA (mm2), cortical BMC (mg/mm), total BMD (mg/cm3), total CSA (mm2), marrow cavity (mm2), cortical thickness (mm), periosteal circumference (mm), endosteal circumference (mm), polar moment of inertia (mm4) and moment of resistance (mm3)were used. In addition, a three-point bending test (servo hydraulic material testing machine MTS miniBionix, Minneapolis, MN, USA) was conducted to determine the strength of the bones, with the load being applied to the mid-diaphyseal measure point (50 %). Displacement (mm) and load (N) were recorded and energy absorption (surface under the curve, N*mm), stiffness (slope of the curve, N/mm) and max stiffness were calculated from the load-displacement curves.
Statistical analyses.
The results obtained were evaluated by one-way ANOVA. Differences were considered significant at $p < 0.05$.

Results and Discussion
The results of this study indicate disturbance of developing bone tissue in female and male sheep offspring after *in utero* and lactational exposure to PCB 118 but not PCB 153.
The coefficient of variation (CV) was low (0.17 - 4.18 %), and these results are comparable with a recent study on goat [1]. There were no significant differences in the variables at the metaphyseal measure point for neither PCB153 nor PCB118 between high and low exposed lambs. However when using the pQCT method on the diaphysis an increased polar moment of inertia and an increased moment of resistance was found in PCB 118 exposed lambs (Table 2) but not in PCB 153 exposed lambs. The biomechanical test (three point bending), however, showed no significant effects on mechanical characteristics at the diaphysis.

Table 2. *Effects of PCB 118 exposure on diaphysis characteristics in 60 days old sheep. Results (mean value ± SEM and p-value) from analysis with pQCT of femur. The number of the animals in each group is shown in parenthesis.*

Mid-diaphysis, 50 %	"Low" exposure (18)			"High" exposure (42)			p-value
Cortical BMD (mg/cm^3)	1165.5	±	7.3	1165.8	±	5.6	1.0
Cortical CSA (mm^2)	112.7	±	5.2	115.2	±	3.2	0.7
Cortical BMC	131.6	±	6.4	134.7	±	4.0	0.7
Cortical thickness (mm)	2.5	±	0.1	2.5	±	0.1	1.0
Total BMD (mg/cm^3)	665.3	±	17.8	661.9	±	10.4	0.9
Total CSA (mm^2)	227.6	±	7.7	236.1	±	5.5	0.4
Total BMC (mg/mm)	151.8	±	6.8	156.3	±	4.3	0.6
Marrow cavity (mm^2)	114.9	±	4.6	120.8	±	3.2	0.3
Periosteal circumference (mm)	53.3	±	0.9	54.3	±	0.6	0.4
Endosteal circumference (mm)	37.9	±	0.8	38.8	±	0.5	0.3
Polar moment of inertia (mm^4)	7903.2	±	568.8	9449.5	±	411.6	0.04
Moment of resistance (mm^3)	484.9	±	26.5	550.8	±	18.4	0.05

Acknowledgements
This study was partly supported by grant 216/2006/1306 from the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (Project: Bone tissue as a target for endocrine disrupting persistent organic pollutants) and by grant 127534/720 from the Norwegian Research Council. Frida Broman is thanked for her contributions to the laboratory work related to the bone analysis.

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